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Reversible Adsorption of Enzymes as a Possible Allosteric Control Mechanism

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In view of the advantages of allosteric phenomena in relation to metabolic control, it is noteworthy that sigmoidal kinetic data have been obtained with many enzyme systems (see, e.g., Stadtman, 1966). We wish to emphasize that failure to observe allosteric effects with an enzyme *in vitro* does not necessarily preclude this form of metabolic control in biological systems, where reversible adsorption on cell-membrane structures could provide the desired regulatory mechanism. From theoretical studies (Monod, Wyman & Changeux, 1965; Nichol, Jackson & Winzor, 1967) it is evident that sigmoidal kinetics are likely to be observed with any system comprising an equilibrium mixture of macromolecular species with different binding affinities for substrate. Results on rabbit muscle aldolase (EC 4.1.2.13) adsorbed to cellulose phosphate are presented to illustrate the feasibility of this form of control.

Selection of the particular model system was governed by the observation (Penhoet, Kochman, Valentine & Rutter, 1967) that aldolase is eluted from cellulose phosphate columns by substrate, fructose 1,6-diphosphate, a result that suggests the likelihood of competition between the adsorbent and substrate for the four active sites of the enzyme. However, a disadvantage of the system is that the more sensitive aldolase assays involve additional linked reactions, either chemical or enzymic, to detect the products resulting from breakdown of fructose diphosphate (Jagannathan, Singh & Damodaran, 1956; Richards & Rutter, 1961). To ensure that our results reflected the effect of adsorbent on the enzymic reaction, and not on the assay procedure, indirect evidence of the kinetic behaviour of the aldolase–cellulose phosphate system was obtained by the following procedure.

Cellulose phosphate was equilibrated with 0·05 M-tris–HCl buffer, pH 7·4, by passage of buffer through a column filled with adsorbent; the cellulose phosphate was then removed from the column and mixed with more buffer to form a slurry. To 0·4 ml. portions (30 mg. of adsorbent) of this thoroughly stirred slurry were added 10 μl. portions (24 μg.) of rabbit muscle aldolase (Sigma Chemical Co., St Louis, Mo., U.S.A.) and sufficient fructose diphosphate (Sigma Chemical Co.) to give final concentrations of the latter in the range 0–0·25 mM, tris–HCl buffer, pH 7·4, being added to give a total volume of 5·0 ml. After equilibration for 30 sec. at 30° each reaction mixture was filtered rapidly through a glass sinter, and the constituent concentration of enzyme in the filtrate $[\bar{E}_s]$ estimated by the colorimetric procedure of Jagannathan *et al.* (1956), a Unicam SP. 800 recording spectrophotometer being used for this purpose. Each assay solution (3·0 ml.) contained a 2·85 ml. portion of filtrate, 0·1 ml. of 0·2 M-fructose diphosphate and 50 μl. (3·6 mg.) of hydrazine, the velocity of the reaction approximating to the maximal value under these conditions.

Results of these experiments are shown in Fig. 1(*a*), the constituent concentration of enzyme in the solution phase of the reaction mixture $[\bar{E}_s]$ being expressed as a fraction of the concentration of adsorbed enzyme $[\bar{E}_a]$ present. In the absence of substrate approx. 11% of the aldolase is not bound by the exchanger, but this proportion is considered to comprise mainly enzyme incapable of being adsorbed rather than the amount of free aldolase in reversible equilibrium with adsorbed form. Since purification of the enzyme by adsorption on cellulose phosphate (Penhoet *et al.* 1967) involves the removal of protein contaminants by elution with 0·05 M-tris–HCl, pH 7·4, it is unlikely that the amount of adsorbable enzyme in solution would exceed 1%; similar behaviour has been reported for liver aldolase (EC 4.1.2.7), approx. 10% of which is not adsorbed by cellulose phosphate under these conditions (Rajkumar, Woodfin & Rutter, 1966). A second point evident from Fig. 1(*a*) is that the phenomena of enzyme adsorption and substrate binding are competitive, the proportion of enzyme in the solution phase being increased by the addition of substrate.

Kinetic data obtained in the absence of cellulose phosphate could be described adequately by the Michaelis mechanism and a value of $1{\cdot}5 \times 10^{-4}$ M for K_m. The variation of velocity (expressed as a fraction of the maximal value V) with substrate concentration for such a system is represented by the continuous line in Fig. 1(*b*), the circles referring to experimental data obtained in the absence of

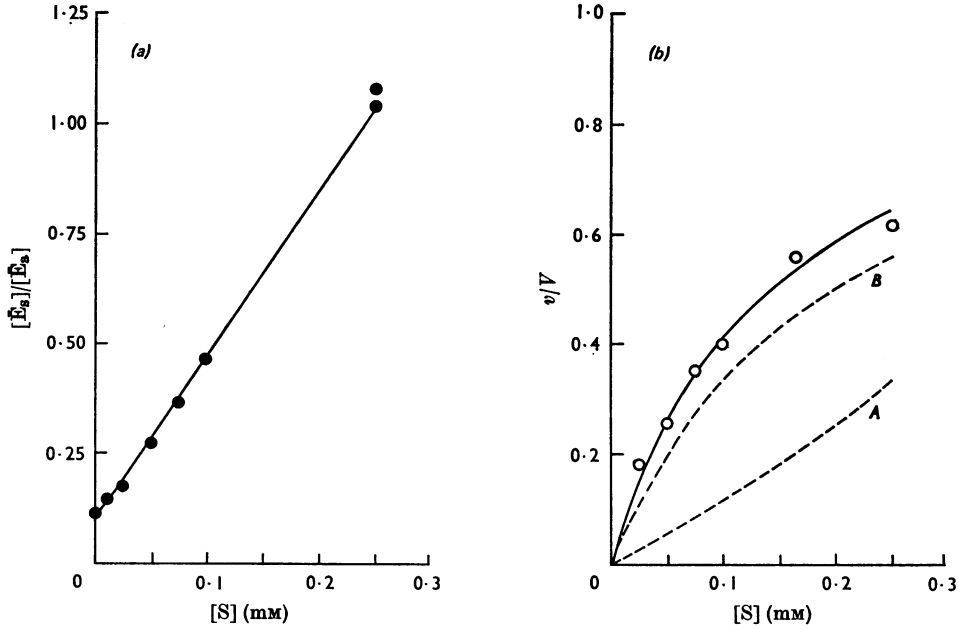

Fig. 1. Effect of substrate concentration on the reversible adsorption of rabbit muscle aldolase to cellulose phosphate in 0·05 M-tris–HCl buffer, pH 7·4: (*a*) its effect on the total concentration of enzyme in the solution phase; (*b*) experimental and theoretical relationships for the dependence of initial velocity on [S], the continuous line and experimental points referring to enzyme in the absence of adsorbent. Curve *A* is based on the assumption that adsorbed aldolase possesses no activity, and curve *B* refers to a model in which the adsorbed form of enzyme retains three fully active sites (see the text).

adsorbent. Also included in Fig. 1(*b*) are v/V versus [S] relationships for the aldolase–cellulose phosphate system calculated on the basis of Fig. 1(*a*) and the assumption that adsorption involves either complete inactivation of the enzyme (curve *A*), or inactivation of a single site/molecule without affecting the other three sites (curve *B*). From the former curve it is clear that reversible adsorption with complete inactivation would lead to pronounced sigmoidality of the v–[S] curve, no such effect being apparent with the alternative model (curve *B*), which, of course, describes the situation prevailing in conventional competitive inhibition.

Of these two extreme models, selected for purposes of illustration, the second represents the more probable situation in the aldolase–cellulose phosphate system because of the approximately linear variation of $[\bar{E}_s]/[\bar{E}_a]$ with concentration of fructose diphosphate (Fig. 1*a*). In either model adsorption is considered to affect the number but not activity of active sites, whereupon we may consider kinetic data in terms of the interaction between enzyme and substrate, the same association constant, K_A, applying to all active sites on both enzymic forms. The constituent concentrations of enzyme in the solution ($[\bar{E}_s]$) and solid ($[\bar{E}_a]$) phases are given by eqns. (1) and (2) respectively (Klotz, 1946):

$$[\bar{E}_s] = [\bar{E}](1+K_A[S])^p \quad (1)$$

$$[\bar{E}_a] = X[\bar{E}](1+K_A[S])^q \quad (2)$$

Square brackets denote molar concentrations, p and q the respective numbers of sites for substrate binding on the solution and adsorbed forms of enzyme, and X represents the partition coefficient describing the equilibrium between free and adsorbed enzyme; [E] and [S] are the equilibrium concentrations of free enzyme and free substrate respectively in the solution phase. From eqns. (1) and (2) it is apparent that:

$$[\bar{E}_s]/[\bar{E}_a] = (1/X)(1+K_A[S])^{p-q} \quad (3)$$

Thus the observed linear relationship between $[\bar{E}_s]/[\bar{E}_a]$ and [S] (Fig. 1*a*) implicates the model with the loss of a single active site on adsorption ($p-q=1$) rather than that in which adsorption is considered to lead to complete loss of activity ($p-q=4$). Combination of the slope of this plot with the value $8\times10^5\,\text{M}^{-1}$ reported for K_A on the basis of

equilibrium-dialysis experiments with hexitol diphosphate (Ginsburg & Mehler, 1966) leads to an estimate of approx. 200 for X. A partition coefficient of this magnitude would be consistent with the use of cellulose phosphate for the chromatographic purification of aldolase (Penhoet *et al.* 1967), and would also vindicate our supposition that approx. 11% of the crystalline aldolase was not adsorbable by cellulose phosphate.

In summary, the present investigation emphasizes the feasibility of reversible adsorption to cell-membrane structures as an allosteric control mechanism for enzymes with more than one active site/molecule (Fig. 1*b*, curve *A*). In the particular model system selected to test this hypothesis, namely aldolase in the presence of cellulose phosphate, the results may be described in terms of classical competitive inhibition rather than of allosteric modification, because only one of the four active sites is affected by adsorption; but it is clear that inactivation of more than one active site would have rendered the biphasic system subject to allosteric control. Finally, although it is not possible to make any deductions about aldolase *in vivo* from investigations of the above synthetic system, it is noteworthy that the coexistence of free and adsorbed forms of this enzyme in muscle has been suggested (Starlinger, 1967) and supported by a recent investigation of the aldolase–actin interaction (Arnold & Pette, 1968).

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