

Derivation of the Rate Equation for the Aspartate Aminotransferase Mechanism from the Michaelis-Menten Assumptions

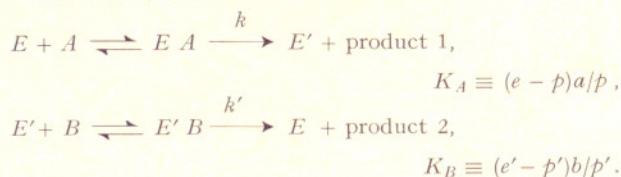
The rate equation for the enzymatic mechanism exemplified by aspartate aminotransferase has been derived from steady-state theory (ALBERTY¹, VELICK and VAVRA²), but it has not been reported before that the derivation can be made using the Michaelis-Menten conditions.

Symbols are those recommended by the Enzyme Commission of the International Union of Biochemistry, modified in that the 2 half-reactions are treated separately, the symbols referring to the second enzyme form being distinguished where necessary by the prime ('), and in that certain additions are required, namely:

$$e_t = e + e' \text{ (total enzyme concentration),}$$

K_a , K_b are the 'true K_m ' for the substrates A and B , as defined by VELICK and VAVRA².

The 2 half-reactions are:



For any constant overall reaction rate, the rates of the 2 half-reactions must be equal, for if they were not, the enzyme form produced by the faster would accumulate, and the enzyme form consumed by it would be depleted until the 2 rates became equal, i.e. $v = k p = k' p'$. Therefore $(p/p') = (k'/k) (= R, \text{ say})$. Thus the concentrations of the 2 enzyme-substrate complexes are in a fixed ratio during any period of constant reaction rate, irrespective of substrate concentrations. The value of R could be altered if k and k' themselves were altered but not otherwise.

Note that,

$$e = p(1 + K_A/a), \quad e' = p(1 + K_B/b)/R.$$

Therefore,

$$e = \frac{e_t R (1 + K_A/a)}{1 + K_B/b + R (1 + K_A/a)},$$

$$v = k p = k' e/R (1 + K_A/a) = \frac{k' e_t}{1 + K_B/b + R (1 + K_A/a)}.$$

The rate equation derived from steady-state theory may be written,

$$V_{max}/v = 1 + K_a/a + K_b/b,$$

and the following correspondences are readily obtained:

$$V_{max} = k' e_t/(1 + R), \quad K_a = R K_A/(1 + R),$$

$$K_b = K_B/(1 + R).$$

The new equation predicts half-maximal velocity when half the total enzyme exists as total enzyme-substrate complex. This may be achieved in an infinite variety of combinations of substrate concentrations, but fixing the concentration of one, fixes the other.

The factors $1/(1 + R)$ and $R/(1 + R)$ express the concentration of $E'B$ and EA respectively as proportions of the total enzyme-substrate complex. It should be noted that R cannot be equated with the ratio of pyridoxal to pyridoxamine forms of aspartate aminotransferase. Indeed it has no simple physical interpretation, since there are, in reality, more than 2 forms of enzyme-substrate complex.

Résumé. On peut déduire l'équation de vitesse de la réaction catalysée par la transaminase glutamique - oxalacétique (EC 2.6. 1.1) en utilisant les postulats de Michaelis et Menten, ainsi que l'hypothèse de «Steady-State».

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¹ R. A. ALBERTY, J. Am. chem. Soc. 75, 1928 (1953).

² S. F. VELICK and J. VAVRA, J. biol. Chem. 237, 2109 (1962).