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Product inhibition of reversible enzyme-catalysed reactions

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Abstract

Product inhibition studies of reversible reactions can give unusual kinetic patterns. A plot of rate versus substrate concentration may appear to be sigmoidal and the double reciprocal plot will show upward curvature. This apparent positive cooperativity is due to the simultaneous occurrence of both the forward and reverse reaction. By fitting the appropriate rate equation to the data it is possible to determine the maximum velocity and Michaelis constant for both forward and reverse reaction from experiments conducted in one direction only.

Keywords: Enzyme kinetics; Product inhibition; Reversible reaction; Cooperative interaction

1. Introduction

Enzyme-catalysed reactions are often inhibited by their products, and there are at least three reasons for this effect. First, the products usually bear a structural resemblance to one or more of the substrates and will compete for binding at the point in the catalytic sequence where that substrate normally binds. Secondly, the dissociation of the enzyme-product complex is a reversible process whose net rate will be slowed by the presence of the product. Sometimes, the distinction between these two reasons is meaningless because the enzyme form resulting from dissociation of the product is the same as that to which the analogous substrate binds. Finally, the product may bind at a site different from the active site to give a complex that binds substrate less readily or has a lower catalytic activity.

The study of inhibition has proved to be a useful tool for elucidating the kinetic mechanism of an enzyme [1]. In most instances, there is an hyperbolic dependence of rate upon substrate concentration and this hyperbolic form is retained when the inhibitor is added. This hyperbolic relationship is usually manifested in the linearity of a plot of 1/rate versus 1/[substrate] where the effect of the inhibitor is to increase the slope and/or intercept of this straight line. Enzymes may exhibit departures from linearity for a variety of reasons. These include inhibition by the substrate at high concentration, the existence of alternative reaction pathways, and interaction between the subunits of oligomeric enzymes. Whatever the cause, these nonlinear effects may be difficult to analyse and interpret in terms of the underlying molecular events.

There is an extensive literature on what are loosely described as 'allosteric' enzymes, that have a sigmoidal dependence of rate upon substrate concentration. In the double reciprocal plot, this is seen as upward curvature. Those enzymes showing this behaviour are usually supposed to have some regulatory rôle in the cell because the sigmoidicity is affected by metabolites, resulting in activation of inhibition. Enzymes displaying these unusual kinetics are usually oligomeric and the sigmoidal substrate saturation curve may be explained in terms of interaction between the subunits. As a result, binding of substrate to one subunit appears to assist binding to a second, a phenomenon that is termed positive cooperativity. This interaction may be modulated by activators or inhibitors and the sigmoidicity may be absent under some conditions.

No reaction is truly irreversible and for many enzyme-catalysed reactions the reversibility is readily observable. This may complicate product inhibition experiments owing to the simultaneous occurrence of the forward and reverse reactions [2–4]. In such circumstances, measurements of initial velocities have often been avoided in favour either of following the progress curves for the approach to equilibrium [2,5–7] or by perturbations of the equilibrium using the 'induced transport' technique [8,9].

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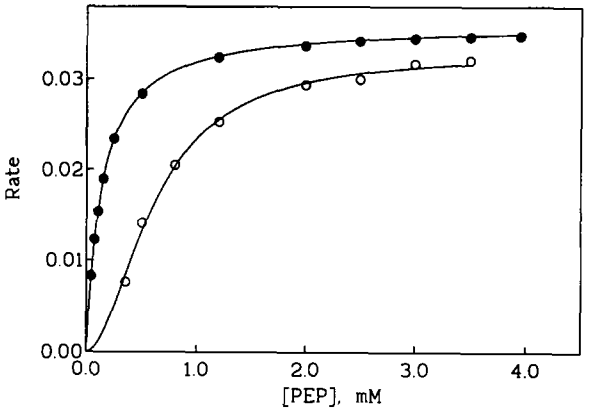

Fig. 1. Apparent cooperativity of enolase induced by 2PG. Enolase activity (μM/min) was measured as a function of PEP concentration in the absence (●) and presence (○) of 50 μM 2PG. The lines represent best fits to the data of Eq. (3) with n_H fixed at a value of 1.0 for the data in the absence of 2PG.

In this paper it is shown that for a reversible reaction, initial velocity measurements in the presence of a product inhibitor can give rise to apparently sigmoidal substrate saturation curves. This effect results solely from the simultaneous occurrence of forward and reverse reactions and, by appropriate analysis of the data, it is possible to obtain the kinetic parameters for the enzyme in both directions.

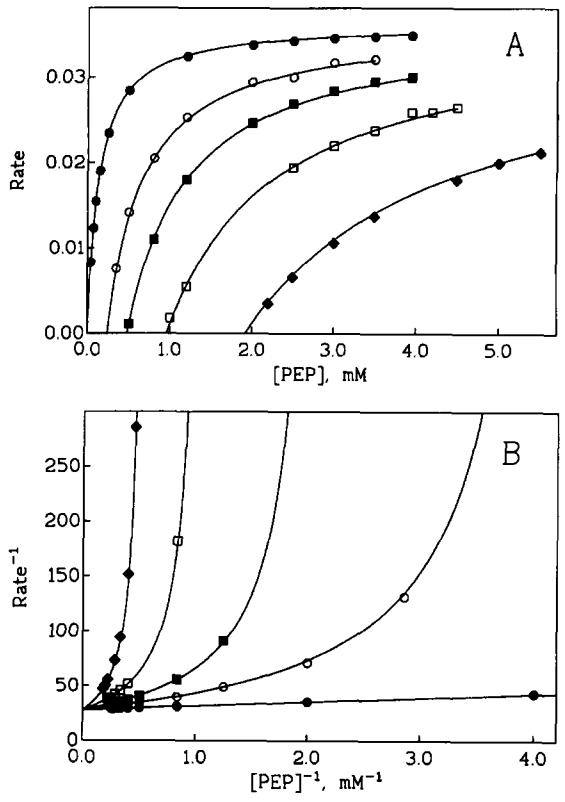

Fig. 2. Inhibition of enolase by 2PG. Enolase activity was measured as a function of PEP concentration in the absence (●) and presence of 2PG at concentrations of 50 μM (○), 100 μM (■), 200 μM (□) and 400 μM (◆). In panel A, the rate (μM/min) is plotted against [PEP] together with the lines of best fit of Eq. (1) to the data. Panel B shows the same data and fitted lines in a double reciprocal transform but with points below 250 μM PEP or giving rates less than 0.0033 μM/min being omitted for the sake of clarity.

2. Materials and methods

Yeast enolase (2-phospho-D-glycerate hydrolase; EC 4.2.1.11) and 2-phospho-D-glycerate (2PG) were purchased from the Sigma Chemical Company, while phospho-*enol*pyruvate (PEP) was obtained from Boehringer-Mannheim. The enzyme assay was performed at pH 7.5 and 30°C in a buffer containing 0.1 M imidazole-HCl, 0.5 M KCl and 2 mM $MgSO_4$. The reaction was started by addition of 14.7 nM enzyme and followed by observing the decrease in absorbance at 235 nm (40 to 350 μM PEP; $\epsilon_M = 2314$) or 250 nm (500 to 5500 μM PEP; $\epsilon_M = 399$).

Rates were determined from the initial portion of the curve and the results were analysed by fitting the appropriate equation to the data using the DNRP53 computer program [10].

3. Results and discussion

Enolase catalyses the reversible hydration of PEP to 2PG and, neglecting the water, the rate equation for this reaction is given as Eq. (1) and in double reciprocal form as Eq. (2), where V_f and V_r are the maximum velocities in the forward and reverse directions, respectively, while K_f and K_r are the corresponding Michaelis constants.

$$v = \frac{V_f[\mathrm{PEP}]/K_f - V_r[\mathrm{2PG}]/K_r}{1 + [\mathrm{PEP}]/K_f + [\mathrm{2PG}]/K_r} \quad (1)$$

$$\frac{1}{v} = \frac{1/V_f + (K_f/V_f)(1 + [\mathrm{2PG}]/K_r)(1/[\mathrm{PEP}])}{1 - (V_r K_f[\mathrm{2PG}]/V_f K_r)(1/[\mathrm{PEP}])} \quad (2)$$

It is evident from Eq. 2 that, unless [2PG] is zero, the double reciprocal plot will be nonlinear. As shown in Fig. 1, the departure from an hyperbolic curve in the plot of rate versus [PEP] might easily be mistaken for a sigmoidal curve. In the absence of 2PG (filled circles), the substrate saturation curve is strictly hyperbolic but addition of 50 μM 2PG (open circles) displaces the curve to the right. As is illustrated by the fitted line, the data in the presence of the product are described well by Eq. (3) with a maximum velocity (V_m) of 32.9 μM/min, a half-saturating substrate concentration ($S_{0.5}$) of 616 μM and a Hill coefficient (n_H) of 1.88. It should be noted that this $S_{0.5}$ cannot be interpreted in terms of specific molecular events and should not be equated to the Michaelis constant for the reaction.

$$v = V_m[\mathrm{PEP}]^{n_H}/([\mathrm{PEP}]^{n_H} + S_{0.5}^{n_H}) \quad (3)$$

The line drawn through the open circles in Fig. 1 is misleading in that it suggests that the rate would be zero in

the absence of PEP. In fact, examination of Eq. (1) reveals that the rate would be negative under these conditions, due to conversion of 2PG to PEP. The rate is only zero at the equilibrium concentration of PEP, 240 μM in this instance.

Fig. 2A shows the true dependence of rate upon [PEP] at a series of 2PG concentrations. Eq. (1) was fitted to these data by nonlinear regression to yield values of 36.2 ± 0.1 μM/min and 158 ± 15 μM/min for V_f and V_r, while K_f and K_r were 135 ± 2 μM and 124 ± 11 μM, respectively. The lines in Fig. 2A illustrate that the fit to the data is excellent. It is of interest that the maximum velocity and the Michaelis constant for both the forward and reverse reaction can be determined by studying the reaction in one direction only.

The double reciprocal plot of these data (Fig. 2B) shows the upward curvature that is expected from Eq. (2) but the shape of these curves might easily be mistaken for positive cooperativity. The reaction catalysed by enolase is readily reversible and, in the direction of PEP hydration examined in the present study, favours PEP by a factor of about 4.8. This makes it rather easy to observe the effect that is described here and, indeed, this system was chosen deliberately to illustrate what happens when the forward and reverse reactions occur simultaneously.

For enzymes that have only one product, data such as those illustrated in Fig. 2B are inevitable although the ease with which the curvature is observed will depend on the equilibrium position and the concentrations of substrates and products. Similar effects may be observed when there is more than one product if all are added, or if all but one of the products are likely to be present in the assay. Enolase is an example of such a system where, in the reaction direction that was studied, the other product is water. The circumstances necessary to observe the phenomenon described here are not particularly restrictive and numerous other enzymes would be expected to show the same effect. Indeed, similar results have been obtained in this laboratory using D-glucose-6-phosphate ketol-isomerase (EC 5.3.1.9) with fructose 6-phosphate as substrate, fumarate hydratase (EC 4.2.1.2) in both directions, as well as with the enolase-catalysed hydration of 2PG. While no case is known to this author where reversibility has been wrongly identified as positive cooperativity, the results presented here may serve as a useful warning to enzymologists.

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References

[1] Cleland, W.W. (1963) Biochim. Biophys. Acta 67, 173–187.
[2] Ray, W.F., Jr., and Roscelli, G.A. (1964) J. Biol. Chem. 239, 3935–3941.
[3] Cennamo, C. (1969) J. Theor. Biol. 23, 53–71.
[4] Darvey, I.G. (1972) Biochem. J. 128, 383–387.
[5] Darvey, I.G., Shrager, R. and Kohn, L.D. (1975) J. Biol. Chem. 250, 4696–4701.
[6] Duggleby, R.G. and Wood, C. (1989) Biochem. J. 258, 397–402.
[7] Northrop, D.B. and Rebholz, K.L. (1994) Anal. Biochem. 216, 285–290.
[8] Britton, H.G. (1966) Arch. Biochem. Biophys. 117, 167–183.
[9] Britton, H.G. (1973) Biochem. J. 133, 255–261.
[10] Duggleby, R.G. (1984) Comput. Biol. Med. 14, 447–455.