BASIC ENZYMOLOGY

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INTRODUCTION

Enzymes are synthesized by all living organisms including man. These life essential substances accelerate the numerous metabolic reactions upon which human life depends. A knowledge of enzyme activity in serum or plasma, blood cells. Homogenates and extracts of tissue and urine assists the physician in determining the origin of tissue damage and organ disease.

Certain metabolites can be made to decompose to desired end products in a test tube. However, extremes in temperature, pH or salt are incompatible with life

The same metabolic processes occur in the body under physiologic conditions: a pH near neutrality and a temperature near 37° centigrade. This occurs through the action of enzymes --catalysts of biologic origin.

A chemical catalyst (enzyme) is a substance which increases the rate of a particular reaction without itself being consumed or permanently changed. A catalyst affects only the rate at which a reaction proceeds, that is, the rate at which a reaction reaches equilibrium, it does not enable the reaction to proceed past its normal equilibrium point. Because catalysts are not altered during the reaction, they are capable of carrying on their catalytic effects repeatedly. Consequently, very small quantities of catalysts are capable of affecting relatively large quantities of the reactants,

Enzymes are proteins which catalyze specific metabolic reactions. They are synthesized in the cell and are important in its functions. Under normal conditions the activities of many enzymes in the blood are held at relatively constant levels by the balance between enzyme synthesis and breakdown. The blood serum levels of a particular enzyme may be increased or decreased by diseases that lead to increased rates of enzyme release; to increased amounts available for release; or to decreased rate of enzyme breakdown Changes in blood serum enzyme activity can thus be indicative of bodily disorders as well as in diagnosis, prognosis, and in assessing therapy effectiveness, enzyme determinations in tissue are generally still relegated to the research laboratory. However. Enzyme determinations in serum, and less often in other body fluids are now routine in many clinical laboratories

As a biologic catalysts, enzymes retain the characteristics of chemical catalysts: they increase the reaction rate, they remain unchanged after the reaction, and they are highly efficient. Because they are unaltered, a single enzyme molecule may interact with an astronomical number of reactant molecules. For example, one mole of enzyme may interact with 10,000 to 1,000.000 moles of reactant molecules per minute. Some enzymes are so specific that they catalyze a single type of reactant. For example, urease, which acts only on urea, others may act on many substrates. For example, alkaline phosphatase which may act on phenyl phosphate, beta glycine phosphate, etc,

As proteins, enzymes possess all the properties in common with other proteins, having molecular weights on the order of 10,000 to well over a million. The ribonuclease a molecule shown at the beginning of this presentation is relatively "small", having a molecular weight of "only" 12,700. Enzymes are labile: subtle changes in their structure, called ***denaturation***, can cause them to lose their activity. Consequently, they must be handled quite carefully. Adverse conditions of temperature, pH, and salt concentration are only a few factors known to cause denaturation of an enzyme.

As proteins, enzymes are multivalent electrolytes containing ionizable groups. The ionization state, which affects the enzyme activity, depends on the hydrogen ion concentration, that is, pH. As electrolytes, enzymes migrate in an electrical field. Positive molecules migrating towards the cathode: negative molecules migrating towards the anode. The greater the net charge, the faster the migration, this property is used in diagnostic enzymology to separate iso-enzymes and will be discussed later in this presentation.

NAMING OF AND TYPES OF ENZYME REACTIONS

Enzymes are named according to the starting material Or chemical group they act upon by adding the suffix “ASE”. Consequently, the enzyme hydrolyzing urea is called urease. That acting on phosphate esters is called phosphatase, and so on. Enzymes of diagnostic value may be classified as transferases: Oxidoreductases. Hydrolases, and lyases.

Transferases catalyze the transfer of chemical groups, such as an amino or phosphate group, from one compound to another.

Oxidoreductases, also referred to as dehydrogenases. Catalyze electron transfer.

Hydrolases catalyze substrate breakdown with water fixation.

Lyases are enzymes that split carbon-to-carbon bonds without the transfer of chemical groups

ENZYME-SUBSTRATE COMPLEX

Described simply, enzymatic reactions involve two steps, the first step is a combination of the enzyme (E) with the substrate (S) to form an enzyme-substrate complex (ES), the second step is the decomposition of the enzyme substrate complex to yield the reaction product (P) and the unchanged enzyme.

The velocity of an enzyme reaction will depend on the time required for the enzyme-substrate combination, and the time required for the enzyme to form the product. Obviously, the time required for the formation of the enzyme-substrate complex will depend on the enzyme concentration, and the substrate concentration.

ACTIVITY

The ability of an enzyme to catalyze a specific reaction is called its "activity". Activity is a measure of the rate at which the reaction proceeds. Enzymes are measured in terms of activity rather than concentration because of their chemical similarities, and their extremely low concentrations in biological fluids. The international unit of enzyme activity is defined as the amount of enzyme which will catalyze the transformation of one micro-mole of substrate per minute under certain specified conditions such as pH and temperature. A milli-unit is a thousandth of an international unit. Enzyme activity is normally expressed in international units

per milliliter of enzyme-containing solution, or where This value is inconveniently small: as milli-units per milliliter.

TEMPERATURE

Enzyme activity depends on a number of factors such as temperature, pH, substrate, coenzymes and inhibitors. Increasing the temperature increases the rate of enzyme activity. At the same time, denaturation of the protein can result in the loss of enzyme activity, this thermal deactivation depends upon the length of time an enzyme is exposed to the particular temperature. Thermal denaturation is negligible for most enzymes below about 30°C . However, between 30° and 40° some denaturation occurs. At 60° centigrade, most enzymes are denatured after only a few minutes.

In 1961 the commission of enzymes of the international union of biochemistry recommended that all enzyme measurements be made at 30° centigrade. However, for historical reasons, convenience, or where low enzyme activity and small samples are involved, other temperatures such as ambient. 25°, 32°, and 37° continue to be used.

Generally speaking, for each 10° rise in temperature, many enzymes will exhibit a two-fold increase in activity (Q_{10}). Temperature difference of only 1°C changes the activity of some enzymes by as much as 4-10%. Therefore, it is essential to carefully control the temperature of specimens, solutions, and spectrophotometer cells during enzyme assays.

A characteristic bell shaped curve is obtained when enzyme activity is plotted against pH. The pH optimum, the pH at which greatest activity occurs, corresponds to the vertex of the curve. This pH optimum depends on the environment in which. The reaction takes place. For example, the nature of the buffer, the nature and concentration of the substrate, and the presence of activators or inhibitors can all affect the optimum pH.

VELOCITY

If all the other variables in an enzyme reaction remain constant, the reaction velocity depends on the substrate concentration (S). At relatively large substrate concentrations the enzyme becomes saturated with substrate and the reaction velocity reaches a maximum (V_{max}). Any further addition of substrate will not significantly increase the reaction velocity.

During the period where the substrate concentration is low with respect to the enzyme concentration, the reaction is termed "first order". Under these conditions the reaction rate will be greatly influenced by the substrate concentration. At high substrate concentrations, the reaction rate reaches a maximum and the reaction is termed "zero order". Under these conditions, the reaction rate becomes independent of the substrate concentration.

When measuring enzyme activity only the zero order part of the curve should be used so that the assay is a reflection of enzyme activity only.

The Michaelis-Menton constant, k_m, can be determined from the substrate concentration curve examined earlier. K_m is numerically equal to the concentration of the substrate, in moles per liter, when the velocity of the enzyme reaction reaches one-half its maximum velocity, V_{max}. It is a number characteristic of each enzyme pair. In addition to having practical applications in the clinical laboratory, this constant can be useful in the development of new methods for measuring enzyme activity. For example, the affinity of a non-specific enzyme for different substrates, as defined by their $K_{m's}$, can help in selecting the appropriate substrate.

COENZYMES

Enzymes often require the presence of certain organic. But non-protein factors called "coenzymes". They are relatively low molecular weight with respect to enzyme proteins and are often required for the correct positioning of the enzyme and substrate so that a reaction can proceed. Many coenzymes are derived from dietary vitamins, especially the b-complex vitamins. For example, niacin is the precursor of two coenzymes, nicotinamide adenine denucleotide or nap, and nicotinamide adenine dinucleotide phosphate or NADP. NAD and NADP are essential for the activity of a group of dehydrogenases, inorganic ions such as magnesium. Manganese, zinc, and iron are also sometimes regarded as coenzymes since these metal ions have been found

necessary to activate certain enzymes.

In either case, coenzymes must be added to many enzyme assay systems in sufficient amounts so as not to be limiting factors in the reaction

INHIBITORS

Inhibitors can cause enzyme reactions to proceed more slowly than expected for given conditions of pH, temperature, and substrate-coenzyme concentrations. Inhibitors may be substances which compete for the active sites on enzymes and generally have chemical structures very similar to those of the substrates. That is, the inhibitors compete with the substrates for the enzymes. For example, hexokinase, which acts upon d-glucose is inhibited by d-xylase or 6-deoxy d-glucose.

An inhibitor may also slow a reaction by binding to the enzyme at a point other than that at which the substrate is bound, in this case, the inhibitor alters the enzyme structure so as to decrease, or even stop the enzyme activity. An important example of this is fluoride ion which inactivates enolase. Enolase is involved in the metabolism of glucose. Consequently, fluoride ion is often used as a preservative in glucose determinations. Inhibitors have been used to gain information on complex enzyme systems, and to better understand single reaction mechanisms.

Naturally occurring inhibitors in blood serum do not have a significant influence on in vitro assays of enzymes. However, urine frequently contains inhibitors which dampen certain enzyme assays and the urine must be dialyzed prior to enzymatic analysis.

ISO-ENZYMES

Enzymes which catalyze the same chemical reaction but exhibit differences in their biochemical, physical, and immunological properties are termed ***iso-enzymes***. Chief among these differences are differences in their rates of electrophoretic migration. For example, under electrophoresis the LDH enzyme can be separated into five distinct fractions.

The various bodily organs and tissues contain characteristic proportions of the different iso-enzymes. When a specific organ or tissue is damaged, the cellular enzymes are released into the blood plasma where they superimpose their iso-enzyme patterns on those normally present:

case in point - cardiac muscle release iso-enzymes following a heart attack and, thus, are used to clinically confirm such a medical event. Consequently, a measure of the serum iso-enzymes patterns obtained by electrophoresis can often reveal the damaged tissue.

MODERN EQUIPMENT

Modern ultraviolet visible spectrophotometers now permit enzyme activities to be measured conveniently, quickly, and accurately. If the substrate acted upon or the product formed are colored, or can be coupled to secondary reactions which produce colored products, then the reaction can be monitored with a colorimeter, that is, a spectrophotometer measuring changes in visible light. Quantitative ultraviolet measurements of enzyme activity are becoming the more accepted approach, however. This preference arises for a number of reasons, but mainly because ultraviolet methods avoid the additional steps, reagents, and undesirable colored side reactions usually required to produce colored reactants and products. For example, a very common ultraviolet approach involves NAD and NADH . As shown in this ultraviolet spectrum, NAHD has a characteristic and relatively high absorption at 340 nanometers. NAD has virtually no absorption at the same wavelength. Consequently, any changes in the NADH concentration, either increasing or decreasing, can easily be monitored with little or no interference due to corresponding changes in NAD concentrations.

A specific example of the usefulness of these ultra violet absorption characteristics is in the assay of LDH enzyme. In one case, pyruvate and NADH are catalyzed by LDH-P to produce lactate and NAD. The NADH concentration would decrease and produce decreasing ultraviolet absorption. In the other case, the opposite reaction is catalyzed by LDH-L to produce increasing concentrations of NADH.

Enzyme reactions which do not involve NADH or NAD may often be coupled with other reactions which do involve these compounds; for example, in GOT assays. Aspartate and alpha-ketoglutarate may be catalyzed by GOT to produce glutamate and oxalacetate. Coupled to the first reaction, involves the generated oxalacetate, NADH, and a second enzyme, malic dehydrogenases to produce malate. Consequently, for every mole of oxalacetate produced by GOT, a mole of NADH is consumed and the absorbance change is directly proportional to GOT

activity. There are several ultra-violet visible spectrophotometric methods which are commonly used to determine enzyme activity levels. The two-point procedure involves measuring the amount of product formed, or the decrease in substrate, over a given interval over time. For example, blood serum samples are added to substrate solutions of known concentration and, after a period of incubation, the substrate or product is again measured. The difference then represents the amount of substrate transformed during the incubation period and provides an indirect measure of enzyme activity.