INTERMEDIARY METABOLISM

Carbohydrate Metabolism

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Digestion of Carbohydrates

Carbohydrates form the staple food stuffs of man especially in the poor countries of the world and constitute one of the primary sources of calorific energy in the form of cereals. Most of the carbohydrate in cereal grains is in the form of insoluble starch granules, which are rendered partially soluble by cooking. Sucrose and milk sugar lactose is also an important constituent of the carbohydrate diet.

The two different polysaccharides in starch, the straight chain amylose consisting of α-D-glucose units in 1,4-linkage and the branched-chain amylopectin consisting of 1,4-linked α-D-glucose units interlinked with α,1,6-linkages are rapidly by the salivary and pancreatic amylases. The α-1,6-linkages in the small-chain oligosaccharides are hydrolyzed by oligo 1,6-glucosidase, maltose by maltase specific disaccharides, lactase (β-galactosidase) and sucrose or invertase are present within the brush border of the intestinal mucosal cells and bring about intracellular hydrolysis of these disaccharides. The final products of the digestive action of the carbohydrates in the human beings are glucose, fructose and galactose.

Fermentation and Respiration

We now begin consideration of the multienzyme systems that catalyze the degradation of the fuel molecules and the recovery of part of their chemical energy as ATP. We shall first examine glycolysis, the anaerobic degradation of glucose to yield lactic acid. Glycolysis is one of the several catabolic pathways known generically as "anaerobic fermentation" by which many organisms extract chemical energy from various organic fuels in the absence of molecular oxygen.

Oxidation – Reduction

All heterotrophic organisms ultimately obtain their energy from oxidation-reduction reactions i.e. reaction in which electrons are transferred from one compound, the electron donor or "reducing agent" to an electron acceptor or "oxidizing agent". "Aerobic" organisms obtain most of their energy from "respiration" defined as the oxidation of organic fuels by molecular oxygen, oxygen thus serves as the final electron acceptor in respiration.

Anaerobic hetrotrophs also obtain most of their energy from oxidation-reduction reactions but in this case electrons pass from one organic intermediate of sugar breakdown, the electron donor to some other organic intermediate in the fermentation process which serves as the electron acceptor. In anaerobic fermentation processes however, there is no net oxidation of the fuel.

Anaerobic and Aerobic

It is conceptually useful to separate the "Intermediary metabolism**"** of carbohydrates into the "Anaerobic process" and the "Aerobic process". Anaerobic glycolysis encompasses the conversion of glucose to ethanol plus carbon dioxide in the alcoholic fermentation by yeast and secondly the conversion of glucose or a glucose unit (provided by glycogen) to lactic acid in the muscle of animals. Neither of these processes require oxygen, and both can take place in the absence of oxygen and provide the requisite cellular energy under completely anaerobic conditions. The anaerobic process by which glucose is degraded into two molecules of lactic acid is known as glycolysis. This process, which occurs in most microorganisms, as well as in most of the cells of higher plants and animals, represents a simple yet elegant mechanism for recovering some of the energy in the glucose molecule as ATP.

Polysaccharides as well as numerous other carbohydrates, that serve as fuel molecules are also degraded by the glycolytic sequence of reactions after they have been converted into a compound in that sequence. Alcoholic fermentation by which yeast converts glucose into ethanol and CO_2, is identical to glycolysis, except for two reactions at the end of the glycolytic sequence.

Discovery

The sequence of reactions of glycolysis and alcoholic fermentation as it exists today was developed by the pioneers in Enzymology. Buchner, in Germany (1897) obtained a cell free extract of yeast which fermented sugars to CO_2 and ethanol shortly, thereafter Harden and Young in England utilized phosphorylated derivatives of the sugars in alcoholic fermentation. A list of pioneers in the field who were the architects of the scheme of the pathway includes Embden, Meyerhof, Robison, Neuberg, Coris, Lipmann, Parnas, and Warburg. Most higher organisms have retained the capacity for anaerobic degradation of glucose to lactate. Moreover in most animals, glycolysis serves as an important emergency mechanism capable of yielding energy for short periods when oxygen is not available.

Stages of glycolysis

Glycolysis is catalyzed by the consecutive action of a group of eleven enzymes, most of which have been crystallized and thoroughly studied. Since they are easily extracted in soluble form from cells, they are believed to be localized in the soluble portion of the cytoplasm. It is also thought that the individual enzymes catalyzing the steps in glycolysis have no physical dependence on each other that is they appear, not to be associated with a stable multienzyme complex.

First Stage of Glycolysis

Enzymatic steps in the first stages of glycolysis:

1. Phosphorylation of D-glucose by ATP mediated by hexokinases (HK)

This is the first of the two priming steps of glycolysis in which ATP is utilized. In it the neutral D-glucose molecule is prepared for the subsequent enzymatic steps by its phosphorylation to a negatively charged molecule at the expense of ATP. There is relatively little free D-glucose in cells, most of the intracellular glucose exists in the phosphorylated form. The phosphorylation of glucose at the 6-position by ATP to yield D-glucose-6-phosphate is catalyzed by two types of enzyme, hexokinase and glucokinase which differ in their sugar specificity and affinity for D-glucose. The reaction for both enzymes is:

$$\text{ATP} + \alpha\text{-D-glucose} \xrightarrow{Mg^{++}} \text{ADP} + \alpha\text{-D glucose 6-p} \quad \text{(- vely charged)}$$

$[\Delta G^{o} = -4 \text{ Kcals mol}^{-1}]$.

Distribution and specificity

Hexokinase (HK) is the more widely distributed and is the enzyme normally employed by most cells. It catalyzes the phosphorylation not only of D-glucose but also of many other hexoses and hexose derivatives including D-fructose, D-mannose and D-glucosamine. It has a higher affinity for aldohexoses and their ketohexoses. Hexokinases are found in yeast and bacteria and in many animal and plant tissues. The enzyme has been crystallized from yeast and has a Mol. Wt. of 111,000. The yeast enzyme can be dissociated into two subunits, two-polypeptide chains of Mol.

Wt. 55,000 each containing an active site. Liver contains a fructokinase that produces fructose 1-phosphate rather than the 6-ester.

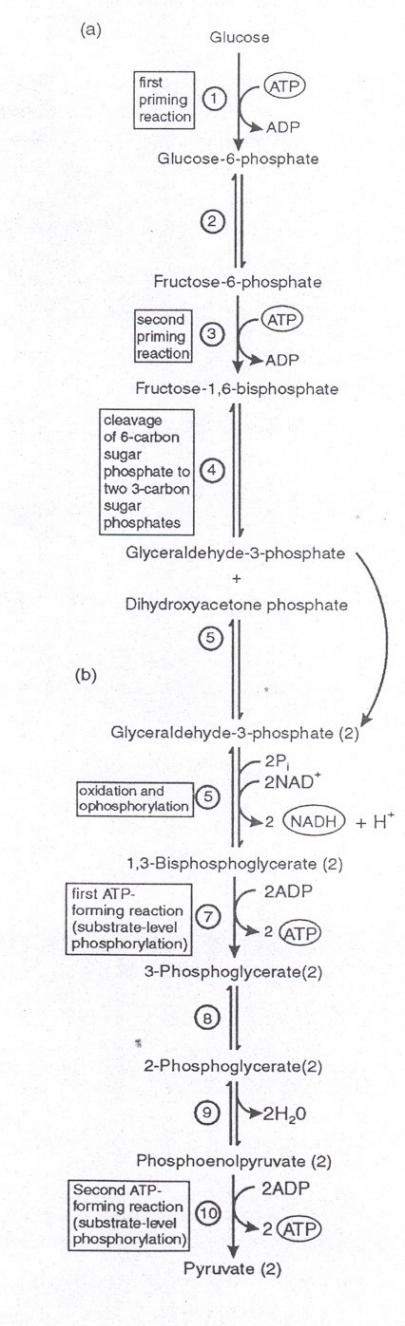


Figure 1: The two phases of Glycolysis. For each molecule of glucose that passes through the preparatory phase: (a) two molecules of glyceraldehydes are formed; both pass through the payoff phase; (b) pyruvate is the end product of the second phase under aerobic conditions, but under anaerobic conditions pyruvate is reduced to lactate to regenerate NAD^+. For each glucose molecule, two ATP are consumed in the preparatory phase, giving a net yield of two molecules of ATP per one of glucose converted to pyruvate.

Isoenzymes (Regulation)

The hexokinase of animal tissues occurs in the form of three isoenzymes that differ in their affinity for glucose. The hexokinase of animal tissues is a regulatory enzyme. It is inhibited by its own product glucose 6-phosphate. Whenever the cell has a high concentration of glucose 6-phosphate and requires no more for its energy demands, hexokinase is inhibited, thus preventing the formation of further G-6-p.

The second type of glucose phosphorylating enzyme, glucokinase (GK), phosphorylates only D-glucose and does not act on other hexoses. Glucokinase has a much higher Km for D-glucose (Km = 10mM) and thus requires a vary high glucose concentration to become fully active, than hexokinase (Km = 100mM). It differs from hexokinase in another respect, it is not inhibited by G6p GK present in liver, where it predominates over hexokinase but it is absent in muscle. It normally comes into play when the blood glucose 01 concentration is temporarily high, as it is following a meal rich in sugar, however this enzyme is deficient in patients suffering from diabetes mellitus in which there is a high blood sugar concentration as a consequence of failure to secrete the pancreatic hormone insulin.

Metal requirement

Both HK and GK require a divalent cation (Mg^{++} or Mn^{++}), which first combines with ATP to form the true substrate, Mg-ATP^{2-} or Mn-ATP^{2-}. Hexokinase is inhibited by certain sulfhydryl reagents. The phosphorylation of glucose by either hexokinase or GK is not reversible under intracellular conditions.

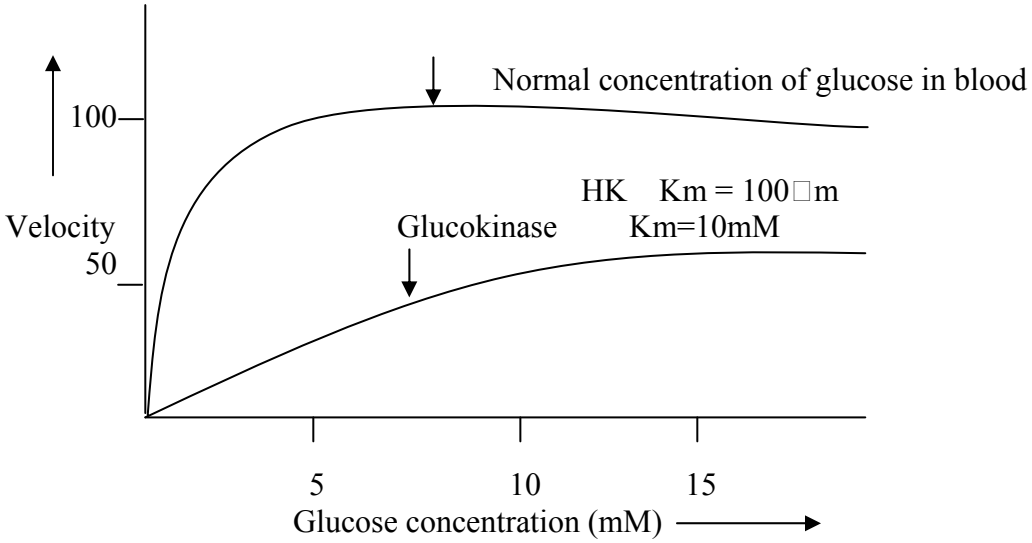


Figure 2: Level of glucose and glucokinase in blood. At normal concentration of blood glucose (5mM) HK is fully saturated. When blood glucose concentration becomes higher GK is more active.

2. Conversion of glucose 6-phosphate to fructose 6-phosphate by phosphohexoisomerase (PGI)

The next reaction in glycolysis is the isomerization of glucose 6 phosphate catalyzed by phosphoglucoisomerase (PGI). The enzyme which has been extensively purified from skeletal muscle and crystallized from yeast does not require a cofactor. Keq of the reaction from left to

right is approximately 0.5. The human skeletal enzyme has a molecular weight of 130,000. It can be dissociated into two subunits of 61,000.

G-6-p ⟶ F-6-p ⟶ Mannose-6-p

An enzyme isomerase that catalyzes the conversion of mannose 6-phosphate to fructose 6-phosphate has been isolated from rabbit muscle.

3. Phosphorylation of D-fructose 6-phosphate to fructose 1,6-diphosphate, phosphofructokinase or fructose 6-phosphate kinase

In this the second of the two "priming reactions" of glycolysis, a second molecule of ATP is required to phosphorylate fructose 6-p in the 1-position to yield fructose 1,6-diphosphate by the action of 6-phosphofructokinase.

D-Fructose-6-p + ATP ⟶ ADP + D-Fructose 1,6-di-p

$\triangle G^{o} = -3.4$ Kcal mol^{-1}

Mg^{++} is required presumably because the true substrate is $Mg\text{-}ATP^{-2}$. Although f-6-p is the specific phosphate acceptor in the reaction UTP and ITP may replace ATP as phosphate donors. Phosphofructokinase is an allosteric enzyme; the phosphorylation of fructose 6-phosphate is the most important control point in the glycolytic sequence. Like many allosteric enzymes it has a high Mol. Wt. 380,000, contains a number of subunits, and shows a complex dependence of its reaction velocity on the concentration of its substrates. PFK has multiple allosteric affectors (modulators).

PFK is inhibited by high concentration of ATP, citrate and long chain fatty acids, but is stimulated by ADP, AMP and (NH_4) ions. Therefore whenever the cell has a high concentration of ATP, or whenever other fuels such as fatty acids or citrate are available, PFK is inhibited and turns off glycolysis. Conversely when the ATP concentration is low, and AMP and ADP thus predominate or whenever the concentration of other fuels such as citrate or fatty acids is low PFK activity is stimulated. Thus the kinetic behaviour of PFK, though very complex is extraordinarily well adapted for this important step of glycolysis.
The positive and negative allosteric modulation of this enzyme may vary from one type of cell to another. The PFK reaction is essentially irreversible in the cell. It will be recalled that most regulatory enzymes catalyze irreversible reactions.

By a separate enzymatic pathway D-fructose 1,6-diphosphate may be converted back to fructose 6-phosphate through a hydrolytic reaction catalyzed by hexose diphosphatase also an allosteric enzyme whose role in the regulation of glucose biosynthesis will be considered later.

Fructose-1,6 di-p + H_2O ⟶ Fructose-6-p + Pi

4. Conversion of fructose 1,6-diphosphate to dihydroxyacetone phosphate and glyceraldehyde 3-phosphate by aldolase (ALD)
The next reaction in the glycolytic sequence involves the cleavage of fructose 1,6-diphosphate to form the two triose phosphate sugars, (1) dihydroxyacetone phosphate and (2) D-glyceraldehyde-3-phosphate. The enzyme aldolase, which catalyzes this reaction, was first extensively purified

from yeast and studied by Warburg. It has now been purified from numerous animal, plant and microbial sources, and is wide spread in nature. Indeed the finding of this enzyme in high concentrations in a particular tissue is indicative of a functioning glycolytic pathway.

Fructose-1,6 di-p ⟷ DHAP + GAP

Specificity
Aldolase will catalyze the cleavage of a number of ketose di- and monophosphates e.g. FDP, S,1,7,P F1-P, erythylose 1-P. In each case however, dihydroxy acetone phosphate is one of the products.
Several different types of aldolases have been isolated and purified from different cells and their properties studied. The prototype of the Class I enzyme is the enzyme from rabbit skeletal muscle.

Aldolase A
The active form is a tetramer consisting of four polypeptide chains.

5. The interconversion of the triosephosphates by triose phosphate isomerase (TIM)
Only one of the two triosephosphates, namely glyceraldehydes-3-phosphate, can be directly degraded in the further reaction of glycolysis. However, the other, dihydroxyacetone phosphate, is reversibly converted into glyceraldehyde-3-phosphate by the enzyme triose phosphate isomerase

DHAP ⟷ (TIM) D.Gly-3-p ---→ into Glycolysis

If cells were unable to convert dihydroxyacetone phosphate to glyceraldehydes-3-phosphate, half of the glucose molecule would accumulate in the cell as the ketone phosphate or be disposed off by other reactions hence the presence of the triose phosphate isomerase, which catalyzes the interconversion of these two trioses and permits the subsequent metabolism of all the glucose molecules.

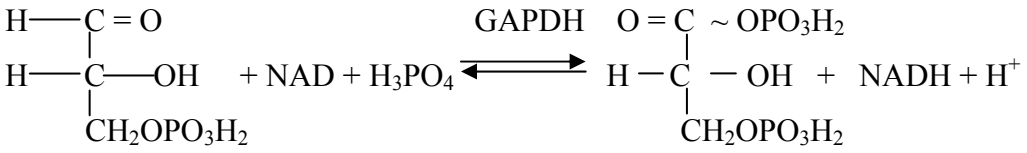
"Meyerhof" was the first one to describe the equilibrium between the triose phosphates. The reaction is analogous to the isomerization of hexose phosphates in that the interconversion of a ketose and an aldose takes place.

Triose phosphate isomerase is an extremely active enzyme. If a molecular weight of 100,000 is assumed, one can demonstrate that 1mole would catalyze the isomerization of 945,000 moles of substrate per min. Thus although the "equilibrium" constant (Keq=22) "favours the ketone derivative", the presence of even a small amount of the isomerase will ensure an immediate conversion of the acetone phosphate into the aldehyde isomer for subsequent degradation.

Enzymatic steps in second stage of glycolysis
This stage includes the oxidation-reduction steps as well as the phosphorylation steps in which ATP is generated from ADP. Since one molecule of glucose form two of glyceraldehydes-3-phosphate, both halves of the glucose molecule follow the same pathway.

6. Oxidation of glyceraldehydes-3-phosphate to 3-phosphoglyceroyl phosphate by Gly-3-p dehydehydrogenase {GAPDH)

This is one of the most important steps of the glycolytic sequence, since it conserves the "energy of oxidation" of the aldehyde group of glyc-3-p in its oxidation product 3-phosphoglyceroyl phosphate. This reaction is the first in the energy yielding or "second" phase of glycolysis and it is also the first reaction in the glycolytic sequence to involve oxidation-reduction in which a high-energy phosphate compound has been formed where none previously existed.



Gly-3-p 1,3-Di-p glycerate

As a result of the oxidation of an aldehyde group to the level of a carboxylic acid, some of the energy which presumably would have been released in the form of heat has been conserved in the formation of the acyl phosphate group of 1,3-diphosphoglyceric acid. The oxidizing agent involved is NAD^+. The reaction is "readily reversible". This is to be expected since the cell has modified the strong exergonic oxidation of an aldehyde to a carboxylic acid, into a reaction in which much of that energy is conserved as an acyl phosphate.

The enzyme has been crystallized from rabbit muscle and yeast and has a molecular weight of 145,000. The enzyme appears to be a tetramer consisting of four identical subunits of approximately 35,000 molecular weight each. Each subunit tightly binds one molecule of NAD^+ making four NAD for the intact oligomer. These NAD^+ molecules are intimately involved in the enzymes catalytic action. Thus glyc-3-p dehydrogenase (triose phosphate dehydrogenase) constitutes an important exception to the generalization that nicotinamide nucleotide dehydrogenases are readily isolated free of their coenzyme molecule.

Triose phosphate dehydrogenase possesses –SH groups which must be free (reduced) for catalytic activity. The well known ability of iodoacetamide to inhibit glycolysis, is due to the covalent and irreversible binding of this reagent with the –SH groups of the dehydrogenase, thereby irreversibly blocking its catalytic action

$$R\text{-}SH + ICH_2\,CONH_2 \dashrightarrow R\text{-}S\text{-}CH_2\,CO\,NH_2 + HI$$

It is also inhibited by heavy metals.

Specificity

The enzyme requires NAD^+ specifically as oxidant, although it is most active with D-3-phosphoglyceraldehyde, it also oxidizes D+L–glyceraldehydes and even acetaldehyde but at a very low rate. The enzyme can also utilize arsenate instead of phosphate, presumably forming 3-phosphoglyceroyl arsenate, a highly unstable compound that immediately and spontaneously decomposes into 3-phosphoglycerate and arsenate in acqueous systems. In the presence of arsenate, no high-energy phosphate compound is generated by the dehydrogenase, although the

overall oxidation takes place. In this way arsenate can "uncouple" oxidation from phosphorylation.

7. Transfer of phosphate from 3-phosphoglyceryl phosphate to ADP by phosphoglycerate kinase (PGK)

Warburg and his colleagues showed that 3-phosphoglyceroyl phosphate formed in the preceding reaction now reacts enzymatically with ADP, with transfer of the acyl phosphate group to ADP and 3-phosphoglycerate catalyzed by phosphoglycerate kinase.

$$\text{3-phosphoglyceroyl phosphate} + \text{ADP} \xrightarrow{\text{PGK}} \text{3-phosphoglycerate} + \text{ATP}$$

This reaction is highly exergonic and serves to "pull" the preceding reaction towards completion. The phosphate-transforming enzyme has an extremely high affinity for 3-phosphoglyceroyl phosphate. The overall equation for the two reactions, the first involving oxidation of glyceraldehydes 3-p to 3-phosphoglyceroyl phosphate by the action of 3-phosphoglyceroyl dehydrogenase and the second involving transfer of the acyl phosphate group to ADP catalyzed by PGK is as follows:

Overall reaction (6 & 7) GAPDH and PGK

$$\text{Glyc-3-p} + \text{Pi} + \text{NAD} + \text{ADP} \longleftrightarrow \text{3PG} + \text{ATP} + \text{NADH} + H^+$$

Through these two consecutive reactions the energy of oxidation of an aldehyde group to a carboxylate group has been conserved in the form of ATP.

8. Conversion of 3-phosphoglycerate to 2-phosphoglycerate by phosphoglyceromutase (PGlyM)

This reaction is catalyzed by the enzyme phosphoglyceromutase

$$\text{3 PG} \xrightarrow{Mg^{++}} \text{2 PG}$$

Mg^{++} is essential for this reaction, which involves the transfer of the phosphate group from 3 to the 2-position. The reaction has only a small standard free energy change and is freely reversible in the cell. There are two forms of this enzyme, the form in animal tissues appears to require 2,3-diphosphoglycerate as an intermediate.

$$\text{2,3-diphosphoglycerate} + \text{3PG} \longleftrightarrow \text{2PG} + \text{2,3 diPG}.$$

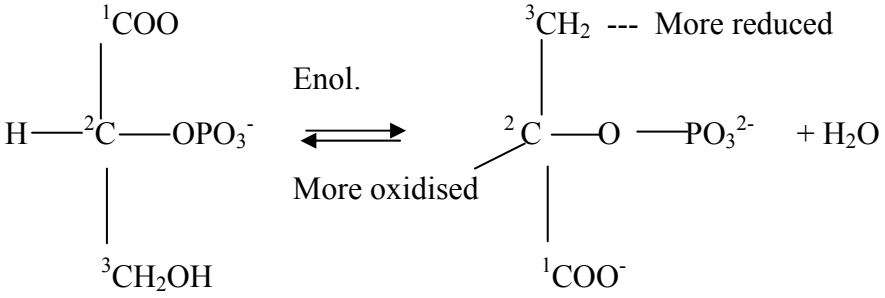
This enzyme falls in the group of catalysts called "phosphomutases" which catalyze the transfer of a phosphoryl group from one carbon atom of the same organic compound. The mechanism of action of this group of enzymes is still under active study, with the information on phosphoglucomutase being voluminous. In the case of the crystalline phosphoglyceroyl mutase from muscle, both require 2,3-diphosphoglycerate as cofactors for activity.

9. Dehydration of 2-phosphoglycerate to phosphoenol pyruvate (PEP) by enolase (Enol)

The conversion of 2-phosphoglycerate to PEP is the second reaction of the glycolytic sequence in which a high energy phosphate compound is generated. It is catalyzed by enolase.

$$\text{2-phosphoglycerate} \longleftrightarrow \text{PEP} + H_2O$$

Enolase has been obtained in pure crystalline form from several sources (Mol. Wt. 85,000). It has an absolute requirement for a divalent cation (Mg^{++} or Mn^{++}), which makes a complex with the enzyme before the substrate is bound. The enzyme is strongly inhibited by fluoride particularly if phosphate is present, the inhibitory species being the phosphofluoridate ion, which forms a complex with Mg^{++}. Although the reaction catalyzed by enolase is formally an elimination of a molecule of water from carbon atom 2,3 of 2-phosphoglycerate, it may also be regarded as an intramolecular oxidation-reduction, since the removal of water causes carbon atom 2 to become more oxidized and carbon atom 3 more reduced.



2 PG PEP

10. Transfer of phosphate from PEP to ADP by pyruvate kinase (PK)
The transfer of the phosphate group from PEP to ADP, yielding free pyruvate is catalyzed by the enzyme pyruvate kinase.

PEP + ADP ---→ Pyruvate + ATP + -7.5 Kcals mol^{-1}

PK has been obtained in pure crystalline form (Mol. Wt. 250,000). The reaction is highly exergonic and it has been found to be irreversible under intracellular conditions. The enzyme requires Mg^{++} or Mn^{++} with which it must form a complex before binding the substrate. Ca^{++} complexes with Mn^{++} or Mg^{++} and forms an inactive complex.

The enzyme also requires an alkali metal cation, which may be K^+, Rb^+, Cs^+; K^+ is the physiological activator. It is believed that the binding of K^+ causes a conformational change of the enzyme to produce a more active form.

11. Reduction of pyruvate to lactate by lactate dehydrogenase (LDH)
In the last step of glycolysis, pyruvate is reduced to lactate at the expense of electrons originally donated by 3-phosphoglyceraldehyde. These electrons are carried by NADH. The reaction is catalyzed by lactate dehydrogenase.

Pyruvate + NADH + H^+ ⟷ Lactate + NAD^+

The overall equilibrium of the reaction is far to the right. LDH exists in atleast five different molecular forms or isoenzymes in higher animals which differ in the rate at which they bring

about reduction of pyruvate at low pyruvate concentrations. This reaction completes the internal oxidation-reduction cycle of glycolysis.

Lactate the end product of the glycolytic sequence under anaerobic conditions, diffuses through the cell membrane to the surroundings as waste. When muscles, function anaerobically during vigorous activity etc. the lactate from muscle goes to blood and liver and is rebuilt to form blood glucose (gluconeogenesis).

The overall balance sheet

The balance sheet for glycolysis can now be constructed to amount for the fate of the

1. Carbon skeleton of glucose.
2. The oxidation-reduction reactions.
3. The input and output of the phosphate, ADP + ATP.

The left hand part of the following equation shows all the inputs of the glycolytic sequence and the right, all the outputs, adjusted for the fact that each molecule of 1-glucose yields two molecules of glyceraldehydes-3-phosphate.

$$\text{Glucose} + 2\text{ATP} + 2\text{NAD} + 2\text{Pi} + 4\text{ADP} + 2\text{NADH} + 2\text{H+} \longrightarrow 2 \text{ lactate} + 2\text{ADP} + 2\text{NADH} + 2\text{H}^{+} + 4 \text{ ATP} + 2\text{NAD} + 2\text{H}_2\text{O}$$

By cancelling out common items on both sides of the equation we get

$$\text{Glucose} + 2\text{Pi} + 2\text{ADP} \dashrightarrow 2 \text{ lactate} + 2 \text{ ATP} + 2\text{H}_2\text{O}$$

In the overall process, D-glucose is converted into two molecules of lactate, two molecules of ADP and phosphate which are converted to ATP and four electrons are transferred from glyceraldehydes 3-p to pyruvate via $2\text{NADH} + \text{H}^{+}$. Two molecules of ATP must be fed into the scheme to prime it, and four molecules of ATP are produced, resulting in a net yield of two ATP's per molecule of glucose transformed.

To summarize

$$1 \text{ Glucose} \longrightarrow 2 \text{ Lactate}$$

$$2 \text{ ADP} + 2\text{Pi} \longrightarrow 2 \text{ ATP}$$

$$4 \text{ electrons from Glyc-3-p to Pyruvate} \longrightarrow 2\text{NADH} + \text{H}$$

Net yield of 2 ATP's

Balance sheet for high-energy bonds of ATP:

- 2 ATP expended
- 4 ATP produced (2 from each of two 3C fragments from glucose). Net production of 2 ~ P bonds of ATP per glucose

Entry of other carbohydrates into the glycolytic sequence and alcoholic fermentation

The storage polysaccharides glycogen and starch and simple sugars other than D-glucose are channeled into the first stage of glycolysis by feeder pathways catalyzed by ancillary enzymes.

Glycogen and Starch

The D-glucose units of glycogen and starch gain entrance into the glycolytic sequence through the sequential action of two enzymes

(1) Glycogen phosphorylase (or starch phosphorylase in plants)

(2) Phosphoglucomutase

Glycogen and starch phosphorylase are members of a general class of enzymes designated as α-1-4 glucan phosphorylases.

$$(\text{Glucose})_n + HPO_4^{2-} \longrightarrow \text{glucose}_{(n-1)} + \text{g1-p}$$

In this reaction the terminal α(1-4) glycosidic linkage at the non-reducing end of a glycogen side chain undergoes phosphorylysis. The cleavage of a terminal glycosidic bond results in the removal of the terminal glucose as g1-p, leaving behind a glycogen chain with one less glucose unit. The enzyme acts repetitively on the non-reducing end of the glycogen chain until it meets the α(1-6) linkage (branch points) which it cannot attack. This produces limit dextrin which may be further degraded after the action of a debranching enzyme, amylo 1,6-glucosidase, which hydrolyzes the α-1-6 linkages at the branch points thus making another length of the polysaccharide chain available to the action of glycogen phosphorylase.

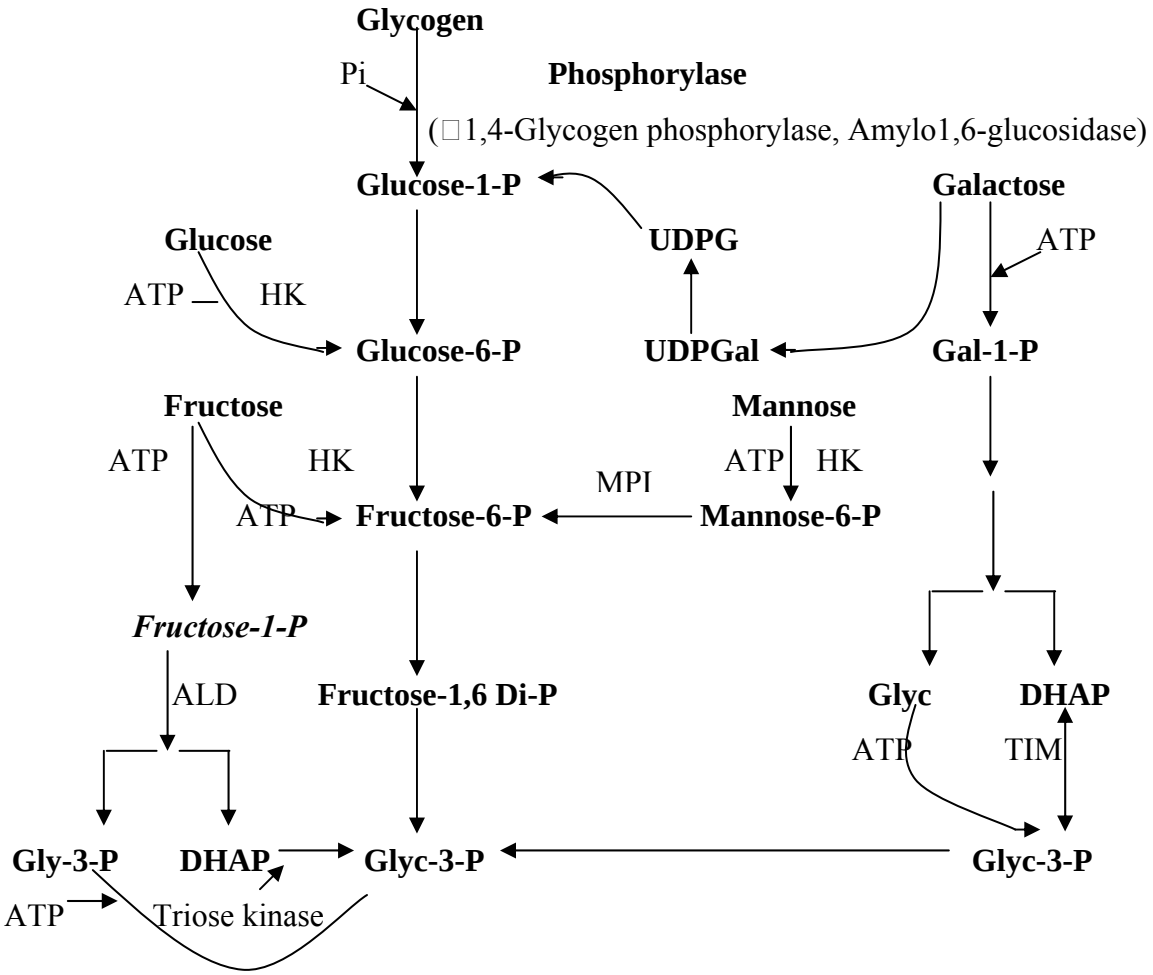


Figure 3: Entry of glycogen and hexoses into glycolysis

Phosphorylase is situated at a strategically important point between the fuel reservoir (glycogen and starch) and the glycolytic sequence that utilizes the fuel. Its activity in muscle and liver is under the regulation of an elaborate set of controls.

Glucose-1-phosphate, the end product of the glycogen and starch phosphorylase reactions is converted into glucose 6-phosphate by the enzyme phosphoglucomutase, which has been obtained in pure form from many sources. It catalyzes the readily reversible reaction.

$$\text{G-1-P} \xleftarrow[\text{Mg}^{++}\text{, glu-1, 6-dip.}]{\text{PGM}} \text{G 6 P}$$

Although it can also catalyze the conversion of D-mannose 1-P to D-mannose 6-P, the rate is only 1/100 to that of g1-p. It requires Mg^{++} and glucose 1,6 dipo for activity. The role of g1,6-dip in the PGM reaction is similar to the role of 2,3 di-P glycerate reaction. G6P formed as the product of the PGM reaction may now enter the glycolytic sequence.

Entry of disaccharides

Disaccharides ingested by higher animals are usually hydrolysed to their monosaccharides components before absorption in the intestines.

1. $\text{Sucrose} + H_2O \xrightarrow[\text{sidase (sucrose or invertase)}]{\beta\text{-fructofurano}} \text{D-glucose} + \text{D-fructose}$

2. $\text{Maltose} + H_2O \xrightarrow[\text{(maltase)}]{\alpha\text{-glucosidase}} \text{2-G glucose}$

3. $\text{Lactose} + H_2O \xrightarrow[\text{(Lactase)}]{\beta\text{-galactosidase}} \text{D-glucose} + \text{D-galactose}$

Entry of monosaccharides

In the liver of vertebrates fructose gains entry into glycolysis by the action of fructokinase.

$$\text{D-fructose} + \text{ATP} \xrightarrow{\text{FK}} \text{D-fructose-1-P} + \text{ADP}$$

the resulting fructose 1-P is then cleaved into D-glyceraldehyde and dihydroxyacetone-P by aldolase.

$$\text{Fructose 1-P} \xrightarrow{\text{ALD}} \text{D-glyceraldehyde} + \text{DHAP}$$

The free D-glyceraldehyde formed is phosphorylated to glyceraldehydes 3-P

$$\text{D-glyceraldehyde} + \text{ATP} \xrightarrow{\text{Glyc K}} \text{D-glyceraldehyde} - 3 - \text{P} + \text{ADP}$$

The DHAP + Gly-3-P so formed are intermediates in glycolysis

ATP + galactose ⟶ ADP + D-gal-1-p

D-gal-1-p ⟶ DHAP + Glyc-3-P

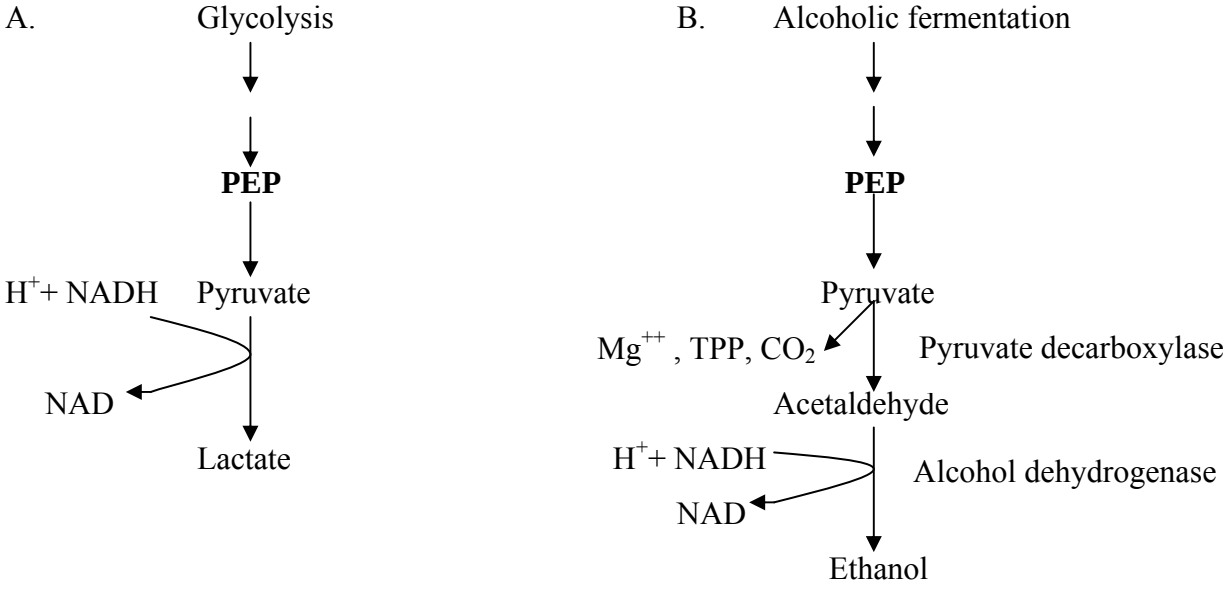
D-Mannose + ATP ⟶ D-mannose 6 p + ADP

D-mannose-6-P $\xrightarrow{\text{Mannose-P-Isomerase}}$ fructose -6-P

Alcoholic fermentation

In organisms like bevers yeast which ferment glucose to ethanol and CO_2, rather than to lactic acid, the fermentation pathway in identical to that described for glycolysis except for the terminal step catalyzed by lactate dehydrogenase, which is replaced by two other enzymatic steps.

In the first step, pyruvate is decarboxylated to acetaldehyde and CO_2 by the enzyme pyruvate decarboxylase. This is an essentially irreversible reaction. Pyruvate decarboxylase requires Mg^{++} and has a tightly bound coenzyme thiamine pyrophosphate. The decarboxylation of pyruvate proceeds through a series of intermediates covalently bound to the thiamine pyrophosphate.



In the final step of alcoholic fermentation acetaldehyde is reduced to ethanol with NADH + H^+ furnishing the reducing power, through the enzyme alcohol dehydrogenase. Ethanol and CO_2 are thus the end products of alcoholic fermentation. The overall equation of alcoholic fermentation can therefore be written as

$$\text{Glucose} + 2\text{Pi} + 2\ \text{ADP} \longrightarrow 2\ \text{ethanol} + CO_2 + 2\text{ATP} + 2H_2O$$

The energy conserving steps leading to ATP formation are identical in both glycolysis and alcoholic fermentation.

Summary

Glycolysis takes place in two stages. In the first D-glucose is enzymatically phosphorylated by ATP and ultimately cleaved to yield two molecules of D-glyceraldehyde-3-P. Other hexoses, pentoses and glycerol are also collected and converted into glyceraldehydes-3-p following their phosphorylation.

In the second stage of glycolysis, the glyceraldehydes-3-p is oxidized by NAD^+ with uptake of inorganic-P by the action of glyceraldehyde phosphate dehydrogenase to from 3,phosphoglyceroyl-P (1,3-di P-Gly). The latter donates its acyl phosphate group to ADP to yield ATP and 3-phosphoglycerate, which is then isomerized to 2PG after dehydration of the latter by enolase, the PEP formed donates its phosphate group to ADP. The other product, free pyruvate, is reduced to lactate by the NADH formed in the dehydrogenation of glyceraldehydes-3-P.

Two molecules of ATP enter the first stage of glycolysis and four are formed from ADP in the second stage, giving a net yield of 2ATPs from one molecule of glucose.

There are three essentially irreversible steps in glycolysis, catalyzed by hexokinase (HK), phosphofructokinase (PFK) and pyruvate kinase (PK). The reaction catalyzed by PFK, a regulatory enzyme, is the major rate limiting step in glycolysis. The entry of glucose residues of glycogen and starch into glycolysis is made possible by glycogen phosphorylase and PGM. Glycogen phosphorylase which catalyzes the conversion of glycogen to g1-p is a regulatory enzyme existing in active (a) and less active (b) form.

Balance sheets for glycolysis and alcoholic fermentation

The pathways for glycolysis and alcoholic fermentation describe the fate of the carbon atom of glucose and tell us nothing about the energetics of the process. Actually during both glycolysis and alcoholic fermentation ATP is generated from ADP and Pi. The complete balance equation for glycolysis and alcoholic fermentation, including the energy conserving steps are as follows:

Glycolysis

$$C_6H_{12}O_6 + 2\ \text{Pi} + 2\ \text{ADP} \longrightarrow 2\ CH_3CHOHCOOH + 2\ \text{ATP} + 2HO_2$$
$$\longrightarrow 2\ \text{Lactic acid}$$

Alcoholic fermentation

$$C_6H_{12}O_6 + 2\text{Pi} + 2\text{ADP} \longrightarrow 2\ CH_3CH_2OH + 2CO_2 + 2\text{ATP} + 2H_2O$$
$$\longrightarrow \text{Ethanol}$$

$$\text{Glucose} \longrightarrow 2\ \text{lactate --- exergonic}$$
$$\triangle G_1^{\circ} = -47\ \text{k cals mol}^{-1}$$

$$2Pi + 2ADP \longrightarrow 2ATP + 2H_2O \text{ --- endergonic}$$
$$\Delta G_2^o = 2x7.3 = 14.6 \text{ k cals mol}^{-1}$$

Summary

$$\text{Glucose} + 2\ Pi + 2\ ADP \longrightarrow 2\ \text{lactate} + 2\ ATP + 2H_2O$$
$$\Delta G^o s = G_1^o + G_2^o$$
$$= 47.0 + 14.6$$
$$= 32.4 \text{ K cals mol}^{-1}$$

From the standard free energy changes shown it is clear that the breakdown of glucose to lactate ($\Delta G^o = -47.0$ K cals mol^{-1}) provides more than sufficient energy to cause the phosphorylation of two molecules of ADP to ATP ($\Delta G^o = +14.6$ K cals mol^{-1}). Therefore 14.6/47.0 x 100, or about 31% of the free energy decreased during the breakdown of glucose to lactate is conserved in the form of ATP.

The overall process of glycolysis, even after making allowance for the coupled formation of ATP, still proceeds with a very large net decrease in free energy –32.4 kcals mol^{-1}. Glycolysis is thus an essentially "irreversible" reaction, with its equilibrium overwhelmingly in the direction of lactate formation.

Tricarboxylic acid cycle

In glycolysis the cells obtain ATP from the breakdown of glucose in the absence of oxygen. However most animal and plant cells normally are aerobic acid oxidize their N2B organic fuels completely to CO_2 and H_2O. Under these conditions the pyruvate is oxidized the CO_2 and water in the aerobic phase of catabolism, called Respiration, a molecular processes involved in O_2 consumption and CO_2 formation by cells. "TCA cycle" is the common central pathway for the degradation of the 2-carbon "acetyl residue" derived not only from carbohydrates but also from fatty acids and amino acids.

The TCA cycle is a cyclic sequence of reactions of almost universal occurance in aerobic cells. It is catalyzed by a multi enzyme system that accepts the acetyl group of acetyl CoA as fuel and disembers it to yield CO_2 and hydrogen atoms which are fed into the respiratory chain. The latter are then led via a sequence of electron carrying proteins to molecular oxygen, which is reduced to form water.

It was known from the work of Thunberg *et al*. that an aerobic suspension of minced animal tissues catalyze the transfer of hydrogen atoms from certain organic acids known to occur in cells especially succinic, malic and citric acid to the reducible dye methylene blue giving it colourless reduced form. Enzymes catalyzing such reactions were called dehydrogenases. By early 1930 several investigators using nanometric measurements of the O_2 utilization rate of minced tissue suspensions found that succinate, fumarate and malate and citrate are rapidly oxidized to CO_2 by molecular O_2.

With the elaboration of the glycolytic sequence in yeast and muscle, it appeared that the compounds which were being metabolized (oxidized) to CO_2 and H_2O by animal tissues were "pyruvate and lactate". The biochemists Keilin, Martin, Knoop, Bauman, Ochoa and Lipmann

have also contributed to an understanding of the metabolic pathway which accomplishes the aerobic oxidation of pyruvate and lactate.

The most important single contributor was the distinguished English Biochemist Sir Hans Krebs. His extensive studies allowed him to postulate in 1937 the cycle of reactions which accounted for the oxidation of pyruvic acid to CO_2 and water. His contribution to the problem was of such magnitude that the scheme is frequently referred to as the Krebs Cycle. In 1953 he was awarded the Nobel Prize for his important discovery.

In 1948 Kennedy and Lehninger found that rat liver mitochondria would catalyze the oxidation of pyruvate and all the intermediates of the TCA cycle by molecular oxygen. Since only Mg^{++} and an adenylic acid had to be added (ATP, ADP or AMP), this finding meant that mitochondria contained not only all the enzymes of the TCA cycle, but also those required to transport the electrons from the substrate to the molecular oxygen.

Cell respiration occurs in three major stages as shown: In the first stage organic fuel molecules, carbohydrates, fatty acids and some amino acids are oxidized to yield 2-C fragments, the acetyl group of acetyl CoA, in the second stage these acetyl groups are fed into the citric acid cycle which enzymatically degrades them to yield energy rich hydrogen atoms and to set free CO_2, the final oxidation product of organic fuels.

Intracellular localization of the enzymes of the TCA cycle

Kennedy and Lehninger (1948) found that isolated rat liver mitochondria containing phosphate, adenine nucleotides and Mg^{++} catalyzes the oxidation of pyruvate and all the intermediates of the TCA cycle at the expense of molecular O_2. The overall rate of O_2 consumption and pyruvate utilization by the isolated liver mitochondria accounted for the rate of respiration of the liver cell. On the other hand the nuclei, microsomes and the soluble cytoplasm were inactive. The liver mitochondria thus contain all the enzymes required for the TCA cycle as well as the components required for the electron transport. The enzymatic reactions of the TCA cycle take place within the inner compartment of the mitochondria. Some of the cycle enzymes occur in the soluble matrix of the inner compartment whereas others are attached to the inner mitochondria membrane and not readily extracted in soluble form. Some of the enzymes of TCA cycle like aconitate hydratase, NADP-isocitrate dehydrogenase and malate dehydrogenase also occur in the cytosol.

Oxidation of pyruvate to acetyl CoA, pyruvate dehydrogenase, PDH

Pyruvate is first oxidized with the loss of CO_2 to acetyl-CoA which then reacts enzymatically with oxaloacetate to form citrate.

The oxidation of pyruvate to acetyl CoA, catalyzed by PDH-complex is a very complicated process.

Overall reaction

$$\text{Pyruvate} + \text{NAD} + \text{CoA} \longrightarrow \text{Acetyl CoA} + \text{NADH}\ H^+ + CO_2$$

$\triangle G^o = -8.0$ Kcals.

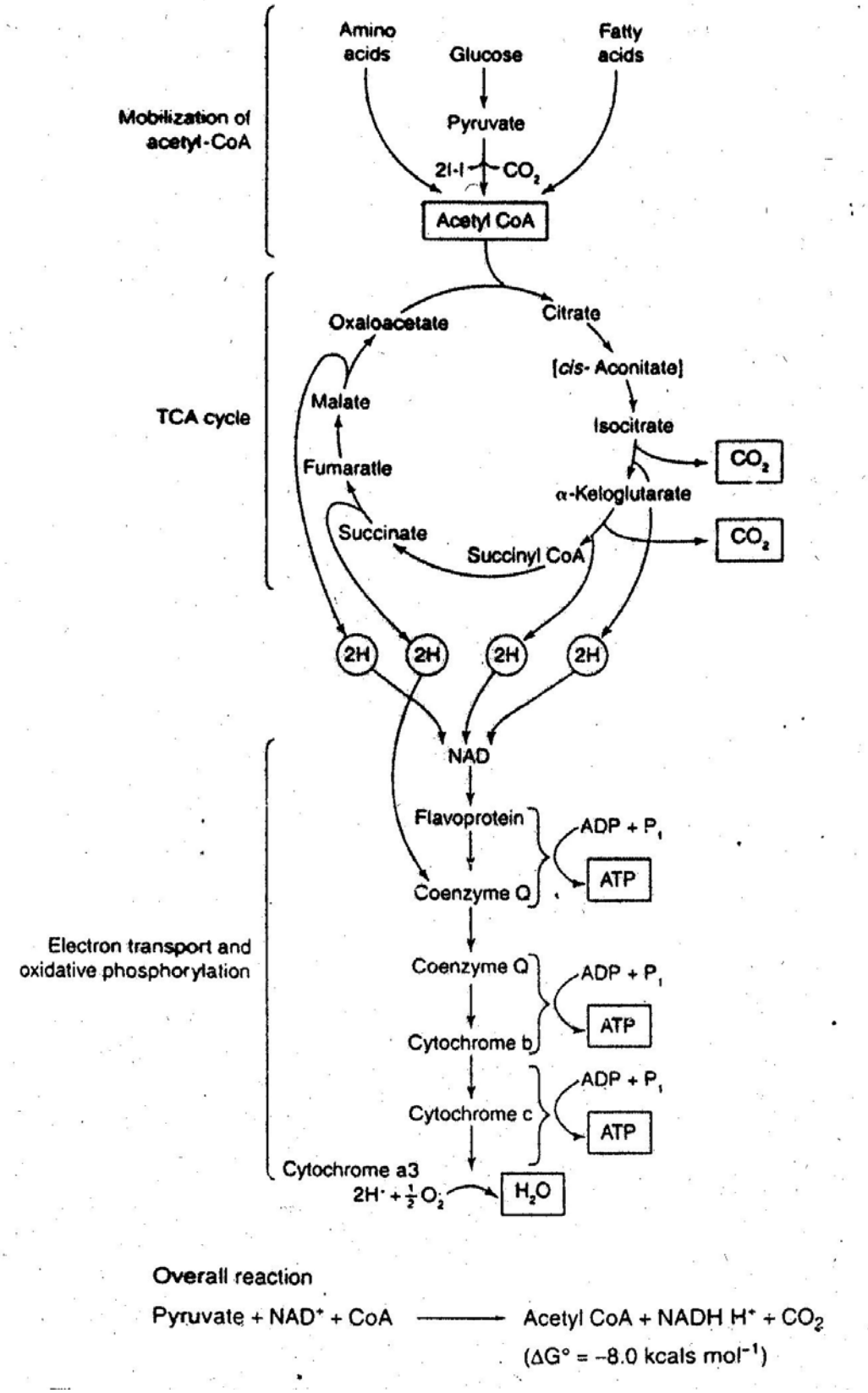


Figure 4: Cell respiration

This reaction, which is irreversible in animal tissues, is not itself a part of the TCA cycle but is obligatory for the entry of all carbohydrates (via pyruvate) into the TCA cycle.

The oxidative decarboxylation of pyruvate to acetyl-CoA and CO_2 requires three different enzymes and five different coenzymes organized into a multienzyme complex.

Step 1 - It is catalyzed by PDH whose prosthetic group is TPP. This reaction is similar to the reaction for the non-oxidative decarboxylation of pyruvate during alcoholic fermentation.

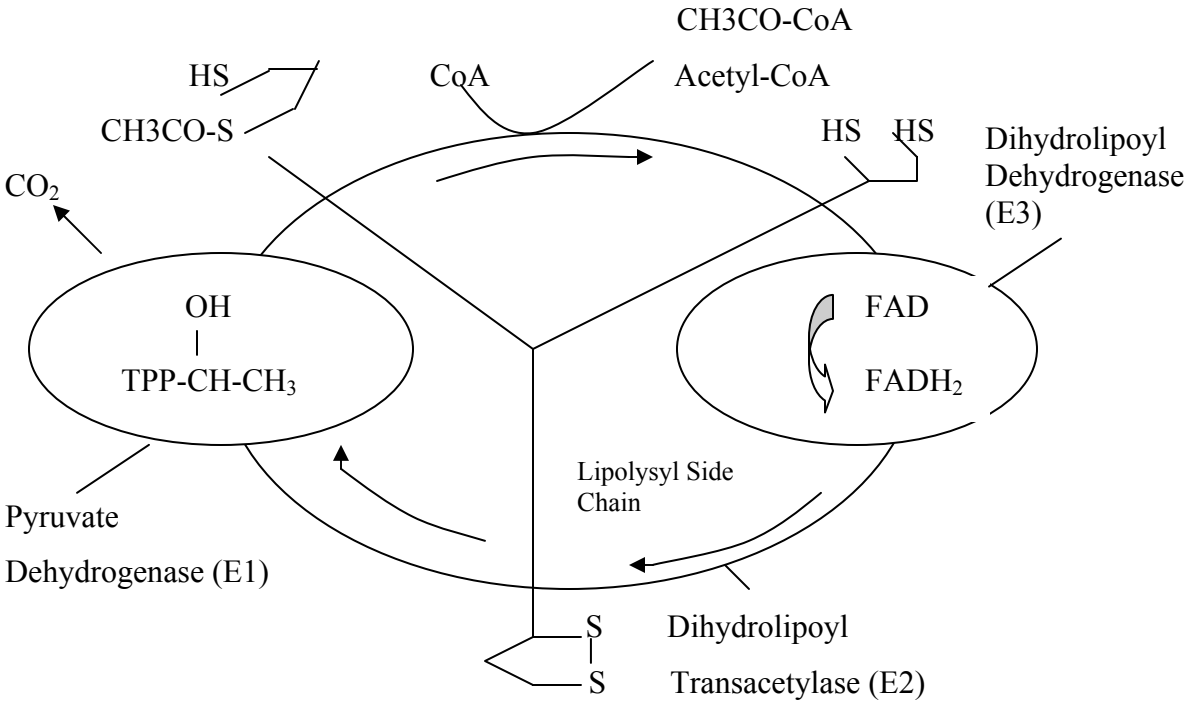


Figure 5: Role of lipoyllpyl group in the pyruvate dehydrogenase complex, the long lipoylhysyl side chain of the dihydrolipoyl transacetylane services as a swinging to transfer electrons from PDH [E_1] to E_3 and to transfer the acetyl group from E_1 to CoA.

Step II - The hydroxyethyl group is dehydrogenated and the resulting acetyl group is transferred to the sulfur atom of carbon 6 of lipoic acid, which is the prosthetic group of the second enzyme of the complex dihydrolipoyltransacetylase.

Step III - The acetyl group is enzymatically transferred from the lipoyl group of dihydrolipoic acid to the thiol group of CoA. The acetyl CoA so formed then leaves the enzyme complex in free form.

Step IV - Tthe dithiol form of the lipoyl group of dihydrolipoyl transacetylase is reoxidized to its disulfide form by transfer of hydrogen atoms to the third enzyme of the complex known as dihydrolipoyl dehydrogenase, whose reducible prosthetic group is tightly bound FAD. The

resulting $FADH_2$, which remains bound to the enzyme is reoxidized in step V by NAD with the formation of NADH.

All of the partial reactions catalyzed by pyruvate dehydrogenase complex are reversible except the initial decarboxylation. The irreversible nature of this process marks the overall reaction irreversible. (-8000 cals)

The pyruvic dehydrogenase complex has been isolated from pig heart and *E.coli* where it has a molecular weight of $4.8x10^6$.

Enzymes:

1. Pyruvate dehydrogenase E_1, TPP
2. Dihydrolipoyl transacetylane E_2, Lipoic acid
3. Dihydrolipoyl dehydrogenase $E_3 – FA_3$

Coenzymes: CoA, NAD, TPP, Lipoic acid, FAD

E_1 E_2 E_3

Reactions of the TCA cycle

The tricarboxylic acid cycle as originally postulated in 1937 was a skeleton. It has since been filled in with many details from the study of highly purified preparation of enzymes catalyzing the individual steps.

1. Conversion of acetyl CoA to citrate (Citrate synthase)

Citric acid, the first tricarboxylic intermediate of the cycle, is formed by the condensation of acetyl-CoA with oxaloacetate:

$$\text{Acetyl CoA} + \text{oxalacetate} + H_2O \longrightarrow \text{Citrate} + \text{CoA}$$

$$\text{Citroyl – CoA-Intermediate} \longrightarrow (\text{monofluoroacetyl CoA} \longrightarrow \text{monofluorocitrate}$$

This reaction is catalyzed by citrate synthase discovered and first called condensing enzyme by "Ochoa". This enzyme, of molecular.weight 100,000 catalyzes the aldol condensation between the methyl group of acetyl CoA and the carbonyl group of oxaloacetate, with hydrolysis of the thioester bond and formation of free CoA-SH. Citroyl-CoA is believed to be formed as a non-dissociating intermediate on the active site, synthetic citroyl-CoA is dissociated (hydrolyzed) by the enzyme to yield citrate and CoA.

The citrate synthase reaction proceeds far in the direction of citrate formation because of the exergonic hydrolysis of the high-energy thioester linkage of citroyl CoA. Citrate synthase also catalyzes formation of monofluorocitrate from monofluoroacetyl CoA, this is an example of

lethal synthesis, since fluoroacetate is not itself toxic whereas fluorocitrate is a potent inhibitor of aconitase, the next enzyme in the TCA cycle.

The citrate synthase reaction is the primary pacemaker step of the TCA cycle, its rate is largely determined by the availability of acetyl-CoA and oxaloacetate and by the concentration of succinyl CoA that competes with acetyl CoA and inhibits citrate synthase. The enzyme is also inhibited by ATP, NADH and by long chain fatty acyl CoA esters.

2. Conversion of citrate to isocitrate (Aconitase)
The enzyme aconitate hydratase, more commonly known as aconitase, catalyzes the reversible interconversion of citrate and isocitrate via the enzyme bound intermediate cis-aconitate.

$$\text{Citrate} \underset{\text{ACON}}{\rightleftharpoons} [\text{ Cis-aconitate}] \rightleftharpoons \text{Isocitrate}$$

The equilibrium mixture at pH 7.4 and 25^{o}C contains about 93% citrate and only 7% isocitrate. However isocitrate is very quickly oxidized in the next step of the cycle, thus pulling the aconitase reaction towards isocitrate formation.

Aconitase contains an Fe (II) and requires a thiol like -SH such as cysteine or reduced glutathione. Aconitase is present in animal tissues in two isozyme forms, one in the mitochondria and the other in the cytosol fraction.

3. Oxidation of isocitrate to α-ketoglutarate (Isocitrate dehydrogenase)

$$\text{Isocitrate} + \text{NAD (NADP)} \underset{\text{ICDH}}{\rightleftharpoons} \alpha\text{-ketoglutarate} + CO_2 + \text{NADH(NADPH)}$$

$$\Delta G^0 = -5.0 \text{ Kcal mol}^{-1}$$

Most microorganisms and tissues of higher animals and plants contain two types of isocitrate dehydrogenase. For years a controversy has raged over which is responsible for the oxidation of isocitrate to α-ketoglutarate in the tricarboxylic and cycle. One type of isocitrate dehydrogenases require NAD^+ as electron acceptor and the other requires $NADP^+$.

Both the NAD^+ and $NADP^+$ linked isocitrate dehydrogenase occur in mitochondria of animal tissues but the former (NAD^+) is found only in the mitochondria whereas the latter ($NADP^+$) is present both in the mitochondria and the cytosol. Most of the available evidence indicates that the NAD linked isocitrate dehyhdrogenase is the major catalyst for isocitrate oxidation in the TCA cycle. This distrinction between NAD and NADP specific isocitrate dehydrogenase was long observed because the NAD linked enzyme is an “allosteric” enzyme that requires ADP as a specific modulator. Until the stimulating effect of ADP was discovered, it was thought that the NAD linked dehydrogenase, as measured in extracts of mitochondria was only feebly active and unable to account for the known high rate of isocitrate oxidation.

The NAD^+ specific isocitrate dehydrogenase of mitochondria requires Mg^{++} for activity. It has eight identical subunits and a molecular weight of about 380,000. It is an exergonic process

When the NAD specific ICDH of animal tissues is stimulated by ADP the latter (ADP) undergoes no enzymatic alterations and can be recovered again at the end of the reaction. No other nucleoside 5'-diphosphate can activate the enzyme nor can AMP or ATP.

The allosteric nature of the enzyme is also apparent from its characteristic reaction kinetics (plot of velocity vs isocitrate concentration of rat liver enzyme as a function of ADP concentration). The NADP-linked isocitrate dehydrogenase may under some circumstances participate in oxidation of isocitrate by mitochondria.

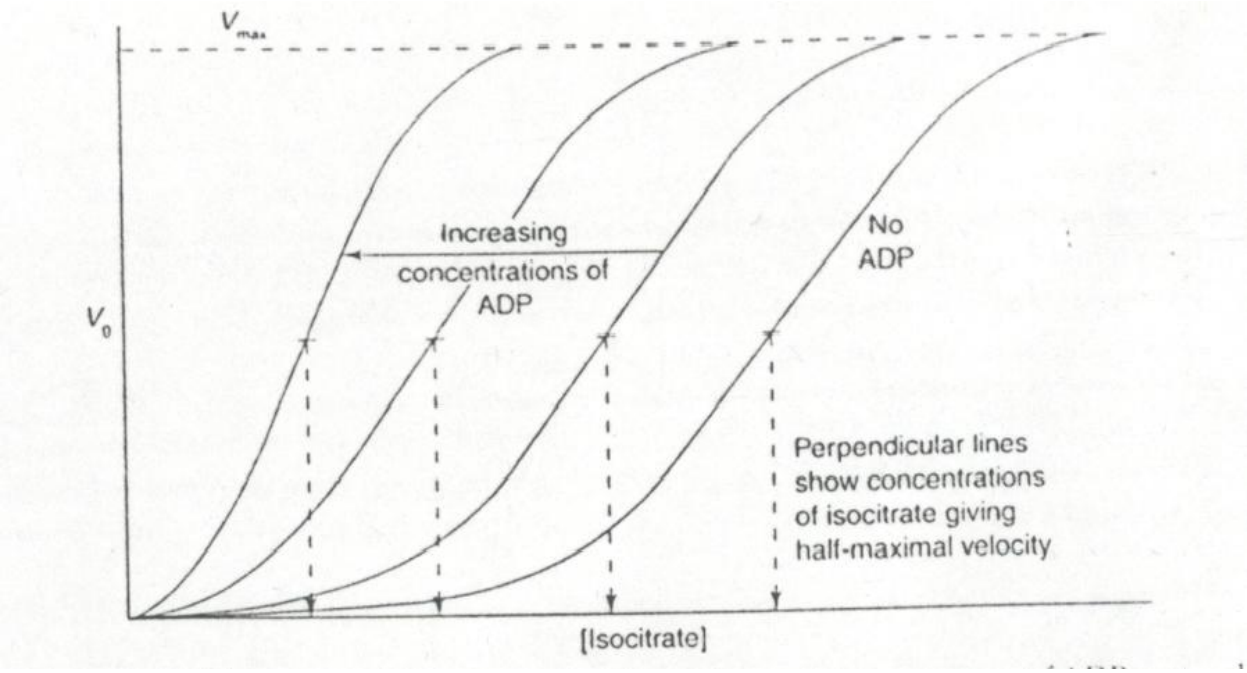


Figure 6: Allosteric stimulation of isocitrate dehydrogenase by ADP. Increasing concentration of ADP cause the apparent Km to decrease, without change in Vmax, thus activating the enzyme when substrate concentration is low

4. Oxidation of α-ketoglutarate to succinyl-CoA (α-Ketoglutarate dehydrogenase complex)

The oxidation of α-KG to succinyl-CoA, which is biologically irreversible in animal cells, is carried out by the α-ketoglutarate dehydrogenase complex

$$\alpha\text{-ketoglutarate} + NAD^+ \xrightarrow{\alpha\text{-KGD complex}} \text{succinyl-CoA} + CO_2 + NADH + H^+$$

$$\Delta G^0 = -8.0 \text{ Kcal mol}^{-1}$$

This reaction is analogous to the oxidation of pyruvate to acetyl CoA and CO_2, and occurs by the same mechanism with thiamine pyrophosphate, lipoic acid, CoA, FAD and NAD^+ participates as coenzymes. The α-ketoglutarate dehydrogenase complex is very similar in structure and properties to the pyruvate dehydrogenase complex. It has been isolated from animal tissues and *E.coli*. The product of the α-ketoglutarate oxidation step is succinyl~ CoA, a high-energy thioester of one carboxyl group of succinic acid.

5. Deacylation of Succinyl CoA (Succinyl–CoA synthetase) or succinic thiokinase

Succinyl CoA undergoes loss of its CoA group not by a simple hydrolysis but by an energy conserving reaction with guanosine diphosphate (GDP) and phosphate.

$$\text{Succinyl\sim CoA} + \text{Pi} + \text{GDP} \xrightarrow[\text{succinic thiokinase}]{} \text{Succinate} + \text{GTP} + \text{CoA}$$

The enzyme catalyzing the reaction, succinyl-CoA synthetase causes the formation of the high-energy phosphate bond of GTP from GDP and Pi at the expense of the high-energy thioester bond of succinyl CoA. The enzyme from animal tissues is specific for GDP as phosphate acceptor that from *E.coli* utilizes ADP. A covalent phosphoenzyme is formed as an intermediate step in this reaction. A histidine residue of the enzyme protein becomes phosphorylated when the enzyme is incubated with ^{32}Pi, succinyl-CoA and Mg^{++}, in the absence of GDP or with ^{32}P-labelled GTP in the absence of succinate. The 3-phosphohistidine residue is believed to donate its phosphate group to GDP in the last step of the overall reaction. This and other evidence suggest the following sequence, in which E desigenates the enzyme protein.

$$\text{Succinyl\sim CoA} + {}^{32}Pi^{*} + E \rightleftharpoons \text{E- Succinly-P} + \text{CoA}$$

$$\underset{\text{(Mixed anhydride)}}{\text{E- Succinly-P}^{*}} \rightleftharpoons \underset{\text{(Phosphoenzyme)}}{\text{E\sim P} + \text{Succinate}}$$

$$\text{E\sim P} + \text{GDP} \rightleftharpoons \text{E} + \text{GTP}$$

In this formulation succinyl phosphate, a mixed anhydride, a mixed anhydride of succinic and phosphoric acids is postulated to be formed on the active site. The enzyme bound succinyl phosphate does not exchange readily with added free succinyl phosphate.

The GTP formed in this reaction then donates its terminal phosphate group to ADP to form ATP in the nucleoside diphosphate kinase reaction.

$$\text{GTP} + \text{ADP} \xrightarrow[\text{NDP Kinase}]{} \text{GDP} + \text{ATP}$$

GTP and ATP have approximately the same standard free energy of hydrolysis.

The formation of GTP or ATP coupled to deacylation of succinyl CoA is called substrate level phosphorylation, to distinguish it form phosphorylations linked to respiratory chain. The formation of ATP coupled to oxidation of 3-phosphoglyceral dehyde during glycolysis is another example of a substrate level phosphorylation. The formation of GTP during the oxidation of α-ketoglutarate to succinate is not inhibited by 2, 4-dinitrophenol, the characteristic uncoupling agent for oxidative phosphorylation.

Succinyl-CoA can be deacylated to succinate by certain other reactions important in anxillary formation of the TCA cycle. The succinate resulting from the α-ketoglutarate dehydrogenase reaction contains carbon atoms from the acetyl CoA fed into the cycle.

6. Oxidation of succinate by succinate dehydrogenase

Succinate is oxidized to furmarate by the flavoprotein enzyme succinate dehydrogenase which contains covalently bound flavin adenine dinucletide (FAD). This enzyme, tightly bound to the inner mitochondrial membrane, is rather difficult to extract from the membrane in soluble form. Many years of intensive research have been required to analyse its composition, properties and mechanism. Its reducible coenzyme FAD functions as hydrogen acceptor in the reaction.

$$\text{Succinate} + \text{E-FAD} \rightleftharpoons \text{Fumarate} + \text{E- FADH}_2$$

The "reduced enzyme" can donate electrons to various artificial electron acceptors e.g. reducible dyes like methylene blue. The normal electron acceptor is not known with certainty. As extracted from beef heart mitochondria, succinate dehydrogenase has a molecular weight of 100,000 and contains one molecule of FAD, eight atoms of iron and eight labile sulfur atoms. The highly purified enzyme appears to have two subunits, of 30,000 and 70,000 molecular weight. The larger subunit of succinate dehydrogenase contains the FAD, four atoms of iron and four of acid labile sulfur. The smaller subunit is an iron sulfur protein containing four iron atoms and four acid labile sulfur atoms. The FAD is covalently bound and can be released on tryptic digestion of the larger subunit. Probably the iron atom of both subunits of succinate dehydrogenase undergoes $Fe^{-2} - Fe^{-3}$ valence changes during electron transfer from succinate to the respiratory chain. A number of iron sulfur proteins are known to function in electron transfer reactions.

Succinate dehydrogenase has some of the attributes of an allosteric enzyme:

(i) It is activated by succinate, phosphate, ATP and by reduced coenzyme Q.
(ii) It is inhibited by very low concentrations of oxaloacetate.
(iii) Malonate is a specific competitive inhibitor of succinate dehydrogenase. When malonate was added to muscle suspension to block succinate dehydrogenase, succinate accumulated quantitatively following the oxidation of added citrate, isocitrate, cisaconitate and α-ketaglutarate.

However, it is not certain that these effects play a role in getting the overall rate of tricarboxylic acid cycle, since succinate dehydrogenase activity in mitochondria is usually far greater than the activity of the electron transport chain.

7. Hydration of fumarate by fumarase
The reversible hydration of formarate to L-malate is catalyzed by the enzyme fumarate hydratase more commonly known as Fumarase.

$$\text{Fumarate} + H_2O \rightleftharpoons \text{L - Malate}$$

This enzyme has been obtained in crystalline form from pig heart. The reaction is freely reversible *in vivo*. Fumarate has a molecular weight of about 200,000 and contains four polypeptide chain subunits, which are inactive in separated form. It requires no coenzyme, ATP decreases the apparent affinity of the enzyme for fumarate.

8. Oxidation of malate to oxaloacetate by malate dehydrogenase
In the last reaction of the cycle the NAD-linked L-malate dehydrogenase catalyzes the oxidation of L-malate to oxaloacetate

$$\text{L - malate} + NAD \rightleftharpoons \text{Oxaloacetate} + NADH + H^+$$
$$\Delta G^0 = +7.1.0 \text{ Kcal mol}^{-1}$$

Although the reaction is endergonic as written it goes in the forward direction very readily in the cell because of the rapid removal of the reaction products oxaloacetate and NADH in subsequent steps. $NADP^+$ is only feebly reduced by the enzyme. The malate dehydrogenase reaction is strictly stereospecific for the L-stereoisomer of malate and for the A side of the pyridine ring of the NAD^+. The cells of higher animals contain two forms of malate dehydrogenase one in the mitochondria and the other in the extramitochondrial cytoplasm.

Summary
We can now sum up the output of one turn around the TCA cycle.

1. For each acetyl group entering, two carbon atoms appear as CO_2. However, these carbon atoms are not the same ones that entered as acetyl groups.
2. Four pairs of hydrogen atoms are yielded by enzymatic dehydrogenation; three pairs have been used to reduced NAD^+ and one pair to reduce the bound FAD of succinate dehydrogenase. These four pairs of hydrogen atom become H^+ ions, the corresponding electrons combine with oxygen, following their transport down the respiratory chain.

Amphibolic nature of the TCA cycle
Amphibolic pathway (amphi = means, both in greek) is a pathway which has a dual function. The amphibolic route in the TCA can be used catabolically to bring about completion of the degradation of small molecules derived from catabolic steps, or it can be used anabolically to furnish small molecules as precursors in biosynthetic reactions.

1. Transamination
The TCA cycle is an amphibolic pathway and functions not only in catabolism but also to generate precursors for anabolic pathways. Certain intermediates of the cycle particularly α-ketoglutarate and oxaloacetate serve as precursors of amino acids, to which they are converted by enzymic transamination reactions

$$\alpha\text{-ketoglutarate} + \text{Alanine} \xrightleftharpoons{\text{GPT}} \text{Glutamate} + \text{Pyruvate}$$

$$\text{Oxaloacetate} + \text{Alanine} \xrightleftharpoons{\text{GOT}} \text{Aspartate} + \text{Pyruvate}$$

2. Citrate can also be removed from the cycle to serve as a precursor of extra mitochondrial acetyl-CoA for fatty acid biosynthesis, through the ATP-citrate lyase reaction.

$$\text{Citrate} + \text{ATP} + \text{Co A} \xrightarrow{\text{Citrate lyase}} \text{Acetyl Co A} + \text{OAA} + \text{ADP} + \text{Pi}$$

Moreover, succinyl CoA can also be removed from the cycle for heme biosynthesis. Thus the TCA cycle can be drained of intermediates for biosynthetic reactions.

Anaplerotic Reactions

TCA cycle intermediates can in turn be replenished by special enzymatic reactions called "anaplerotic reactions" (filling up). The most important is the enzymatic carboxylation of pyruvate to form oxalacetate, first discovered by H.G. Wood and C Werkman in bacteria. The pathway of this carboxylation in animal tissues and the identity of the enzyme involved proved to be exceptionally difficult problems which required many years of research to clarify. Ultimately M.F. Utter and his colleagues showed that in the liver oxalacetate formation from pyruvate is catalyzed by pyruvate carboxylase, a mitochondrial enzyme.

$$\text{Pyruvate} + CO_2 + \text{ATP} + H_2O \xrightleftharpoons[\text{PC}]{Mn^{++}} \text{OAA} + \text{ADP} + \text{Pi}$$

In the liver and other animal tissues pyruvate may be carboxylated to produce more oxalacetic acid whenever the tricarboxylic acid cycle is deficient in oxalacetate or its precursors. Conversely when oxalacetate is in excess it is disposed off by reversal of this reaction, the pyruvate so formed is oxidized to completion via the cycle.

(i) Pyruvate carboxylase has a molecular weight of about 650,000
(ii) It is inactivated at 0^oC at which it dissociates into four subunits.
(iii) The native enzyme contains four molecules of biotin covalently attached to the four subunits through amide linkages, with the amino groups of specific lysine residues at the active site.
(iv) Each subunit also binds one Mn^{++} ion.

The biotin prosthetic group on the enzyme active site serves as an intermediate carrier of the carboxyl group, which it transfers to pyruvate to form oxalacetate. This reaction occurs in two steps:

$$\text{E-biotin} + \text{ATP} + CO_2 + H_2O \rightleftharpoons \text{E- Carboxy biotin} + \text{ADP} + \text{Pi}$$
$$\text{E- Carboxy biotin} + \text{Pyruvate} \rightleftharpoons \text{E- biotin} + \text{OAA}$$

Sum:

$$\text{ATP} + CO_2 + \text{Pyruvate} + H_2O \rightleftharpoons \text{OAA} + \text{ADP} + \text{Pi}$$

Pyruvate carboxylase is an allosteric enzyme. The rate of its forward reaction, leading to oxalocetate formation is high, unless acetyl – CoA its positive modulator is present. Thus whenever acetyl CoA, the fuel of the tricarboxylic acid cycle accumulates, it stimulates the pyruvate carboxylase reaction to produce more oxalocetate, thus enabling the cycle to oxidize more acetyl CoA. Although the pyruvate carboxylase reaction is the most important anaplerotic reaction in the liver and kidney of higher animals other reactions may also participate.

One such reaction is that catalyzed by malic enzyme, also called malate dehydrogenase (decarboxylating; NADP). It is present in the soluble fraction of the cell and requires Mn^{++}.
Intermediates of the TCA cycle may also be generated from aspartate and glutamate, which are converted into oxalacetic acid and α-ketoglutanic acids respectively by transaminase reactions. They are present in both soluble and mitochondrial fractions.

Glutamate + Pyruvate $\rightleftharpoons$ α- Ketoglutarate + Alanine

Aspartate + Pyruvate $\rightleftharpoons$ Oxaloacetate + Alanine

Glyoxalate Cycle

The glyoxalate cycle, a modified form of the TCA cycle, takes place in most plants and microorganisms but not in higher animals. The primary purpose of the glyoxalate cycle, first delineated by H.Krebs and H.R. Kornberg is to enable plants and microorganisms to utilize fatty acids or acetate in the form of acetyl CoA as sole source of carbon particularly for the net biosynthesis of carbohydrates from fatty acids. Animals cannot bring about net synthesis of glucose from acetate or fatty acids because the two carbon atoms are lost as CO_2 in the reaction of the TCA cycle from acetyl CoA to oxalacetate. The glyoxalate cycle bypasses the CO_2 evolving steps of the TCA cycle.

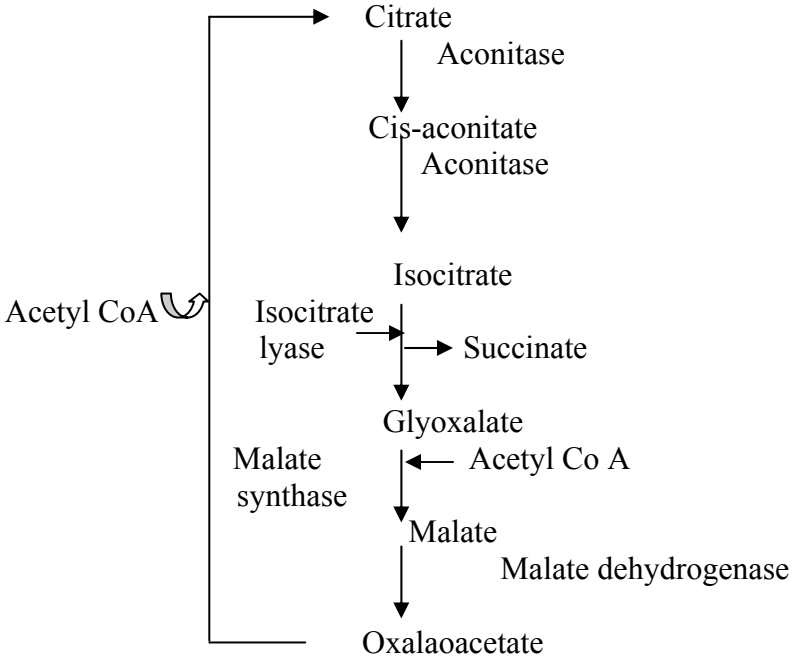


Figure 7: The Glyoxalate cycle

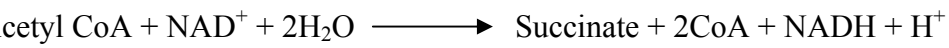
Acetyl CoA first condenses with oxalacetate to form citrate, which is then converted by the action of aconitase into isocitrate as in the TCA cycle. However, the breakdown of isocitrate occurs by a pathway in which three reactions of the TCA cycle are bypassed, namely isocitrate dehydrogenase, α-keto glutarate dehydrogenase and succinyl CoA synthetase

Isocitrate is first cleaved by isocitrate lyase to form succinate and glyoxalate.

Malate synthase then catalyzes the condensation of glyoxalate with another molecule of acetyl CoA to form malate which is then oxidized to oxalacetate by malate dehyrogenase. The oxalacetate may then condense with acetyl CoA to form citrate to start another turn of the glyoxalate cycle.

In each turn of the cycle two molecules of acetyl-CoA enter and one molecule of succinate is formed. The Succinate is used for biosynthetic purposes particularly in gluconeogenesis, the biosynthesis of new sugar.

The overall reaction of the glyoxalate cycle is:



In higher plants and microorganisms both the TCA cycle and the glyoxalate cycle may operate simultaneously, the TCA to provide energy needs via oxidative phosphorylation and the latter to provide succinate for the formation of new carbohydrates from fat.

Localization

Although the TCA cycle reaction in higher plants are localized in the mitochondria the two characteristic enzymes of the glyoxalate cycle isocitrate lyase and malate synthase are localized in another class of cytoplasmic organelles called **glyoxysomes**.

These membrane surrounded organelles lack most of the enzymes of the TCA cycle and have no cytochrome system. They are found only in plant cells capable of converting fatty acids into sugar.

Regulation

The glyoxalate cycle is under allosteric regulation. Isocitrate lyase is strongly inhibited by PEP, a key intermediate in the biosynthesis of glucose from non-sugar precursors. Isocitrate lyase and malate synthase are inducible enzymes synthesized by plant cells only when they are needed.

The glyoxalate cycle is especially prominent in plant seeds, which can convert acetyl residues derived from the oxidation of fats into carbohydrates via succinic acid. Higher animals lack isocitrate lyase and malate synthase.

Alternate routes of glucose catabolism

The Embeden-Meyerhof scheme is a major route for the anaerobic degradation of hexoses to pyruvate. It should be, however, stressed that this is not the only pathway for the degradation of hexoses. For example in all the forms of plant and animal life, which have now been studied, it is evident that there are several routes for the metabolism of glucose. One of the more important routes is called the 'Pentose Phosphate Pathway', also known as the hexose-monophosphate shunt or the phosphogluconate pathway.

Pentose phosphate pathway

It was early recognized that an alternate route existed for the metabolism of glucose. Its existence was indicated by the fact that in some tissues the classical inhibitors of glycolysis, iodoacetate and fluoride had no effect on the utilization of glucose.

In addition, the experiments of Warburg resulting in the discovery of $NADP^+$ and the oxidation of glucose 6-phosphate to 6-phospho gluconic acid led the glucose molecule into an unfamiliar area of metabolism.

Moreover, with the advent of carbon-14 it could be shown in some instances that glucose labelled in the C-1 carbon atom was more readily oxidised to $^{14}CO_2$ than was glucose labeled at C_6 position. If the glycolytic sequence was the only means whereby glucose could be converted to Pyruvate-3-C^{14} and subsequently broken down to CO_2 then CO_2 should have been produced at an equal rate from glucose 1-^{14}C and glucose-6-^{14}C. Their observation stimulated work and the work has resulted in the delineation of the pentose phosphate pathway.

The chief architects of the pathway are B.L. Horecker and E. Racker; among the earlier workers three should be mentioned are Warburg, Lipmann and Dickens.

Localization

The reactions of the pentose phosphate pathway take place in the soluble portion of the extra mitochondrial cytoplasm of animal cells. All the enzymes required in the sequence have been highly purified and extensively studied, particularly by Racker and Horecker.

Oxidative Pathway

1. Glucose-6-phosphate dehydrogenase

The first reaction of the pathway is the enzymatic dehydrogenation of glucose 6- phosphate to form 6-phosphogluconate by glucose 6-phosphate dehydrogenase also known as Zwischenferment

Glucose-6-phosphate + NADP $\rightleftharpoons$ 6- phosphogluconolactone + NADPH+ H^+

This enzyme discovered by O. Warburg was the first dehydrogenase found to be specific for NADP as electron acceptor. It carries out dehydrogenation of carbon atom 1 of the pyranose form of glucose 6-phosphate to yield the corresponding 6-phosphate which is hydrolysed by a specific lactonase.

$$\text{6- phosphogluconolactone} + H_2O \longrightarrow \text{6- phosphogluconate}$$

The over all equilibrium of these two reactions lies far in the direction of formation of NADPH. The enzyme is strongly inhibited by NADPH.

2. 6- Phosphogluconate dehydrogenase

In the next step 6-phosphogluconate goes under oxidation and decarboxylation by 6-phosphogluconate dehydrogenase, a Mg^{++} dependent enzyme to form D-ribulose 5-phosphate, a reaction that generates a second molecule of NADPH. The reaction is not reversible.

$$\text{6-phosphogluconic acid} + \text{NADP} \xrightarrow{Mg^{++}} \text{D-ribulose-5-p} + \text{NADPH} + CO_2 + H^+$$

This enzyme is also inhibited by NADPH. The inhibiton can be reversed by addition of artificial electron acceptors like phenazine methosulphate (PMS) or oxidized glutathione (GSSG)

3. Phospho ribose isomerase

At the level of ribulose 5-phosphate the carbon atoms of glucose enter the second or reversible part of the pentose phosphate pathway. All subsequent reactions of this part are readily reversible.

Initially ribulose 5-phosphate undergoes two isomerization reactions to form products subsequently utilized in the pathway. Phosphoribose isomerase catalyzes the interconversion of the keto sugar and the aldopentose phosphate, ribose 5-phosphate. This reaction is analogous in its action to phosphohexose isomerase encountered in glycolysis. The Keq of the reaction from left to right is approximately 3.

$$\text{D-ribulose 5-phosphate} \overset{\text{isomerase}}{\rightleftharpoons} \text{D-ribose-5-p}$$

4. Phosphoketopentose epimerase

The second isomerization involving ribulose 5- phosphate is catalyzed by the enzyme phosphoketopentose epimerase. The K eq. is 0.8

$$\text{D-ribulose 5-phosphate} \rightleftharpoons \text{D-xylulose 5-phosphate}$$

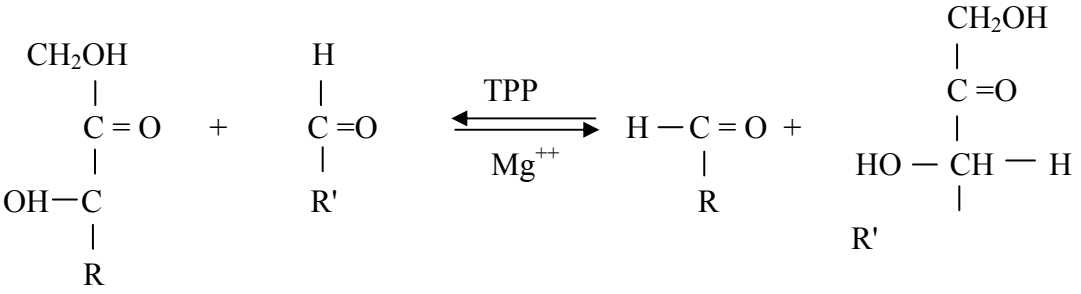
The mechanism of this reaction is not known although it probably involves the enediol as an intermediate. The enzymes 1-4 belong to the oxidative part of the pathway. Subsequent enzymes are non oxidative.

Non-Oxidative Pathway

5. Transketolase

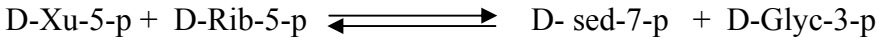
Upto this point the enzymes have dealt with the oxidative degradation of the hexose chain of glucose 6 phosphate and the subsequent interrelations of the pentose phosphate produced. During the period in which these reactions are being studied it was apparent that other sugars including heptoses, tetroses and trioses were also formed. Some clarification of the relations

between the pentoses and these other sugars resulted when the enzyme transketolase was discovered and described. This enzyme catalyzes the transfer of ketol group from a donor molecule to an acceptor aldehyde. The generalized reaction may be written as:

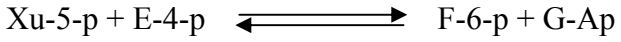


Ketol donor Acceptor aldehyde Product Aldehyde Product Keto donor

In the specific instance, transketolase catalyzes the transfer of a ketol group from xylulose 5-phosphate to ribose 5-p to form sedoheptulose 7-p and glyceraldehyde 3-p.



Transketolase requires TPP and Mg^{++} as cofactors. Transketolase may also catalyze the transfer of a ketol group from xylulose 5-phosphate to erythrose 4-phosphate to form fructose 6-phosphate and glyceraldehyde 3-p.



6. Transaldolase

This enzyme, like transketolase functions as a transferring enzyme by catalyzing the transfer of the dihydroxy acetone moiety of fructose 6 phosphate or seduheptulose 7 phosphate to a suitable aldose. The acceptor aldose may be glyceraldehyde 3-p or in the reverse reaction erythrose 4-p.

Ribose 5-p may also be an acceptor in which case an octose, octulose 8-p is formed. Finally to complete the pentose phosphate pathway the E-4-p produced in the above reaction can accept a C_2 unit from Xu5-p in a reaction also catalyzed by TK to form F6-p and GAP

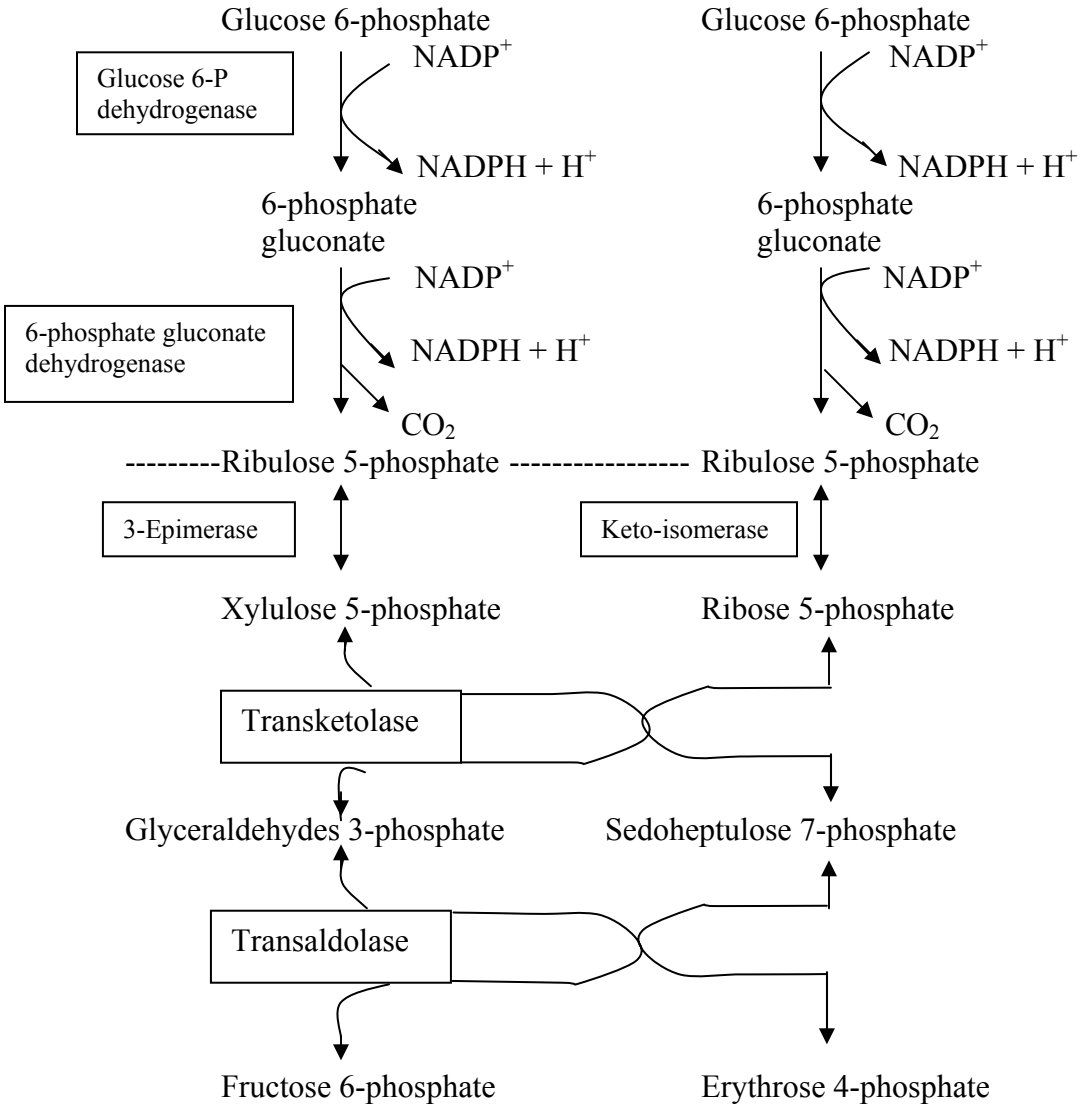


Figure 8. The flow chart of pentose phosphate pathway

Significance of the pentose phosphate pathway

Although many of the reactions of the pentose phosphate pathway were initially worked out in yeast and bacteria, the pathway exists in mammals as well. The pentose phosphate pathway is a multifunctional pathway specialized to carry out four main activities depending on the organism and its metabolic state.

1. Its primary purpose in most cells is to generate reducing power in the extramitrochondrial cytoplasm in the form of NADPH. This function is especially prominent in tissues e.g. liver, mammary gland and the adrenal cortex, the tissues which carry out the reductive synthesis of fatty acids and steroids from acetyl CoA. Skeletal muscle which is not active in synthesizing fatty acids, virtually lacks this pathway.

2. The second special function of the phosphogluconate pathway is to convert hexoses into pentoses, particularly D-ribose 5-p which is an essential component of nucleotides and RNA.

3. The third function is the complete oxidative degradation of pentoses by converting them into hexoses, which can then enter the glycolytic sequence.

4. In the fourth function synthesis of the glucose from CO_2 the phosphogluconate pathway is modified so as to participate in the formation of glucose from CO_2 in the dark reactions of photosynthesis by supplying NADPH. NADPH and ATP are used as the source of energy to bring about the reduction of CO_2 to yield glucose, simultaneously NADPH is reoxidized to NADP and ATP is broken down again into ADP and phosphate.

Summary

The phosphogluconate pathway serves to carry out the complete oxidation of glucose 6-phosphate to CO_2 with simultaneous reduction of $NADP^+$ to NADPH by a complex sequence of reaction in which six molecules of glucose 6-p are oxidized to six molecules each of ribulose 5-phosphate and CO_2 . Five molecules of glucose 6-phophate are then regenerated from the six molecules of ribulose 5-phosphate.

The over all equation is:

$$6\text{- Glucose-6-p}+12\ NADP+7H_2O \longrightarrow 5\ \text{Glucose-6-p} + 6\ CO_2 + 12\ NADPH + 12\ H^+ + Pi$$

If we cancel out common terms, we get

$$\text{Glucose-6-phosphate} + 12\ NADP+7H_2O \longrightarrow 6\ CO_2 + 12\ NADPH + 12\ H^+ + Pi$$

The direction of the flow and the path taken by glucose 6-phosphate after entry into the phosphogluconate pathway reactions are determined largely by the relative requirements of the cell for NADPH and ribose 5-phosphate. If the requirement for NADPH exceeds that for ribose 5-p the excess pentose phosphate can be converted back into hexose phosphate. Only in certain cells and tissues such as microorganism active in biosynthesis of fat, or the lactating mammary gland does the complete oxidative pathway leading exclusively to NADPH prevail.

An isotopic approach can be made to assess the fraction of glucose catabolism in a given cell or tissue proceeding via the glycolytic vs the phosphogluconate pathways. The cells are divided into two batches, one being incubated with G-1-^{14}C and the other with G-6-^{14}C, a comparison is made of the initial rate at which ^{14}C appears in $^{14}CO_2$ formed by oxidation of glucose. The combined action of glycolytic sequence and the TCA cycle yields CO_2 from both types of labelled glucose at equal initial rates, whereas the phosphogluconate pathway initially yields $^{14}CO_2$ only from G-1^{14}C. In rat liver as much as 20% of the CO_2 may come from the phosphogluconate pathway depending on the metabolic state. A much larger fraction of glucose enters the PPP in the mammary gland, where fatty acid synthesis, requiring NADPH is a major metabolic activity. However, in heart and skeletal muscle relatively little glucose oxidation occurs via the phosphogluconate pathway.

Biosynthesis of carbohydrates

We shall now have the enzymatic pathways involved in the biosynthesis of glucose and other hexoses from simpler precursors, the conversion of monosaccharides into disaccharides and the biosynthesis of the various storage and structural polysaccharides and the key regulatory mechanism for controlling the rates of key enzymatic steps.

Major pathways in carbohydrate synthesis

In most cells, the conversion of glucose 6-phosphate to pyruvate, catalyzed by the glycolyte enzymes is the central pathway of carbohydrate catabolism under either aerobic or anaerobic conditions. In a comparable manner the reverse process the conversion of pyruvate to glucose 6-phosphate is the central pathway in the biosynthesis of carbohydrates by many different organisms. One such feeder pathways consist of the reaction sequences by which intermediates of the TCA cycle are transferred into precursors of glucose. This pathway is also employed when the carbon chains of certain amino acids are converted into glucose. Another major feeder pathway consists of reaction bringing about the net reduction of CO_2 to form precursors of glucose into photosynthetic cells.

We shall begin by examining the central biosynthetic pathway leading from pyruvate to glucose 6-phosphate, which is utilized in the process of gluconeogenesis, the synthesis of new glucose from such precursors as pyruvate, lactate certain amino acids and intermediates of the TCA cycle (Figure 9).

Biosynthetic pathway from pyruvate to glucose 6-phosphate

Most of the reaction steps in the heavily travelled central pathway from pyruvate to glucose 6-phosphate are catalyzed by enzymes of the glycolytic sequence and thus proceed by reversal of steps employed in glycolysis. However, there are two irreversible steps in the normal downhill glycolytic pathway, which cannot be utilized, in the uphill conversion of pyruvate to glucose 6-phosphate. In the biosynthetic direction these steps are bypassed by alternative reactions which are thermodynamically favourable in the direction of synthesis.

Conversion of pyruvate to PEP

The first of these bypass steps is the phosphorylation of pyruvate to PEP which does not occur at any significant rate by direct reversal of the pyruvate kinase reaction, presumably because of the large positive standard free energy change.

$$\text{Pyruvate} + \text{ATP} \xrightleftharpoons{\text{PK}} \text{Phosphoenolpyruvate} + \text{ADP}$$
$$\Delta G^0 = +7.5 \text{Kcal mol}^{-1}$$

During gluconeogeneis the phosphorylation of pyruvate is achieved by an alternate pathway through a somewhat round about sequence of reaction that require the co-operation of enzymes both in the cytosol and mitochondrial compartments in the liver of rat and certain other species.

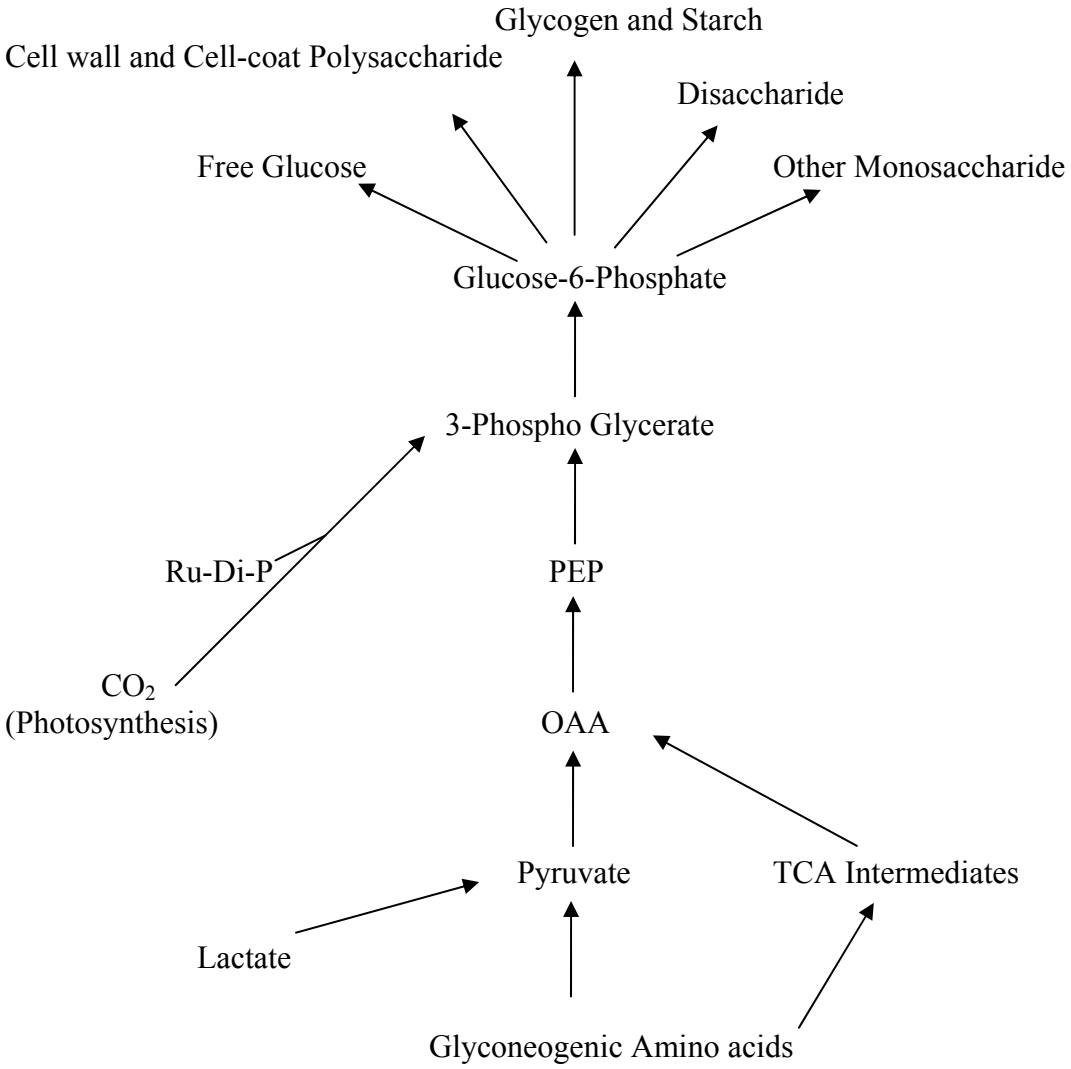


Figure 9: Central Pathway of Hexose Biosynthesis - The auxiliary pathway feeding into the central pathway from CO_2, lactate and amino acids as are the diverging pathway leading from glucose-6-phosphate to other carbohydrate. RuDP is ribulose 1, 5- diphosphate, an important intermediate in photosynthetic formation of hexose.

The first step is catalyzed by pyruvate carboxylase (PC) of mitochondria, which catalyzes the major anaplerotic reaction by which TCA cycle intermediates are generated from pyruvate. The reaction is freely reversible as shown by the ΔG^0 value

1. $\text{Pyruvate} + CO_2 + \text{ATP} \underset{Mn^{++}}{\overset{PC}{\rightleftharpoons}} \text{OAA} + \text{ADP} + \text{Pi}$

$\Delta G^0 = -0.5$ Kcal mol^{-1}

However, this reaction is tightly regulated since pyruvate carboxylase, an allosteric enzyme is completely inactive in the absence of its specific positive modulator acetyl-CoA. Mn^{++} is required for the reaction. The oxaloacetate formed in this mitochondrial reaction is then reduced to malate at the expense of NADH by the mitochondrial form of malate dehydrogenase.

2. $OAA + NADH + H^+ \xrightleftharpoons{MDH\ (m)} NAD + Malate$
$\Delta G^0 = -6.7$ Kcal mol^{-1}

The malate so formed then leaves the mitochondria via the dicarboxylate transport system of the inner mitochondrial membrane, in the cytosol the malate is then reoxidized by the cytoplasmic form of NAD-linked malate dehydrogenase to form extra mitochondrial oxaloacetate

3. $Malate + NAD \xrightleftharpoons{MDH\ (c)} OAA + NADH + H^+$
$\Delta G^0 = +6.7$ Kcal mol^{-1}

Although this reaction is strongly endergonic it proceeds to the right because the end products are quickly removed. In the last step of the bypass, oxaloacetate is acted upon by phosphoenol pyruvate carboxykinase (GTP) to yield PEP and CO_2 a reaction in which GTP or ITP serves as the phosphate donor.

4. $Oxaloacetate + GTP \xrightarrow[PEPCK]{} PEP + CO_2 + GDP$
$\Delta G^0 = +1.0$ Kcal mol^{-1}

PEPCK has a molecular weight of about 75,000. Since it has a very low affinity for CO_2 the reactive chemical species the enzyme is biologically active only in the direction of PEP formation.

The overall equation sum of 1-4 for the formation of PEP is

$Pyruvate + ATP + GTP \xrightleftharpoons{} PEP + ADP + GDP + Pi$
$\Delta G^0 = +0.5$ Kcal mol^{-1}

The overall reaction is thermodynamically reversible because of its small free energy change. It will tend to go to the right whenever the ATP/ADP ratio is high and excess pyruvate is present.

Conversion of phosphoenol pyruvate to fructose diphosphate

PEP generated from pyruvate by the above reactions is now easily converted into fructose 1,6-diphosphate by reversal of the glycolytic reaction beginning with that catalyzed by enolase and ending with that catalyzed by fructose diphosphate aldolase

PEP $\xrightarrow[\text{Reversal of Glycolysis}]{}$ F1, 6 di-p

Conversion of fructose 1, 6 diphosphate to fructose 6 phosphate

We now come to the second crucial point in gluconeogenesis in which a reaction of the downhill glycolytic sequence is bypassed by an enzyme functioning primarily in the direction of synthesis. The downhill reaction of glycolysis at this point is that catalyzed by PFK.

$$\text{Fructose-6-phosphate} + \text{ATP} \xrightarrow{\text{PFK}} \text{FDP} + \text{ADP}$$
$$\Delta G^0 = -3.4 \text{ Kcal mol}^{-1}$$

It does not function in the reverse direction biologically, in part because of the unfavourable ΔG^0. During gluconeogenesis this reaction is bypassed by the cytosol enzyme hexose diphosphatase more commonly known as fructose diphosphatase, which carries out the essentially irreversible hydrolytic removal of the 1-phosphate group.

$$\text{Frucotose} -1,6 \text{ di-p} + H_2O \xrightleftharpoons{\text{FDPase}} \text{Fructose 6-phosphate} + \text{Pi}$$
$$\Delta G^0 = -4.0 \text{ Kcal mol}^{-1}$$

Hexose diphosphatase is an allosteric enzyme. It is strongly inhibited by the negative modulator AMP and stimulated by 3-phosphoglycerate and citrate. The enzyme has at least three binding sites for AMP, which are distinct from the substrate binding sites. It contains four or more subunits. The enzyme is maximally active, and thus favours formation of glucose when the concentration of certain glucose precursors is high and the AMP concentration is low i.e. when the energy is high.

Formation of glucose 6 phosphate from fructose 6 phosphate

In the last step, fructose 6-phosphate is reversibly converted into glucose 6-phosphate by glucose phosphate isomerase (PGI) which functions reversibly in both glycolysis and gluconeogenesis.

$$\text{F-6-P} \xrightleftharpoons{\text{PGI}} \text{G-6-P}$$

We can now sum up the reactions in gluconeogenesis, leading from pyruvate to glucose 6-phosphate

Uphill

$$2 \text{ Pyruvate} + 4\text{ATP} + 2\text{GTP} + 2 \text{ NADH} + 2\text{H} + 6 \text{ } H_2O \longrightarrow$$
$$\text{Glucose-6-phosphate} + 4 \text{ ADP} + 2 \text{ GDP} + 2\text{NAD} + 5 \text{ Pi}$$

For each molecule of glucose 6-phosphate formed six high energy phosphate bonds are consumed and two molecules of NADH are required as reductant, the overall reaction is exergonic. This equation is very different from that for the down hill conversion of glucose 6-phosphate into pyruvate which generates three molecules of ATP.

<u>Downhill</u>

$$\text{Glucose-6-phosphate} + 3\text{ADP} + 2 \text{ Pi} + 2\text{NAD}^+ \longrightarrow$$
$$2 \text{ Pyruvate} + 3\text{ATP} + 2 \text{ NADH} + 2\text{H}^+ + 3 \text{ } H_2O$$

Glucose 6 phosphatase converts glucose 6-phosphate to glucose
In some animal tissues, particularly the liver, kidney and intestinal epithelium glucose 6-phosphate may be dephosphorylated to form free glucose. The liver is the major site of the formation of blood glucose. The hydrolytic cleavage of the glucose 6-phosphate does not occur by reversal of the hexokinase reaction but is brought about by the enzyme glucose 6-phosphatase which catalyzes the exergonic hydrolytic reaction

$$\text{Glucose 6-phosphate} + H_2O \longrightarrow \text{Glucose} + Pi$$
$$\Delta G^0 = -3.3 \text{ Kcal mol}^{-1}$$

This Mg^{++} dependent enzyme is characteristically found in the endoplasmic reticulum of the liver of vertebrates. Its activity is dependent on lipids and on the intactness of the membrane. It has not been demonstrated in muscle or brain, which thus cannot donate free glucose to the blood.

Gluconeogenesis from tricarboxylic acid cycle intermediates
The pathways from pyruvate to glucose allow the net synthesis of glucose from various precursors of pyruvate or PEP. Chief among them are the TCA cycle intermediates, which may undergo oxidation to OAA The oxaloacetate is then converted into PEP by the action of PEP CK. By this pathway three carbon atoms of the various tricarboxylic acid cycle intermediates are ultimately convertible into the three carbon atoms of PEP.

Gluconeogenesis from amino acids
Some or all of the carbon atoms of certain amino acids are ultimately convertible by vertebrates either into pyruvate or into intermediates of the TCA cycle which in turn are precursors of PEP. Such amino acids, which are thus also precursors of glucose, are called glycogenic amino acids. Two examples are glutamic and aspartic acids, which are directly convertible by transamination into TCA cycle intermediates, alpha ketoglutarate and oxaloacetate

Glycogenic	**Ketogenic**	**Gycogenic and ketogenic**
Alanine	Leucine	Isoleucine
Arginine		Lysine
Aspartic acid		Phenylalanine
Asparagine		Tyrosine
Cysteine		Histidine
Glutamic acid		Methionine
Glutamine		
Glycine		Proline
		Serine
		Threonine
		Tryptophan
		Valine

The list shows the amino acids that are glycogenic in mammals. Also shown are the amino acids that are both glycogenic and ketogenic such as phenylalanine and tyrosine, which on degradation are cleaved to form fumaric acid, which is glycogenic, and acetoacetate one of the ketone bodies.

The amino acid leucine yields neither pyruvate nor a TCA cycle intermediate during its oxidative degradation. Since it does yield acetyl-CoA, which can be converted into ketone bodies but not into pyruvate, it is ketogenic amino acids. In plants and many microorganisms no distinction can be made between glycogenic and non-glycogenic amino acids because all the amino acids may ultimately contribute to the net formation of glucose through the combined reaction of the TCA cycle and glyoxalate cycles.

Biosynthetic pathways leading from glucose 6-phosphate: Nucleoside diphosphate glucose

Now that we have seen how glucose or glucose 6-phosphate is formed from simple precursors in both animals and plants we shall trace the biosynthetic pathways leading from glucose 6-phosphate to:

(i) Other hexoses and hexose derivatives
(ii) Disaccharide
(iii) Storage polysaccharides
(iv) The complex structural polysaccharides of cell walls, cell coats and intracellular spaces

In these pathways hexose residues must often be transformed into hexose derivatives or transformed to other monosaccharides or to the ends of polysaccharide chains.

Such reactions show a common pattern in that they employ as the energized glycosyl donor a nucleoside diphosphate sugar (NDP-sugar) e.g. uridine diphosphate glucose, UDPG.

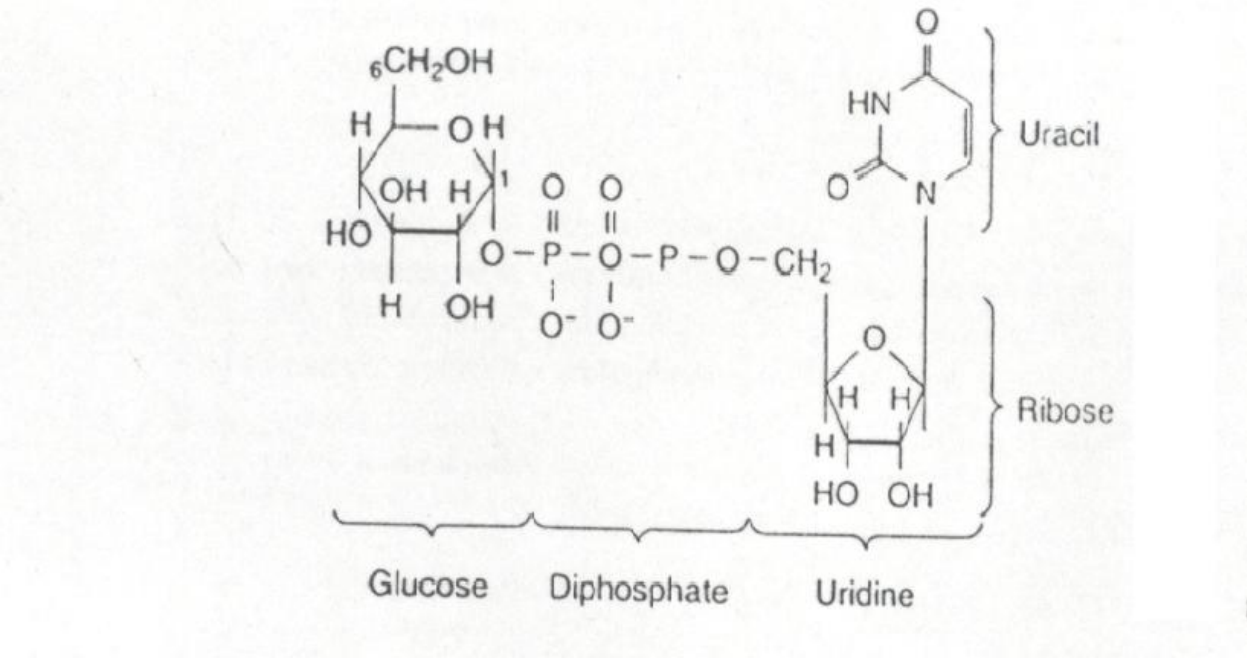


Figure 10: Uridine diphosphate glucose (UDPGlc)

The role of the nucleoside diphosphate sugar was first discovered by L.F.Leloir and his colleagues. They showed that a nucleotide diphosphate sugar is formed by the action of the enzyme generally called glycosyl-1-phosphate nucleotidyl transferase or pyrophosphorylase.

NTP + Sugar-1-P ⟷ NDP-Sugar + PPi

The pyrophosphate formed is derived from the two terminal phosphate groups of NTP. Although uridine diphosphate usually serves as the glycosyl carrier in higher animals ADP, CDP and GDP also function as specific sugar carries in various enzymatic transfer reactions in different plants, microorganisms and animal tissues.

Once an NDP-sugar has been formed its sugar residue may undergo a variety of enzymatic reactions including "oxidation", "reduction" and "epimerization" as well as transfer to other sugars or sugar polymers

Monosaccharides – interconversion – does not requires a NDP sugar for the interconversion.

Biosynthesis of disaccharides and other glycosides

In some plants such as sugarcane, sucrose is formed from glucose and fructose by the following overall reaction

2ATP + UTP + glucose + fructose ⟶ sucrose + 2ADP +UDP+3Pi + PPi

Using the following enzymes

1.	Hexokinase	ATP + Glucose ⟶ G-6-P + ADP
2.	Phosphoglucomutase	G-6-P ⟷ G-1-P
3.	Glucose 1-p-Uridyl transferase	UTP +G-1-P ⟶ UDPG + PPi
4.	Fructokinase	ATP + Fructose ⟶ F-1-P + ADP
5.	Sucrose phosphate synthase	UDPG + F-1-P ⟶ UDP + Sucrose-6-P
6.	Sucrose phosphatase	Sucrose 6p + H_2O ⟶ Sucrose + Pi

Thus three high-energy phosphate bonds are required to form the single glycosidic bond of sucrose. The overal reaction of the formation of sucrose from glucose and fructose is quite irreversible.

Lactose Synthase System

The disaccharide lactose is formed in the mammary gland from D-glucose and UDP galactose by the action of two enzyme proteins, which together constitute the Lactose synthase system. The first protein A, found in the mammary gland and also in the liver and small intestine catalyzes a reaction between UDP-galactose and various acceptors particularly N-acetyl-D-glucosamine.

UDP-gal+N-Acetyl D-glucosamine ⟶ (Protein A) UDP +N-Acetylactose amine

Protein A by itself is only weakly active with D-glucose as acceptor because the km for D-glucose is very high. The second component protein B has long been known as the alpha lactalbumin of milk, it has no catalytic activity of its own. This protein has been found to decrease greatly, the km of protein A for D-glucose so that the enzyme will utilize D-glucose in prefernece to N-acetyl-D-glucosamine as galactose acceptor causing it to make lactose instead of N-acetyl-lactosamine

$$\text{UDP-gal+D-glucose} \longrightarrow \text{UDP + lactose}$$

Alpha lactalbumin is remarkable in another respect, it has a striking homology to the enzyme lysozyme in its amino acid sequence.

Synthesis of glycogen and starch and the role of nucleoside diphosphate sugars

A very heavily travelled biosynthetic pathway starting form g-6-p leads to the formation of the storage polymers glycogen in animals and starch in higher plants. This path begins with the conversion of g-6-p to g-1-p catalyzed by PGM.

$$\text{G-6-P} \xleftrightarrow{\text{PGM}} \text{G-1-P} \xleftarrow{\text{Degrading}} \text{Glycogen}$$

We have seen that G-1-p is a product of the action of the glycogen degrading enzyme phosphorylase. It was once thought that glycogen phosphorylase catalyzes both the synthesis and degradation of glycogen since the phosphorylase reaction is easily reversed *in vitro*. However under intra cellular conditions glycogen phosphoryase catalyzes only the breakdown of glycogen. For the conversion of g-l-p to glycogen a different enzyme is involved which employs UDP glucose as glucosyl donor.

The first step in glycogen synthesis in animals is catalyzed by G-1-p-uridyl transferase

$$\text{G-1-P} + \text{UTP} \xleftrightarrow[\text{Uridyl Transferase}]{} \text{UDPG} + \text{PPi}$$

The structure of UDPG has been mentioned earlier.

In the second step the glucosyl group of UDPG is transferred to the terminal glucose residue at the non-reducing end of an amylase chain to form an alpha-1,4-glycosidie linkage between carbon atom 1 of the added glucosyl residue and the 4-hydroxyl of the terminal glucose residue of the chain. This reaction is catalyzed by glycogen synthase:

Glycogen synthase

$$\text{UDPG} + \underset{\text{(Primer)}}{(\text{Glucose})_n} \xrightarrow[\text{Glycogen Synthase}]{} \text{UDP} + (\text{Glucose})_{n+1}$$

The overall equilibrium greatly favours synthesis of glycogen. Glycogen synthase requires as a primer an alpha (1-4) polyglucose chain having at least four glucose residues to which it adds successive glucosyl groups, however it is far more active with long chain glucose polymers such as amylose. In higher animals UDPG is the most active glucosyl donor; ADP glucose is only about 50% active. In some lower organisms, however, ADP glucose is the preferred substrate for glycogen synthase.

Properties
Glycogen synthase of mammalian liver and muscles contain 4-subunits and has a total molecular weight of about 360,000. The enzyme is usually associated with glycogen granules in the cell.

Glycogen synthase cannot make the alpha-1,6-bonds found in the branch points of glycogen. However 1,4-alpha glucan branching enzyme which is present in many animal tissues, catalyzes transfer of a terminal oligosaccharide fragment of six or seven glucosyl residues from the end of the main glycogen chain to the 6-hydroxyl group of a glucose residue of the same or of another glycogen chain in such manner as to form alpha (1-6) linkage and thus create a new branch point.

Metabolic disorders
In human beings several hereditary disorders of glycogen metabolism have been recognized in which abnormally large amounts of glycogen are accumulated particularly in the liver. The genetically deficient enzymes in such patients have been detected by assay of small samples of tissues obtained by surgical biopsies. In one of these disordres 1.Von GierKe's disease g-6-phosphatase activity is defective. In Andersons disease the branching enzymes defective and the glycogen has abnormally long unbranched chains. In McArdles disease muscle glycogen phosphorylase is defective leading to excessive glycogen depositionin the muscles. In Coris disease there is a deficiency of the debranching enzyme.

Starch
In plants and some bacteria starch synthesis occurs by a pathway similar to that in glycogen synthesis catalyzed by starch synthase. However, ADP-glucose rather than UDP-glucose is the more active glucose donor in many plants. The sequence is

$$\text{ATP} + \alpha\text{ D-g-1-p} \xleftrightarrow{\text{G-1-P Adenyl transferase}} \text{ADP-glucose} + \text{Ppi}$$

$$\text{ADP-glucose} + (\text{Glucose})_n \xleftrightarrow{\text{Starch Synthase}} \text{ADP} + (\text{Glucose})_{n+1}$$

The first of these reactions is catalyzed by glucose 1-phosphate adenyl transferase, an allosteric enzyme that is stimulated by 3 PG and FDP.

Polysaccharide biosynthesis

Structural polysaccharides of cell walls and coats
In the biosynthesis of the structural polysaccharides of cell walls and coats much more complex pathways operate than for the storage polysaccharides. Some cell wall and cell coats

polysaccharides are hetero rather than homopolysaccharides. Moreover, the precursor or intermediates must be extruded through the cell membrane and assembled outside the cell.

As in glycogen synthesis nucleoside diphosphate sugar functions as glycosyl donor in biosynthesis of cell walls and coats. Glycosyl units are added to one end of a pre-existing polysaccharide molecule to lengthen it by one unit with liberation of the free nucleoside diphosphate. The extrusion of precursors form the cell wall and the assembly of wall outside the membrane is affected by the participation of the lipid intermediates phosphate esters of long isoprenoid alcohols which aim to carry hydrophilic sugar residue across the membranes. This area of biosynthesis is still very much a frontier and provides many challenges in analysis of the strategies cells have perfected to build their external walls and coats from internally generated precursors. In animals there are different types of polysaccharides found in the intracellular space of animal tissues like glycogen.

Hyaluronic the simplest acid mucopolysaccharide which is a major component of the ground substance or intracellular filler between cells consists of alternating D-glucuronic acid and N-acetyl glucosamine residues. It is formed by successive alternating reactions of UDP-glucuronic acid and N-acetyl glucosamine at the growing end of the chain in a manner similar to the formation of homopolysaccharides.

Chondroitin, dermatin and keratin sulphates are formed with similar reaction. However these acid mucopolysaccharides normally occur covalently attached to polypeptide chains as glycoproteins or protein polysaccharides. Although the structure and biosynthesis of such glycoproteins are extremely complex some reaction steps have been worked out.

Mucopolysaccharidoses

There are a number of genetic diseases in man called mucopolysaccharidoses in which excessive accumulation of mucopolysaccharides occur in tissues. Skeletal defects and severe mental retardation are the major symptoms. The question arises as to whether the excessive deposition of these mucopolysaccharides is the result of abnormally high rates of synthesis or abnormally low rates of breakdown. Intensive study of the complex biosynthetic and degradative pathways of mucopolysaccharide metabolism has revealed that these disorders are probably the result of genetic deficiency in specific hydrolytic enzymes responsible for the degradation of different polysaccharides.

Suggested Reading

1. Mathews, Harry, Richard Freeland and Robert L. Miesfield. Biochemistry – A short course. John Wiley and Sons.
2. Harpers Biochemistry. Prentice Hall International.
3. Conn, E.E. and Stumpf, P.K. Outline of Biochemistry. Wiley International.
4. Lehninger, A.L., Nelson, D.L. and Cox, M.M. Principles of Biochemistry. CBS Publishers.
5. Berg, J.M., Tymoczko, J.L. and Styryer, L. Biochemistry. W.H. Freeman & Co.