Biochimica et Biophysica Acta, 615 (1980) 143—146

BBA 69101

RATE MEASUREMENTS IN CASES OF SUBSTRATE INHIBITION

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(Received February 6th, 1980)

Key words: Initial velocity; Substrate inhibition; Kinetic parameter

Summary

Analysis of a simple model for substrate inhibition of an enzyme shows that the methods of Lee and Wilson (Lee, H.-J. and Wilson, I.B. (1971) Biochim. Biophys. Acta 242, 519—522), and Yun and Suelter (Yun, S.-L. and Suelter, C.H. (1977) Biochim. Biophys. Acta 480, 1—13), may be used to derive initial rate data from the sigmoidal-reaction progess curves that may be generated in such cases and in a number of instances, may be used to derive the kinetic parameters defining the rate equation from single-reaction progress curves.

Introduction

In the course of studies on an aryl-sulfate sulfohydrolase (EC 3.1.6.1) from the mollusc *Haliotis iris*, we have observed a pronounced substrate inhibition with the commonly used substrate 2-hydroxy-5-nitrophenyl sulfate (Clark, A.G. and Jowett, D.A., unpublished data). We have also observed that the reaction progress curves have a marked sigmoidal character. This latter behaviour we ascribe to the progressive decrease in the substrate inhibition during the course of the reaction. This characteristic appeared to present a problem when attempting kinetic studies on the enzyme as the constantly changing gradient renders it difficult to draw a tangent to the curve which will lead to a valid estimate of the initial velocity. However, from the study of a number of simple models for substrate inhibition it appears that some modes of substrate inhibition yield equations to which the approximation of Lee and Wilson [1] may be applied, and hence velocities and corrected substrate concentrations terms may be derived by drawing chords rather than tangents to the progress curves.

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Theory and Discussion

We propose a simple, general model for substrate inhibition, as is shown in Fig. 1. Rapid equilibrium is assumed for all association-dissociation reactions. Reaction parameters are defined as follows:

$$K_s = \frac{[E][S]}{[ES]}, \quad \alpha K_s = \frac{[SE][S]}{[SES]}, \quad K_i = \frac{[E][S]}{[SE]}, \quad \alpha K_i = \frac{[ES][S]}{[SES]}$$

Given a starting concentration S_0, the velocity at time t is:

$$\frac{dp}{dt} = \frac{V}{1 + \frac{K_s}{K_i} + \frac{K_s}{S_0 - p} + \frac{S_0 - p}{\alpha K_i}} \tag{1}$$

where p is product concentration. Product inhibition is assumed to be negligible.

The integrated form of this equation is:

$$V \cdot t = \left(1 + \frac{K_s}{K_i} + \frac{S_0}{\alpha K_i}\right) p + K_s \ln \frac{S_0}{S_0 - p} - \frac{p^2}{2\alpha K_i} \tag{2}$$

Taking two points on the progress curve at times t_1 and t_2, when the product concentrations are p_1 and p_2, the change in time and product concentration are related as shown in the equation below.

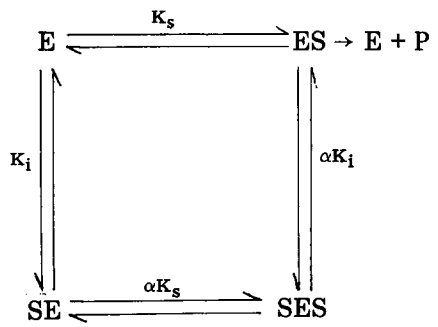
$$V \cdot \Delta t = \left(1 + \frac{K_s}{K_i} + \frac{S_0}{\alpha K_i}\right) \cdot \Delta p + K_s \ln \frac{S_0 - p_1}{S_0 - p_2} - \frac{(p_1 + p_2)\,\Delta p}{2\alpha K_i} \tag{3}$$

Given that $\Delta p = p_2 - p_1 = S_1 - S_2$ and $p_1 + p_2 = 2S_0 - S_1 - S_2$:

$$V \cdot \frac{\Delta t}{\Delta p} = 1 + \frac{K_s}{K_i} + \frac{K_s \cdot \ln(S_1/S_2)}{S_1 - S_2} + \frac{S_1 + S_2}{2} \cdot \frac{1}{\alpha K_i} \tag{4}$$

By the approximation used by Lee and Wilson [1] and Yun and Suelter [2]:

$$\frac{2}{S_1 + S_2} = \frac{\ln(S_1/S_2)}{S_1 - S_2} \quad \text{if} \quad \frac{S_1 - S_2}{S_1} < 0.3$$



Scheme I. A model for inhibition of an enzyme by its substrate.

Thus, provided that this condition is met, Eqn. 4 may be rewritten as:

$$\frac{1}{\bar{v}} = \frac{1}{V}\left(1 + \frac{K_s}{K_i} + \frac{K_s}{\bar{s}} + \frac{\bar{s}}{\alpha K_i}\right)$$

where

$$\bar{s} = \frac{S_1 + S_2}{2} \quad \text{and} \quad \bar{v} = \frac{\Delta p}{\Delta t} \tag{5}$$

Thus, in cases of substrate inhibition conforming to the above model rate data obtained by drawing chords to the progress curve may be used in the determination of values for the parameters defining Eqn. 5. Variations on this model may be obtained by assigning extreme values to α and K_i and for these the approximation still holds.

Yun and Suelter [2] have shown that the approximation of Lee and Wilson [1] may be applied to a variety of mechanisms of enzyme action and of inhibition, including product inhibition. They did not, however, examine the case of substrate inhibition. We feel that it is worth using these methods in this case since it is difficult to draw valid tangents to the markedly sigmoidal rate curves which may be generated in such a situation.

In theory, entire rate curves may be analysed by the methods of the above workers, taking successive chords along the progress curves. The velocity and substrate concentration terms thus derived should be compatible with Eqn. 5 and may be used to obtain values for the apparent K_m, V and K_i terms governing the equation. In the application of this model in its most general form, it will not be possible to use these apparent parameters to assign values to the true V, K_s, K_i and α. In some restricted cases, this may be possible. Thus, if K_i is very much larger than K_s but αK_i has a small value, then Eqn. 5 reduces to the form derived by Haldane [3] and V, K_s and αK_i may be determined by the methods proposed by Dixon and Webb [4]. On the other hand, if α has a value of 1 the equation reduces to

$$\bar{v} = V \Big/ \left(1 + \frac{K_s}{K_i} + \frac{\bar{s}}{K_i} + \frac{K_s}{\bar{s}}\right) \tag{6}$$

Analysis of the vertical intercepts and gradient terms obtained when plotting the reciprocal rate data against $\bar{s}$ and $1/\bar{s}$ as in Ref. 4, shows that they may again be processed to yield values for V, K_s and K_i.

It should be stressed that Eqn. 5 does not depend on the value of the initial substrate concentration S_0. Superimposable curves should be obtained regardless of the initial substrate concentration and regardless (within the constraints cited above) of the size of the chords taken.

Matters are different if product inhibition also occurs, a situation we have had to consider as the aryl-sulfate sulfohydrolase from *H. iris* is inhibited by SO_4^{2-} (Clark, A.G. and Jowett, D.A., unpublished data). If it is assumed that free enzyme may form a dead-end complex with the product of the enzyme system shown in Fig. 1, governed by the dissociation constant K_p, then the rate equation obtained is;

$$v = V \Big/ \left(1 + \frac{K_s}{K_i} + \frac{S_0 - p}{\alpha K_i} + \frac{K_s}{S_0 - p} \cdot \left[1 + \frac{p}{K_p}\right]\right) \tag{7}$$

integration yields

$$Vt = p\left(1 + \frac{S_0}{\alpha K_i} + \frac{K_s}{K_i} - \frac{K_s}{K_p}\right) + \frac{K_s}{K_p} \cdot S_0 - \frac{p^2}{\alpha K_i} - K_s\left(1 + \frac{S_0}{K_p}\right) \ln(S_0 - p) \qquad (8)$$

and taking the difference in p_1 and p_2 at times t_1 and t_2 as before, we obtain

$$\frac{\Delta t}{\Delta p} = \frac{1}{V} \cdot \left\{1 - \frac{K_s}{K_p} + \frac{K_s}{K_i} + \frac{\bar{s}}{\alpha K_i} + \frac{K_s}{\bar{s}}\left(1 + \frac{S_0}{K_p}\right)\right\} \qquad (9)$$

an equation exactly analogous to that derived by Yun and Suelter [2] for simple product inhibition. Puttin $\bar{p} = S_0 - \bar{s}$ we obtain

$$\bar{v} = V \Big/ \left\{1 + \frac{K_s}{K_i} + \frac{\bar{s}}{\alpha K_i} + \frac{K_s}{\bar{s}}\left(1 + \frac{\bar{p}}{K_p}\right)\right\} \qquad (10)$$

It is thus clear that, from Eqn. 10, even in the presence of concerted substrate and product inhibition, chords to the reaction curve will generate data compatible with Eqn. 10. Such data are analogous to the initial velocity data implicitly required in Eqn. 7. Thus, even with this complex type of inhibition, chords to the reaction curve generate data which are compatible with the initial velocity Eqn. 7, even though the data are not strictly initial velocities.

Taking successive chords along a reaction curve will, as before, generate data defining a curve. In contrast with the previous case however, the character of this curve is a function of the initial substrate concentration, as is clearly shown in Eqn. 9. Given a range of initial substrate concentrations, we obtain not a single curve as in the case of simple substrate inhibition, but a family of curves. These curves may be used to derive values for apparent kinetic parameters governing the behaviour of the system. The extent to which these values reflect those of the true parameters will depend on the detail of the model. The type of behaviour described above is analogous to that described previously [2] for simple product inhibition and, even in cases where precise values cannot be assigned to the theoretical kinetic parameters, provides the basis for a useful, rapid test for product inhibition which is valid even in the presence of substrate inhibition.

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