The regulatory properties of rabbit muscle pyruvate kinase

The influence of effector concentrations

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The initial velocity of the reaction catalysed by rabbit muscle pyruvate kinase was studied as a function of the concentrations of the modifiers phenylalanine and fructose 1,6-bisphosphate under conditions where the relationships between the initial velocities and the concentrations of substrates are non-hyperbolic. It is shown that these data can be represented by the exponential model for a regulatory enzyme.

It has been established by earlier reports that rabbit muscle pyruvate kinase (EC 2.7.1.40) displays regulatory properties at pH 7.4, and in particular becomes susceptible to allosteric activation by fructose 1,6-bisphosphate and inhibition by phenylalanine (Phillips & Ainsworth, 1977; Carminatti *et al.*, 1971). The influence of substrate concentrations on the regulatory behaviour has been studied in detail by Ainsworth *et al.* (1983), and interpreted by the exponential model (Ainsworth, 1977; Ainsworth & Gregory, 1978). The purpose of the present paper is to report further results on the influence of effectors.

Experimental

Experimental methods, materials and assays are as described in the preceding paper (Ainsworth *et al.*, 1983).

Correction of data

Initial-velocity data were corrected for dead-end inhibition by MgADP by using the equation:

$$v_{\text{corr.}} = v_{\text{obs.}} \cdot (1 + [\text{ADP}][\text{Mg}^{2+}] \alpha_D / K_1) \quad (1)$$

where the concentrations are in mM units and α_D / K_1 has the value of 0.1 mM⁻² (Ainsworth *et al.*, 1983).

Analysis of data

The two-ligand exponential model (Ainsworth & Gregory, 1978) was employed to analyse non-hyperbolic relationships between the initial velocity of the catalysed reaction and a substrate concentration. According to this model, the initial velocity, v , measured as a function of the con-

centration of ADP (A) and an effector (B , either phenylalanine or fructose 1,6-bisphosphate), is represented by:

$$\frac{v}{V} = \frac{A \cdot \alpha_{ab}}{1 + A \cdot \alpha_{ab}} \cdot \frac{1 + \gamma \cdot B \cdot \beta_{ab}}{1 + B \cdot \beta_{ab}} \quad (2)$$

where V represents the rate of product formation when the enzyme is completely saturated by A in the absence of B . A different rate is measured when the enzyme is also saturated by B , and the change is defined by multiplying V by the constant γ : inhibition is associated with values $0 < \gamma < 1$, and activation with values $\gamma > 1$. The association binding constants α_{ab} and β_{ab} relate to particular values of the fractional saturation of the enzyme by the two ligands ($p_A = a$) and ($p_B = b$), and are defined by:

$$\ln \alpha_{ab} = \ln \alpha_{00} + k_{AA} \cdot p_A + k_{BA} \cdot p_B \quad (3)$$

$$\ln \beta_{ab} = \ln \beta_{00} + k_{AB} \cdot p_A + k_{BB} \cdot p_B \quad (4)$$

and, assuming allosteric linkage, $k_{AB} = k_{BA}$ (Ainsworth & Gregory, 1978; Ainsworth *et al.*, 1983). Note that the effector influences the reaction through its effect on α_{ab} (eqn. 3) as well as through γ . The values of γ were determined as described by Kinderlerer *et al.* (1981), and were found equal to 0 (for phenylalanine) and 1 (for fructose 1,6-bisphosphate) within the errors of the estimates. The remaining constants of the exponential model were obtained by using computer programs described by Kinderlerer *et al.* (1981) and Ainsworth *et al.* (1983): in particular, the program 'DESCENT' was found to be especially useful. A justification for using the two-ligand model to describe a system where more than two ligands present in the assay are

known to exert allosteric effects has been provided by Ainsworth *et al.* (1983).

Results and discussion

Inhibition by phenylalanine

Phenylalanine has long been known to inhibit the pyruvate kinase reaction (Carminatti *et al.*, 1971). However, this observation was made in experiments where the functions $v = f[\text{ADP}]$ and $v = f[\text{phosphoenolpyruvate}]$ remained hyperbolic. By contrast, we have examined the inhibition in conditions where $v = f[\text{ADP}]$ displays positive cooperativity. The data are shown in Fig. 1, together with curves calculated from the corresponding exponential model constants given in Table 1. The deviations that may be observed are haphazard and probably arise from experimental error. The value of k_{BB} indicates that the binding of phenylalanine is positively co-operative, in agreement with findings obtained by Kayne & Price (1972). The reciprocal heterotropic interactions are small and positive, and thus phenylalanine increases the affinity of the enzyme for ADP while simultaneously forming a catalytically inactive complex, which leads to complete inactivation when phenylalanine is saturating ($\gamma = 0$).

Activation by fructose 1,6-bisphosphate

Phillips & Ainsworth (1977) demonstrated that the enhancement of pyruvate kinase activity brought about by fructose 1,6-bisphosphate is a hyperbolic function of the activator concentration, which indicates an apparent binding constant of 0.8 mM^{-1} .

Table 1. Values of two-ligand exponential model constants for the corrected initial values of the pyruvate kinase-catalysed reaction illustrated in Figs. 1 and 2. The units of V are $\mu\text{mol/min per mg}$. The error parameters RSS and MD% are defined in Ainsworth *et al.* (1983); the values of the constants are given to a precision consistent with these errors.

Constant	Concentration variables ...	A = ADP; B = phenylalanine	A = ADP; B = fructose 1,6-bisphosphate
$\ln[\alpha_{00} (\text{mM}^{-1})]$		-1.474	-1.466
k_{AA}		1.669	1.126
$\ln[\beta_{00} (\text{mM}^{-1})]$		-2.253	-0.176
k_{BB}		1.874	-0.497
k_{AB}		0.336	0.956
V		141.628	160.298
γ		0	1
RSS		2.164	3.874
MD%		7.775%	7.945%

Phillips & Ainsworth (1977) also showed that the same final velocity was achieved at high concentrations of ADP with or without addition of the activator. It appeared on this basis that $\gamma = 1$. Additional data examined by Ainsworth & Gregory (1978) confirmed this view, and the data were fitted by assuming (because of the hyperbolic enhancement) that k_{BB} and k_{AB} were zero. The data reported by Ainsworth & Gregory (1978) have now been re-examined by the computational procedures described above (not then available), and a marginally improved fit was obtained. The exponential model constants are given in Table 1, and the fit they provide is illustrated in Fig. 2. It is seen that evidence of systematic error still remains. The activator's binding constant, β_{00} , has the value 0.84 mM^{-1} , in good agreement with that provided by enhancement, and it appears probable that its apparently hyperbolic binding stems from the opposing contributions of k_{BB} and k_{AB} to the measured binding constant, β_{ab} .

The binding constant for ADP has a value in

Fig. 1. Effect of [phenylalanine] on the corrected initial velocity of pyruvate formation at several fixed values of [ADP] at pH 7.4 at 25°C .

Reaction solutions contained 0.1 M-K^+ , 0.1 mM-Mg^{2+} and $0.2 \text{ mM-phosphoenolpyruvate}$. ADP concentrations (mM) were: A, 0.5; B, 0.7; C, 1.0; D, 2.0; E, 3.0; F, 5.0. In this Figure and Fig. 2, velocities (represented by letters) are corrected for dead-end inhibition by eqn. (1) with $\alpha_{\text{D}}/K_1 = 0.1 \text{ mM}^{-2}$ and fitted by dotted curves calculated in accordance with the two-ligand exponential model (eqns. 2-4) with the constants given in Table 1.

Fig. 2. Effect of [ADP] on the corrected initial velocity of pyruvate formation at several fixed values of [fructose 1,6-bisphosphate] at pH 7.4 at 25°C

Reaction solutions contained 0.1M-K⁺, 0.1mM-Mg²⁺ and 0.2mM-phosphoenolpyruvate. Fructose 1,6-bisphosphate concentrations (mM) were: A, 0; B, 0.25; C, 0.5; D, 1.0; E, 2.5; F, 5.0. Other conditions and calculations were as described in the legend to Fig. 1.

good agreement with that provided by the phenylalanine inhibition experiment (Table 1), and also with values obtained in experiments without effec-

tors (Ainsworth *et al.*, 1983). The values of the homotropic interaction constant, k_{AA} , are both positive but more variable in magnitude; the same effect is observed in values from experiments without effectors.

Conclusion

These studies have shown that the allosteric effects of phenylalanine and fructose 1,6-bisphosphate can be described by the exponential model, the description of the effects of substrate concentration and pH in these terms having been given in previous papers (Gregory & Ainsworth, 1981; Ainsworth *et al.*, 1983).

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