Appendix A

Enzyme Kinetics

Enzyme (E) reacts reversibly with substrate (S) to form a complex (ES), with rate constant k_1 for the forward reaction and k_{-1} for the reverse reaction. ES also breaks down reversibly to form product (P) and the free enzyme (E). At the beginning of the reaction the amount of ES is maximal and the amount of P formed is so small that the backward reaction is negligible, so that this stage may be regarded as irreversible with a rate constant k_2.

Therefore $E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} P + E$ ..... (1)

The rate of a reaction equals the product of the rate constant and the concentration(s) of the reactant(s). At the beginning of the reaction the concentration of ES quickly reaches a relatively constant value, its rate of breakdown, $k_2[ES] + k_{-1}[ES]$, equalling its rate of formation $k_1[E][S]$, where square brackets indicate concentration, ie,

$$k_1[E][S] = k_{-1}[ES] + k_2[ES]$$
$$= [ES](k_{-1} + k_2)$$
$$\text{and } [E] = \frac{[ES](k_{-1} + k_2)}{k_1[S]}$$
$$= \frac{[ES]}{[S]} \cdot \frac{(k_{-1} + k_2)}{k_1}$$
$$\text{or } [E] = \frac{[ES]K_m}{[S]} \quad \ldots\ldots\ldots\ldots\ldots (2)$$

where K_m (Michaelis constant) equals $\frac{(k_{-1} + k_2)}{k_1}$.

As total enzyme concentration $[E_t] = [E] + [ES]$, then $[E] = [E_t] - [ES]$, and, substituting for [E] in (2), we get

$$[E_t] - [ES] = \frac{[ES]\,K_m}{[S]}$$
$$\text{therefore } [E_t] = \frac{[ES]\,K_m}{[S]} + [ES]$$
$$= [ES]\left(\frac{K_m}{[S]} + 1\right)$$
$$= \frac{[ES]\,(K_m + [S])}{[S]}$$
$$\text{therefore } [ES] = \frac{[E_t]\,[S]}{(K_m + [S])} \quad \ldots\ldots (3).$$

When the rate of formation of ES equals the rate of breakdown to P + E, the overall reaction rate will be equal to the rate of the latter reaction, ie, $v = k_2[ES]$, so that $[ES] = \frac{v}{k_2}$ ..... (4).

When the concentration of substrate is sufficient to saturate the enzyme, virtually all the enzyme will be in the form ES, ie, $[ES] = [E_t]$, and the reaction rate will be maximal (V_{max}), so that $V_{max} = k_2[E_t]$

$$\text{or } [E_t] = \frac{V_{max}}{k_2} \quad \ldots\ldots\ldots\ldots\ldots (5).$$

Substituting in equation 3 the values for [ES] and $[E_t]$ in equations 4 and 5 respectively

$$\frac{v}{k_2} = \frac{\frac{V_{max}}{k_2} \cdot [S]}{K_m + [S]}$$

multiplying both sides by k_2

$$v = \frac{V_{max}\,[S]}{K_m + [S]} \quad \ldots\ldots\ldots\ldots (6).$$

Equation 6 is the Michaelis-Menten equation which relates v, V_{max}, and [S] in terms of K_m. The significance of K_m is illustrated if we make [S] equal to K_m; then $v = \frac{V_{max}}{2}$, that is, K_m is equal to the substrate concentration which gives a reaction rate equal to half the maximal reaction rate.

If v, the initial reaction rate, is plotted against [S], the result is a curve showing a rapid rise of reaction rate with increasing [S] at low substrate concentrations, whereas at high [S] the enzyme becomes saturated with substrate and the rate reaches a plateau representing V_{max}, which is dependent on enzyme concentration (Fig. 2, page 15). K_m can be read off as the value for [S] where v is $\frac{1}{2}V_{max}$. To define a curve accurately it is necessary to determine many points, and there are advantages in manipulating the Michaelis-Menten equation into the form of a straight-line equation $y = ax + b$ where a is the slope and b is the intercept on the y axis. One method is as follows:

Taking the reciprocal of both sides of equation 6

$$\frac{1}{v} = \frac{K_m + [S]}{V_{max}\,[S]}$$
$$\text{or } \frac{1}{v} = \frac{K_m}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}} \quad \ldots\ldots\ldots\ldots (7).$$

This is the Lineweaver-Burk equation, and a plot of $\frac{1}{v}$ against $\frac{1}{[S]}$ will give a straight line with a slope of

$\frac{K_m}{V_{max}}$ and a y-intercept of $\frac{1}{V_{max}}$ (Fig. 3, page 15).

Thus, when $\frac{1}{[S]} = 0$ (ie, [S] is infinitely high) then

$$\frac{1}{v} = \frac{1}{V_{max}}$$

Furthermore, when $\frac{1}{v} = 0$, then from equation 7

$$-\frac{K_m}{V_{max}} \cdot \frac{1}{[S]} = \frac{1}{V_{max}}$$

$$\text{therefore } -\frac{1}{[S]} = \frac{1}{K_m}$$

and thus $\frac{1}{K_m}$ can be read off where the line cuts the $\frac{1}{[S]}$ axis.

Enzyme Inhibition

Enzyme inhibitors are usually classified as competitive, non-competitive, and uncompetitive, and these all have different properties which can conveniently be illustrated by means of the Lineweaver-Burk plot.

Competitive inhibitors act by competing with the substrate for the active site of the enzyme, so that their effect depends on the concentration of both inhibitor and substrate. At infinitely high substrate concentration ($1/[S] = 0$) the substrate will completely overcome the effect of the inhibitor so that V_{max} (and therefore the y-intercept) will be unaffected. But at lower substrate concentrations, the concentration of ES will be less than in the absence of an inhibitor so that for any given substrate concentration v will be lower (1/v will be higher) than in the absence of the inhibitor; hence the substrate concentration required for $\frac{1}{2}V_{max}$ will be higher, ie, K_m rises ($1/K_m$, and hence the x-intercept, will be lower). The Lineweaver-Burk plot will resemble line B in Fig. 4 (page 16) as compared with the uninhibited enzyme line A.

Non-competitive inhibitors result in a reduction of enzyme activity irrespective of [S], probably by combining with a non-active site on the enzyme. This produces a proportional reduction in velocity (rise in 1/v) at all substrate concentrations, including that which gives V_{max}, which is therefore reduced (y-intercept increased). The residual enzyme activity is normal so that Km (or $-$ 1/Km, the x-intercept), is unaffected. The resulting Lineweaver-Burk plot is shown in Figure 4, line C page 16.

Uncompetitive inhibitors also produce a fall in V_{max} (ie, rise in $1/V_{max}$) and, in addition, a proportional fall in K_m (ie, rise in $1/K_m$) so that the Lineweaver-Burk plot lies above and parallel to that of the uninhibited enzyme (Figure 4, line D page 16). It is considered that such inhibitors react with the enzyme-substrate complex in such a way as to reduce its rate of breakdown into product at all substrate concentrations, thus reducing V_{max} (ie, $1/V_{max}$ higher). However, the reaction velocity is still proportional to the concentration of enzyme-substrate complex ES and a reduction in the rate of breakdown of ES will result in there being more ES present for any given substrate concentration, therefore a smaller concentration of substrate will be needed to produce the ES concentration corresponding to $\frac{1}{2}$ V_{max}, ie, K_m will be lower ($1/K_m$ higher).

Enzyme Assays

When an enzyme is assayed, the substrate concentration should be high enough to saturate the enzyme, so that the reaction rate, which is proportional to [ES], is dependent on the enzyme concentration and not upon substrate concentration. Also the initial reaction velocity should be studied so that product does not accumulate in sufficient quantity to cause a significant reversal of the reaction $ES \rightarrow P + E$.

Other conditions affecting enzyme activity, eg, temperature and *p*H, should if possible be optimal or, if not optimal, at least rigidly standardized.

The amount of enzyme present cannot as a rule be expressed in terms of concentration because we rarely have the information necessary to convert enzyme activity into absolute terms such as milligrams or moles of enzyme. It is therefore necessary to use arbitrary units of activity and these, for the sake of comparability between different methods, should be standardized whenever possible. The standard unit (U) is the amount of any enzyme which will catalyse the transformation of one micromole of substrate per minute under standard conditions such as have been indicated above, and the temperature should always be specified. It must be emphasized that two different methods will not necessarily give the same values for a given enzyme preparation even when activity is defined in a standard manner and assayed at standard temperature with conditions designed to ensure V_{max}; for instance, a difference in buffer systems may affect enzyme activity, or one method may measure the forward reaction whereas another may measure the reverse reaction. It is therefore desirable to quote the method of assay and state whether the forward or reverse reaction is measured.

Appendix B

Trivial Name of Enzyme	*IUB Code Number*
Acetylcholinesterase	3.1.1.7
β-Acetylglucosaminidase	3.2.1.30
Acid phosphatase	3.1.3.2
Adenylate kinase	2.7.4.3
Alanine aminotransferase	2.6.1.2
Alkaline phosphatase	3.1.3.1
Amylase	3.2.1.1
Arginase	3.5.3.1
Argininosuccinate lyase	4.3.2.1
Argininosuccinate synthetase	6.3.4.5
Arylsulphatase	3.1.6.1
Aspartate aminotransferase	2.6.1.1
ATPase	3.6.1.3
Carbonic anhydrase	4.2.1.1
Carboxylesterase	3.1.1.1
Carboxypeptidase A	3.4.2.1
Catalase	1.11.1.6
Cholinesterase	3.1.1.8
Creatine kinase	2.7.3.2
Diamine oxidase	1.4.3.6
O-Diphenol oxidase (Dopa oxidase)	1.10.3.1
Diphosphoglyceromutase	2.7.5.4
Fructosediphosphate aldolase	4.1.2.13
Galactose 1-phosphate uridylyltransferase	2.7.7.10
β-Galactosidase	3.2.1.23
Glucokinase	2.7.1.2
Glucose 6-phosphatase	3.1.3.9
Glucose 6-phosphate dehydrogenase	1.1.1.49
Glucosephosphate isomerase	5.3.1.9
β-Glucuronidase	3.2.1.31
Glutamate dehydrogenase	1.4.1.3
D-Glutamyltransferase	2.3.2.1
Glutathione peroxidase	1.11.1.9
Glutathione reductase	1.6.4.2
Glycerolphosphate dehydrogenase	1.1.99.5
Glycogen phosphorylase	2.4.1.1
Glycoside hydrolase	3.2.1
Hexokinase	2.7.1.1
Hyaluronidase	3.2.1.35
Hypoxanthine phosphoribosyltransferase	2.4.2.8
Isocitrate dehydrogenase	1.1.1.42
Ketose 1-phosphate aldolase	4.1.2.7
Lactate dehydrogenase	1.1.1.27
Leucine aminopeptidase	3.4.1.1
Lipase	3.1.1.3
Lysozyme	3.2.1.17
Malate dehydrogenase	1.1.1.37
Methylmalonyl-CoA mutase	5.4.99.2
5'-Nucleotidase	3.1.3.5
Ornithine carbamoyltransferase	2.1.3.3
Phosphoglucomutase	2.7.5.1
Phosphogluconate dehydrogenase	1.1.1.43
Phosphoglycerate kinase	2.7.2.3
Pyruvate kinase	2.7.1.40
Reduced NAD dehydrogenase	1.6.99.3
Renin	3.4.4.15
Serine dehydratase	4.2.1.13
Succinate dehydrogenase	1.3.99.1
Transketolase	2.2.1.1
Triosephosphate isomerase	5.3.1.1
UDP glucose epimerase	5.1.3.2

Table *List of enzymes by their trivial names with IUB code numbers*