

BIOCHEMICAL JOURNAL LETTERS

Specificity constants for two-substrate reactions

Engel [1] has proposed a “true combined specificity constant” for two-substrate reactions. Unfortunately, his proposal is only applicable to reactions which proceed through a ternary complex. There is no equivalent constant for reactions which proceed through a substituted enzyme (ping-pong) mechanism [2,3 (page 112)]. Although specificity constants are often said to measure catalytic efficiency and to have fundamental significance, these statements are only true in the context of measurements *in vitro* when initial velocities are measured with enzyme concentrations much less than those of the substrate and much less than the K_m , and when product is absent, conditions not always found *in vivo* ([4] and references therein). Because of the arbitrary aspect of this constant, a consistent definition is desirable.

The definition of specificity constant given by the IUB Nomenclature Committee [5] for a single-substrate reaction is the apparent second-order rate constant for a reaction at very low substrate concentrations, and thus defined it is applicable whatever kinetics the enzyme displays with higher substrate concentrations. For Michaelis–Menten kinetics, this second-order rate constant is identical with the ratio k_{cat}/K_m . Cornish-Bowden [3 (page 84)] has pointed out that for a pair of alternative substrates A and B present together (and Michaelis–Menten kinetics) the ratio of velocities is given by:

$$\frac{v_A}{v_B} = \frac{V^A K_m^B [A]}{V^B K_m^A [B]} \quad (1)$$

where V and K are the parameters obtained for each substrate separately at the same enzyme concentration, so that for *any* equal substrate concentrations the ratio of velocities is the ratio of specificity constants.

For two-substrate reactions, when a second substrate B is competing with A in its reaction with C, equations for initial velocities are readily derived using steady-state assumptions (except for the random order/ternary complex mechanism which requires equilibrium assumptions to force a Michaelis–Menten outcome). The equations are a little more complex than those for competing one-substrate reactions, but the ratio of velocities reduces to eqn. (1). Except when C is the second substrate to add in an ordered reaction forming a ternary complex, the result is independent of [C]. In the exceptional case it is true only when C is saturating. (It should be stressed, as Engel has done [1], that K_m^A and K_m^B are K_m values properly determined for saturating C [3 (page 111)], even though they are then applied more generally.) For the Theorell–Chance mechanism [6], eqn. (1) is valid when the competing substrates are the first to add to form an enzyme–substrate complex (at any concentration of C), but when the competing substrates are the second to react the concept of specificity constant has no meaning. In this case the ratio of velocities is simply $k_A[A]/k_B[B]$, where k_A and k_B are second-order rate constants for the reaction of the enzyme–C complex with A and B respectively.

Because Cornish-Bowden’s competition concept [3] for

enzymes displaying Michaelis–Menten kinetics is applicable to two-substrate reactions at any equal concentrations of the competing substrates, is applicable to all two-substrate reaction mechanisms, and uses the common operationally defined K_m for a substrate, I suggest that the specificity constant defined by k_{cat}/K_m be retained for all reactions. For some two-substrate reactions we may have to consider two alternative pairs of substrates: for example, two competing substrates for a dehydrogenase which can react with two competing cofactors (commonly ordered addition/ternary complex mechanisms), or two amino acids competing for transamination with two competing 2-oxoacids (substituted enzyme mechanisms when the transaminase has a tightly bound pyridoxal phosphate cofactor). If A or its alternative B can react with C or its alternative D, the substrates may be considered three at a time. For example, if any equal concentrations of A and B compete in a reaction with C and give velocities v_{AC} and v_{BC} respectively, these will have the ratio $k_{cat}^{AC} K_m^{BC} / k_{cat}^{BC} K_m^{AC}$. The superscripts identify the reactions for which k_{cat} and K_m have to be separately determined, and there will be three other ratios (v_{AD}/v_{BD} , v_{AC}/v_{AD} , and v_{BC}/v_{BD}) each with its appropriate k_{cat} and K_m values. Specificities will then be apparent from a comparison of the four velocity ratios. (The actual work involved is not greater than that required for Engel’s proposed combined constant [1].)

This treatment does not consider relative velocities when all four substrates are present together, but that is not the purpose of specificity constants as used, for example, in studies on the effects of point mutations on catalytic efficiency and substrate specificity. The effects of mutations *in vivo* may require evaluation of rates with all four substrates and products present, but the effects of changes in individual rate constants (to which changes in k_{cat} and K_m are secondary) have not yet been analysed fully ([7] and references therein).

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Enzyme specificity in reactions of more than one co-substrate

In a recent letter, Engel (1992) discusses the meaning of the specificity constant of an enzyme with two co-substrates. He makes several important points, especially in relation to the ‘overall catalytic efficiency’ introduced by Feeney et al. (1990)