19.2.2 Enzymology

General terminology (See also Ch.15, kinetic methods of analysis)

Activation energy

An operationally defined quantity expressing the dependence of a rate coefficient on temperature. In enzymology it is usually considered to be the energy required for a molecule to form an activated complex which is in the transition of making or breaking a chemical bond. In an enzyme-catalyzed reaction, this corresponds to the formation of the activated enzyme-substrate complex.

Activator

An effector molecule that increases the catalytic activity of an enzyme when it binds to a specific site.

Active centre

The term is often applied to the sites that are the effective sites for a particular heterogeneous catalytic reaction. In enzymology it is considered to be that part of enzyme or other protein at which the initial binding of substrate and enzyme occurs to form the intermediate enzyme-substrate complex and at which location further chemical change characteristic of the catalyzed reaction takes place.

Allostery

A phenomenon whereby the conformation of an enzyme or other protein is altered by combination, at a site other than the substrate-binding site, with a small molecule, referred to as an *effector*, which results in either increased or decreased activity by the enzyme.

Apoenzyme

The protein part of an enzyme without the cofactor necessary for catalysis. The cofactor can be a metal ion, an organic molecule (coenzyme), or a combination of both.

Catalyst

A catalyst is a substance that increases the rate of a reaction without modifying the overall standard Gibbs energy change in the reaction; the process is called *catalysis*, and a reaction

in which a catalyst is involved is known as a *catalyzed reaction*.

Note: An enzyme is a biocatalyst.

Catalytic activity

The property of a catalyst which is measured by the catalyzed rate of conversion of a specified chemical reaction, produced in a specified assay system.

Catalytic activity concentration

The property of an enzyme obtained by dividing the catalytic activity by the volume of the original system from which the sample comes.

Note: In actual practice, the name may be shortened to "catalytic concentration."

Coenzyme

The dissociable, low-relative-molecular-mass active group of an enzyme which transfers chemical groups, hydrogen, or electrons. A coenzyme binds with its associated protein (apoenzyme) to form the active enzyme (holoenzyme).

Denaturation

The partial or total alteration of the structure of a protein without change in covalent structure by the action of certain physical procedures (heating, agitation) or chemical agents. Denaturation is the result of the disruption of tertiary bonding, which causes the opening of the folded structure of a protein and the loss of characteristic physiologic, enzymatic, or physicochemical properties; it can be either reversible or irreversible.

Enzyme

An enzyme is a protein that acts as a catalyst.

Holoenzyme

An active enzyme consisting of the apoenzyme and coenzyme.

Immobilized Enzymes

Soluble enzymes bound to an insoluble organic or inorganic matrix, or encapsulated within a membrane in order to increase their stability and make possible their repeated or continued use.

Induction

In enzymology, induction is a biological process which results in an increased biosynthesis of an enzyme thereby increasing its apparent activity. It results from the presence of an inducer.

Inhibition

An inhibitor is a substance that diminishes the rate of a chemical reaction; the process is called *inhibition*.

Isoenzyme

One of a group of related enzymes catalyzing the same reaction but having different molecular structures and characterized by varying physical, biochemical, and immunological properties.

Lineweaver-Burk plot

A plot of the reciprocal of velocity of an enzyme-catalyzed reaction (ordinate) versus the reciprocal of substrate concentration (abscissa). The plot is used to graphically define the maximum velocity of an enzyme-catalyzed reaction and the Michaelis constant for the enzyme.

Michaelis-Menten kinetics

Sometimes the relationship between the rate of an enzyme-catalyzed reaction [υ] and the substrate concentration [A] takes the form

$$v=\frac{V[A]}{K_{mA}+[A]} \qquad (1)$$

where V and K_{mA} are constants at a given temperature and a given enzyme concentration. The reaction is then said to display Michaelis-Menten kinetics. (The term hyperbolic kinetics is also sometimes used because a plot of υ against [A] has the form of a rectangular

hyperbola through the origin with asymptotes $v = V$ and $[A] = -K_{mA}$). This term, and others that imply the use of particular kinds of plot, should be used with care to avoid ambiguity, as they can be misleading if used out of context.

Note: The quantity $k_0[E]_0$ is given the symbol V and the name *limiting rate*. It is particularly useful when k_0 cannot be calculated because the total catalytic-centre concentration is unknown, as in studies of enzymes of unknown purity, sub-unit structure and molecular mass. The symbol V_{max} and the names *maximum rate* and *maximum velocity* are also in widespread use although under normal circumstances there is no finite substrate concentration at which $v = V$ and hence no maximum in the mathematical sense. The form V_{max} is convenient in speech as it avoids the need for a cumbersome distinction between "capital V" and "lower case v". When a true maximum does occur (as in substrate inhibition: the symbol v_{max} (not V_{max}) and the name maximum rate may be used for the true maximum value of v but care should be taken to avoid confusion with the limiting rate).

The second constant K_{mA} is known as the Michaelis constant for A; the alternative name *Michaelis concentration* may also be used and has the advantage of emphasizing that the quantity concerned has the dimensions of a concentration and is not, in general, an equilibrium constant. When only one substrate is being considered, the qualifier A may be omitted, so that the symbol becomes K_m.

When the qualifier is included, its location is a matter of typographical convenience; no particular significance attaches to such variants as K_m or K_{mA}. The Michaelis constant (or Michaelis concentration) is the substrate concentration at which $v = 0.5\ V$, and its usual unit is mol dm^{-3} which may be written as mol l^{-1}. The term Michaelis constant and the symbol K_m should not be used when Michaelis-Menten kinetics are not obeyed.

Product

The substance produced by the enzyme-catalyzed conversion of a substrate.

Substrate

A substrate is a reactant (other than the catalyst itself) in a catalyzed reaction.

Types of kinetic conditions

Pseudo-zero-order reaction

A zero-order reaction is one in which the rate of reaction is independent of the concentration of reactant. A reaction may be described as pseudo-zero order if it is independent of the reaction of the particular substrate being varied (e.g., the concentration

of the enzyme is held constant). This may apply when the enzyme is saturated with substrate over the range of substrate concentration studied.

Pseudo-first-order reaction

A first-order reaction is one in which the rate of reaction is proportional to the concentration of reactant. A second-order reaction, whose rate is proportional to the product of the concentrations of two reactants, may be described as pseudo-first order when the concentration of one is held constant, so that its rate is directly proportional to the concentration of the other.

Units Used to Express Enzyme Activity

International Unit

The unit enzyme activity proposed by the International Union of Biochemistry in 1964. Specifically, it is the amount of enzyme that catalyzes the conversion of one micromole of substrate per minute under the specified conditions of the assay method.

Note: This unit is no longer recommended because the term does not indicate what physical quantity it refers to, and because the minute is not the SI unit of time.

Katal

The amount of enzyme activity that converts one mole of substrate per second under specified reaction conditions.

Note: The katal is now the recommended unit to express enzyme activity. It is also called the mole per second.

Measurement Modes

One Point: A reaction mode in which only one measurement is made during the progress of the reaction. The measurement can be made either after stopping the reaction or when the reaction is in progress.

Two Point: A reaction mode in which individual measurements are taken at time t_1 and t_2. These times may either be fixed or variable.

Fixed Time: A two-point reaction mode in which measurements are taken at specified (i.e., "fixed") times. This mode is preferred for assays in which the reaction rate is first order in regard to the initial substrate concentration.

Variable Time: A two-point reaction mode in which the first measurement is taken at a specified time (t_1) and the second at a time after which a fixed signal change has occurred. This mode is preferred for the determination of the catalytic activity concentration of enzymes or other catalysts.

Multipoint: A reaction mode in which at least three data points are obtained during the time course of the reaction.

Sequential two point: A reaction mode in which a series of data points are accumulated by measuring the signal changes occurring in an assigned sequence of time intervals (consecutive or overlapping). Various algorithms can then be used to convert the series of intervals of signal change into units of enzyme activity.

Multipoint with average rate: A reaction mode in which enzyme activity is determined by either averaging the rate for each interval measured or averaging selected intervals after some have been rejected.

Multipoint with computer analysis: A reaction mode in which multiple points are obtained and the rate obtained by applying a mathematical model to data.

Continuous: A reaction mode in which the reaction is monitored continuously and the data presented in either an analog or digital mode.