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Inhibition of adenine phosphoribosyltransferase by pyrophosphate

The kinetic mechanism of adenine phosphoribosyltransferase (adenylate:pyrophosphate phosphoribosyltransferase, EC 2.4.2.7) from Ehrlich ascites tumor cells[1] and from human erythrocytes[2,3] has been concluded from initial velocity studies to be of the so-called ping-pong bi-bi[4] type, with binary enzyme–substrate complexes. Product inhibition experiments with AMP were compatible with this conclusion, but those with PP_i were not[1]. In this study, two types of interaction of PP_i with this enzyme are demonstrated, and a different kinetic mechanism is proposed.

The initial velocity of radioactive AMP synthesis from *PP*-ribose-*P* and [^{14}C]-adenine was measured as described elsewhere[1] with an enzyme preparation partially purified from Ehrlich ascites tumor cells[5]. Isotope exchange between [^{14}C]adenine and AMP was measured by the same method, but in the absence of *PP*-ribose-*P*.

The ping-pong bi-bi kinetic mechanism proposed previously (in which *PP*-ribose-*P* binds to the enzyme first, followed by release of PP_i, addition of adenine, and finally release of AMP) predicts that PP_i should not bind to the free enzyme. However, this product has been shown previously partially to protect free adenine phosphoribosyltransferase against inactivation by heating[1]. In the present study PP_i was also found to decrease the rate of enzyme inactivation by *p*-hydroxymercuribenzoate. $1 \cdot 10^{-3}$ M PP_i and $1 \cdot 10^{-5}$ M *PP*-ribose-*P* afforded 57% and 100% protection, respectively, against inactivation by $5 \cdot 10^{-6}$ M *p*-hydroxymercuribenzoate. This "dead-end" binding of PP_i to free adenine phosphoribosyltransferase is probably at the same site to which *PP*-ribose-*P* binds.

The ping-pong bi-bi mechanism also predicts that PP_i should not, as a product, be inhibitory when *PP*-ribose-*P* concentration is varied at saturating levels of adenine, but should be a competitive inhibitor when adenine concentration is varied at saturating levels of *PP*-ribose-*P*[6]. In the presence of additional binding of PP_i to free enzyme, this product should be a competitive inhibitor with respect to both substrates. Earlier

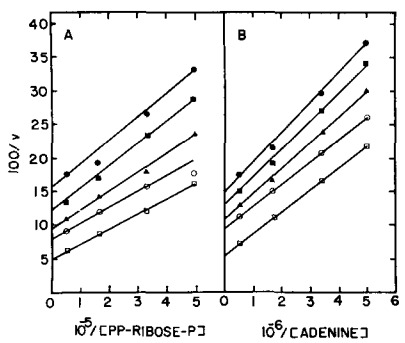

Fig. 1. Product inhibition by PP_i. A. Double reciprocal plot of initial velocity against *PP*-ribose-*P* concentration at an [^{14}C]adenine concentration of $1 \cdot 10^{-4}$ M. B. Double reciprocal plot of initial velocity against adenine concentration at a *PP*-ribose-*P* concentration of $3 \cdot 10^{-4}$ M. The concentrations of PP_i were: 0 (□), $1 \cdot 10^{-4}$ M (○), $4 \cdot 10^{-4}$ M (▲), $7 \cdot 10^{-4}$ M (■), and $1 \cdot 10^{-3}$ M (●). In each case an amount of Mg^{2+} equal to that of PP_i was added in addition to that normally present during assay.

experiments[1] were ambiguous because saturating levels of the fixed substrates were not used.

Fig. 1 shows that PP_i was a noncompetitive inhibitor with respect both to *PP*-ribose-*P* when the concentration of adenine was 100 times its Michaelis constant, and to adenine when the concentration of *PP*-ribose-*P* was 50 times its Michaelis constant. These results clearly do not support the proposed ping-pong bi-bi mechanism.

The possibility was considered next that the kinetic mechanism of the adenine phosphoribosyltransferase reaction was of the ordered bi-bi[4] type (in which *PP*-ribose-*P* binds first to the enzyme, followed by adenine; PP_i dissociates first, and then AMP) and that the initial velocity data were misleading; this has been shown to be the case with hypoxanthine–guanine phosphoribosyltransferase[6] and, under certain conditions, with quinolinate phosphoribosyltransferase[7]. In this case, under the conditions of Fig. 1, PP_i should be a noncompetitive inhibitor with respect to *PP*-ribose-*P* and a uncompetitive inhibitor with respect to adenine. These results were not obtained. Eqns. 1a and 1b, however, which are the double reciprocal forms of the rate equation for an ordered bi-bi mechanism with PP_i acting both as product inhibitor and as a dead-end inhibitor binding to free enzyme, predict that inhibition by PP_i should be noncompetitive with respect to both substrates, as was observed in Fig. 1.

$$\frac{E_0}{v} = \frac{K_A}{V_1 A}\left[\frac{K_{iA}K_B}{K_A B}\left(1 + \frac{P}{K_{ii}P}\right) + 1 + \frac{P}{K_{ii}P} + \frac{K_{iA}K_B K_Q P}{K_A K_P K_{iQ} B}\left(1 + \frac{P}{K_{ii}P}\right)\right] + \frac{1}{V_1}\left[1 + \frac{K_B}{B} + \frac{K_Q K_B P}{K_P K_{iQ} B} + \frac{P}{K_{iP}}\right] \quad (1a)$$

$$\frac{E_0}{v} = \frac{K_B}{V_1 B}\left[\frac{K_{iA}}{A}\left(1 + \frac{P}{K_{ii}P}\right) + 1 + \frac{K_{iA}K_Q P}{K_P K_{iQ} A}\left(1 + \frac{P}{K_{ii}P}\right) + \frac{K_Q P}{K_P K_{iQ}}\right] + \frac{1}{V_1}\left[\left(1 + \frac{P}{K_{ii}P}\right)\frac{K_A}{A} + \frac{P}{K_{ii}P} + 1\right] \quad (1b)$$

A, B, P, and Q are *PP*-ribose-*P*, adenine, pyrophosphate, and adenylate, respectively; K_{iA}, K_{iB}, K_{iP}, and K_{iQ} are inhibitor constants for *PP*-ribose-*P*, adenine, pyrophosphate, and adenylate, respectively; v and V_1 are initial velocity and maximum forward velocity, respectively; E_0 is total enzyme.

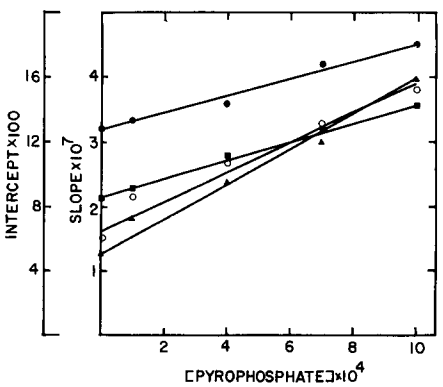

Fig. 2. Plot of slopes and intercepts from Fig. 1 against PP_i concentration. ■ and ▲, slopes and intercepts, respectively, when *PP*-ribose-*P* was the variable substrate; ● and ○, slopes and intercepts, respectively, when adenine was the variable substrate.

Fig. 2 shows replots of slopes and intercepts from Fig. 1 against PP_i concentration. The replot of slopes from Fig. 1A gives on the abscissa the negative value of the dissociation constant of the free enzyme–PP_i complex; $K_{iiP} = 1.55 \cdot 10^{-3}$ M. The replot of intercepts from Fig. 1B gives on the abscissa the negative value of the product inhibition constant; $K_{iP} = 4.7 \cdot 10^{-4}$ M.

Binding of PP_i to free enzyme introduces terms in P^2 into the rate equation (Eqn. 1) for product inhibition, and inhibition plots (Fig. 1) and replots of slopes (Fig. 2) should consequently be parabolic instead of linear. The slope term may be represented by the general equation, slope $= 1 + a + bP + cP^2$, and if $bP \gg cP^2$, the replot will approach linearity. It can be calculated that the maximum deviation from linearity would be 1.5% when *PP*-ribose-*P* was the variable substrate and 2.3% when adenine was the variable substrate. These deviations could not be detected by the graphical methods used.

Ping-pong bi-bi and ordered bi-bi mechanisms can also be distinguished in [^{14}C]adenine–AMP isotope exchange experiments. In the former case this exchange should occur in the absence of PP_i, but none was observed. In the latter case isotope exchange should require PP_i; in the presence of $2 \cdot 10^{-4}$ M PP_i, the rate of exchange was 5.2 pmoles/min per μg protein. PP_i-dependent adenine–AMP exchange was also observed using yeast adenine phosphoribosyltransferase[8].

Both the product inhibition and isotope exchange data are therefore consistent with an ordered bi-bi mechanism for adenine phosphoribosyltransferase. PP_i also binds to the free enzyme as a dead-end inhibitor. These data cannot be reconciled with a ping-pong bi-bi mechanism. Initial velocity data[1] which were first interpreted in support of the latter mechanism, can be reconciled with the ordered bi-bi mechanism if the dissociation constant of the enzyme–*PP*-ribose-*P* complex is much lower than the Michaelis constant for this substrate. This has been confirmed (unpublished results). Hypoxanthine–guanine phosphoribosyltransferase gives very similar initial velocity and product inhibition data[6].

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