Enzyme Activity and Assays

Introductory article

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Enzyme activity refers to the general catalytic properties of an enzyme, and enzyme assays are standardized procedures for measuring the amounts of specific enzymes in a sample.

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Factors that Affect Enzymatic Analysis

Enzyme activity is measured *in vitro* under conditions that often do not closely resemble those *in vivo*. The objective of measuring enzyme activity is normally to determine the amount of enzyme present under defined conditions, so that activity can be compared between one sample and another, and between one laboratory and another. The conditions chosen are usually at the optimum pH, 'saturating' substrate concentrations, and at a temperature that is convenient to control. In many cases the activity is measured in the opposite direction to that of the enzyme's natural function. Nevertheless, with a complete study of the parameters that affect enzyme activity it should be possible to extrapolate to the activity expected to be occurring *in vivo*.

The factors that affect the activity of an enzyme include substrate concentration, pH, ionic strength and nature of salts present, and temperature. Activity is measured as the initial rate of substrate utilization when no products are present (a situation that rarely occurs *in vivo*). There are many compounds that may act as inhibitors which repress the activity, so they should not be present. The subject of enzyme inhibitors is a complex one which will not be dealt with here. However, it is worth noting that the converse, namely the involvement of nonsubstrate activators, must be attended to with many enzymes, since they can be totally inactive without an activator present.

The effect of substrate concentration

The traditional enzyme has a hyperbolic response to substrate concentration, according to the Michaelis–Menten equation:

$$v = \frac{V_{max} \cdot [S]}{K_m + [S]} \qquad [1]$$

where [S] is the substrate concentration, v is the rate measured, V_{max} is the maximum theoretical rate at infinite substrate concentration, and K_m is the Michaelis constant. Applying this formula we find that the rate v is one-half of V_{max} when $[S] = K_m$, and 91% of V_{max} when $[S] = 10 \times K_m$. The substrate concentration that is used in enzyme assays is chosen according to parameters such as the K_m, the solubility of the substrate, whether high concentrations may inhibit, and the cost of the substrate. If values much less than $2 \times K_m$ are used, it becomes more critical to know exactly what the concentration is (some preparations of unusual substrates may be impure, or the exact amount present may not be known). This is because the rate measured varies with substrate concentration more rapidly as the substrate concentration decreases, as can be seen in **Figure 1**. In most cases an enzyme assay has already been established, and the substrate concentration, buffers and other parameters used previously should be used again.

There are many enzymes which do not obey the simple Michaelis–Menten formula. The most extreme deviations are with those enzymes known as 'allosteric', in which a sigmoid shape of response is found (**Figure 1**). With many allosteric enzymes an activator is required, and this can convert the sigmoid shape to a hyperbolic curve. Each allosteric enzyme has its own specific characteristics, so we cannot generalize about their behaviour.

The effect of pH on enzyme activity

Enzymes are active only within a limited range of pH. But the limits may be wide, e.g. pH 5 to 10, or narrow, e.g. over 1 pH unit. Within the range there will be an optimum at which the maximum activity (the highest value for V_{max}) is attained: this could be a short range in itself. The activity can also be affected by the nature of the buffer used. There could be a discontinuity in activity over the pH range tested because of the use of different buffers. Alternative buffers for a given pH should be tested.

The effect of pH is generally tested at high substrate concentration. However, if tested at low concentration, it is

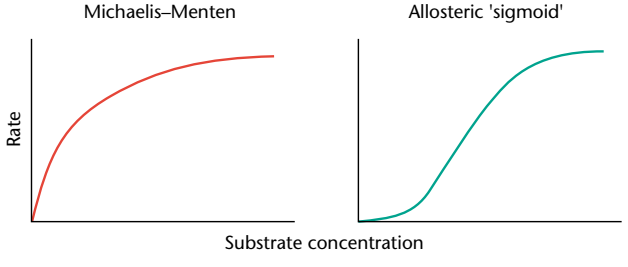

Figure 1 Comparison of a conventional Michaelis–Menten enzyme with an allosteric enzyme: the rate variation with substrate concentration.

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