A KINETIC INTERPRETATION OF THE ALLOSTERIC MODEL OF MONOD, WYMAN AND CHANGEUX

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A plausible model for allosteric proteins was described by Monod, Wyman and Changeux [1] based on the postulate that an oligomeric protein exists in two forms in equilibrium which possess different affinities for ligands. Both forms consist of two or more identical protomers each having one binding site for a given ligand; the microscopic dissociation constant for any one ligand is the same for all homologous sites in each of the two states of the oligomer, the sites being identical and independent of one another.

The possibilities of homotropic and heterotropic cooperative interactions in the model, arising solely from the effects of ligands on the spontaneous equilibrium between the two states of the protein, were demonstrated theoretically by the calculation of saturation functions for ligands, and the conditions favouring sigmoid functions were derived. The theory can be applied directly to haemoglobin, since saturation functions can be determined experimentally. In principle, saturation functions can also be determined directly for one substrate of a two-substrate enzyme by stoichiometric studies. This has been done for a number of pyridine nucleotide-linked dehydrogenases, and allows distinction between allosteric models involving multiple sites [1, 2] and purely kinetic models involving alternative reaction pathways [3, 4]. For most enzymes however the experimental evidence of allosteric behaviour is a sigmoid relation between initial rate and substrate concentration. As initial rates are not necessarily simply related to saturation functions or dissociation constants of enzyme-substrate compounds, the extrapolation of the model of Monod et al. to initial rate data for enzymes lacks rigour. Initial rate equations were not derived, and it was considered that the kinetics of most enzyme systems would exhibit appreciable deviations from the theoretical functions.

It seemed to be of some interest, therefore, to evaluate this model for an allosteric enzyme in terms of steady state rates. The kinetic model is shown in scheme 1 for an enzyme with 4 active centres per molecule. Let $L = T/R$ be the equilibrium constant for the two states of the enzyme, k_1 and l_1 be the microscopic velocity constants for the combination of substrate at any one binding site of R and T respectively, k_{-1} and l_{-1} be the corresponding microscopic dissociation velocity constants of the compounds, and k_2 and l_2 be the microscopic velocity constants for the steps to products. Then the model requires that the specific rates of the various steps in the scheme take the statistical values shown, since all the sites in each form of the enzyme are assumed to be identical and independent of one another. It is also convenient to define 'microscopic Michaelis constants' of the Briggs-Haldane [5] type for the two enzyme states by $K_m^R = (k_{-1}+k_2)/k_1$ and $K_m^T = (l_{-1}+l_2)/l_1$.

The steady state concentrations of the enzyme-substrate complexes derived in the usual way, in terms of free R and T and the rate constants, are $RS_4 = R(S/K_m^R)^4$, $RS_3 = 4R(S/K_m^R)^3$, $RS_2 = 6R(S/K_m^R)^2$, $RS = 4R(S/K_m^k)$, and $TS_4 = T(S/K_m^T)^4$, etc. These steady state relations are the same as the equilibrium relations [1] except that 'microscopic Michaelis constants' replace microscopic dissociation constants. The total concentrations of enzyme and complexes of the R form is $R(1+S/K_m^R)^4$ and of the T form,

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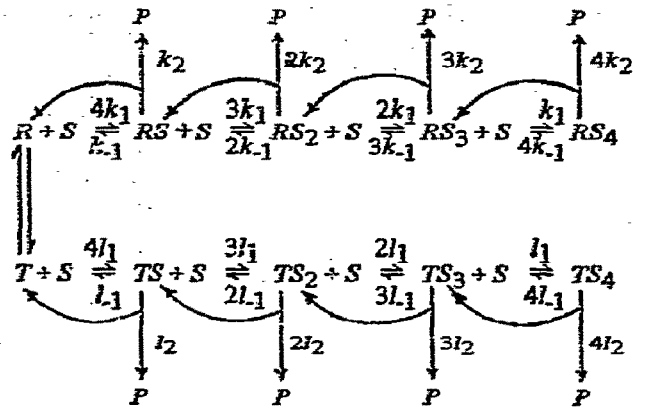

Scheme 1.

$T(1+S/K_m^T)^4$. Moreover the steady state concentrations of the free forms are found to be in the equilibrium ratio, $T/R = L$, and the total molar concentration of enzyme, e, is therefore related to that of the free R form by the conservation equation:

$$e = R[(1+S/K_m^R)^4 + L(1+S/K_m^T)^4].$$

Finally, the initial velocity in the steady state is

$$v_0 = dP/dt = k_2(RS+2RS_2+3RS_3+4RS_4) + l_2(TS+2TS_2+3TS_3+4TS_4) \quad (1)$$

$$= \frac{4e[k_2(S/K_m^R)(1+S/K_m^R)^3 + Ll_2(S/K_m^T)(1+S/K_m^T)^3]}{(1+S/K_m^R)^4 + L(1+S/K_m^T)^4}.$$

For the general case of n sites, and setting $S/K_m^R = a$ and $K_m^R/K_m^T = c$,

$$v_0 = \frac{ne[k_2a(1+a)^{n-1} + Ll_2ac(1+ca)^{n-1}]}{(1+a)^n + L(1+ca)^n}.$$

This rate equation is analogous to the saturation function derived by Monod et al. [1], but dissociation constants are replaced by Michaelis constants, and provision is made for the general case that the maximum specific rates per site or catalytic activities of the two enzyme forms, k_2 and l_2, are different. For the special case that $k_2 \ll k_{-1}$ and $l_2 \ll l_{-1}$, so that Michaelis constants are dissociation constants, and that $k_2 = l_2$, the rate equation can of course be obtained by multiplying the saturation function [1] by the total concentration of sites, ne, and the maximum specific rate k_2 [6].

In scheme 1, the transitions $RS \rightleftharpoons TS$ etc. are neglected; that is, it is assumed either that the concerted configurational change of the protomers requires prior dissociation of the substrate, or that the transitions are slow compared with the rates of the other reactions, which is not unlikely [7]. Inclusion of these paths gives a very complex rate equation. The complete rate equation for a two-substrate enzyme would also be complex, but it can be seen at once that for a compulsory order of combination of the two substrates eq. (1) would describe the variation of rate with the leading substrate concentration ($S = S_1$) if S_2 is present in saturating concentration. Then $K_m^R = k_2/k_1$ and $K_m^T = l_2/l_1$. For the simple case in which ternary complexes are not rate-limiting (Theorell-Chance mechanism) k_2 and l_2 would be dissociation velocity constants for the complexes RS_1' and TS_1', where S_1' is the leading substrate in the reverse reaction [8].

Like the saturation curves, the rate curves will of course exhibit the most marked homotropic interactions when K_m^R and K_m^T differ greatly, and, if K_m^T is the smaller, when L is large. If $K_m^T = K_m^R$, eq. (1) simplifies to

$$v_0 = \frac{neS(k_2 + Ll_2)/(1+L)}{S + K_m^R} \quad (2)$$

which is of the same form as the Michaelis-Menten equation. This represents a 'V system' of Monod et al [1]: no homotropic or heterotropic interactions would be observed, but an effector having different affinity for R and T would alter the maximum rate, $(k_2+Ll_2)/(1+L)$, by modifying L, as will be shown. The substrate concentration which gives half the maximum rate is equal to the Michaelis constant as previously defined and would be unchanged by an effector.

If also $k_2 = l_2$ or if $L = 0$, then eq. (2) simplifies to the Michaelis-Menten equation for a single enzyme species with n identical and independent active centres, of course.

To account for heterotropic interactions in the presence of an allosteric effector F, scheme 1 must

be extended to include species RF, $RF_1 \ldots RF_4$, and RSF, $RSF_1 \ldots RSF_4$, etc., and the corresponding T forms. In spite of the apparent complexity of the full scheme, the steady state rate equation is easily derived because the assumption that the effector acts only by displacing the $R \rightleftharpoons T$ equilibrium, and does not directly affect reactions at the active centre [1], means that the same microscopic velocity constants for the enzymic reaction will apply to all enzyme species of one form, regardless of the binding of F. It follows that in the steady state all the complexes will be in equilibrium with free ligand and that $R+RF+\ldots RF_4 = R(1+F/K_F^R)$; $T+TF+\ldots TF_4 = T(1+F/K_F^T)$; $RS+RSF+\ldots RSF_4 = RS(1+F/K_F^R)$, etc., where K_F^R and K_F^T are the microscopic dissociation constants for effector for the two enzyme forms. It also follows that the relative steady state concentrations of the effector-free species will be the same as in the absence of effector. The initial rate equation is found to be identical with eq. (1) except that L is replaced by

$$L' = L(1+F/K_F^T)^4/(1+F/K_F^R)^4.$$

If $K_F^R < K_F^T$, then the effector will be an activator if $K_F^R < K_F^T$ and an inhibitor if $K_F^R > K_F^T$. For the simpler case considered in terms of saturation functions by Monod et al. [1], in which the substrate combined only with the R form, i.e. $K_m^T = \infty$, the rate equation simplifies to

$$v_0 = \frac{4ek_2S}{S+K_m^R[L+(1+S/K_m^R)^3]/(1+S/K_m^R)^3}.$$

Thus the effector would not alter the maximum rate, $4ek_2$, but L', and therefore the substrate concentration which gives half the maximum rate, and also the substrate homotropic interactions, would be increased by the effector if $K_F^R > K_F^T$, and decreased if $K_F^R < K_F^T$. In the latter case F is an activator, and the v_0/S plot would approach a rectangular hyperbola as F increases and L' becomes small. These are fundamental properties of the model considered in terms of saturation functions by Monod et al. [1] and need not be further elaborated here.

The present treatment shows that the predictions of the model may be applied to enzyme velocities in the steady state as well as to saturation functions, and may provide a basis for the interpretation of rate data. The only assumptions involved are that the enzyme concentration is small compared with the substrate and effector concentrations, and that allosteric transitions of substrate-containing species are either forbidden or slow.

It may be added that in eq. (2) with $L = L'$, the effector may be a proton. It might be expected that as a result of a change in conformation of the oligomer and of constraints between the protomers accompanying the transition $R \rightleftharpoons T$, a change of dissociation constants of acidic groups in the protomers would occur. The transition would then be pH dependent and the proton would act as an effector. The finding with some enzymes that sigmoid v_0/S relations are observed at some pH values and not at others could be explained on the basis of this model, therefore. This explanation does not require that the ionising groups be close to the active centre or involved in substrate binding or the catalytic step, which are the usual interpretations of pH effects on enzyme reactions.

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