Remarks on the Kinetics of Enzymes with Interacting Effector Molecules. Tests of a Configurational Hypothesis in a Quasi-Equilibrium Model*

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ABSTRACT: A rather general theory for the interaction of effector molecules bound to oligomeric enzymes is proposed and formulated mathematically. The essential postulates of the theory are that the binding (and in the case of a substrate, the catalysis) of an effector by a protomer depend (apart from possible steric interactions) entirely upon what configuration the protomer has adopted, and that this configuration influences the configuration adopted by other subunits in the oligomer. The nature of this interaction is not further specified.

Thus the theory includes the models of Monod *et al.* (*J. Mol. Biol. 12*, 88 (1965)) and Koshland *et al.* (*Biochemistry 5*, 365 (1966)) as special cases. In the context of this theory simple verifiable consequences of the hypothesis that two ligands be bound to one configuration, and to that configuration only, are derived. Six different cases arise according to whether either effector is or is not a substrate of the enzyme, and to whether the two effectors are bound to the same site on a protomer or to two different sites. The predictions take the form of equations involving (a) an arbitrary function immediately obtainable from the experimental data and (b) one or two arbitrary constants according to whether one or two sites are involved. Methods for comparing the predictions with experimental data are given. They are easy to apply. The predictions are independent of the nature of the interaction between the subunits and are applicable without any knowledge about these interactions in any given system. It is also shown that if the differences between a set of members of a set of configurations of a protomer are such as not to be recognized by other protomers in the enzyme molecule, then the kinetic behavior of such a set greatly resembles, but is not identical with, that of a single configuration. Aspects of this behavior are investigated in detail.

Present thinking about the mechanisms of interactions between molecules bound to enzymes emphasizes the idea of changes in the three-dimensional conformation of all or part of the enzyme molecule as mediating these interactions. It is assumed that an enzyme molecule is capable of existing in more than one conformation, and that the different conformations behave differently toward the ligands. The view, rather widely accepted at present, that the enzymes which show biologically significant interactions between ligands are as a rule oligomeric, and that the conformation adopted by any subunit influences that adopted by others in the same molecule, allows and explains interaction between ligands bound to sites on different polypeptide chains.

In order to relate these ideas to experimental data (we shall be concerned here mostly with kinetic data) in a quantitative way, it is necessary to make further assumptions concerning the nature and properties of these configurations, and to examine the consequences of these assumptions mathematically. Monod *et al.* (1965) and Koshland *et al.* (1966) have made important contributions in this sense. The unambiguous comparison of the mathematical predictions of such theories with experimental findings can, however, be a difficult matter.

In this and subsequent papers we formulate the problem in a more general way than those authors and obtain predictions which, if tested, would seem to permit practically unambiguous inferences about certain underlying events at a molecular level.

To see the difference between the present approach and that of the above-cited authors, it is necessary to formulate our model and the main aims of our mathematical treatment.

Section 1. The Model

The following are the assumptions of the model. Consider an enzyme with q protomers. Any protomer is capable of existing in an unspecified number of configurations, each different in affinities for ligands, catalytic constants, and other properties. For a given configuration, T, of a protomer there exist $(q + 1)$ species, T_i, where i protomers are in the configuration T. In the absence of ligand, the concentration of each species T_i, is specified by an equilibrium constant, L_i, where $(T_i) = L_i(T_0)$ (note $L_0 = 1$). We shall make no special assumptions concerning these constants. The rate constants of interconversion between different configurations are supposed rapid in comparison with that of decomposition of bound

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substrate into product of the reaction catalyzed by the enzyme. Except where specifically stated, there are no interactions between ligands other than those due to a tendency for a protomer being in a particular conformation to influence the conformations adopted by other subunits in the same molecule. This influence is expressed in a series of constants such as L_i.

A ligand binding to a given conformation of a subunit naturally stabilizes that conformation. This influences the conformation adopted by other subunits in the same molecule, which in turn determines the interactions between those subunits and ligands. The result is interaction between ligand molecules bound to different subunits.

The hypotheses that have to be made in theoretical discussions if we want to apply our model to the complete description of the kinetics of a particular system can be analyzed into three types.

Hypotheses about the Constants L_i. In general, one will seek from physicogeometrical considerations to express all the L_i in terms of a single or a limited number of arbitrary constants. For instance, Monod *et al.* (1965) make the simplest possible assumption, that $L_i = 0$ for all i except $i = 0$, and $i = q$. The model of Koshland *et al.* (1966) uses physicogeometrical assumptions about the interactions at the surfaces of contact between protomers in a tetramer to obtain the L_i in terms of two underlying constants. There are many alternative assumptions that could be made (Whitehead, 1970a). The models of Monod *et al.* (1965) and Koshland *et al.* (1966) are thus special cases of our generalized model, where we make no particular assumption about the L_i (and, in consequence, cannot give a complete description of the kinetics of any system). In order for there to be interaction between sites on different protomers it is necessary that the distribution of any configuration be other than binomial, *i.e.*, that

$$L_i \neq \binom{q}{i}(L)^i$$

where L is a constant for the equilibrium of any protomer, the same in all species (T_i), between the T state and all other states.

Hypotheses about the Number of Configurations, Their Properties and Their Relationships. Examples of such hypotheses might be that a given configuration binds two given ligands ω and ψ, or that a configuration which binds ω fails to bind ψ, or that only some of the configurations which bind ω bind ψ, etc.

Hypotheses about the Binding Sites of Ligands. For two given ligands we shall in general have in mind two possibilities: (i) that there exists, per protomer, one site capable of binding either of the ligands (the *one-site hypothesis*); and (ii) that there exist, per protomer, two sites, each specific for one of the ligands (the *two-site hypothesis*). We shall ignore the possibility that there exist more than one specific site for any ligand in the protomer. The reasons for this are: firstly, that this hypothesis does not in general give rise to the simple and practical equations that we are able to deduce from the hypothesis of one site per ligand per protomer; secondly, symmetry considerations make it impossible in principle that there can be more than one site with given properties in a protomer. Admittedly there may well be cases where there is more than one site per protomer for a given ligand, each site having different properties (and notably different specificity) but these cases will in general have the unfortunate mathematical properties mentioned above, and are probably rather the exception.

It will be convenient to be able to refer easily to each of these three types of hypothesis, and we shall designate them, respectively, interaction- (I), configuration- (C) and site- (S) type hypotheses.

To obtain analytic equations for the velocity in terms of the concentration of substrates and effectors (and a certain number of constants), it is necessary to make hypotheses of types I, C, and S all at once. The aim of the mathematical development of this and future communications is to show that our generalized configurational model where we do not make any hypotheses of type I makes precise predictions for appropriate hypotheses C and S. The predictions made concern the *relations* of the velocities observed under different circumstances and consist of equations containing arbitrary functions f and g, which functions can be determined experimentally (they are velocities, v, expressed as a fraction of the velocity under the limit condition either of substrate saturation, v_s, or the absence of effector, v_0, under some standard conditions). The advantages of this method of proceeding are (1) it gives equations which are relatively simple and easy to test when the appropriate data are gathered; (2) comparison of these equations with data allows a conclusion about the application of C, S hypotheses to a given system which is independent of any I hypothesis. At the present state of the science such hypotheses (*e.g.*, the configurational symmetry hypothesis; Monod *et al.*, 1965) are still controversial. Of course, all the present results could be obtained from some narrower theory such as that of Monod *et al.* (1965), and should some such theory become established as universally valid the full generality of the present treatment would become in a sense superfluous, though its results would probably retain some usefulness. Even then, it would still be important to know upon which assumptions the results we shall obtain depend, and not to take any agreement between the predictions and experiment as support for any particular I-type hypothesis.

Mathematical Development

The plan of the following treatment is: in section 2 we shall consider the mathematical expression of the above postulates as applied to a single ligand binding to a configuration T. The equations obtained in this section are necessary in the development of the theory, though not themselves greatly useful in direct application to experimental situations. In section 3 we consider various instances of the hypothesis that two ligands are bound to the same configuration, T, of the protomers. This gives the essential results of this paper, namely, simple relations easily applicable to experimental data. (In future papers we shall examine other possible relationships between configurations binding two ligands.) Having established the usefulness of the approach, we examine in more detail and generalize in section 4 the notion of a configuration. (Readers who find the mathematics troublesome might do well to skip the mathematical arguments (some of which are presented in appendices), reading this section for its conclusions first.)

Section 2. Mathematical Formulation of the Binding to Enzyme of a Single Ligand Bound to T Only

A ligand ω is supposed bound to the configuration T of a protomer only. Consider the following reaction between $T\Omega_1$ and $T\Omega_0$, a protomer with one and with zero molecules of ω bound, respectively

$$T\Omega_0 + \omega \rightleftharpoons T\Omega_1$$

then

$$(T\Omega_1) = (T\Omega_0)B_\omega\omega$$

where B_ω is an association constant for ω, the same for *all* protomers in the T state. It follows that if T is the total concentration of protomers in the T state

$$\frac{(T\Omega_0)}{(T)} = \frac{1}{1 + B_\omega\omega} \tag{1}$$

and

$$\frac{(T\Omega_1)}{(T)} = \frac{B_\omega\omega}{1 + B_\omega\omega} \tag{2}$$

Now consider the species T_i. The probability that all of the i available sites will be unoccupied is the product of the probabilities for each separate protomer, *i.e.*

$$\left(\frac{1}{1 + B_\omega\omega}\right)^i = \frac{(T_i\Omega_0)}{(T_i)}$$

$(T_i\Omega_r)$ is the concentration of molecules (T_i) with r molecules of ω bound, thus $(T_i\Omega_0)$ is that with no molecules of ω bound.

$$(T)_i = (T_i\Omega_0)(1 + B_\omega\omega)^i \tag{3}$$

(There is in fact a binomial distribution of ω on the i available sites such that

$$(T_i\Omega_r) = (T_i\Omega_0)\binom{i}{r}(B_\omega\omega)^r$$

and eq 3 can be obtained by summation of the binomial series.) From the definition of L_i in the preceding section, 3 becomes

$$(T_i) = (T_0)L_i(1 + B_\omega\omega)^i \tag{4}$$

We may now easily obtain equations expressing, as a function of ω, the fraction $(\bar{T})$ of the total number of protomers which are in the configuration T, the fractional saturation $\bar{\Omega}$ of the enzyme by ω, and the velocity, v.

$$(\bar{T}) = \frac{\sum_{i=0}^{q} i(T_i)}{q\sum_{i=0}^{q}(T_i)}$$

from eq 4

$$(\bar{T}) = \frac{\sum_{i=0}^{q} iL_i(1 + B_\omega\omega)^i}{q\sum_{i=0}^{q} L_i(1 + B_\omega\omega)^i} \tag{5}$$

$\bar{\Omega}$, the fractional saturation of the enzyme by ω, is equal to the fraction of protomers capable of binding ω, *i.e.*, $(\bar{T})$, multiplied by the fractional saturation of such protomers, given by eq 2.

$$\bar{\Omega} = \frac{B_\omega\omega}{1 + B_\omega\omega}(\bar{T}) \tag{6}$$

which can be written as

$$\bar{\Omega} = \frac{\sum_{i=0}^{q} iL_iB_\omega\omega(1 + B_\omega\omega)^{i-1}}{q\sum_{i=0}^{q} L_i(1 + B_\omega\omega)^i} \tag{7}$$

We now seek equations for the effect of the ligand on the velocity of the enzyme-catalyzed reaction.

First we take the case where the ligand is not a substrate (such a ligand will be denoted y). Let the species T_i be characterized by a velocity constant, k_i, at any given concentration of substrate (this in general can include a contribution both from the protomers in the T state and from those in states other than T—here we will refer to the ensemble of all such states as the "R state"). Then

$$\frac{v}{v_0} = \frac{\sum_{i=0}^{q}(k_iT_i)}{k\sum_{i=0}^{q}(T_i)}$$

where v_0 is the velocity in the absence of y and k is a velocity constant characteristic of the enzyme unoccupied by y; equal in fact to

$$\sum_{i=0}^{q} k_iL_i \Big/ \sum_{i=0}^{q} L_i$$

Substitution of eq 4 in the above equation gives us, for the effect of y on v

$$\frac{v}{v_0} = \frac{\sum_{i=0}^{q} k_iL_i(1 + B_y(y))^i}{k\sum_{i=0}^{q} L_i(1 + B_y(y))^i} \tag{8}$$

The assumption here that T_i can be characterized by a single velocity constant as a function of i at constant substrate concentration is not obviously appropriate when y is a competitor for the substrate site. In this case, and assuming y to be bound only at substrate sites, the velocity of catalysis by T_i can be decomposed into two components: (1) That

due to the protomers not in the T configuration; this will be characterized by a single velocity constant we shall call ρ_i. The velocity due to these protomers in T_i is then $\rho_i(T_i)$. (2) That due to protomers in the T configuration. A protomer in this configuration and unoccupied by y will have a characteristic velocity constant τ. The contribution of protomers in the T form in T_i would then be $i\tau(T_i)$ were it not for the steric effect of y. If there is full competition, such that a protomer binding y is without activity, then this quantity must be multiplied by the fraction of sites unoccupied by y, *i.e.*, $1/(1 + B'_y(y))$, where B'_y is the effective association constant for y, equal in fact to $B_y/(1 + B_x(x))$, x being the substrate concentration. Thus, for the total velocity v_{T_i} due to T_i

$$v_{T_i} = \rho_i(T_i) + \frac{i\tau(T_i)}{1 + B_y'(y)}$$

It follows that

$$\frac{v}{v_0} = \frac{\sum_{i=0}^{q} L_i[\rho_i(1 + B_y'(y))^i + i\tau(1 + B_y'(y))^{i-1}]}{k\sum_{i=0}^{q} L_i(1 + B_y'(y))^i} \quad (9)$$

(Note that the k_i used in eq 8 are equal simply to $\rho_i + i\tau$. k in eq 9 has the same significance and value as before.)

If the steric effect on substrate binding is not absolute, so that a protomer combined with y still retains a finite velocity constant, $\tau\sigma$ say, then instead of eq 9 we have

$$\frac{v}{v_0} = \frac{\sum_{i=0}^{q} L_i[\rho_i(1 + B_y'(y))^i + i\tau(1 + B_y'(y))^{i-1}(1 + \sigma B_y'(y))]}{k\sum_{i=0}^{q} L_i(1 + B_y'(y))^i} \quad (10)$$

Finally, if the ligand whose effect on v we are examining is the substrate, x, then v/v_s (where v_s is v at saturating concentrations of x) is equal simply to the fractional saturation of the enzyme by x, *i.e.*, an expression of the form of that in eq 6.

$$\frac{v}{v_s} = \frac{B_x(x)}{1 + B_x(x)}(\bar{T}) \quad (11)$$

$$\frac{v}{v_s} = \frac{\sum_{i=0}^{q} iL_1B_x(x)(1 + B_x(x))^{i-1}}{q\sum_{i=0}^{q} L_i(1 + B_x(x))^i} \quad (12)$$

Section 3. Two Ligands Bound to the Same Configuration

In this section we consider the question: What, in the context of our model, are the observable consequences of the binding of two ligands to the same configuration T of the protomers and to that configuration only?

We have to consider six cases, according to whether the two ligands being compared are (1) two modifiers, defined as nonsubstrate effectors; (2) a substrate and a modifier, or (3) two substrates; in each case (i) the one-site and (ii) the two-site hypothesis must be considered.

The following argument immediately tells us what behavior to expect in cases of the one-site hypothesis. A molecule of either of the two ligands if bound by T and T only will, once bound to an enzyme with a given fraction $\bar{T}$ of its protomers in that state, shift to exactly the same extent the equilibrium between T and other configurations. Since the nonsteric interactions between ligands are supposed due to these shifts, the corresponding free energies of interaction between bound molecules would be the same for each of the two ligands. The equations that follow from just such a situation can be derived without reference to the underlying mechanism and are eq 17–19, 21, and 23–27 of this paper (E. P. Whitehead, unpublished work). Here however we shall derive these equations from the configurational theory and also establish the formulas for the corresponding two-site case which cannot be done from the above thermodynamic postulates alone.

Effects on $\bar{T}$ of Two Effectors

The common basis of the results for all six cases is best understood by considering first of all the effects on $\bar{T}$. Call $\bar{T}$ in the presence of ω, $(\bar{T})_\omega$, and define a function $\mathfrak{I}$ such that

$$(\bar{T})_\omega = \mathfrak{I}(\omega) \quad (13)$$

Then $\mathfrak{I}(\omega)$ is equal to the expression on the right-hand side of eq 5. Then in the absence of ω and presence of another ligand ψ, bound also to T, $(\bar{T})_\psi$ will be given by the identical eq 5 except that everywhere in place of ω we must write ψ. That is, starting from eq 5, we obtain $(\bar{T})_\psi$ by means of the transformation

$$\omega \longrightarrow \frac{B_\psi(\psi)}{B_\omega}$$

In other terms

$$(\bar{T})_\psi = \mathfrak{I}(a\psi) \quad (14)$$

where $a = B_\psi/B_\omega$. This is true for both the one-site and the two-site hypotheses. The behavior is different for these two cases, however, when both ligands are present together.

(*i*) *The One-Site Hypothesis.* In this case it is readily seen that

$$\frac{(T\Omega_0\Psi_0)}{(T)} = \frac{1}{1 + B_\omega\omega + B_\psi\psi}$$

and by the same argument as in section 1 it follows that

$$(\bar{T})_{\omega,\psi} = \frac{\sum_{i=0}^{q} iL_i(1 + B_\omega\omega + B_\psi\psi)^i}{q\sum_{i=0}^{q} L_i(1 + B_\omega\omega + B_\psi\psi)^i}$$

Thus, for the one-site hypothesis

$$(\bar{T})_{\omega,\psi} = \mathfrak{I}(\omega + a\psi) \quad (15)$$

(*ii*) *The Two-Site Hypothesis.* In this case

$$\frac{(T\Omega_0\Psi_0)}{(T)} = \frac{1}{(1 + B_\omega\omega)(1 + B_\psi\psi)}$$

$$(\bar{T})_{\omega,\psi} = \frac{\sum_{i=0}^{q} iL_i(1 + B_\omega\omega)^i(1 + B_\psi\psi)^i}{q\sum_{i=0}^{q} L_i(1 + B_\omega\omega)^i(1 + B_\psi\psi)^i}$$

therefore

$$(\bar{T})_{\omega,\psi} = \mathfrak{I}(\omega + a\psi + B_\psi\omega\psi) \tag{16}$$

The presence at constant concentration in the system of any number of ligands besides ω and ψ does not have any effect on eq 13–16 (though these ligands in general influence the function $\mathfrak{I}$). A change in the concentration of any such ligand may change $\mathfrak{I}$ but will not change the constants in these equations except in the two-site case if it is bound to the ω or ψ sites. In the first case B_ψ will be unaffected but $a_\chi = a_0(1 + B_\chi\chi)$, where a_χ is the value of a at concentration χ of a third ligand. If χ is bound to the ψ site then B_ψ/a does not change with χ but

$$a_\chi = \frac{a_0}{1 + B_\chi\chi}$$

and similarly

$$(B_\psi)_\chi = \frac{(B_\psi)_0}{1 + B_\chi\chi}$$

We shall now use these results in consideration of the six cases that arise.

Section 3a. The Equations for v

Case 1. Two Modifiers

If the two modifiers are not bound to the substrate site, v/v_0 depends uniquely on $\bar{T}$, and it follows immediately that if, for the velocity v_y in the presence of modifier y alone, we define a function g such that

$$\frac{v_y}{v_0} = g(y) \tag{17}$$

then, for a modifier z bound to T only

$$\frac{v_z}{v_0} = g(a(z)) \tag{18}$$

where here $a = B_z/B_y$, and for the *one-site hypothesis* (case 1 (i))

$$\frac{v_{y,z}}{v_0} = g((y) + a(z)) \tag{19}$$

whereas for the *two-site hypothesis* (case 1 (ii))

$$\frac{v_{y,z}}{v_0} = g((y) + a(z) + B_z(yz)) \tag{20}$$

These results could also have been obtained by obtaining the equations analogous to eq 8 and comparing them. Using eq 10, this procedure shows that eq 17–20 are also true when the modifiers are bound to the substrate site, provided that σ is the same for the two modifiers. In other words, the condition of the above relations is that not only the corresponding free energies of intersite (allosteric) interaction but also those of steric interaction with the substrate must be equal for the two modifiers.

Equations 19 and 20 contain a function g. The value of g for any argument y is known (or of $g^{-1}(v/v_0)$ for any argument v/v_0) experimentally; from eq 17 it can be read off the curve of v/v_0 *vs.* y in the absence of z. Apart from this numerically known function, there are only one and two arbitrary constants appearing in eq 19 and 20, respectively. The same is true in the other four cases we shall now treat. It should not be too difficult to test such simple relationships and to evaluate the arbitrary constants. The tests are discussed in subsection 3b.

Case 2. Substrate and Modifier

By being bound to the configuration which binds the substrate, the effector displaces the equilibrium in favor of that configuration (at least if the cooperation between protomers is positive) and such a modifier is, therefore, in the appropriate conditions, an activator.

(*i*) *In the one-site case* we shall have an instance of the well-known effects of activation by a substrate competitor at sufficiently low concentrations of both and inhibition at sufficiently high concentrations of either (see Whitehead, 1970a, p 366). The activation is purely an effect on $\bar{T}$, while the inhibition is an effect (competition) on the activity of protomers in the T state. v/v_s is given by multiplying the expression for competitive inhibition by $\bar{T}$, the fraction of the enzyme available for catalysis.

$$\frac{v}{v_s} = \frac{B_x(x)}{1 + B_x(x) + B_y(y)}\mathfrak{I}((x) + a(y))$$

where here $a = B_y/B_x$.

$$= \frac{B_x(x)}{1 + B_x(x) + B_y(y)} \frac{\sum_{i=0}^{q} iL_i(1 + B_x(x) + B_y(y))^i}{q\sum_{i=0}^{q} L_i(1 + B_x(x) + B_y(y))^i}$$

If we call v/v_s in the absence of y, $f(x)$, this function is equal to the right-hand side of eq 12. Comparison of this expression with the above equation shows that

$$\frac{v}{v_s} = \frac{(x)}{(x) + a(y)} f((x) + a(y)) \tag{21}$$

(ii) *In the two-site case*, the complication of competition

does not arise and eq 11 holds. Therefore, in the presence of the allosteric activator

$$\frac{v}{v_s} = \frac{B_x(x)}{1 + B_x(x)} \mathfrak{J}((x) + a(y) + B_y(xy)) \tag{22}$$

Case 3. Two Substrates

(*i*) *The One-Site Case.* This corresponds to a case of two alternative substrates. From the point of view of one substrate, the other is simply our activator–inhibitor acting in the same way as the modifier considered in case 2 (i). It easily follows from the considerations of case 2 (i) that if for x alone

$$\frac{v}{v_{sx}} = f(x) \tag{23}$$

then for w present alone

$$\frac{v}{v_{sw}} = f(a(w)) \tag{24}$$

$$(a = B_w/B_x)$$

In the presence of both substrates, we have for the rate of decomposition of x

$$\frac{(v)_x}{v_{sx}} = \frac{(x)}{(x) + a(w)} f((x) + a(w)) \tag{25}$$

and for that of w

$$\frac{(v)_w}{v_{sw}} = \frac{a(w)}{(x) + a(w)} f((x) + a(w)) \tag{26}$$

and for the total rate v

$$v = \frac{(x)v_{sx} + a(w)v_{sw}}{(x) + a(w)} f((x) + a(w)) \tag{27}$$

(*ii*) *The Two-Site Case.* We could consider here the case of two different substrates whose reaction could be catalyzed separately at two different sites. The treatment of such a case is obvious from cases 2 (ii) and 3 (i). The more natural and interesting example of the two-site hypothesis for two substrates, however, is that of two mutually reacting substrates. We shall consider here only the case of a direct reaction between two substrates, where there is a random order of addition and the simultaneous presence of both substrates on a protomer is necessary for reaction. This will suffice to illustrate the principle; naturally, many of the possible variations of mechanism for reaction between two substrates whose kinetics for enzymes with noninteracting protomers have been analyzed (*e.g.*, Cleland, 1967) are possible here too. For our case the velocity of catalysis of a protomer in the T state, as a fraction of its maximum rate, is

$$\frac{v_T}{v_{T_{max}}} = \frac{B_x(x)B_w(w)}{(1 + B_x(x))(1 + B_w(w))}$$

and hence for v/v_s, which we shall call $f(x,w)$

$$\frac{v}{v_s} = f(x, w) = \frac{B_x(x)B_w(w)}{(1 + B_x(x))(1 + B_w(w))} \cdot (\bar{T}) = B_x(x)B_w(w) \frac{\sum_{i=0}^{q} iL_i[(1 + B_x(x))(1 + B_w(w))]^{i-1}}{q\sum_{i=0}^{q} L_i[(1 + B_x(x))(1 + B_w(w))]^i} \tag{28}$$

The expression in 28 is unchanged by the simultaneous substitutions

$$B_x(x) \longrightarrow B_w(w),\ B_w(w) \longrightarrow B_x(x)$$

This fact is expressed by the equation

$$f(x,w) = f(a(w),(x)/a) \tag{29}$$

where $a = B_w/B_x$.

3b. Application of the Results of 3a

We now discuss how the equations of the previous sections can be used in the analysis of experimental kinetic data.

Case 1. We first consider the case of the two effectors present each in the absence of the other. We have already concluded that the free energies of interaction between y molecules and z molecules (and between these and the substrate) are the same. Therefore, v plotted *vs.* log (y) or log (z) will give parallel curves. This equally follows from eq 17 and 18, for these equations can be written as

$$(y) = g^{-1}(v_y/v_0),\ a(z) = g^{-1}(v_z/v_0)$$

whence

$$\log (y) = \phi(v_y/v_0),\ \log (z) = \phi(v_z/v_0) - \log a$$

where

$$\phi \equiv \log g^{-1}$$

We see that the distance apart of the parallel curves is log a (see Figure 1).

(i) Although once a has been determined from the above, then knowing the curve g, the value $g((y) + a(z))$ predicted for the *one-site case* (eq 19) is easily obtained for any y and z and the prediction tested, this calculation is not even necessary. At a fixed concentration of z the curve of v/v_0 *vs.* y when eq 19 is obeyed is simply a section of the curve $g(y)$, the origin of the y axis being shifted a distance $(a(z))$ along the original y axis. It will be seen from Figure 2 that after such a shift the point on the vertical axis corresponding to a concentration y_1 of y is $g((y_1) + a(z))$.

The quantity n, "Hill's exponent," defined for a modifier y by

$$n_y \equiv \mathrm{d} \log\left(\frac{v - v_0}{v_{sy} - v}\right) \Big/ \mathrm{d} \log y$$

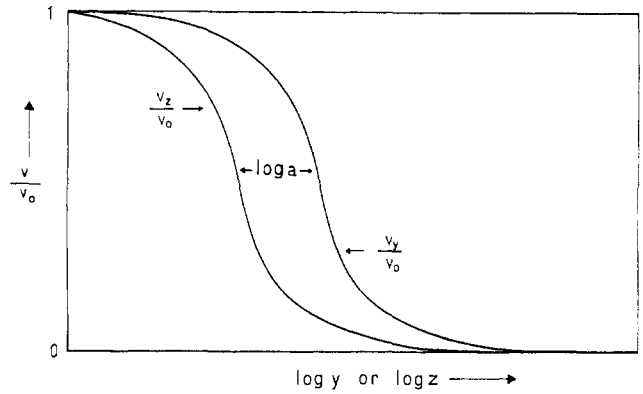

FIGURE 1: The effects on v/v_0 of two modifiers bound to the same configuration (case 1) when plotted logarithmically.

where v_{sy} is the velocity at saturation by y, is often used in reporting the kinetics of enzymes with interacting sites. It is a consequence of eq 19 that for any value of v/v_0 there exists a single-valued function of v/v_0, $n(v/v_0)$ for which

$$n_y + n_z = n(v/v_0)$$

whatever the concentrations of y and z.

(ii) The equation for the *two-site case*, eq 20, may be verified as follows: let eq 20 be written in its inverse form

$$g^{-1}\left(\frac{v}{v_0}\right) = (y) + \left(\frac{B_z}{B_y} + B_z(y)\right)(z)$$

When y is fixed, a plot of $g^{-1}(v/v_0)$ *vs.* (z) is linear, and this plot, at several values of y, generates a family of straight lines. (For any value of v/v_0, we must again emphasize, the value of $g^{-1}(v/v_0)$ can be read off the curve of v/v_0 *vs.* (y) in the absence of z.) From the above equation, when (y) = 0 we shall have a straight line through the origin, *viz.*

$$g^{-1}\left(\frac{v}{v_0}\right) = \frac{B_z}{B_y}(z)$$

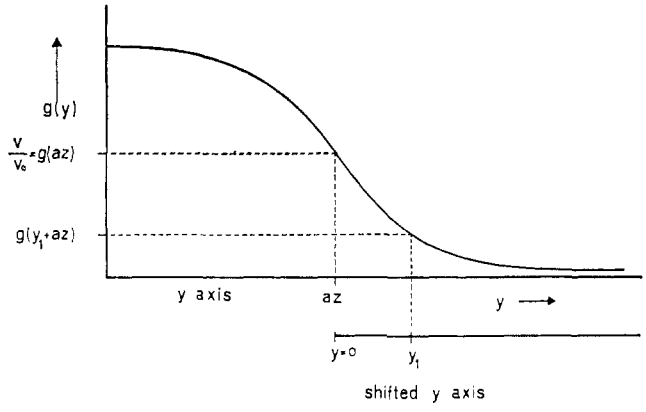

FIGURE 2: The effect, when present together, of two modifiers bound to the same configuration and the same site. See text (section 3b, case 1 (i) for explanation.

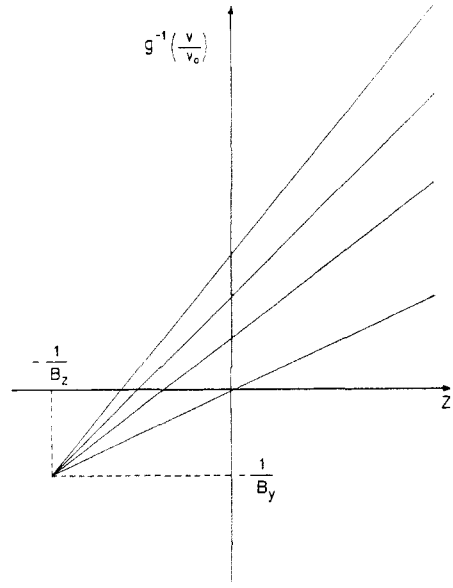

FIGURE 3: Plot of $g^{-1}(v/v_0)$ *vs.* (z) when two modifiers are bound to different sites, but to the same configuration of the enzyme. See text (section 3b, case 1 (ii)) for explanation.

and this line meets any other where

$$\frac{B_z}{B_y}(z) = (y) + \left(\frac{B_z}{B_y} + B_z(y)\right)(z)$$

i.e., where $(z) = -1/B_z$. This means that all of the lines meet in a point (see Figure 3); the projection of this point on the z axis gives $-1/B_z$. Moreover, since the slope of the line through the origin is B_z/B_y, the projection of the same point on the vertical ($g^{-1}(v/v_0)$) axis is $-1/B_y$. We thus have an easy means of recognizing the kind of behavior predicted and determining the constants B_y and B_z.

Case 2 (i). In order to confront experimental data with eq 21, it is necessary to determine the constant, a. This is best done by a series of measurements at saturating concentrations of either effector, when eq 21 reduces to

$$\frac{v}{v_s} = \frac{(x)}{(x) + a(y)}$$

and a could be determined from, for example, the slope of a plot of v_s/v *vs.* (y) at fixed x, which should be linear. Once a is known, the velocity for any value of x and y can be predicted using eq 21. From the definition of f, its value for any value of the argument is known empirically from the curve of v/v_s *vs.* (x) in the absence of y.

(ii) We now discuss eq 22. In the first place we may notice that eq 22 predicts that the activator does not change the velocity at saturating concentrations of substrate, that in general y has the effect of decreasing the cooperativity of the substrate, and that at sufficiently high concentrations of y, $\bar{T} = 1$, and for v_{sy}, the velocity at saturation by y, we have

$$\frac{v_{sy}}{v_s} = \frac{B_x(x)}{1 + B_x(x)}$$

TABLE I[a]

T	a configuration (or set of configurations) of a protomer; also the concentration of protomers in the state T	v_s	v at saturation by substrate
		$v_{s\omega}$	v at saturation by ω
(T^j)	a member of the set T	v_T, v_R, v_{T_1}, etc.	the velocity due to T, to R, to T_1, etc.
R	all configurations other than T	B_ω	the association constant for binding of ω to a protomer in the state T
T_i	the species of enzyme molecule with i subunits in the state T; also the concentration of that species	$B_\omega{}^j$	the association constant for binding of ω to a protomer in the state T^j
$(T_i{}^j)$	the concentration of protomers in the state T^j contained in molecules T_i	a	the ratio of the association constants for two ligands, *e.g.*, B_ψ/B_ω, defined separately in each particular case
$(T\Omega_r)$, $(T\Omega_r\Psi_s)$	a protomer in state T, with r molecules of ω, or r molecules of ω and s of ψ bound, respectively; also the concentration of that species	b	an effective association constant, defined in section 4
		a_χ, b_χ	the effective a and b at concentration χ of a ligand χ
$(T_i\Omega_r)$, $(T_i\Omega_r\Psi_s)$	T_i with r molecules of ω or r molecules of ω and s of ψ bound, respectively; also the concentration of that species	k_t	the velocity constant of catalysis by T_t
		k	a velocity constant for catalysis by the enzyme in the absence of ligands (see section 2)
ω, ψ, χ	Ligands (whether substrate or nonsubstrate undefined); also concentration of those ligands	ρ_t	the velocity constant of catalysis by the protomers in the state R in T_t
x, w	substrates	τ	the velocity constant of catalysis by a protomer in the state T
y, z	nonsubstrate effectors	τ^j	the velocity constant of catalysis by a protomer in the state T^j
$\bar{T}$	the fraction of protomers in the state T	$\bar{\tau}$	an effective velocity constant defined in Appendix A
$(\bar{T})_\omega$	$\bar{T}$ at concentration ω of the ligand ω	τ'^j	the velocity constant for decomposition into product of the complex (T^jX)
$\bar{\Omega}$	the fractional saturation of the enzyme by ω (similarly $\bar{X}$ = fractional saturation by x, etc.)	$\mathfrak{J}$	functional operator expressing the dependence of $\bar{T}$ on ligand concentration, defined in section 2
L_t	a constant of equilibrium between (T_t) and (T_0) (see section 1)	$\mathfrak{R}$	a functional operator expressing the contribution to v/v_0 of the protomers in the R state, as a function of ligand concentration
$L_t{}^j$	a constant of equilibrium between $(T_t{}^j)$ and (T_0) (see section 4)	α, β, γ, δ, α', β'	overall constants appearing in equations for v, defined in Appendix 1
q	the total number of protomers per enzyme molecule	μ	an arbitrary variable
(E)	the concentration of enzyme molecules		
v	reaction velocity		
v_y, $v_{y.z}$	the velocity in the presence of y, or of both y and z, respectively		
v_0	v at zero concentration of a given ligand		

[a] An index placed immediately next to an unbracketed symbol, *e.g.*, $L_t{}^j$, $B_y{}^j$, etc., never signifies, in this paper, an exponent, but always the specialized meaning given in this table.

i.e., the action of the substrate obeys the Michaelis law. This fact allows the easy determination of B_x, and hence the function $\mathfrak{J}$ can be obtained since, in absence of y,

$$\mathfrak{J}(x) = \frac{v}{v_s}\left(1 + \frac{1}{B_x(x)}\right)$$

B_y can be evaluated, and the equation checked by essentially the same method as for eq 20

$$\mathfrak{J}^{-1}\left\{\frac{v}{v_s}\left(1 + \frac{1}{B_x(x)}\right)\right\}$$

should be plotted against y. This will give a family of straight lines with a common point whose projection on the y axis is $-1/B_y$.

Instead of determining B_x first, we could equally define $\mathfrak{J}(x) = v/v_{sy}$ when y is absent and plot $\mathfrak{J}^{-1}(v/v_{sy})$ *vs.* (y), though the first method seems preferable.[1]

[1] The plot proposed and used by Buc (1967) and Buc and Buc (1967) for the analysis of the same configurational hypothesis as here, but in the context of the particular interaction hypothesis of Monod *et al.* (1965), is essentially a special case of the method proposed here. Instead of using the information contained in the experimental curve of $(\bar{X})$ *vs.* (x) in the absence of y, they use an analytical function which fits that curve. Provided this one curve can be fitted by a function of this form, the configuration hypothesis alone will (if true) ensure the linearity of the other curves in the plot and their convergence to a point. Thus the latter properties of the plot do not provide independent evidence in favor of this particular *interaction* hypothesis.

Case 3 (i). *The One-Site Case.* As already observed, the second substrate in this case acts in exactly the same way as the modifier in case 2 (i). If the two velocities of each substrate can be determined separately, then the same analysis as for case 2 (i) can be applied.

(ii) The Two-Site Case. The constants B_x and B_w in the two-site case are easily estimated since eq 28 predicts that at a sufficiently high concentration of (x), v/v_s as a function of (w) is expressed by the Michaelis expression and *vice versa.*

$$\frac{v}{v_s} = \frac{B_w(w)}{1 + B_w(w)}$$

Once therefore these constants are known, experiments to verify eq 29 are easily planned. In expt 1, let a curve of v/v_s as a function of y at a fixed concentration x_1 of x be obtained. Then in expt 2 at a fixed concentration of w equal to $B_x(x_1)/B_w$, let the curve of v/v_s as a function of x be obtained. Then the observations of the two experiments are connected by a relation of the form

$$\frac{v_1}{v_s} = f_1(w), \frac{v_2}{v_s} = f_1\left\{\left(\frac{B_x}{B_w}\right)(x)\right\}$$

if eq 29 holds. This relation may then be tested for as many different values of x_1 as we please and will hold for each of them if eq 29 is true (though f_1 will change with x_1).

Section 4

Having established that useful formulas can be obtained by the consideration of binding to *one* configuration, T, we must now ask whether this concept of a single configuration can be generalized. It seems in fact unlikely that there would exist a single configuration binding a given ligand; rather, we should expect the ligand to be bound more or less by each of a *population* of configurations. We must then ask if such a population can behave in essentially the same way as a single configuration. If so, the results developed above will remain useful and we can usefully speak of "a configuration T," it being understood that T is really a population of configurations. It is, for instance, obvious that an ensemble of different configurations all binding a ligand with the same constant of binding can be regarded as a single configuration from the point of view of the treatment of section 2. If a second ligand were also bound to this same set of configurations, its association constant also being equal for all the configurations, then the whole of the results of section 3a obviously apply. What other conditions allow that the results established above for a single configuration T remain exactly true when T is in reality a population of configurations?

Consider the population of protomers in the state T then to consist of protomers in different states (T^j), where j can take as many different values as there are configurations composing T. Let the constant of binding to a protomer in the state (T^j) of ligands ω and ψ be called $B_\omega{}^j$, $B_\psi{}^j$ and let, in the absence of ligand, the equilibria between (T_0) and (T^j) be characterized by equilibrium constants $L_t{}^j$ such that

$$(T_t{}^j) = L_t{}^j(T_0)$$

where $(T_t{}^j)$ is the concentration of protomers in the configuration (T^j) in the population T_t.

Then relations 13–16 hold in the one-site case when either of the following conditions, and in the two-site case when the second holds: (a) $B_\psi{}^j/B_\omega{}^j$ is independent of j, *i.e.*, these configurations select in the same way between the two ligands; (b) other protomers do not recognize the differences between the configurations (T^j) so that the relative proportions $(T^1):(T^2):(T^3)\ldots\ldots$ are the same in (T_1), $(T_2),\ldots\ldots\ldots(T_q)$. (Any configuration ($T^j$) is then distributed among the i protomers of T_i binomially.) One of the consequences of this hypothesis is that for any pair of configurations (T^{j_1}) and (T^{j_2}), $(L_t{}^{j_1})/(L_t{}^{j_2})$ is independent of t.

A proof of the statement concerning hypothesis a above need not be given here, but a formal proof of that concerning hypothesis b will be given before considering it from an intuitive point of view.

On hypothesis b, since the configuration of any protomer in T_t does not influence that taken by any T protomer, the concentration of a T_t molecule with its subunits in configurations (T^{j_1}), $(T^{j_2})\ldots\ldots(T^{j_i})$, where the j_r are not necessarily all different, will be equal to

$$(T_0)\cdot L_t{}^{j_1}\cdot L_t{}^{j_2}\ldots\ldots\ldots\ldots L_t{}^{j_i}$$

in the absence of ω and

$$(T_0)\cdot L_t{}^{j_1}(1 + B_\omega{}^{j_1}\omega)\cdot L_t{}^{j_2}(1 + B_\omega{}^{j_2}\omega)\ldots\ldots\ldots \times L_t{}^{j_i}(1 + B_\omega{}^{j_i}\omega)$$

in its presence. Since T_t is the sum of all possible such species, in the presence of ω

$$(T_t) = (T_0)\sum_{j_1,\ldots\ldots j_i}\sum L_t{}^{j_1}\ldots\ldots L_t{}^{j_i}(1 + B_\omega{}^{j_i}\omega)\ldots\ldots\ldots \times (1 + B_\omega{}^{j_i}\omega)$$

Each j_r takes all of the possible values of j, so that this reduces to

$$(T_t) = (T_0)\left[\sum_j L_t{}^j(1 + B_\omega{}^j\omega)\right]^i$$

$$= (T_0)\frac{L_t{}^1}{L_1{}^1}\left[\sum_j L_1{}^j(1 + B_\omega{}^j\omega)\right]^i$$

from our initial remarks. Hence

$$(\bar{T})_\omega = \frac{\sum_{i=0}^{q} i\left[\frac{L_t{}^1}{L_1{}^1}\sum_j L_1{}^j(1 + B_\omega{}^j\omega)\right]^i}{q\sum_{i=0}^{q}\left[\frac{L_t{}^1}{L_1{}^1}\sum_j L_1{}^j(1 + B_\omega{}^j\omega)\right]^i} \qquad (30)$$

note that this is of the same form as eq 5. Similarly, in the one-site case

$$(\bar{T})_{\omega,\psi} = \frac{\sum_{i=0}^{q} i\left[\frac{L_t{}^1}{L_1{}^1}\sum_j L_1{}^j(1 + B_\omega{}^j\omega + B_\psi{}^j\psi)\right]^i}{q\sum_{i=0}^{q}\left[\frac{L_t{}^1}{L_1{}^1}\sum_j L_1{}^j(1 + B_\omega{}^j\omega + B_\psi{}^j\psi)\right]^i} \qquad (31)$$

and likewise for the two-site case

$$(\bar{T})_{\omega,\psi} = \frac{\sum_{i=0}^{q} i\left[\frac{L_i^1}{L_1^1}\sum_j L_1^j(1 + B_\omega^j\omega)(1 + B_\psi^j\psi)\right]^i}{q\sum_{i=0}^{q}\left[\frac{L_i^1}{L_1^1}\sum_j L_1^j(1 + B_\omega^j\omega)(1 + B_\psi^j\psi)\right]^i} \quad (32)$$

eq 31 is obtained from eq 30 by the operation of the substitution

$$\omega \rightarrow \omega + \psi\frac{\sum_j L_1^j B_\psi^j}{\sum_j L_1^j B_\omega^j} \quad (33)$$

and eq 32 by the substitution

$$\omega \rightarrow \omega + \psi\frac{\sum_j L_1^j B_\psi^j}{\sum_j L_1^j B_\omega^j} + \omega\psi\frac{\sum_j L_1^j B_\psi^j B_\omega^j}{\sum_j L_1^j B_\omega^j} \quad (34)$$

The coefficients of ψ in eq 33 and of ψ and $\omega\psi$ in eq 34 are constants, so that eq 15 and 16 are obeyed, where

$$a = \frac{\sum_j L_1^j B_\psi^j}{\sum_j L_1^j B_\omega^j}$$

and where it will be preferable to write in place of B_ψ a constant b.

$$b = \frac{\sum_j L_1^j B_\psi^j B_\omega^j}{\sum_j L_1^j B_\omega^j}$$

We may note here that the presence in the system at constant concentration of ligands other than ω and ψ does not affect the essential eq 13–16, but a change in the concentration of these ligands may affect the constants a and b as detailed in Appendix 2.

A special case of the two-site situation crops up when there is no configuration binding both ω and ψ, *i.e.*, for all j either $B_\omega^j = 0$ or $B_\psi^j = 0$. Then $b = 0$ and, as could be expected intuitively, a behaviour identical to that of the one-site case results.

The above arguments, then, establish the validity of eq 13–16 for $(\bar{T})$ under the stated hypotheses. For the relationships concerning v which are the main result of section 3, to hold it is necessary that v depend in a sufficiently simple way on $(\bar{T})$. Detailed investigation (by methods illustrated in the appendices to this paper) shows that of the six cases that arise, the equations obtained in section 3a remain true in all one-site cases on hypothesis a,[2] and on hypothesis b in cases 2 (i), 3 (i), and 3 (ii). In the remaining three cases new equations are obtained but they are closely related to those already obtained, to which in a number of special cases they reduce.

[2] In case 3 (i), however, for relations 23–27 to hold on hypothesis a, it is also necessary that the ratios of catalytic constants for the two configurations, k_x^j/k_w^j, be independent of j.

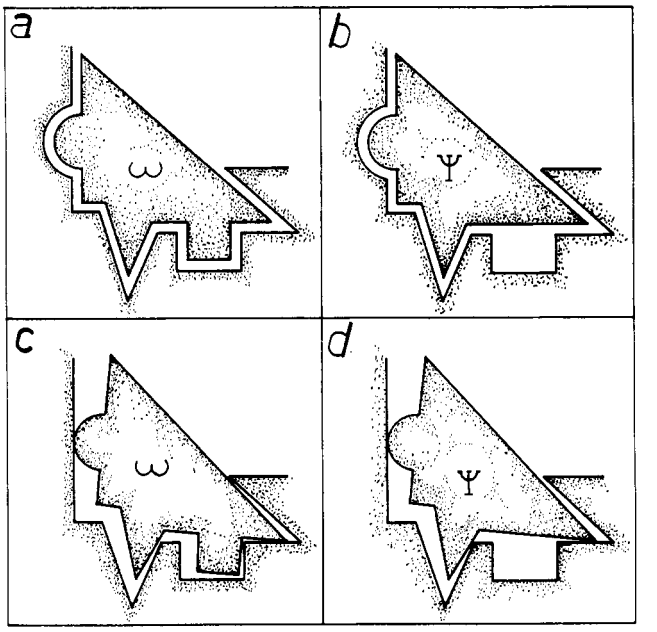

FIGURE 4: Illustration of possible physical basis for hypothesis a of section 4. ω and ψ represent two sterically related ligands, pictured bound by one configuration in part a,b and a different configuration in part c,d. The configurations are not different in the region of steric difference between the ligands.

It is, for instance, intuitively apparent that in case 1 (two nonsubstrate effectors), if the T state is inactive or if all the configurations (T^j) have an identical activity, then v/v_0 will depend uniquely on $(\bar{T})$, and hence the appropriate relations of section 3a remain unmodified. As the practical interest of the full equations for the cases in question seems (with the possible exception of case 2 (ii)) limited, they are dealt with in the appendices to this paper.

A physicogeometrical basis for hypothesis a could occur if the two ligands were sterically related and the different configurations (T^j) not different in the region of the protomer in contact with the sterically different parts of the ligands (see Figure 4). As regards hypothesis b, it must be assumed that the differences between the configurations (T^j) are not sufficient in the region of effective contact between the protomers to affect other protomers differentially.

Note that on hypothesis a, the behavior of the population resembles that of a single configuration only in the rather superficial sense of giving agreement with eq 13–15 and the corresponding relations of section 3a. Thus, if the differences between configurations binding ω are recognized by other protomers, then we can only write equations of a form which is not in general of the same form as eq 5. On the other hand, hypothesis b gives equations like 30 which is identical in form with the corresponding eq 5 for one single configuration T.

That T, under the second and more interesting condition, should behave like a single configuration is understandable intuitively. Consider an isolated protomer capable of adopting different configurations (T^j) in accord with the initial assumptions of our model. Ligands would displace equilibria between different configurations, but the results of this would all be of a type familiar from the standard kinetics of protomers, partial or complete competitive and/or noncompetitive

effects described by already well-known equations. If now the protomer is not isolated but present in an oligomeric structure, changes between configurations (T^j) produce, by our hypothesis, no effect on other protomers. A protomer free to adopt all the configurations (T^j) therefore behaves like a protomer in a single configuration T, upon which competitive and noncompetitive effects like those mentioned above can take place while it is this configuration. For this reason, the equations describing these effects contain expressions of a classical type which appear as multipliers of the function giving ($\bar{T}$); as shown above, this function remains of the same form as in (eq 5). ($\bar{T}$) fulfills the same role here as (E_T), the total quantity of enzyme, in the classical kinetics of isolated protomers. All of this may however be more clearly understood after a study of Appendix 1.

A characteristic form of behavior of systems obeying hypothesis b is that at saturating concentrations of at least one of the ligands binding to T only (*i.e.*, to configurations (T^j) only), no cooperativity is observed for the kinetics of any of the ligands bound to T only. This is because all protomers are then practically always in one or other of the configurations (T^j), the configuration of any protomer not under these conditions, however, affecting that of any other. The behavior is therefore identical with that (at saturating concentrations of one of the ligands) of a system of isolated protomers able to adopt a series (T^j) of configurations. This reasoning is confirmed in the results arrived at in Appendix 1.

Discussion

For all of the six cases that arise, we have been able to arrive at a simple kinetic test of the hypothesis that two ligands are bound to the same configuration of the enzyme.

These tests have several advantages. Firstly, they are simple. They involve only one or two arbitrary constants. Moreover, in cases where the equations are obeyed, it is possible to obtain a ratio of association constants in the one-site cases and, more usefully still, two association constants in the two-site cases. In the further kinetic investigation of mechanism of intersite interaction (*i.e.*, of "I" hypotheses), it could be useful to have these constants, deduced in a manner independent of any specific I hypothesis. A point related to the advantage of simplicity is that the tests can be made highly exacting. Consider eq 17–20, for instance. They may be tested under any given conditions by the methods of section 3b. If they hold, one may then change the concentration of some third effector, or the substrate, and under these new conditions again test the relations. The function g will change but the relations should remain true if the initial hypothesis is. Moreover, the constants will (save exceptions already noted) retain the values already found. If the relations and the values of the constants can be shown to hold over a whole range of conditions, the hypothesis can be said to have survived an exacting test. Of course, it would be possible for the relationships to hold under certain conditions and to fail under others. This would be an indication of the presence, in negligible amounts in some conditions but in significant amounts in others, due to stabilization by an effector, of configurations which differentiate between the two ligands in a manner different from the configuration T—this would be information worth having.

The approach used here could, of course, be used for other configurational hypotheses. The one investigated in this paper is simply an illustration of the approach, a useful (because simple) and important one. But for other configurational hypotheses, relations of the kind illustrated here could be obtained and in many cases would be easily applicable.

The application of the methods suggested in this paper need not be confined to kinetic studies. Instead of v, any conveniently measurable physical parameter which is likely to be affected by changes of configuration could be measured. It would be necessary simply to decide whether the parameter is expected to depend upon ($\bar{T}$), upon $\bar{\Omega}$ or some other function. The present equations could then be used, or, in the last case, other appropriate ones derived. The results obtained for different parameters would not necessarily confirm each other in a simple manner. Thus, a set of configurations which had exactly the same properties toward ligands and so, from a kinetic point of view, could be regarded as a single configuration, might have quite different properties with respect to some other parameter, and *vice versa*. The exact relation between configurations as studied kinetically and as revealed by other physical measurements is in itself an exciting and as yet little explored domain of experimental research.

Granted that the assumptions of the model are very general, there are nevertheless different assumptions that could be made, and there is no theoretical or experimental assurance that those made here are necessarily true. A question that must therefore be asked is: Would agreement with the predictions of section 2 constitute support for the general assumptions of the model as well as for the specific C hypothesis they are designed to test?

It is first as well to be clear about the role of the notion of a "configuration" in the kinetics of ligand interactions in enzymes. As this notion is used in kinetics, it means no more than a state of a protomer with a characteristic set of physical constants expressing its interaction with ligands and with other configurations. On this operational definition of a "configuration," it is practically an *a priori* necessity to invoke the concept in theories of interaction (probably few workers at this time, however, would hesitate to go beyond the operational definition in regarding a configuration as signifying a three-dimensional conformation of a polypeptide chain). Therefore, C hypotheses will arise in all possible models.

To answer the question posed would require the definition of other models and the investigation of their properties. This is to be the subject of a later paper. It can however for the present be stated that the binding of two ligands to the same configuration will give the same formulas as obtained here in a wide category of models, probably all of those where the interaction of a protomer with a ligand is determined uniquely by the former's configuration. Therefore, while the formulas derived from the present type of analysis do not help in discriminating between different models, they are of very general utility for the testing of C hypotheses.

Appendix 1

The Equations Given by Hypothesis b of Section 4

In this appendix we shall state and discuss those equations given by hypothesis b of section 4 which are different from those arrived at in section 3 (*i.e.*, cases 1 (i), 1 (ii), and 2 (ii)). We shall then illustrate their derivation by means of one

example only, that of case 1 (i). Essentially the same methods can be used for all six cases.

In the general case where the appropriate interaction hypothesis for a system is unknown, and where there are no other simplifying factors, testing of these equations may be discouragingly complicated. For this reason we shall not enter here into the question of testing, though some suggestions are given in a manuscript deposited in the National Auxiliary Publications Service. The interested reader may care to note that, even in the general case, the constants a and b can always be obtained, provided that the two saturating velocities always involved are different, since at high enough concentrations of the effectors the equations assume a simple form containing these constants. The equations may be most useful in cases where there is more information available, *e.g.*, information about interaction hypotheses, information from nonkinetic methods about some of the constants involved, and in cases where there are simplifying factors, some of which we shall note here, where the equations simplify.

Case 1 (i). The general equation for this case is most conveniently written in the form

$$v = (\mathrm{E})\Re((\mathrm{y}) + a(\mathrm{z})) + \left(\frac{\bar{\tau}(\mathrm{E})}{\beta} + v_{s\mathrm{y}}(\mathrm{y}) + av_{s\mathrm{z}}(\mathrm{z})\right) \times \frac{\Im((\mathrm{y}) + a(\mathrm{z}))}{\frac{1}{\beta} + (\mathrm{y}) + a(\mathrm{z})} \quad \text{(A1)}$$

where $\Re$ is an arbitrary function expressing the contribution to v of the protomers in the R state, E is the total concentration of enzyme, $\bar{\tau}$ and β are constants

$$\bar{\tau} = \frac{\sum_j \tau^j L_1^j}{\sum_j L_1^j}, \beta = \frac{\sum_j L_1^j B_y^j}{\sum_j L_1^j}$$

τ_j being the velocity constant for catalysis by a protomer in the state (T^j). $v_{s\mathrm{y}}$ and $v_{s\mathrm{z}}$ are the limiting values of v at saturating concentrations of the respective effectors, values which, as the form of eq A1 shows, are independent of the concentration of the other effector. In terms of the properties of the configurations

$$v_{s\mathrm{y}} = \frac{\sum_j \tau^j L_1^j B_y^j \sum_j L_1^j(\mathrm{E})}{\sum_j L_1^j B_y^j}, v_{s\mathrm{z}} = \frac{\sum_j \tau^j L_1^j B_z^j \sum_j L_1^j(\mathrm{E})}{\sum_j L_1^j B_z^j} \quad \text{(A2)}$$

We now note some special cases. First, when the T state is a catalytically inactive state (whether or not the substrate is bound to it), $\bar{\tau} = v_{s\mathrm{y}} = v_{s\mathrm{z}} = 0$ and the second term of A1 vanishes and we are left with eq 17–19 without modification. This is a case where y and z are inhibitors, capable of total inhibition at sufficiently high concentration. Secondly, if τ^j is independent of j, then $v_{s\mathrm{y}} = v_{s\mathrm{z}}$ and we again have eq 17–19.

When the R state is completely inactive, y and z are activators and the first term may be omitted from A1. The resulting equation can, in principle, be tested (Whitehead, 1970b).

Case 1 (ii). The general equation for this case is, analogously to 1 (i), of the form

$$v = (\mathrm{E})\Re((\mathrm{y}) + a(\mathrm{z}) + b(\mathrm{yz})) + \left(\frac{\bar{\tau}(\mathrm{E})}{\beta} + v'_{s\mathrm{y}} + av'_{s\mathrm{z}}(\mathrm{z}) + bv_{s\mathrm{yz}}(\mathrm{yz})\right) \frac{\Im((\mathrm{y}) + a(\mathrm{z}) + b(\mathrm{yz}))}{\frac{1}{\beta} + (\mathrm{y}) + a(\mathrm{z}) + b(\mathrm{yz})} \quad \text{(A3)}$$

where $v'_{s\mathrm{y}}$ and $v'_{s\mathrm{z}}$ represent the saturating velocities in the presence of the respective ligand alone. These, of course, are given by the expressions in eq A2 above. In contradistinction to case 1 (i), however, the limiting velocity obtained at saturating concentrations of the one ligand is in general dependent on the concentration of the other, thus

$$v_{s\mathrm{y}} = \frac{v'_{s\mathrm{y}} + bv_{s\mathrm{yz}}(\mathrm{z})}{1 + b(\mathrm{z})}, v_{s\mathrm{z}} = \frac{v'_{s\mathrm{z}} + \frac{b}{a}v_{s\mathrm{yz}}(\mathrm{y})}{1 + \frac{b}{a}(\mathrm{y})}$$

$v_{s\mathrm{yz}}$ is the velocity at saturating concentrations of both ligands. In terms of the properties of the configurations

$$v_{s\mathrm{yz}} = \frac{\sum_j \tau^j L_1^j B_y^j B_z^j \sum_j L_1^j(\mathrm{E})}{\sum_j L_1^j B_y^j}$$

Essentially the same special cases arise as in case 1 (i): if the T state is inactive, or if τ^j is independent of j we get eq 17, 18, and 20. In the case where the R state is inactive, the first term of A3 vanishes and the resulting equation is, in principle, testable though this would be much more difficult than for the corresponding situation in the one-site case.

Case 2 (ii). The general equation for this case (A4) is simpler than for the cases 1 due to the facts that the R state is necessarily inactive and that the T state too is inactive in the absence of x. The equation is

$$\frac{v}{v_{s\mathrm{x}}} = \frac{(\mathrm{x}) + \frac{v_{s\mathrm{xy}}}{v_{s\mathrm{x}}}b(\mathrm{xy})}{\frac{1}{\beta'} + (\mathrm{x}) + a(\mathrm{y}) + b(\mathrm{xy})}\Im((\mathrm{x}) + a(\mathrm{y}) + b(\mathrm{xy})) \quad \text{(A4)}$$

where $v_{s\mathrm{x}}$ is the velocity at saturation by x in the absence of y, $v_{s\mathrm{xy}}$ that at saturation by both x and y. Analogously to previous cases

$$\beta' = \frac{\sum_j L_1^j B_x^j}{\sum_j L_1^j}$$

while

$$\frac{v_{s\mathrm{xy}}}{v_{s\mathrm{x}}} = \frac{\sum_j \tau'^j L_1^j B_x^j B_y^j}{\sum_j \tau'^j L_1^j B_x^j}$$

τ'^j being the rate constant for decomposition into product of the complex TX.

At saturating concentrations of y, the effect of x on v obeys Michaelian kinetics, with a maximum velocity v_{sxy} and a Michaelis constant of a/b. Equation A4 can be tested in the general case.

The expression which multiplies the function $\Im$ in A4 is an expression for a mixed partial competitive, partial noncompetitive effect of y. Special cases that arise are the following: when (1) τ'^j is independent of j, then $v_{sxy} = v_{sx}$ and this expression becomes one for a partial competitive effect j (2) if either B_y^j or B_x^j is independent of j for all j for which $\tau'^j \neq 0$, then $\beta' = b/a$ and the expression becomes one for a partial noncompetitive effect; (3) if both conditions 1 and 2 hold, A4 simplifies to give eq 22.

Methods

We conclude this appendix, giving an example of the methods whereby the equations cited in these appendices may be arrived at, as well as those equations that turn out identical to those derived in section 1. We take as our example case 1 (i) under condition b. We ask first of all, what is the overall rate of catalysis v_T by protomers which are in the state T?

$$v_T = \sum_j \tau^j(T^j)$$

$$(T^j) = (T^jX_0Y_0) + (T^jX_1Y_0) + (T^jX_0Y_1)$$

$$= (T^jX_0Y_0)(1 + B_y^j(y) + B_z^j(z))$$

$$= (T_0)L_1^j(1 + B_y^j(y) + B_z^j(z))$$

$$v_T = (T_0)\sum_j \tau^j L_1^j(1 + B_y^j(y) + B_z^j(z))$$

For the total quantity of T we have

$$(T =) \sum_j (T^j)$$

$$= (T_0)\sum_j L_1^j(1 + B_y^j(y) + B_z^j(z))$$

Hence

$$v_T = (T)\frac{\sum_j \tau^j L_1^j(1 + B_y^j(y) + B_z^j(z))}{\sum_j L_1^j(1 + B_y^j(y) + B_z^j(z))}$$

which is of the form

$$v_T = \bar{\tau}\left(\frac{\frac{1}{\beta} + \alpha(y) + \gamma(z)}{\frac{1}{\beta} + (y) + a(z)}\right)(T)$$

where α and γ are constants. Now $(T) = (E\cdot\bar{T})$, and the constants $\bar{\tau}\alpha E$ and $\bar{\tau}\gamma E$ are readily identified with v_{sy} and v_{sz}. The above expression then gives us the right-hand term of eq A1.

Now the velocity v_R of protomers in states other than T is an arbitrary function of $R = 1 - (\bar{T})$, and hence of $((y) + a(z))$. We shall therefore write

$$v_R = (E)\Re((y) + a(z))$$

where

$$\Re(\mu) = \frac{\sum_{i=0}^{q} \rho_i \left[\frac{L_i^1}{L_1}\sum_j L_1^j(1 + B_\mu^j\mu)\right]^i}{\sum_{i=0}^{q}\left[\frac{L_i^1}{L_1^1}\sum_j L_1^j(1 + B_\mu^j\mu)\right]^i}$$

The total velocity of the enzyme is the sum of that due to the R and T states

$$v = v_R + v_T$$

giving A1.

Appendix 2

Here we state the effects upon the constants a and b of a third ligand χ, bound in an arbitrary fashion to the configurations composing T as well as to other configurations. These effects are as follows.

(i) The One-Site Case. Under either hypothesis a and b the constant a is unchanged by the introduction of χ if the latter is bound by the same site as ω and ψ. If χ is bound to a different site, then a is unchanged under hypothesis a, but under hypothesis b changes according to the equation

$$a = a_0\frac{1 + \epsilon\chi}{1 + \zeta\chi}$$

where ϵ and ζ are constants

$$\epsilon = \frac{\sum_j L_1^j B_\psi^j B_\chi^j}{\sum_j L_1^j B_\psi^j}$$

$$\zeta = \frac{\sum_j L_1^j B_\omega^j B_\chi^j}{\sum_j L_1^j B_\omega^j}$$

(ii) The Two-Site Case under Hypothesis b. If χ is bound to the ω site, then b is unchanged by χ, but a changes according to

$$a_\chi = a_0(1 + \epsilon\chi)$$

if χ is bound to the ψ site then the ratio b/a is unchanged by χ, but

$$a_\chi = \frac{a_0}{1 + \zeta\chi}$$

and similarly

$$b_\chi = \frac{b_0}{1 + \zeta\chi}$$

In the case where χ is bound to a site on the protomer different from both the ω and the ψ sites, a, b, and a/b may all change and

$$a_\chi = a_0\frac{1 + \epsilon\chi}{1 + \zeta\chi}$$

$$b_\chi = b_0\frac{1 + \eta\chi}{1 + \zeta\chi}$$

where

$$\eta = \frac{\sum_j L_1^j B_\psi^j B_\omega^j B_\chi^j}{\sum_j L_1^j B_\omega^j}$$

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Metabolism and Subcellular Location of 25-Hydroxycholecalciferol in Intestinal Mucosa*

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ABSTRACT: The metabolism and subcellular distribution of 25-hydroxycholecalciferol, a biologically active metabolite of cholecalciferol (vitamin D_3) was investigated in the intestinal mucosa of vitamin D deficient rats. The mucosal nuclear fraction accumulates approximately 50 and 25% of the total cellular ^3H after administration of 0.025 and 0.25 μg of [26,27-^{3}H]-25-hydroxycholecalciferol, respectively. Both 1,2-^3H- and 26,27-^3H-labeled 25-hydroxycholecalciferol are converted *inv ivo* by the intestinal mucosa into polar metabolites, referred to as peak V and peak VI. Within 30 min after a 0.025-μg dose of [26,27-^{3}H]-25-hydroxycholecalciferol, 72% of the nuclear radioactivity is peak VI, the more polar of the two metabolites. Two hours after the dose the relative amount of peak VI steadily decreases and after 8 hr peak V has increased to 58% of the total tritium-containing metabolites in the nuclear fraction. The time course of appearance of these metabolites indicates peak VI is the probable precursor of peak V. The paucity of these polar metabolites in the cytoplasmic fractions suggest they are of nuclear origin and hence may be of significance in understanding at the nuclear level the mode of action of vitamin D on the calcium transport mechanism in the small intestine.

The currently accepted model for vitamin D action in the small intestine proposes that specific genetic information is unmasked in response to the vitamin, leading ultimately to the synthesis of specific functional protein(s) involved in a calcium transport system (DeLuca, 1967). A response in calcium absorption is known to lag several hours after administration of vitamin D and recent evidence partially explains the reason for this lag period. First it has been conclusively established that vitamin D_3 (cholecalciferol) must be converted enzymatically by the liver into an "active form" (Ponchon and DeLuca, 1969a; Horsting and DeLuca, 1969) which has been identified as 25-hydroxycholecalciferol (Blunt *et al.*, 1968a).[1] The next important event in vitamin D action occurs at or in the nucleus of the intestinal mucosal cell where the vitamin and its metabolites accumulate (Stohs and DeLuca, 1967). Then by an unknown mechanism, a metabolite of vitamin D stimulates the synthesis of nuclear RNA, as demonstrated by the increased incorporation of [^{3}H]orotic acid into nuclear RNA (Stohs *et al.*, 1967) as well as the increased DNA template activity of these cells (Hallick and DeLuca, 1969). Presumably this nuclear RNA includes specific mRNA which codes for the protein components of the calcium transport system. Both of these nuclear responses occur well before any detectable response in the calcium transport mechanism (Blunt *et al.*, 1968b; Wasserman and Taylor, 1968), but undoubtedly RNA synthesis adds substantially to the lag in vitamin D action.

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[1] This compound may also be referred to as 25-OH vitamin D_3, 25-HCC, or peak IV.