ANTAGONISTIC HOMOTROPIC INTERACTIONS AS A POSSIBLE EXPLANATION OF COENZYME ACTIVATION OF GLUTAMATE DEHYDROGENASE

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Glutamate dehydrogenase does not show the sigmoid relation between velocity and substrate or coenzyme concentrations exhibited by many allosteric enzymes, even in the presence of the allosteric inhibitor GTP. Instead, deviations from Michaelis-Menten behaviour of another kind are seen in Lineweaver-Burk plots of reciprocal rate against reciprocal NAD concentration, which deviate from linearity toward higher rates with increasing coenzyme concentration [1, 2]. We have studied this coenzyme activation in detail, and found that it extends over wide coenzyme concentration ranges, and occurs also with NADP, contrary to earlier reports [1, 3]. A tentative explanation is suggested in terms of negative or antagonistic homotropic interactions between multiple active centres in an Adair-Koshland model [4, 5]. The model of Monod, Wyman and Changeux [6] apparently cannot explain such kinetic behaviour.

Ox-liver glutamate dehydrogenase is very stable in dilute solutions (0.4—4.0 μg/ml) in phosphate buffer, I = 0.25, pH 7, and linear progress curves recorded with a sensitive fluorimeter [7] permitted accurate estimates of initial rates with coenzyme concentrations down to 1 μM. Duplicate or triplicate measurements usually agreed to within 1%. Some results obtained at 25°, with a fixed glutamate concentration of 50 mM, are shown in fig. 1 as Lineweaver-Burk plots covering different NAD concentration ranges. Overall, the plots are concave downwards, but the 'activation' effects extends from much lower concentrations than was previously reported [2]. Moreover, the plots show rather sharp discontinuities and apparently consist of several linear portions with different slopes. Similar results were obtained with the alternative coenzyme NADP. Discontinuities in Lineweaver-Burk plots have occasionally been noted by others [8, 9] and are at first sight difficult to explain as a real kinetic property of a pure enzyme. Attempts to obtain evidence of the presence of more than one enzyme or coenzyme species, or of changes of the sedimentation constant of the enzyme with coenzyme concentration, were unsuccessful. The same results were obtained with commercial and purified [10] coenzyme preparations.

The enzyme is believed to exist under these conditions as a catalytically active subunit of molecular weight about 300 000 with several [4—6] binding sites for coenzyme [11—13]. The fact that the kinetic behaviour described can be considered as the opposite type of deviation from Michaelis-Menten behaviour to a sigmoid velocity/substrate concentration relation led us to consider an explanation in terms of negative homotropic interactions between several identical active sites.

Consider for simplicity a protein with two binding sites per molecule. It is well known that a sigmoid saturation curve relating fractional saturation, y, and free ligand concentration, S, corresponds to a reciprocal plot of $1/y$ against $1/S$ which is concave upwards and has a continuously increasing slope, and can be described by a reciprocal form of Adair's equation with appropriate relations between the constants:

$$\frac{1}{y} = \frac{c + dS + eS^2}{aS + bS^2} \qquad (1)$$

The shape of the reciprocal plot is determined by the sign of the second differential coefficient, $d^2(1/y)/d(1/S)^2 = 2FS^3/(a + bS^3)$ where $F = a^2e + b^2c - abd$.

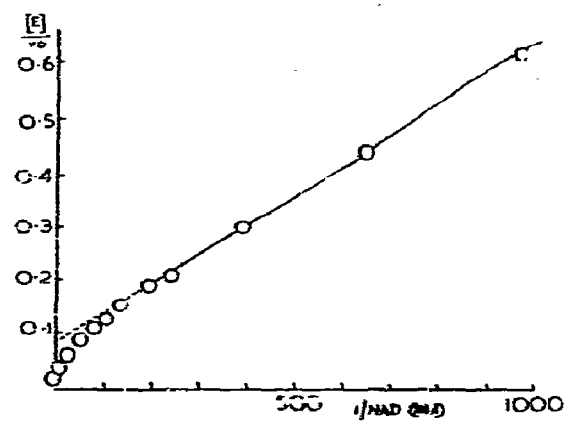


Fig. 1(a)

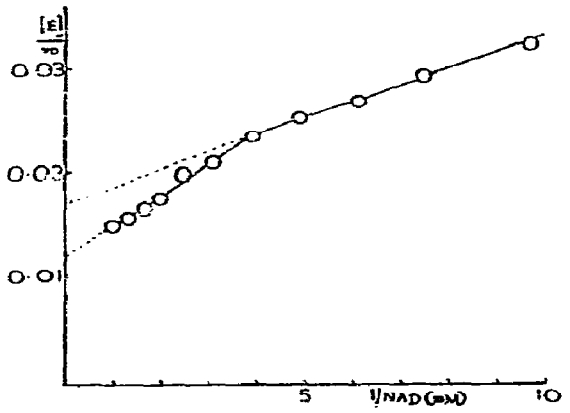


Fig. 1(c)

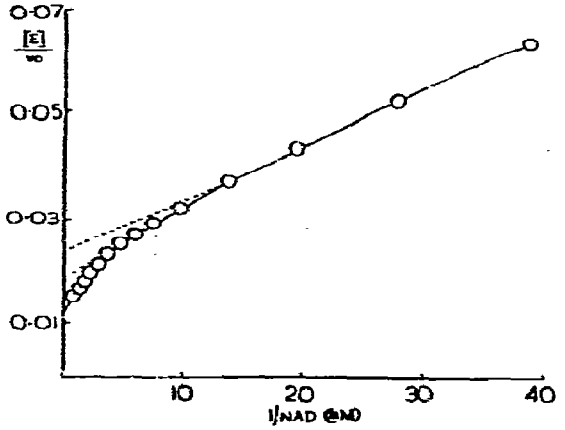


Fig. 1(b)

Fig. 1. Lineweaver-Burk plots for the oxidative deamination of glutamate (50 mM) catalysed by ox-liver glutamate dehydrogenase in phosphate buffer, pH 7.0 at 25°. The reciprocal specific rate ($[E]/v_0$) is plotted against the reciprocal NAD concentration. (a) 0.001–1.0 mM NAD; (b) 0.025–1.0 mM NAD; (c) 0.1–1.0 mM NAD.

If F is positive the reciprocal plot will be concave upwards, and the saturation curve sigmoid. If $F=0$, the reciprocal plot will be linear and the saturation curve a rectangular hyperbola. However, if F is negative, the reciprocal plot will be concave downwards, whilst the saturation curve will differ from a rectangular hyperbola in that it will be steeper below the half-saturation point and less steep above it.

The site-interaction hypothesis proposed by Adair [4] for haemoglobin is symmetrical in that it predicts both types of deviation from a linear reciprocal plot according to whether the interactions are positive or negative. The binding of a ligand S to a protein E with two identical sites is considered to occur in two steps with equilibrium constants, $K_1 = [ES]/[E][S]$ and $K_2 = [ES_2]/[ES][S]$, and the reciprocal saturation function is

$$\frac{1}{y}=\frac{2(1+K_1S+K_1K_2S^2)}{K_1S+2K_1K_2S^2} \qquad (2)$$

Comparison with eq. (1) shows that $F=2K_1^2K_2(4K_2-K_1)$. If the two sites are independent, K_1 and K_2 will have the statistical values of $2K$ and $K/2$, where K is the microscopic equilibrium constant for each site in the free protein. Then $4K_2 = K_1$, and $F=0$, and the reciprocal plot will be linear. If there are positive interactions so that $K_2 > K/2$, F will be positive and the reciprocal plot will be concave upwards (and the saturation function sigmoid). If there are negative interactions, so that $K_2 < K/2$ then $4K_2 < K_1$, and F will be negative. The reciprocal plot will then be concave downwards.

According to the model of Monod et al. [6] an allosteric protein exists in two conformational forms in equilibrium, $R \rightleftharpoons T$, in each of which the two or more binding sites are identical and independent, but the microscopic equilibrium constants for ligand binding, K_r and K_t, are different for the two forms. For two sites, the reciprocal saturation function [6] is

$$\frac{1}{y}=\frac{(L+1)+2(K_r+LK_t)S+(K_r^2+LK_t^2)S^2}{(K_r+LK_t)S+(K_r^2+LK_t^2)S^2} \qquad (3)$$

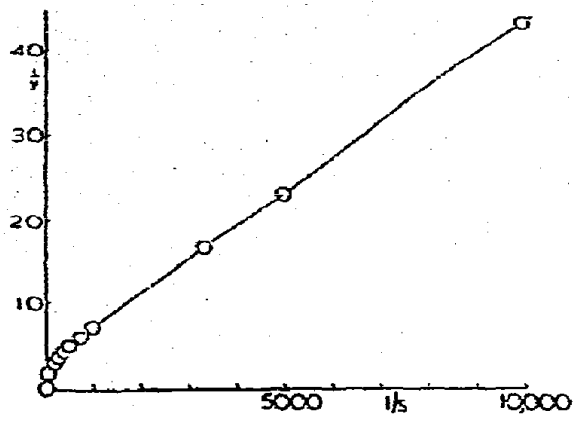


Fig. 2(a)

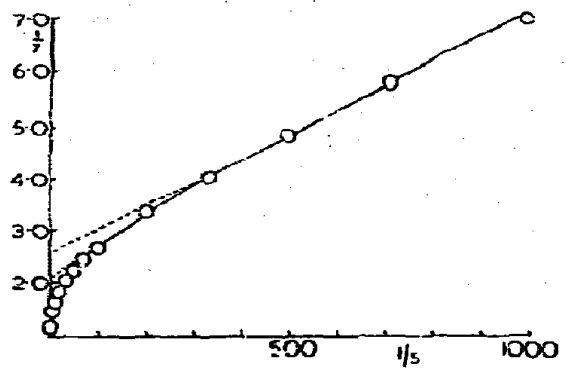


Fig. 2(b)

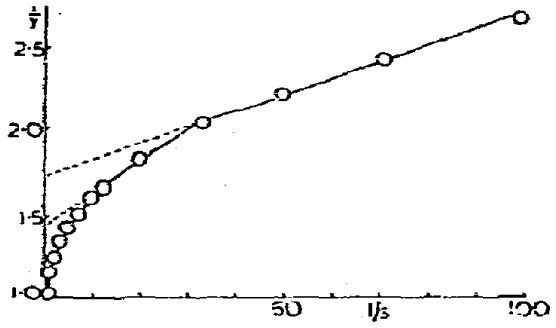


Fig. 2(c)

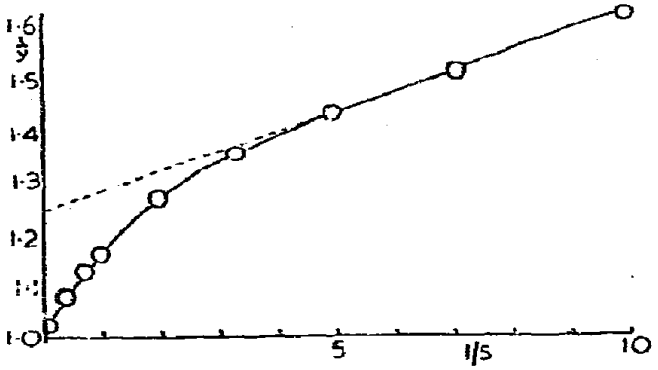


Fig. 2(d)

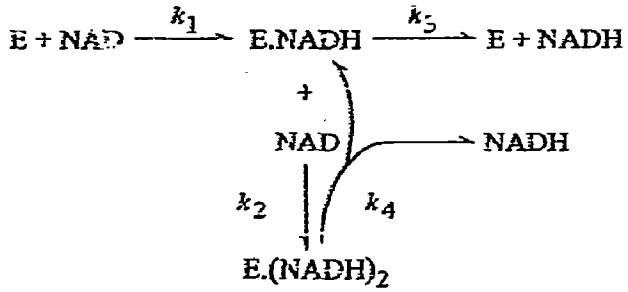
Fig. 2. (a–d). Plots of reciprocal fractional saturation ($1/y$) against reciprocal ligand concentration over four ranges of the latter for a protein with four binding sites, calculated from an Adair model with negative interactions ($K_1 = 10^3$, $K_2 = 10^2$, $K_3 = 10$, $K_4 = 1$).

where L is the conformational equilibrium constant. This equation is also of the same form as eq. (1), and $F = (LK_r^2 + L^2K_t^2)/(K_t - K_r)^2$. Thus, if K_t and K_r differ, F will be positive and the reciprocal plot will be concave upwards. The model is asymmetric in that negative values of F are not possible, and reciprocal plots that are concave downwards cannot be explained by it. Steady state treatment of this model for an enzyme also yields an expression for the reciprocal initial rate of the same form as eq. (1), in terms of Michaelis constants and maximum rates for the two enzyme forms [14], and again it can readily be shown that F is a function of $(K_r - K_t)^2$.

It therefore appears that the model of Adair and Koshland [4, 5] with negative interactions, but not that of Monod et al. [6], provides a feasible explanation of coenzyme or substrate 'activation' of the type observed with glutamate dehydrogenase. Moreover, if the negative interactions are reasonably strong, discontinuities in the plots would be expected. The effect would be as if the number of binding sites increased with the coenzyme concentration. This is illustrated by theoretical plots of the reciprocal saturation function for an Adair model with four binding sites (fig. 2), for which the equilibrium constants for the four successive steps were assumed to be 1000, 100, 10 and 1, compared with statistical values of 1000, 375, 157 and 63.

Saturation functions and velocity curves are not necessarily directly related, but it can be inferred that

the effects of negative binding interactions would be similarly reflected in velocity curves. The precise kinetic model for an enzyme would depend upon the detailed mechanism. A steady state rate equation for the case of a multi-site enzyme with negative interactions can be readily derived for a single-substrate system, or for a coenzyme-substrate reaction in which the reactant coenzyme binds to the enzyme before the substrate, and the rate of dissociation of the product coenzyme determines the maximum rate. For example, in the latter case with saturating substrate concentrations, and assuming for simplicity two active sites per molecule, the reaction may be represented by



Steady state analysis gives the initial rate equation

$$\frac{[E]}{v_0} = \frac{k_3k_4 + k_1k_4S + k_1k_2S^2}{k_1k_3k_4S + k_1k_2k_4S^2}$$

which is of the same form as eq. (1), and for which $F = k_1^2k_2k_3k_4^2\ (k_1k_3 + k_2k_4 - k_1k_4)$. This function will of course be zero when $2k_2 = k_1$ and $k_4 = 2k_3$, which are the statistical relations. The conditions for a Lineweaver-Burk plot which is concave upwards are that $2k_2 < k_1$ and $k_4 > 2k_3$, i.e. that the on-velocity for the second reactant coenzyme molecule is smaller, and the off-velocity for the second product coenzyme molecule is greater, than the statistical value. Obviously the model can be extended to any number of sites, and discontinuities in Lineweaver-Burk plots could be predicted if deviations from the statistical values are large enough.

Coenzyme or substrate activation has previously been explained by postulating specific allosteric sites, other than the active site, for these reactants [2], or by abortive complex formation [15]. For an enzyme with two or more active centres, negative interactions between them provides an alternative explanation which also accounts for the discontinuities occasionally observed in Lineweaver-Burk plots. Substrate activation effects in kinetic studies are not uncommon, and sufficiently detailed and precise initial rate measurements, or binding studies, may reveal evidence of negative interactions with other enzymes.

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