

Biophysical and Biochemical Techniques

Introduction to Biophysical and Biochemical Techniques

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CONTENTS

Introduction

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Introduction

Biochemistry is an interdisciplinary science that systematically integrates principles of mathematics, physics and chemistry to explain distinctive features of life processes in terms of structure-function correlations. Advances in biochemistry owe mainly to exploitation of principles and techniques used in physical sciences. Combination of biochemistry, cell biology and microbiology, in the recent past, to form molecular biology has led to great advances in understanding and control of biological processes in medicine, agriculture and pharmacology and food industries.

Biochemistry is not pure, but an applied discipline and is both analytical and quantitative to explain cause and effect relationship in molecular terms. Analysis means getting to bottom of things. It is used only when combined with synthesis i.e. combining together all pieces obtained by analysis followed by extrapolation of observations to understand the whole system.

In biochemical studies experimental models are first subjected to qualitative analysis i.e. the complex system is broken down and constituents are separated, concentrated and identified. Qualitative analytical biochemistry is concerned mainly at molecular level and at times at electronic level as well. Quantitative analysis is concerned with measurement of concentration of constituents identified by qualitative analysis.

Progress in research depends upon development of technique. No matter how carefully an experiment is planned, a hypothesis can not be converted in to fact unless experimentally proved using adequate technique. Moreover, the results may be meaningless or even misleading if the technical know how is inadequate. During the recent past, biochemical methods have become specialized and sophisticated and proper understanding of the technique in its use in biochemical investigations is very important to arrive at any meaningful result.

Separation and identification of particular compound from complex conglomeration in biological tissues is accomplished by various techniques such as chromatography, electrophoresis, centrifugation, mass spectroscopy, ultraviolet, visible and infrared spectrophotometry, nuclear magnetic resonance and electron spin resonance spectroscopy and so on.

Photometry is one of the powerful tool of analysis and is done at various wavelengths in the ultraviolet, visible and infra red regions of the electromagnetic spectrum. The technique is used both for identification of compounds and their quantitation. Absorption of electromagnetic radiations by compounds is highly specific phenomena. Different wavelengths have different quanta of energy. A compound will absorb only that wavelength of radiant energy which has just the same amount of energy that is needed by the compound for excitation to higher energy level. Energy absorbed in the ultra violet and visible region of the spectrum causes excitations in the electronic state of the compound to a higher level. Since this region contains high amount of energy, the absorbed energy is also capable of causing excitations in the molecular vibration and rotation states, therefore in this region we obtain absorption bands rather than line spectra. The energy requirement for molecular excitations to higher electronic and rotational vibrational levels is fixed and depends upon the structure of the molecule and only that particular wavelength having the same amount of energy as needed by the molecule would be absorbed. This wavelength of absorption is characteristic property of the molecules and is used for their

identification and since amount of radiation absorbed at that wavelength is proportional to the concentration of light absorbing molecule, the technique is widely used for quantitation of the molecule.

In the infra red region, where quanta of energy in the radiations is lesser, the absorbed radiations cause excitations in the vibrational and rotational levels of the molecule. Pure rotational spectra arise in the far infra red region and are of little use to biochemists. Absorption spectra in this region are mainly used to determine specific groups in compounds.

More information about structure of many molecules can be obtained by nuclear magnetic resonance spectroscopy. In this technique the nuclear spins of molecules are excited by placing the sample between the pole pieces of strong magnet and applying variable rotating radio frequency to cause resonance. Depending upon the nuclear spin different nuclei resonate at different frequency. This property is used to distinguish and estimate different compounds having different nuclear conformation. Excellent analytical studies of compounds having unpaired electrons and free radicals is achieved by electron spin resonance spectroscopy where radiations in the microwave region are used to cause electron resonance for the analysis.

Many compounds, especially those having multiple conjugated double bonds with high degree of resonance stability, after absorbing electromagnetic radiations at a particular wavelength, exhibit fluorescence and the latter is used again both for quantitative and qualitative analyses. Atomic absorption spectroscopy is yet another powerful tool to study certain elements such as Na, K and Ca, which otherwise cannot be detected and estimated by classical methods. Mass spectroscopy sorts out ions on the basis of differences in their mass/charge ratio and is widely used to analyze various compounds including oligopeptides.

Centrifugation has been used to separate subcellular components and macromolecules by sedimenting them under enhanced and varying gravitational force. The technique is especially valuable for determination of molecular weight of macromolecules as the sedimentation rate is dependent upon molecular dimension and size.

Isotopes, in particular the radioactive ones have been employed in studying various metabolic pathways. Isotopes differ in physical property but have the same chemical behavior. Radioactive isotopes are easily detectable and are used as tags to decipher metabolic pathways, to study the kinetics of enzyme reactions and in diagnosis and therapy of many types of cancers.

Other routinely used analytical tools are different types of chromatography and electrophoretic techniques. Details of these techniques are given in different sections to follow

Bio-analysis of information processing molecules, DNA and RNA has lead to clearer appreciation of the connection between base sequences in the DNA, and how specific proteins are formed thus allowing to understand how cells differentiate during development. Artificial synthesis of DNA, using polymerase chain reaction and methods for multiplying and transferring DNA to unnatural recipients in genetic engineering have solved many hitherto unknown mechanisms and problems. All these advances that we see in the field of biological

sciences owe to various physical and chemical, qualitative and quantitative techniques that have been employed by researchers.