KINETICS OF REGULATORY ENZYMES: EFFECTORS FOR YEAST PHOSPHOFRUCTOKINASE DO NOT ALTER THE APPARENT KINETIC ORDER OF THE REACTION*

Daniel E. Atkinson, James A. Hathaway, and Eddie C. Smith†

Department of Chemistry
University of California
Los Angeles, California 90024

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Following the discovery of negative feedback controls in biosynthetic sequences by Umbarger (1956) and Yates and Pardee (1956), similar variation in the kinetic behavior of key enzymes in response to changes in concentrations of specific control metabolites (effectors) has been implicated in the regulation of a variety of metabolic sequences, both synthetic and degradative. A sigmoid curve of rate as a function of substrate concentration is now recognized as a very common characteristic of these regulatory enzymes. A positive effector increases the rate of reaction with a given concentration of substrate; addition of such an effector to the reaction mixture will thus move the rate-versus-substrate-concentration curve toward the left in a conventional plot. Concurrently, the curve usually seems to become less sigmoid. Since sigmoid rate curves can arise only from some kind of cooperative interaction between enzyme and more than one molecule of substrate (a kinetic order higher than one with respect to substrate), it has been frequently assumed that positive and negative effectors change the kinetic order of enzymic reactions by decreasing

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† U. S. Public Health Service Postdoctoral Research Fellow.

or increasing the interactions among bound substrate molecules (or between substrate binding sites). The results reported in this paper demonstrate that this assumption is not necessarily true; neither the positive nor the negative effector for yeast phosphofructokinase (PFK) affects the kinetic order of the reaction catalyzed.

Results--Adenosine-5'-monophosphate (AMP) is a powerful and specific positive effector for yeast PFK (Ramaiah et al., 1964). It appears to act by competing with the negative effector, ATP, for one or more regulatory sites on the enzyme surface. Nucleoside triphosphates other than ATP can serve as P donor in the yeast PFK reaction, but only ATP also acts strongly as a negative effector (Viñuela et al., 1963; Ramaiah et al., 1964). These effects are illustrated in Fig. 1A.

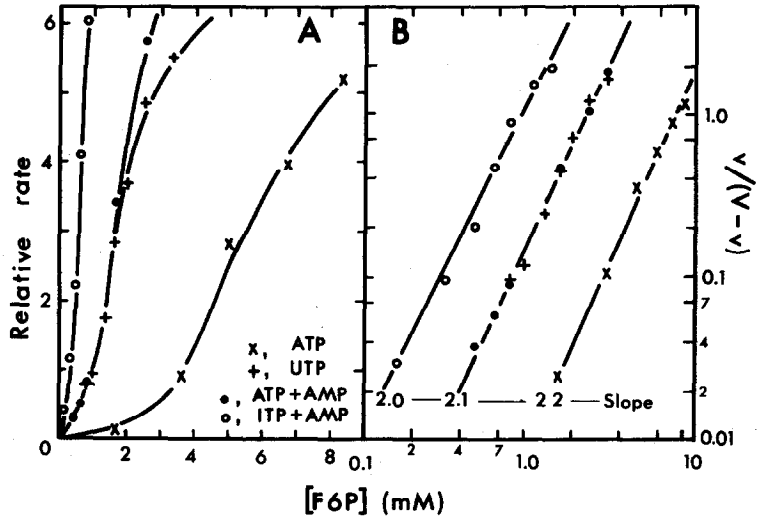

Figure 1. Effect of AMP and ATP on the kinetic behavior of yeast PFK.

The enzyme solution was the 25% to 40% $(NH_4)_2SO_4$ precipitate obtained from a centrifuged crude cell-free preparation of bakers' yeast (Fleischmann). The assay procedure was described previously (Ramaiah et al., 1964). Concentrations in reaction mixtures: $MgCl_2$, 3.3 mM; Tris-HCL, pH 8.5, 33 mM; F6P as indicated on abscissa; nucleotides (when present as indicated on figure): ATP, UTP, or ITP, 0.5 mM; AMP, 5 mM.

A: Direct plot of reaction rate as a function of F6P concentration.

B: Plot of v/V_{max} - v as a function of F6P concentration on logarithmic coordinates. The slope of each line is indicated on the figure.

The plot of rate as a function of fructose-6-phosphate (F6P) concentration is markedly sigmoid when ATP serves as P donor, but appears much less so when AMP is added to the reaction or when UTP replaces ATP. Each curve appears to approach "normal" Michaelis kinetics more closely than do those to the right of it. This appearance is illusory, however, being merely a consequence of compression along the substrate coordinate. The situation is clarified when the same data are used in a plot of log $[v/(V_{max} - v)]$ versus log (S) (Fig. 1B). The slope of such a plot is a function of the number of substrate-binding sites and the strength of interaction between them; if the interactions are strong the slope is numerically equal to the number of sites[1]. It is clear from Fig. 1B that, although the apparent affinity of the enzyme for substrate varies over a considerable range, the kinetic consequences of interactions between sites are essentially identical under all conditions tested. These conditions include the presence and absence, separately or together, of both the negative effector, ATP, and the positive effector, AMP. The results are thus inconsistent with any hypothesis in which the effector is considered to alter interactions among sites, whether by causing the enzyme to dissociate into subunits or by any other mechanism.

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[1] For an enzyme with n sites at which substrate may bind, a Michaelis equilibrium expression can be written in the form

$$(ES_n)/(E) = (S)^n/K,$$

where (S), (E), and (ES_n) are concentrations of substrate, free enzyme, and complex, respectively, and K is the product of the n Michaelis constants. If it is assumed that the concentrations of all complexes containing fewer than n substrate molecules are negligibly small (that is, that binding is strongly cooperative) and that the reaction velocity, v, and the maximal velocity, V_{max}, are proportional to (ES_n) and total enzyme concentration, respectively, it follows that

$$\log [v/(V_{max} - v)] = n \log (S) - \log K.$$

Thus a plot of log $[v/(V_{max} - v)]$ as a function of log (S) will be a line of slope n.

The rate of sedimentation of the PFK activity peak in a sucrose density gradient is not affected by the presence of either AMP or F6P at levels giving maximal kinetic effects.

Viñuela et al. (1963) observed a second-order dependence of rate on F6P concentration when ATP served as P donor in the yeast PFK reaction, but reported apparent first-order kinetics when a non-inhibitory P donor (GTP) was used. This conclusion was based on the linearity of reciprocal plots of velocity against F6P concentration. However, all rates plotted for the GTP experiments were well above $V_{max}/2$. In that region the shape of the double reciprocal plot is an extremely poor index of kinetic order. The difference between the plots presented by Viñuela et al. for ATP and GTP thus is more likely attributable to the much wider range of velocities plotted for the ATP experiments than to any difference in kinetic order.

Comparison with other enzymes--We have compared published rate data for several apparently regulatory enzymes to obtain a preliminary indication as to whether the effector substances for these enzymes affect the apparent order of the reactions catalyzed. The curve of rate versus substrate concentration in the presence of effector was expanded or compressed along the substrate coordinate as necessary to bring its midpoint into coincidence with the midpoint of the corresponding curve for reaction mixtures lacking effector. After this treatment, the curves should superpose if the effector does not modify intersite interactions, but should have different shapes if interactions are significantly weakened or enhanced by the effector. By this simple criterion the following enzymes resemble yeast PFK in that the effector seems not to affect interactions: DPN isocitrate dehydrogenase from yeast (Hathaway and Atkinson, 1963) and from rat muscle (Hathaway and Atkinson, unpublished; PFK from the liver fluke (Mansour and Mansour,1962) and from rabbit muscle (Passonneau and Lowry, 1962). For the enzymes in the following list the curves do not superpose, which suggests that

changes in interactions may be caused by the effector: PFK from heart muscle (Mansour, 1963) and from Escherichia coli (Atkinson and Walton, in press); aspartyl transcarbamylase (Gerhart and Pardee, 1962); biosynthetic threonine dehydrase (Changeaux, 1963); and deoxythymidine kinase (Okazaki and Kornberg, 1964).

Except for yeast PFK and yeast isocitrate dehydrogenase, the placement of any individual enzyme in one or the other group should not be considered to be firmly established. (A more extensive description and discussion of isocitrate dehydrogenase kinetics is in preparation.) Experiments on the other enzymes were not, in general, designed to give a detailed picture of the kinetics at low substrate concentrations, and the number of useful points is therefore usually small. We feel that the comparison nevertheless indicates the existence of at least two types of enzyme-effector interactions.

Summary--The reaction catalyzed by yeast PFK is second order in F6P; this response is not changed by either AMP or ATP, the effectors for this enzyme. Comparison of published results indicates that for some enzymes the effector changes the apparent order with regard to substrate, while others are like yeast PFK in this respect. Thus effector action cannot in general be attributed to alteration of interactions between substrate binding sites.

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