NEW METHOD TO DIFFERENTIATE BETWEEN SOME MECHANISMS OF ACTION OF ENZYMES WITH THREE SUBSTRATES

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1. Introduction

In the usual kinetic analysis of three-substrate reactions the saturation curve of one substrate (first substrate) is measured at three or four different, but in each experiment constant, concentrations of another substrate (second substrate), keeping the concentration of the third substrate constant. This procedure should be performed at 3–4 different concentrations of the third substrate and should be repeated in all combinations of first, second and third substrates. The constants of the elementary steps are obtained from tertiary plots, i.e. after two transformations in the experimental data. Obviously transformations increase the error of the final values. A method which needs only one transformation would be useful.

The systematisation of enzymic mechanisms with three substrates [1] shows that there are some mechanisms where one, two or three two-substrate factors (ϕ_{AB}, ϕ_{BC}, ϕ_{AC}) and/or the three-substrate factor (ϕ_{ABC}) equal zero. The method presented in this paper offers a new possibility to differentiate between these mechanisms.

2. Results and discussion

The primary plots of reciprocal initial velocity *vs.* reciprocal concentration of first substrate have a common intercept the x-coordinates of which are presented in table 1.

Table 1 presents also the rationale of differentiation between cases in which two-substrate factors equal zero.

The plotting of the x-coordinate of the common intercept against the concentration of the third substrate can be used to differentiate between the various mechanisms if: i) One two-substrate factor and the three-substrate factor or any one-substrate factor do not equal zero, i.e. $\phi_{BC}, \phi_{ABC} \neq 0$ or $\phi_{AC}, \phi_{ABC} \neq 0$ or $\phi_{AB}, \phi_{ABC} \neq 0$ as well as ϕ_A or ϕ_B or $\phi_C \neq 0$.

ii) $\phi_{AB}/\phi_B \neq \phi_{ABC}/\phi_{BC}$ (in case 1 of table 1 and analogously in the other cases).

iii) We do not use such a low substrate concentration that $\phi_B[C]$ and $\phi_{AB}[C]$ become negligible compared to ϕ_{BC} and ϕ_{ABC} or vice versa at high substrate concentration (in case 1 of table 1 and analogously in the other cases).

In each case if the x-coordinate of common intercept *vs.* the reciprocal of the concentration of third substrate gives a straight line, then $\phi_{ABC} = 0$, if it is a curved plot $\phi_{ABC} \neq 0$.

It can be shown that if the $\phi_{AB}, \phi_{AC}, \phi_{BC}, \phi_{ABC} \neq 0$ and the primary plots are straight lines, rapid equilibrium random mechanism holds, and in turn if $\phi_{AC} = 0$ compulsory ordered mechanism, if ϕ_{AB}, $\phi_{AC} = 0$ rapid equilibrium partially random BC mechanism, if $\phi_{AB}, \phi_{AC}, \phi_{BC} = 0$ rapid equilibrium partially random AB mechanism, if $\phi_{AB}, \phi_{AC}, \phi_{ABC} = 0$ concerted-substitution mechanism, if $\phi_{AB}, \phi_{AC}, \phi_{BC}$, $\phi_{ABC} = 0$ compulsory ordered ping-pong mechanism with triple transfer and two substituted enzyme species [1–3].

Performing the analysis presented in table 1, i.e. plotting the x-coordinate of the common intercept of primary plots *vs.* the concentration of third substrate, the straight lines go through the origin in cases 3 and 5 if both ϕ_{AB} and $\phi_{AC} = 0$, in cases 1 and

Table 1

The x-coordinates of common intercepts of primary plots and differentiation of three-substrate reactions.

Case	I	II	III	a = x-coordinate of the common intercept of primary plots	a *vs.* concentration of third substrate	
	Substrate				Straight line	Curved plot
1	A	B	C	$-(\phi_B[C] + \phi_{BC})/(\phi_{AB}[C] + \phi_{ABC})$	$\phi_{AB} = 0$	$\phi_{AB} \neq 0$
2	A	C	B	$-(\phi_C[B] + \phi_{BC})/(\phi_{AC}[B] + \phi_{ABC})$	$\phi_{AC} = 0$	$\phi_{AC} \neq 0$
3	B	A	C	$-(\phi_A[C] + \phi_{AC})/(\phi_{AB}[C] + \phi_{ABC})$	$\phi_{AB} = 0$	$\phi_{AB} \neq 0$
4	B	C	A	$-(\phi_C[A] + \phi_{AC})/(\phi_{BC}[A] + \phi_{ABC})$	$\phi_{BC} = 0$	$\phi_{BC} \neq 0$
5	C	A	B	$-(\phi_A[B] + \phi_{AB})/(\phi_{AC}[B] + \phi_{ABC})$	$\phi_{AC} = 0$	$\phi_{AC} \neq 0$
6	C	B	A	$-(\phi_B[A] + \phi_{AB})/(\phi_{BC}[A] + \phi_{ABC})$	$\phi_{BC} = 0$	$\phi_{BC} \neq 0$

General equation: $E/v_0 = \phi_0 + \frac{\phi_A}{[A]} + \frac{\phi_B}{[B]} + \frac{\phi_C}{[C]} + \frac{\phi_{AB}}{[A][B]} + \frac{\phi_{AC}}{[A][C]} + \frac{\phi_{BC}}{[B][C]} + \frac{\phi_{ABC}}{[A][B][C]}$ [1, 3].

and 6 if ϕ_{AB} and $\phi_{BC} = 0$, in cases 2 and 4 if ϕ_{AC} and $\phi_{BC} = 0$. Consequently, all straight lines start from the origin in all cases if ϕ_{AB}, ϕ_{AC} and $\phi_{BC} = 0$. Moreover, this method offers a means to differentiate between the concerted substitution mechanism II.b, II.c and II.d (as defined by Dalziel [1]) which have so far been kinetically undistinguishable. In mechanisms II.b, II.c all straight lines start from the origin except in cases 5 and 6 (cf. table 1), in mechanism II.d all straight lines start from the origin except in cases 1 and 2 (cf. table 1).

This method may be useful in a determining the mechanism of action of three-substrate enzymes in certain cases as defined above. However, this approach cannot replace the original one in the evaluation of the numerical value of the constants. It is to be noted that if a curved plot is obtained in the analysis as shown in table 1, this result is conclusive. But if a straight line is obtained, due to restriction 3 as presented above, higher and lower substrate concentrations should also be used to prove the mechanism. On the other hand, in certain instances not all the analyses should be done, since three cases are enough to show whether or not the four constants, or some of them, equal zero.

References

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