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Enzyme Specificity: Its Meaning in the General Case

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If an enzyme catalyses two competing reactions, their relative rates are determined by the concentrations of the competing substrates and the two specificity constants, i.e. the catalytic constants for the two substrates divided by the corresponding Michaelis constants. The concept of a specificity constant can be extended to reactions that require two or more substrates: in such cases the specificity for any competing substrate is determined by the apparent specificity constant measured at whatever concentrations of co-substrates, inhibitors, etc., exist under the conditions of competition. The partitioning between two competing substrates is independent of the concentration of any species, such as co-substrate, inhibitor, etc., that reacts only in the part of the mechanism that is common between the competing substrates.

1. Introduction

Specificity is a crucial concept in the study of enzymes. Indeed, it would hardly be an exaggeration to say that the specificity of enzyme catalysis was more important for the appearance of life than efficiency of enzyme catalysis. It is surprising, therefore, that the term is used rather loosely by biochemists, being applied, for example, to comparisons of substrates considered in isolation from one another. As Fersht (1977) in particular has emphasized, the only way of considering specificity that has a useful physiological meaning is in relation to discrimination between competing substrates that are simultaneously present in the same reaction mixture.

If A_1 is a substrate in a single-substrate reaction that follows Michaelis–Menten kinetics with catalytic constant k_{01} and Michaelis constant K_{m1}, and A_2 is a substrate for the same enzyme, also reacting according to Michaelis–Menten kinetics but with parameters k_{02} and K_{m2}, the ratio of the rates v_1 and v_2 obtained when A_1 and A_2 are mixed together at concentrations a_1 and a_2 respectively is as follows (Fersht, 1977):

$$v_1/v_2 = \frac{(k_{01}/K_{m1})a_1}{(k_{02}/K_{m2})a_2}. \qquad (1)$$

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This very important result shows that discrimination between two competing substrates is determined neither by the catalytic constants nor by the Michaelis constants, but by their ratio k_0/K_m. In recognition of this, the Nomenclature Committee of the International Union of Biochemistry (1982) has recommended the name *specificity constant* for this quantity, with the symbol k_A, in which the subscript defines the substrate referred to. Thus equation (1) can be written in the following simple form:

$$v_1/v_2 = k_{A_1}a_1/k_{A_2}a_2. \qquad (2)$$

A potential difficulty is that in practice one is rarely concerned with discrimination between competing substrates in a single-substrate reaction, because most enzymes catalyse reactions between two or more substrates. How, for example, should one define the specificity of a hexokinase in catalysing the phosphorylation of two sugars, e.g. glucose and fructose, by ATP? This is an example of an enzyme catalysing two competing reactions $A_1 + B \rightarrow P + Q_1$ and $A_2 + B \rightarrow P + Q_2$, in which there are two competing substrates A_1 and A_2, giving rise to different products Q_1 and Q_2, but with a second substrate-product pair B and P that are common to the two reactions. If both reactions obey Michaelis–Menten kinetics, k_{01}, K_{m1} and k_{A_1} may be defined as the parameters observed for the reaction of A_1 when B is saturating and no products, inhibitors or competing substrates are present; the corresponding parameters with subscript 2 are defined similarly for the reaction of A_2. When B is not saturating, or products, inhibitors or competing substrates are present, the observed values are apparent values, k_{01}^{app}, K_{m1}^{app} etc.

It is not immediately obvious, at least not to me, what equation corresponds to equation (2) in this more general and more physiologically useful case. Must one use equation (2) as it stands, with k_{A1} and k_{A2} referring to the rather artificial and unphysiological case of saturating B, or must they be replaced by $k_{A_1}^{app}$ and $k_{A_2}^{app}$, or is the result the same whichever way the equation is written? I shall show in this paper that the answer to this question is not only very simple but is also experimentally convenient and intuitively satisfying.

2. Theory

Any mechanism containing two competing reactions will contain a part that is unique to one competing reaction, a part that is unique to the other, and a part that is common to both. This common part may consist of a single enzyme form, or it may contain one or more common steps, as in the example shown in Fig. 1(c): the step $E + A \rightleftarrows EA$ is common to the

reactions of both B_1 and B_2, but the remainder of the mechanism is unique to the reaction of B_1 or to that of B_2. The whole mechanism may be regarded as a coalescing of the reaction of B_1 (Fig. 1(a)) with that of B_2 (Fig. 1(b)).

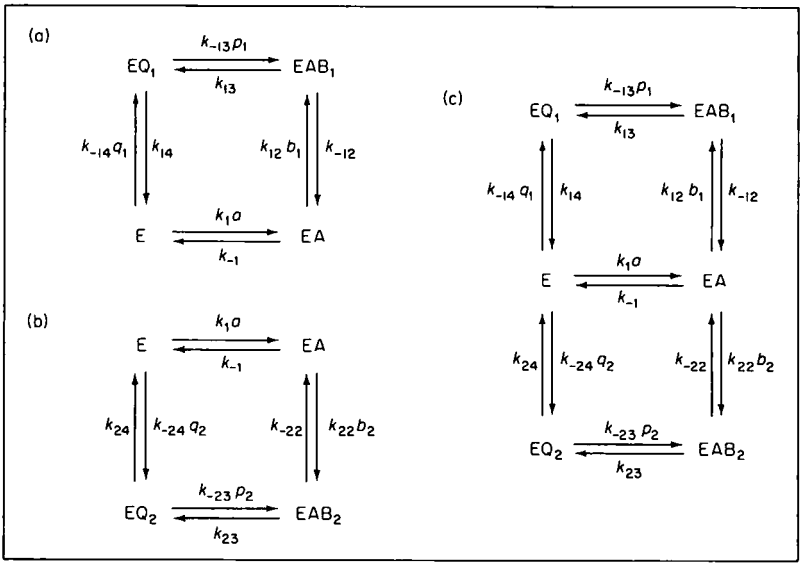

FIG. 1. Mechanism for competition between two substrates in a reaction requiring two substrates, the first substrate A being common to the competing reactions of B_1 and B_2. (a) Reaction between A and B_1 in the absence of B_2; (b) reaction between A and B_2 in the absence of B_1; (c) reaction with both competing second substrates present simultaneously.

Provided that B_i (here representing either B_1 or B_2, i.e. $i = 1$ or 2) enters into only one step of a reaction for its conversion into products, the reaction must obey Michaelis–Menten kinetics with respect to B_i, i.e. the rate v_i for reaction of B_i must be given by an equation of the following form:

$$v_i = N_i e_0 b_i / (CU_i + F_i b_i) \tag{3}$$

in which e_0 is the total enzyme concentration and N_i, CU_i and F_i are independent of b_i, the concentration of B_i. The numerator coefficient N_i is the sum of the products of the rate constants and reactant concentrations other than b_i for all pathways that both contain a step away from each enzyme form and include a complete reaction cycle that converts B_i into products (Wong & Hanes, 1962; Wong, 1975). The independence of N_i from b_i follows from the fact that $N_i b_i$ must be proportional to b_i if B_i enters into only one step of the reaction. The denominator $CU_i + F_i b_i$ is derived by the method of King & Altman (1956) and is the sum of all the products

of rate constants generated by all the King–Altman patterns. If B_i enters into only one step of the reaction this denominator must be a linear function of b_i and hence it must be possible to define CU_i and F_i so that they are both independent of b_i. Moreover, if the mechanism is considered to consist of a unique part, containing the step in which B_i participates and any number of other steps that are connected to this step, and a common part, containing the rest of the mechanism, it must be possible to factorize CU_i into a common factor C that contains only rate constants from the common part and a unique factor U_i that contains only rate constants from the unique part.

For the mechanism of Fig. 1(a), considered in the absence of products, the coefficients have the following definitions in terms of rate constants and the concentration a of A:

$$N_{1,0} = k_1 k_{12} k_{13} k_{14} a \tag{4}$$

$$CU_{1,0} = (k_{-1} + k_1 a)(k_{-12} + k_{13}) k_{14} \tag{5}$$

$$F_{1,0} = (k_1 a + k_{13}) k_{12} k_{14}. \tag{6}$$

In these expressions, the first subscript, 1, specifies the substrate, B_1, referred to, and the second, 0, specifies that no competing substrate is present. If the common part of the mechanism is defined as that part that also appears in Fig. 1(b), i.e. the step interconverting E + A with EA, $CU_{1,0}$ may be factorized to give

$$C = k_{-1} + k_1 a \tag{7}$$

$$U_{1,0} = (k_{-12} + k_{13}) k_{14}. \tag{8}$$

As equation (3) is of the form of the Michaelis–Menten equation, the apparent specificity constant for B_i may be expressed as

$$k_{B_i}^{app} = N_i / CU_i \tag{9}$$

which has the following value for the mechanism of Fig. 1(a):

$$k_{B_{1,0}}^{app} = N_{1,0} / CU_{1,0} = k_1 k_{12} k_{13} a / [(k_{-1} + k_1 a)(k_{-12} + k_{13})]. \tag{10}$$

If the first subscript 1 is replaced throughout by 2, the corresponding equations define the parameters for B_2, i.e. the mechanism of Fig. 1(b), are generated:

$$N_{2,0} = k_1 k_{22} k_{23} k_{24} a \tag{11}$$

$$U_{2,0} = (k_{-22} + k_{23}) k_{24} \tag{12}$$

$$k_{B_{2,0}}^{app} = N_{2,0} / Cu_{2,0} = k_1 k_{22} k_{23} a / [(k_{-1} + k_1)(ak_{-22} + k_{23})]. \tag{13}$$

Consider now the reaction when both competing substrates B_1 and B_2 are present. The same general equation, equation (3), applies. The definitions of the coefficients are more complicated, but in a regular way that can be rationalized by consideration of the methods of King & Altman (1956) and Wong & Hanes (1962). (The ideas of Volkenstein & Goldstein (1966), which are discussed in Cornish-Bowden (1976), are also helpful.) For conversion of B_1, the original numerator coefficient $N_{1,0}$ must be multiplied by the sum of products of rate constants for all pathways that lead into the common part of the mechanism from the part that is unique to B_2. As none of these pathways can contain the step that involves B_2 (because this leads out of the common part of the mechanism), it is obvious that this factor must be $U_{2,0}$, i.e.

$$N_{1,2} = N_{1,0}U_{2,0}. \tag{14}$$

Similarly, for the conversion of B_2 in the presence of B_1 the numerator coefficient must be

$$N_{2,1} = N_{2,0}U_{1,0}. \tag{15}$$

It is not necessary to consider the form of the denominator, because this must be the same for both B_1 and B_2, so that the ratio of rates is simply the ratio of numerators:

$$\frac{v_1}{v_2} = \frac{N_{1,2}b_1}{N_{2,1}b_2} = \frac{N_{1,0}b_1/CU_{1,0}}{N_{2,0}b_2/CU_{2,0}}. \tag{16}$$

However, comparison with equation (9) shows that this result may be expressed more simply in terms of apparent specificity constants:

$$v_1/v_2 = k^{\mathrm{app}}_{\mathrm{B}_1,0}b_1/k^{\mathrm{app}}_{\mathrm{B}_2,0}b_2. \tag{17}$$

This result applies quite generally to all mechanisms in which the competing substrates appear in one step each.

Although C is included explicitly in equation (16) to make the relationship with equation (9) obvious, it can be cancelled between numerator and denominator. A consequence of this is that any species that is involved only in the common part of a mechanism has no effect on the discrimination between competing substrates. In particular, any additional substrates required by the reaction have no effect on the partitioning if they are confined to the common part of the mechanism. In such cases, but only in such cases, equation (17) may be rewritten in terms of actual rather than apparent specificity constants:

$$v_1/v_2 = k_{\mathrm{B}_1}b_1/k_{\mathrm{B}_2}b_2. \tag{18}$$

In the particular case of the mechanism shown in Fig. 1(c), A occurs in the common part, and so equation (18) is valid for this mechanism. However, if we were considering the alternative competition between A_1 and A_2, the substrates that bind first to the enzyme, the second substrate B would react with EA_1 and with EA_2 in two different reactions; so it would not be in the common part of the mechanism and no equation similar to equation (18) would describe the discrimination between A_1 and A_2.

3. Discussion

It appears that the parameter that defines discrimination between competing substrates in a reaction requiring more than one substrate is the ratio of the apparent specificity constants that apply to the competing substrates in the absence of one another. This is a very convenient property from the point of view of considering specificity *in vivo*, because it means that the (apparent) specificity constants not only can but must be measured at the concentrations of additional substrates, effectors, etc. that are thought to exist *in vivo*; it is not necessary, indeed it would be wrong, to vary the concentrations of the other substrate(s) required by the reaction or to extrapolate to saturation or any other physiologically unrealistic state.

Fromm (1964) has suggested the use of competing substrates for distinguishing between kinetic mechanisms for two-substrate reactions, and has shown that this approach works satisfactorily in cases where the mechanism is known independently (Rudolph & Fromm, 1970). More recent discussion of this method may be found in Fromm (1975) and Dixon & Webb (1979). The discussion in the present paper has been concerned primarily with the meaning of specificity rather than with its use as a mechanistic probe, but there is an obvious algebraic correspondence between the two cases and there may be circumstances in which specificity measurements may lead directly to mechanistic information. In principle, one could use the limited applicability of equation (18) as a mechanistic probe, for example in circumstances where it was difficult to make accurate measurements of the initial rates for conventional kinetic analysis but measurement of the relative rates for two competing substrates was possible. Any dependence of the ratio of rates on the concentration of any other species, whether this was another substrate required by the reaction or an inhibitor or other effector, would show that this other species bound to a form of the enzyme containing the competing substrate; if no dependence could be detected it would suggest that the other species bound to the enzyme only in the part of the mechanism that was in common between the two competing substrates.

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