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Co-operative Effects in Enzyme Catalysis: A Possible Kinetic Model Based on Substrate-Induced Conformation Isomerization

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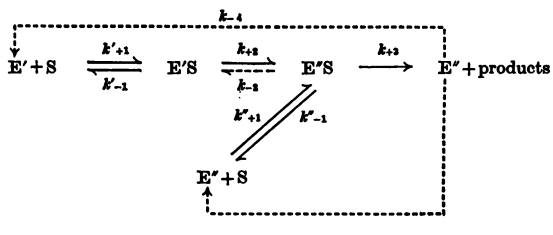
There has recently been much speculation in the literature on the origin of sigmoid velocity–substrate relationships (substrate co-operative effects) observed for some enzymes and some models have been proposed to explain these effects (Monod, Wyman & Changeux, 1965; Atkinson, Hathaway & Smith, 1965; Koshland, Nemethy & Filmer, 1966). Ferdinand (1966) has shown that apparent co-operative effects can arise in reactions involving a pair of substrates if a random non-equilibrium addition of the substrate partners to the enzyme occurs. In a recent verbal communication (British Biophysical Society, Winter Meeting 1965) the present author showed how co-operative effects can arise in a simple way without the need for special assumptions about the quaternary structure of the protein. Atkinson (1966) has also briefly indicated how conformational relaxation might give rise to co-operative protein–ligand interactions. As stated, however, it is not clear that his model is thermodynamically possible. The present communication has been produced in response to several requests for the publication of a formal statement of the author's model, which is summarized in the kinetic scheme shown in Scheme 1. In this scheme the initial process is the combination of the substrate with the enzyme in the form E′ to produce the complex E′S. This then isomerizes to give the complex E″S, which decomposes to give the products of the reaction and regenerates the enzyme in the isomeric form E″. Thus there are two conformational isomers of the protein, of which E′ is thermodynamically more stable than E″. It is assumed that the interconversion process E″ $\rightleftharpoons$ E′ is slow compared with all other reactions in the scheme. It is further assumed that the rate-limiting step in the sequence of reactions starting with the combination of E′ and S is the process E′S $\rightarrow$ E″S. Thus $k_{+2} \ll k_{+3}$. After the first initial turnover of the enzyme, the conformational isomer E″ is produced and this species can react with further substrate molecules to produce E″S, thus by-passing the slow step in the reaction sequence starting from E′. The free energy for the conversion E′$\rightarrow$E″ comes from the first turnover of the substrate and thus derives from the reaction itself.



Scheme 1.

The reverse isomerization of E″ to produce E′ could occur via the reaction sequence E″$\rightarrow$E″S$\rightarrow$E′S$\rightarrow$E′. If $k_{+2} \gg k_{-2}$ this process would be kinetically unimportant. With this restriction it can be shown by simple thermodynamic analysis that $k''_{-1}/k''_{+1} \ll k'_{-1}/k'_{+1}$: the dissociation constant of E″S is then smaller than that of E′S and the binding capacity of the enzyme for the substrate increases with the degree of progression of the reaction. The process E′S$\rightarrow$E″S in these circumstances causes an increase in the strength of attachment of the substrate and the reaction scheme can be regarded as an extension of Koshland's induced-fit hypothesis (Koshland, 1958).

A less restrictive condition for the reverse isomerization to be kinetically insignificant is that $k_{+3} \gg k_{-2}$. In this instance nothing can be said about the relative magnitudes of the dissociation constants of E′S and E″S. It is not therefore a necessary corollary of this model that the binding capacity for the substrate increases as the reaction proceeds.

The reaction scheme can obviously give rise to very complex kinetic behaviour and, since the rate of conversion of E′ into E″ is a function of the substrate concentration, sigmoid progress curves and apparent co-operative effects are possible. It is also possible to interpret the effects of allosteric modifiers within the framework of this scheme. Interaction with a modifier that provides a favourable kinetic pathway for the equilibrium of E″ and E′ will obviously inhibit the reaction by causing the removal of the kinetically favourable, but thermodynamically unfavourable, species E″. Such modifiers would normally be classified as allosteric inhibitors, and the model predicts that these could abolish co-operativity with respect to the substrate. Their effect would essentially be to increase the

rate constant k_{-4}. Modifiers that increase the rate of the isomerization process E'S⇌E''S will cause a rate increase at low substrate concentrations and co-operativity with respect to the substrate would be abolished if the value of k_{+2} is increased sufficiently for the inequality $k_{+2} \gg k_{+3}$ to hold.

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Regulation of the Concentration or Activity of Pyruvate Kinase in Yeasts and its Relationship to Gluconeogenesis

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Marked decreases in the concentration of pyruvate kinase have been observed in certain yeasts grown in conditions of gluconeogenesis (Ruiz-Amil, de Torróntegui, Palacián, Catalina & Losada, 1965; Gancedo, 1966). A similar behaviour with low glucose concentration in the medium has also been claimed (Hommes, 1966). Recently Hess, Haeckel & Brand (1966) found a strong activation by FDP* of the pyruvate kinase of brewer's yeast. We now report that two alternative mechanisms for the regulation of pyruvate kinase occur in different yeasts, one controlling its concentration and the other its activity. Either of these regulatory mechanisms permits a 'shutting off' of pyruvate kinase at the level of the phosphoenolpyruvate crossroad in gluconeogenesis.

Saccharomyces cerevisiae and *Candida utilis* are compared in Table 1. The former does not exhibit changes in the concentration of pyruvate kinase in relation with the carbon source, and the pyruvate kinase in the extracts is utterly insufficient to account for the glycolytic capacity of this yeast. Its activation by FDP qualitatively confirms that found by Hess *et al.* (1966) with brewer's yeast. Quantitatively it is about five times as great; this could be due to difference in the organism or in the test conditions. On the other hand, *C. utilis* has a large amount of pyruvate kinase when grown on glucose, but this amount is markedly smaller when grown on ethanol, and in no case was the activity increased by addition of FDP. Intermediate values of pyruvate kinase have been observed in *C. utilis* grown on glycerol. In *Rhodotorula glutinis* the pyruvate-kinase concentration also changes markedly with the carbon source (Ruiz-Amil *et al.* 1965), and again we have found that the enzyme activity is not affected by FDP.

The low concentration of pyruvate kinase in ethanol-grown *C. utilis* is accompanied by virtual inability to ferment glucose when transferred to a glucose medium (Table 1). The fact that the gradual regaining of the ability to ferment glucose was completely prevented by Actidione suggests that protein synthesis is involved (Siegel & Sisler, 1964), although more work would be required to pinpoint the limiting step.

Reciprocal changes in the concentrations of phosphofructokinase and fructose 1,6-diphosphatase have been observed in yeast and seem to be a factor in the regulation of the shift from glycolysis to gluconeogenesis, or vice versa, at the level of this pair of antagonistic irreversible enzymes (Gancedo, Salas, Giner & Sols, 1965). Gluconeogenesis from oxaloacetate precursors involves in yeast phosphoenolpyruvate carboxykinase (Ruiz-Amil *et al.* 1965). In these conditions phosphoenolpyruvate is a major crossroad, and an active pyruvate kinase would be a considerable hindrance. It appears that in yeasts the required 'shutting off' of pyruvate kinase can be accomplished either by a marked decrease in the concentration of enzyme or through a marked dependence for its activity on a concentration of FDP that could be reached in glycolysis but

* Abbreviation: FDP, fructose 1,6-diphosphate.