Kinetic Analysis of Experiments Involving the Single Turnover of an Enzyme

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The complete solution to the kinetic equation for nucleotide fluorescence quenching on addition of pyruvate to the lactate dehydrogenase–NADH complex modifies previous interpretations of such experiments.

The time-course for the disappearance of enzyme (E) when it is mixed with excess of substrate (S) and reacts according to eqn. (1)

to give an enzyme form (F), which is not used again, is (Capellos & Bielski, 1972):

$$[E]/[E]_0 = C_1 e^{\lambda_1 t} + C_2 e^{\lambda_2 t} \quad (2)$$

Let $[E]_0$ be the total concentration of enzyme active sites used. The boundary conditions are $[E]_{t=0} = [E]_0$ and $(d[E]/dt)_{t=0} = k_{+1}[E]_0[S]$.

All the unknowns in eqn. (2) can be expressed in terms of the kinetic constants of eqn. (1) as follows:

$$\begin{aligned} A &= (k_{+1}[S] + k_{-1} + k_{+2}) \\ B &= k_{+1}k_{+2}[S] \\ \lambda_1 &= -A/2 - (A^2/4 - B)^{1/2} \\ \lambda_2 &= -A/2 + (A^2/4 - B)^{1/2} \\ C_1/[E]_0 &= \frac{\lambda_1 + k_{+1}[S]}{\lambda_1 - \lambda_2} \\ C_2/[E]_0 &= \frac{\lambda_2 + k_{+1}[S]}{\lambda_2 - \lambda_1} \end{aligned}$$

It appears that these equations have not yet been used in connexion with single-turnover reactions of an enzyme.

Two groups of workers (Südi, 1974; Boland & Gutfreund, 1975) have used eqn. (1) as a model for the single turnover of the NADH complex of pig heart lactate dehydrogenase (E^{NADH}) when this is mixed with excess of pyruvate (P) (Scheme 1). Both groups observed that the complete time-course for the decrease in fluorescence can be described by two limiting exponentials, which have observed rate

constants $k_{obs,1}$ and $k_{obs,2}$ (Fig. 5 of Südi, 1974, and Fig. 5 of Boland & Gutfreund, 1975). Südi's (1974) suggestion actually amounted to $k_{obs,1} = k_{+1}[P] + k_{+2}$, whereas Boland & Gutfreund (1975) suggested $k_{obs,1} = k_{+1}[P] + k_{-1} + k_{+2}$. Neither of these linear $k_{obs,1}$ versus $[P]$ functions is the correct solution to eqn. (1). Since λ_1 in eqn. (2) is a larger negative number than λ_2 , one may (as did both groups) subtract the slower reaction from the faster to obtain the initial rapid decrease (D):

$$D = ([E]/[E]_0 - C_2 e^{\lambda_2 t}) = C_1 e^{\lambda_1 t}$$

This decrease will have amplitude C_1 and

$$k_{obs,1} = -\lambda_1 = \frac{1}{2}(k_{+1}[P] + k_{-1} + k_{+2}) + \frac{1}{2}\{(k_{+1}[P] - k_{-1})^2 + k_{-1}(2k_{+2} + k_{-1} + 2k_{+1}[P])\}^{1/2} \quad (3)$$

Thus $k_{obs,1}$ will be a non-linear function of pyruvate concentration with $k_{obs,1} \rightarrow (k_{+2} + k_{-1})$ when $[P] \rightarrow 0$, and $k_{obs,1} \rightarrow k_{+1}[P]$ when $[P] \rightarrow \infty$. $k_{obs,2}$ will be analogous to $k_{obs,1}$, except that the square-root term will be negative.

Eqn. (3) requires the re-interpretation of the $k_{obs,1}$ versus pyruvate concentration plots of both Südi (1974) and Boland & Gutfreund (1975). This is illustrated by the example of the experimental data of Boland & Gutfreund (1975) in Fig. 1. The experimental observations published by Südi (1974,

Scheme 1.

Fig. 1. Dependence of the first-order rate constant for the fast rate of nucleotide fluorescence quenching on pyruvate concentration

The non-linear regression (----) suggested by eqn. (3) is at least as good a representation of the experimental points (from Fig. 6 of Boland & Gutfreund, 1975) as is the linear regression (—) used by these workers and by Südi (1974). The curve (----) is calculated from eqn. (3) by using $k_{+1} = 3.5 \times 10^6 \text{ M}^{-1} \cdot \text{s}^{-1}$, $k_{+2} = 200 \text{ s}^{-1}$ and $k_{-1} = 150 \text{ s}^{-1}$.

Fig. 4a) are obviously insufficient for a non-linear regression analysis.

The broken line in Fig. 1 has been calculated from eqn. (3) with $k_{+1} = 3.5 \times 10^6 \text{ M}^{-1} \cdot \text{s}^{-1}$, $k_{-1} = 150 \text{ s}^{-1}$

and $k_{+2} = 200 \text{ s}^{-1}$. Of these values, the estimate of k_{+2} is based on independent evidence, whereas both k_{-1} and k_{+1} are higher than the values originally chosen by Boland & Gutfreund (1975). The non-linear regression result is in as good agreement with the experimental results of Boland & Gutfreund (1975) as is the linear regression result assumed by both these workers and Südi (1974). It would not be fruitful to use a least-squares-fitting technique to estimate values of k_{-1} and k_{+2} until instrumentation improvements enable more precise experimental results to be obtained.

None of the considerations in the present paper change the conclusion of Boland & Gutfreund (1975) that binding pyruvate at $\text{pH} > 7$ induces proton uptake. Yet these considerations do suggest that the rate at which pyruvate dissociates from enzyme-NADH is the same whether measured in the direction of lactate oxidation (180 s^{-1}) or pyruvate reduction (150 s^{-1}). Thus there is no longer experimental support for the postulate (Scheme 1 of Boland & Gutfreund, 1975) that pig heart lactate dehydrogenase exists in alternative conformations depending on whether it is oxidizing lactate or reducing pyruvate.

References

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