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How to draw kinetic barrier diagrams for enzyme-catalysed reactions

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A modified way to construct kinetic barrier diagrams is presented. Although the diagram superficially resembles a free-energy profile, it is independent of any conception derived from transition-state theory. Some simple calculations referring to the lactate dehydrogenase turnover reaction at equilibrium demonstrate self-consistency of the diagram and its direct relevance to the results of numerical simulations of the detailed course of enzyme-catalysed reactions.

As recently stated by Burbaum *et al.* [1], there are several problems in the illustration of enzyme-catalysed reactions. To cope with these problems, Burbaum *et al.* [1] have recommended the drawing of 'kinetic barrier diagrams', instead of the classical free-energy profiles. Some years ago I presented a complete set of ten rate constants for describing the lactate dehydrogenase (LDH) reaction in terms of a five-step turnover mechanism [2–4]. This kinetic system (Table 1*a*) allows a numerical testing of the kinetic barrier formalism which has not yet been reported. On the basis of this numerical test, I conclude that very much can be gained by basically altering the way in which one plots kinetic barrier diagrams.

The procedure suggested is based on plotting, in a 'peak-and-valley' type barrier diagram, reciprocal fluxes as maxima and reciprocal concentrations as minima. Accordingly, two new constructional features characterize the suggested type of kinetic barrier diagrams: (i) the minima no longer involve direct reference to free substrates and products; each diagram refers to a specific combination of reactant concentrations; and (ii) the conservation equation of classical enzyme kinetics is involved in the calculation of both minima and maxima.

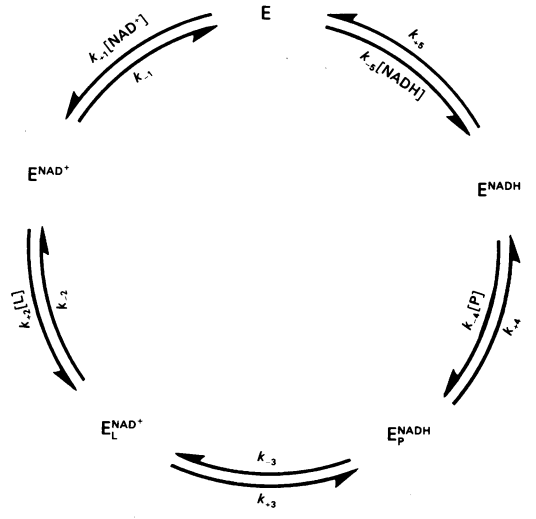
The main argument for introducing the modified diagram is its direct relevance to the results of numerical simulations of the detailed course of enzyme-catalysed reactions. An example, constructed with the parameters listed in Table 2, is shown in Fig. 1. Fig. 1 refers to the equilibrium of the LDH turnover as catalysed by the pig heart enzyme (Scheme 1). This reaction is described, at pH 8.4 and 6.3 °C [2–4], by the ten rate constants found in Table 1. Equilibrium kinetics (Fig. 1) can only be measured by special methods, such as monitoring the rate of isotope exchange between labelled reactants. The isotope-exchange reaction, which involves all five steps of the LDH turnover (Scheme 1), is that between NAD^+ and NADH.

It should be noted that Fig. 1 has two ordinates with different dimensions. Accordingly, Fig. 1 could be regarded as a combination of two diagrams, one for intermediate concentrations (minima) and one for unidirectional fluxes (maxima). On the one hand, neither slopes nor barrier heights can be numerically interpreted with any of the two ordinates [note that the dimensions of barrier height would be time (s)]. On the other hand, the higher kinetic barrier corresponds to a lower valley (i.e. higher intermediate concentration) and/or a higher peak (i.e. lower flux).

The calculations yielding Fig. 1 also involve the equilibrium concentrations of NAD^+, lactate (L), pyruvate (P) and NADH which are listed in Table 1. This specific set of reactant concentrations results in a kinetic situation (see Fig. 1) in which (*a*) the equilibrium one-way fluxes in the five consecutive reaction steps are as closely similar as possible, and (*b*) the concentration of free enzyme ([E]) is equal to the total concentration of liganded enzyme ($[E_a]$). Note that, from the conservation equation:

$$[E]+[E_a] = [E_t]$$

It should be further noted that the numerical values of the rate constants k_{+3} and k_{-2} on the one hand, and k_{+4} and k_{-3} on the other hand, are already very similar ($1200 \sim 1002 \neq 190 \sim 246$). From this it follows that the equilibrium fluxes through the three central steps of Scheme 1 are also very similar. Since this similarity is determined by true first-order constants, it is independent of substrate concentrations. By contrast the absolute values of these three fluxes, as well as their relation to the fluxes in steps 1 and 5 (coenzyme binding and release) are obviously determined by reactant concentrations. The simple calculations which have led to finding those reactant concentrations which yield both $[E_a] = [E]$, and the best approximation to 'uniform flux' are detailed in the following five points.



Scheme 1. The five-step turnover of the LDH reaction

The minimal mechanism is defined by the possible dissociation products of enzyme–substrate intermediates, and a corresponding set of ten macroscopic rate constants.

Abbreviations used: LDH, lactate dehydrogenase; L, lactate; P, pyruvate.

Table 1. Numerical values of the parameters involved in the calculations yielding Fig. 1.

Reaction conditions: pH 8.4, 6.3 °C. (*a*) Numerical values of the ten rate constants defined in Scheme 1 [2–4]. (*b*) Reactant concentrations (related by $K_{eq.} = 2.369 \times 10^{-4}$) derived as described in the text.

(*a*) Rate constants

Parameter	Value	Parameter	Value
k_{+1}	8.74×10^6 M$^{-1}\cdot$s^{-1}	k_{-1}	526 s^{-1}
k_{+2}	6.07×10^4 M$^{-1}\cdot$s^{-1}	k_{-2}	1200 s^{-1}
k_{+3}	1002 s^{-1}	k_{-3}	246 s^{-1}
k_{+4}	190 s^{-1}	k_{-4}	1.21×10^6 M$^{-1}\cdot$s^{-1}
k_{+5}	16 s^{-1}	k_{-5}	3.63×10^7 M$^{-1}\cdot$s^{-1}

(*b*) Equilibrium substrate concns.

Parameter	Value
[NAD$^+$]	1.6124×10^{-6} M
[L]	1.3437×10^{-2} M
[P]	1.3223×10^{-5} M
[NADH]	3.8824×10^{-7} M

Table 2. Numerical values of the five intermediate concentrations and ten elementary reactions coupled in the turnover cycle illustrated in Fig. 1

(*a*) Intermediate concentrations ([E$_a$] = molar concn. of liganded enzyme)

Parameter	Value
[E]	1.0000 [E$_a$]
[E$^{NAD^+}$]	0.0268 [E$_a$]
[E$_L^{NAD^+}$]	0.0182 [E$_a$]
[E$_P^{NADH}$]	0.0742 [E$_a$]
[E^{NADH}]	0.8808 [E$_a$]

(*b*) Elementary reactions (unidirectional fluxes; in units of [E$_a$]$\cdot$s^{-1})

Parameter	Value
[E][NAD$^+$]k_{+1}	14.0932
[E$^{NAD^+}$][L]k_{+2}	21.8526
[E$_L^{NAD^+}$]k_{+3}	18.2469
[E$_P^{NADH}$]k_{+4}	14.0932
[E^{NADH}]k_{+5}	14.0932
[E][NADH]k_{-5}	14.0932
[E^{NADH}][P]k_{-4}	14.0932
[E$_P^{NADH}$]k_{-3}	18.2469
[E$_L^{NAD^+}$]k_{-2}	21.8526
[E$^{NAD^+}$]k_{-1}	14.0932

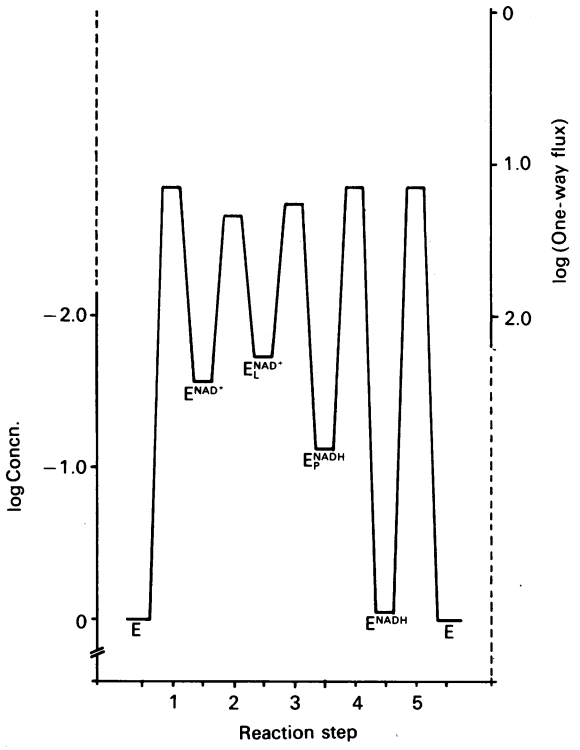


Fig. 1. Kinetic barrier diagram for the LDH reaction as defined by the numerical values of 14 parameters in Table 2

The sequence of reaction steps shown by the abscissae corresponds to one cycle of the turnover reaction (Scheme 1). The ordinate yields the logarithms of both intermediate concentrations (minima; in units of the molar concn. of liganded enzyme, [E$_a$]) and unidirectional fluxes (maxima; in units of [E$_a$]$\cdot$s^{-1}).

(i) Steps 1 and 5 will yield equal fluxes if:

$$[E][NAD^+]k_{+1} = [E][NADH]k_{-5}$$

yielding

$$\frac{[NADH]}{[NAD^+]} = \frac{k_{+1}}{k_{-5}} = 0.2408$$

Referring to the overall equilibrium constant ($K_{eq.} = 2.396 \times 10^{-4}$), one further obtains the concentration ratio of pyruvate to lactate

$$\frac{[P]}{[L]} = K_{eq.}\frac{[NAD^+]}{[NADH]} = 9.8411 \times 10^{-4}$$

(ii) In a similar manner, one can select the pyruvate concentration at which:

$$[E^{NADH}]k_{+5} = [E^{NADH}][P]k_{-4}$$

wherefrom

$$[P] = \frac{k_{+5}}{k_{-4}} = 1.3223 \times 10^{-5}\,\text{M}$$

and

$$[L] = \frac{[P]}{K_{eq.}}\frac{[NADH]}{[NAD^+]} = 1.3437 \times 10^{-2}\,\text{M}$$

(iii) We can now express the concentrations of all four enzyme substrate intermediates with [E$^{NAD^+}$] as follows:

$$[E_L^{NAD^+}] = \frac{[L]}{K_2}[E^{NAD^+}] = 0.6797\,[E^{NAD^+}]$$

$$[E_P^{NADH}] = K_3[E_L^{NAD^+}] = 2.7684\,[E^{NAD^+}]$$

$$[E^{NADH}] = \frac{K_4}{[P]}[E_P^{NADH}] = 32.8750\,[E^{NAD^+}]$$

(iv) If we further introduce the definition for liganded enzyme, we obtain from

$$[E_a] = [E^{NAD^+}] + [E_L^{NAD^+}] + [E_P^{NADH}] + [E^{NADH}]$$

the following

$$[E^{NAD^+}] = 0.0268\,[E_a]$$

$$[E_L^{NAD^+}] = 0.0182\,[E_a]$$

$$[E_P^{NADH}] = 0.0742\,[E_a]$$

and

$$[E^{NADH}] = 0.8808\,[E_a]$$

Finally, derived from the equilibrium condition, we also have the equality:

$$[E][NAD^+]k_{+1} = [E^{NAD^+}]k_{-1} = 14.0932\,[E_a]$$

from which

$$\frac{[E_a]}{[E]} = 6.2016 \times 10^5\,[NAD^+]$$

and if

$$[E] = [E_a],$$

then

$$[NAD^+] = 1.6125 \times 10^{-6}\ \text{M}$$

and

$$[NADH] = 3.8824 \times 10^{-7}\ \text{M}$$

Considering the more general argument derived from Fig. 1, I conclude that it is possible to construct kinetic barrier diagrams which provide a comprehensive quantitative illustration of enzymic turnover reactions. Diagrams such as those shown in Fig. 1 can be numerically derived from phenomenological enzyme kinetics. Although this type of diagram appears to be very useful in displaying the results of numerical simulations of enzyme kinetics, it is not dependent on any of the conceptions derived from transition-state theory (free-energy of activation, transition state, standard state, etc.). The example discussed here refers to the equilibrium of a two-substrate, two-product, five-step mechanism (Scheme 1). However, the suggested procedure is not limited by the number of reactants and/or reaction steps, and can also be used for clearly illustrating the distinction between equilibrium and non-equilibrium reactions.

As to the usefulness of the proposed procedure, the present discussion should be restricted to the choice of ordinate dimensions. I suggest that any quantitative description of the consecutive reaction steps of any enzymic-turnover reaction must yield the numerical values of three groups of dimensionally distinct quantities: (*a*) intermediate concentrations (M); (*b*) velocities with which these intermediates are transformed into each other (one-way fluxes, $\text{M}\cdot\text{s}^{-1}$); (*c*) time constants which relate fluxes to intermediate concentrations (first-order rate constants, s^{-1}).

The usefulness of a diagram which is intended to illustrate such results critically depends on the choice of quantities plotted. The present procedure is based on plotting reciprocal fluxes (as maxima) and reciprocal concentrations (as minima), in a 'peak-and-valley'-type barrier diagram. This diagram defines the kinetic barrier as the reciprocal flux:

$$\frac{1}{v_i} = \frac{1}{[i]k_i^{1st}} \qquad (1)$$

the dimensions of which are $\text{s}\cdot\text{M}^{-1}$. Also plotted in Fig. 1 are the reciprocal concentrations of intermediates. Concentrations and fluxes are, however, related by a time factor, and eqn. (1) might seem to indicate that, by equating the logarithms of reciprocal fluxes, concentrations and rate constants, it is possible to draw a kinetic barrier diagram with a single ordinate from which all three quantities can be read off. However, these three quantities have all got different dimensions. For instance, time is constituent of the dimensions of fluxes and rate constants, but not of concentrations. Accordingly, by changing unit time from s to ms,

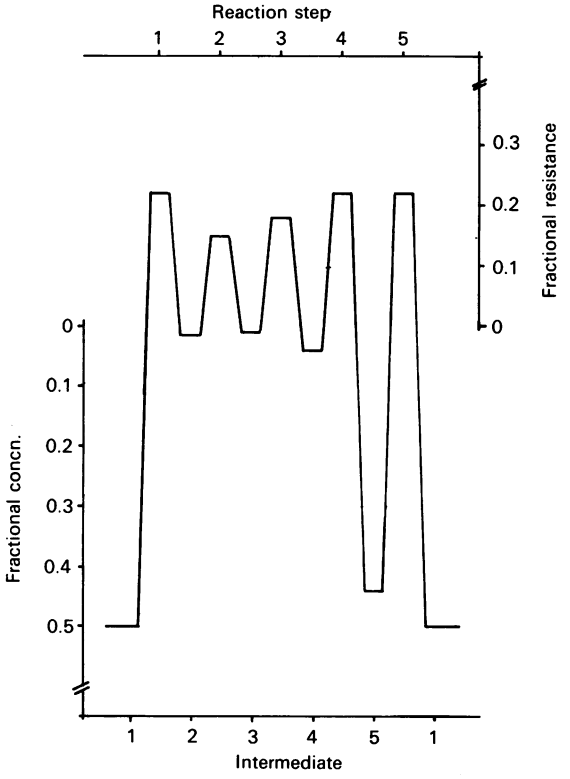


Fig. 2. Kinetic barrier diagram of the same equilibrium system as in Fig. 1

Both ordinate scales are arithmetic. Left: fractional concentrations ($[E_t] = 1$ M); right: fractional resistance ($1/v_t = 0.3127\ \text{s}\cdot\text{M}^{-1}$). Note the usefulness of also drawing two abscissae specifying intermediate and reaction step respectively.

the logarithmic values of fluxes and rate constants are changed by three units, whereas the logarithmic values of concentrations remain unchanged. Consequently, the shape of the diagram can be freely manipulated while retaining its exactness. In other words, the distance between the levels of minima and maxima can in no case yield additional useful information. Quantities with different dimensions basically require different ordinates.

My choice of defining a kinetic barrier by reciprocal flux (eqn. 1) is supported by the analogy of this quantity to resistance in electrical circuits [5]. The analogy reaches so far that even eqn. (2) is valid [5,6]:

$$\frac{1}{v_t} = \frac{1}{v_1} + \frac{1}{v_2} + \ \ldots\ + \frac{1}{v_n} \qquad (2)$$

according to which the barrier to the overall reaction can be calculated as the sum of all the barriers encountered in the constituent reaction steps. Eqn. (2) is obviously analogous to Ohm's Law. For the latter reason, if the kinetic barrier is defined as reciprocal flux ($1/v$), it may also be referred to as resistance.

There are no logarithms involved in eqns. (1) and (2). Indeed, I also find that logarithmic scales are not essential features of kinetic barrier diagrams based on plotting fluxes and concentrations. Especially informative diagrams can be drawn by plotting fractional intermediate concentrations (upside down) as minima, and fractional resistance of individual reaction steps to the overall reaction velocity as maxima (Fig. 2). Fig. 2 illustrates the same kinetic equilibrium as Fig. 1. Accordingly, the overall reaction can be identified as isotope exchange between NAD^+ and NADH at the specified equilibrium substrate concentrations.

The illustrative power of Figs. 1 and 2 can be conceived even

more clearly if one also considers two further isotope-exchange reactions which may potentially be observed in the same equilibrium system. These are, in addition to the [NAD^+]⇌[NADH] exchange reaction, a [lactate]⇌[NADH] exchange, and a [L]⇌[P] exchange (see Yagil & Hoberman [6]). It should be noted that the reciprocal velocities of all three exchange reactions can be read off from Figs. 1 and 2, namely, as the sum of five, four and three of the resistances (barriers) shown in any one of these two Figures. The straightforwardness with which Figs. 1 and 2 depict this result, and the fact that Fig. 2 yields an equivalent substitute for Fig. 1, are strong arguments for defining kinetic barrier as reciprocal flux.

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