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# THE EFFECTS OF MODIFIERS ON ENZYME CATALYSIS: A NON-CLASSICAL NEAREST NEIGHBOR APPROACH *

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We present a nearest neighbor lattice model of the effects of modifiers on two-state enzyme catalysis of the reaction $s \rightleftharpoons p$. We do not in general make the assumptions of the classical approach to cooperative catalysis that yield (1) adsorption isotherms of the same form as those for the corresponding equilibrium system and (2) a rate of the catalyzed reaction proportional to the number of occupied catalytic sites. Closed form results are obtained for two approximations, the Bragg–Williams and the quasi-chemical. The latter requires (1), but is exact for several simple cases, including the concerted model, under this condition. Under (1) it is found that an interaction between modifier and catalytic sites, whether attractive or repulsive, increases the magnitudes of the slopes of the adsorption isotherms but that interactions between identical sites (catalytic or modifier) increase these magnitudes if attractive and decrease them if repulsive. Thus, the former interaction allows for phase transitions if sufficiently attractive or repulsive, but the latter only if sufficiently attractive. Herein also lies the explanation for why the concerted model displays only "positive cooperativity". It is further seen that it is not possible to classify a modifier as an activator or inhibitor of the catalyzed reaction solely on the basis of the sign of the interaction energy between catalytic and modifier sites. For a given energy, the rate of the reaction may increase or decrease in response to the modifier, or it may respond biphasically. Similarly, the rate may respond biphasically to the activities of s or p, leading to instabilities. Thus, possibilities of multiple nonequilibrium stationary states or spatio-temporal patterns are raised.

## 1. Introduction

Many diverse cooperative phenomena, ranging from ferromagnetism [1] to the interaction of social groups [2], have been studied with success in the context of nearest neighbor lattice statistics. Cooperative enzyme catalysis is no exception. It has been shown [3] that the sequential model [4] is identical to the classical Ising model [1] and that the Hill equation [5] follows as a special case. Nearest neighbor lattice statistics have also been used to study the effects of modifiers on catalysis. Thompson [3], for example, considered two classes of sites, those on which the catalysis occurs and others which adsorb a modifier, with state dependent interactions between nearest neighbor catalytic and modifier sites. Such lattices are known in connection with other phenomena as decorated Ising models [6].

The application of nearest neighbor lattice statistics to cooperative enzymes is particularly interesting, since the stationary states of primary importance are nonequilibrium ones, where the rate of the catalyzed reaction is nonzero. Thus, possibilities of nonequilib-

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rium phase transitions and spatio–temporal patterns are raised. In fact, the concerted model [7], which is a special case of the nearest neighbor work presented here, can predict such behavior when coupled appropriately to transport processes [8,9]. However, in spite of these successes in treating nonequilibrium cooperative phenomena, the classical nearest neighbor approach is actually an equilibrium formulation. Kinetic Ising models [10–12] describe transient behavior but still possess only equilibrium stationary states. Only in a recent series of papers [13–15] has Hill extended the Ising model to explicitly allow for nonequilibrium stationary states. He considered a lattice of $n \geqslant 2$ state enzyme molecules with state dependent interactions between nearest neighbors. The transitions among the $n$ states of a single molecule are such that there is at least one cycle in the kinetic diagram [16]. It is the presence of cycles, at least one of which has a nonzero thermodynamic force when the catalyzed reaction is not at equilibrium, which allows for nonequilibrium stationary states.

That the classical equilibrium approach to interactions has been applied to systems with nonequilibrium stationary states (e.g., enzymes) is possibly a consequence of certain analogies which exist between catalysis and equilibrium ligand adsorption in the absence of interactions. In the stationary state, a Michaelis–Menten or Briggs–Haldane noncooperative enzyme displays an adsorption isotherm of the same form as the Langmuir equilibrium ligand adsorption isotherm (with appropriate identifications of parameters and variables, of course). Furthermore, under the rather standard condition that adsorption of product may be neglected, the rate of the catalyzed reaction becomes proportional to the number of occupied catalytic sites. Similarly, the classical approach to enzyme catalysis in the presence of interactions usually takes the stationary state population properties (e.g., adsorption isotherms) to have the form of those for corresponding equilibrium ligand adsorption systems and the rate of catalysis to be proportional to the number of occupied catalytic sites, whether the interactions are among catalytic sites themselves [4], between catalytic sites and enzyme conformations [7], or between catalytic sites and modifier sites [3]. Hill's work, however, has shown that the procedure of calculating the stationary state properties of enzymes through analogies to equilibrium systems requires four assumptions in the presence of interactions, two to assure adsorption isotherms of an equilibrium form and two more to obtain a rate of catalysis proportional to the number of occupied catalytic sites.

Since enzyme catalysis is of lesser importance at equilibrium, where the rate of the catalyzed reaction is zero, it would seem important to study the effects of modifiers on enzyme catalysis without recourse to some of the usual assumptions. Thus, our purpose in this paper is to apply Hill's method of taking into account the nonequilibrium aspects of catalysis to the sorts of models proposed by Thompson for the effects of modifiers. We again consider lattices with two classes of sites, with state dependent interactions between nearest neighbor catalytic and modifier sites, between nearest neighbor catalytic sites, and between nearest neighbor modifier sites. For simplicity in this initial paper, we do not treat transient conditions and allow each site to have only two accessible states. A modifier site may be either occupied by a molecule of the modifier or unoccupied; these two states are connected by a single transition pathway in the kinetic diagram for an isolated site. The kinetic diagram for a single catalytic site, however, has two transition pathways (as for a Michaelis–Menten or Briggs–Haldane enzyme), comprising a cycle and thus allowing for nonequilibrium stationary states. It should be mentioned that a two state catalytic cycle is one of the necessary assumptions for nonequilibrium stationary state population properties of an equilibrium form [13] and that we will be forced to invoke the other necessary assumption to develop one of our two approximations to the general problem. However, even where we make the assumptions necessary for population properties of an equilibrium form, we will not in general make those necessary for the rate of the catalyzed reaction to be proportional to the number of occupied catalytic sites. It should also be noted that catalytic cycles with more than two states may display novel behavior in the presence of interactions [17,18]. Extensions of the model to include any number of classes of sites, catalytic or modifier, are straightforward, at least in the case of two state catalytic cycles, and will be pointed out where appropriate.

Although, with the exception of several special cases (see, for example, ref. [19]), Hill's work has dealt with interactions between identical enzyme molecules, it can also be applied to the problem of the effects of modifiers on catalysis. For example, a molecule could be pictured as consisting of two sites, one catalytic and one modifier. While Hill's approach and ours can be shown to be equivalent in some simple situations, they are not equivalent in general. It is not always possible to group the sites of our lattices into identical subunits so that the structure of the interactions is maintained. Which approach will find greater application to real systems is an open question. We have one process in mind, the activation of striated muscle contraction by calcium ion, in which at least part of the system, the thin filament, seems better represented by our approach than Hill's. Our approach has the further advantage that it can be easily related to previous work using the classical equilibrium formulation. In any case, the model should be of interest in itself, as it represents a further study of nonequilibrium cooperative systems.

We have chosen to discuss this work in terms of enzyme catalysis or its equilibrium equivalent, ligand adsorption, since these are the applications with which we are most familiar. Hopefully, the results will not be so limited. Equilibrium Ising models have found many applications, and, as Hill has pointed out, his nonequilibrium formulation can be applied not only to catalysis, but also to other nonequilibrium processes, such as membrane transport [20–22].

In this paper we consider only theoretical aspects of the problem for the most part. In a subsequent publication we will discuss the activation of striated muscle contraction by calcium ion in terms of our results. We define the model in the next section, considering both the interactions and the nonequilibrium formulation of their effects. Since no closed form solution for the general problem is known even at equilibrium, we then extend two classical approximations to our nonequilibrium situation, the Bragg–Williams approximation [23] in section 3 and the quasi-chemical approximation [24,25] in section 4. It will be seen that the latter is exact for several special cases. Finally, the results, including their relation to previous work on nonequilibrium systems utilizing an equilibrium formulation, are discussed.

## 2. The model

The kinetic diagrams for single isolated (subject to no interactions) sites of each of the two classes we consider are shown in fig. 1. The catalytic sites (fig. 1a) catalyze a reaction s ⇌ p via a two state cycle. The

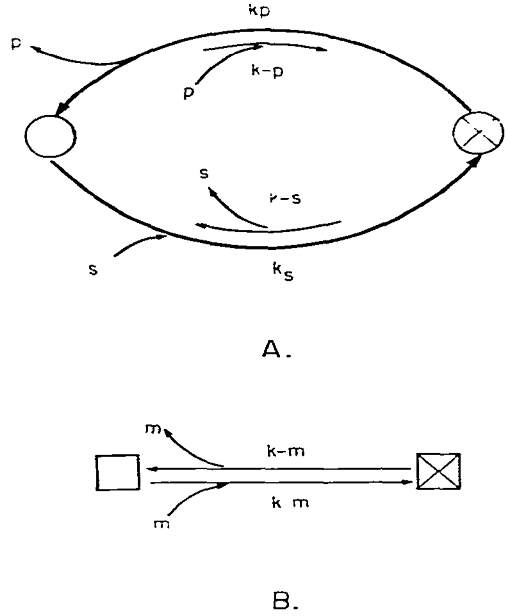

Fig. 1. Kinetic diagrams for isolated catalytic (A) and modifier (B) sites. The circle in (A) represents a catalytic site, and the square in (B), a modifier site. An X inside the symbol for a site indicates that it is occupied; otherwise, it is unoccupied.

circle represents the site itself; an X inside the circle indicates that the site is what we call occupied; otherwise, it is unoccupied. In perhaps more usual terminology, one would refer to the "enzyme–substrate complex" and the "free enzyme", respectively. There are two adsorption-like pathways: one for s, with rate constants $k_s$ and $k_{-s}$ for adsorption and desorption, respectively; and one for p, with rate constants $k_{-p}$ (adsorption) and $k_p$ (desorption). The thermodynamic force $X$, normalized to temperature through the Boltzmann constant, for the catalyzed reaction in the direction s → p is then [16]

$$e^X = \frac{k_p k_s}{k_{-s} k_{-p}} \frac{z_s}{z_p}, \tag{2.1}$$

where $z_s$ and $z_p$ are the chemical activities of s and p, respectively.

The transitions for an isolated modifier site, represented by a square, are shown in fig. 1b. The X indicates that the site is occupied by a molecule of the ligand m; otherwise, it is unoccupied. The rate constant for adsorption is $k_m$ and that for desorption, $k_{-m}$.

Since we will be dealing with transitions between the two states of a site in the presence of interactions, we must take into account the effect of the interactions on the rate constants. So long as we maintain detailed balance at equilibrium, the way in which we accomplish this is somewhat arbitrary. The particular choice we make is that of Hill [13]. Consider a transition pathway between some state A and another state B in the presence of interactions with an environment e. Let $w_{Ae}$ ($w_{Be}$) be the energy of interaction of state A (B) with e. We take all interaction energies to be normalized to temperature through the Boltzmann constant. Hill's assumption is that

$$k_{AB}(e) = \exp [f_{AB}(w_{Ae} - w_{Be})] k_{AB} ,$$
$$k_{BA}(e) = \exp [(1 - f_{AB})(w_{Be} - w_{Ae})] k_{BA} , \qquad (2.2)$$

where $k_{AB}(e)$ and $k_{AB}$ are the rate constants for the A → B transition in the presence and absence, respectively, of the interactions; $k_{BA}(e)$ and $k_{BA}$ are the corresponding quantities for the B → A transition; and $f_{AB}$ is a real constant [26] that may depend on A and B but not e. We should emphasize that we could formulate the effects of interactions on rate constants in other ways; e.g., we could have chosen to use the approach of Glauber [10] and Kimball [12] to the one-dimensional kinetic Ising model. Incidentally, it appears that Hill's formulation can be made equivalent to that of Glauber and Kimball only in the case $f_{AB} = 1/2$.

For reasons noted below we will consider a site to interact with its nearest neighbors explicitly only when it is occupied. The rate constants for the modifier sites in the presence of interactions are then

$$k_m(e) = \exp [-f_m w_m(e)] k^0_m ,$$
$$k_{-m}(e) = \exp [(1 - f_m) w_m(e)] k^0_{-m} , \qquad (2.3)$$

where $w_m(e)$ is the interaction energy of an occupied modifier site with its environment e, and a superscript 0 indicates the rate constants in the absence of interactions. Similarly, for the catalytic sites, we have

$$k_s(e) = \exp [-f_s w_c(e)] k^0_s ,$$
$$k_{-s}(e) = \exp [(1 - f_s) w_c(e)] k^0_{-s} ,$$
$$k_{-p}(e) = \exp [(f_p - 1) w_c(e)] k^0_{-p} , \qquad (2.4)$$
$$k_p(e) = \exp [f_p w_c(e)] k^0_p ,$$

where $w_c(e)$ is the interaction energy of an occupied catalytic site with e. Note that there is a distinct $f$ here for each of the two transition pathways; this follows from the requirement of detailed balance at equilibrium for each pathway separately. However, if $f_s + f_p = 1$, then the interactions affect the rate constants for both pathways to the same extent. In this case, if we formally combine both the s and p pathways into a single one [27], we find a simulated detailed balance formally analogous to that at equilibrium even at nonequilibrium stationary states. In this "quasi-equilibrium" regime, population properties take an equilibrium form at nonequilibrium stationary states with $(k^0_s z_s + k^0_{-p} z_p)/(k^0_p + k^0_{-s})$ playing the role of a chemical activity [13].

The particular interactions with which we will be concerned are as follows. We consider a lattice with $M_1$ catalytic or class 1 sites and $M_2$ modifier or class 2 sites. The lattice is specified by the numbers of nearest neighbors: each class 1 site has $c_1$ other class 1 sites and $c_{12}$ modifier sites as nearest neighbors; each class 2 site has $c_{21}$ catalytic sites and $c_2$ other modifier sites as nearest neighbors. $c_{12}M_1 = c_{21}M_2$, of course. Three such lattices are shown in fig. 2. (Occupied and unoccupied sites are not distinguished in this figure.) When both a catalytic site and a nearest neighbor modifier site are occupied, there is a (free) energy of interaction $w_{12}$; when two nearest neighbor catalytic sites are occupied, there is an interaction energy $w_1$; and when two nearest neighbor modifier sites are occupied, there is an interaction energy $w_2$.

A seemingly more general model could be proposed, in which there is a different interaction energy for each possible state of a pair of nearest neighbor sites. However, for the classical Ising model, in which there is only a single class of sites, it is well known that there are conservation relations between the numbers of nearest neighbor pairs in the possible states [28]. These relations allow the effects of all possible nearest neighbor interactions to be expressed in terms of a single appar-

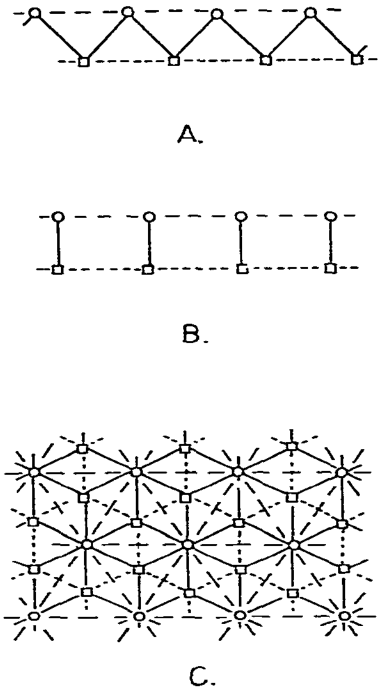

Fig. 2. Example of lattices for two classes of sites. Circles represent class 1 or catalytic sites, and squares, class 2 or modifier sites. The numbers of nearest neighbors are (A) $c_1 = c_2 = c_{12} = c_{21} = 2$; (B) $c_1 = c_2 = 2, c_{12} = c_{21} = 1$; (C) $c_1 = c_{12} = 6$, $c_2 = c_{21} = 3$.

ent interaction energy between nearest neighbor occupied sites. Similar conservation relations apply here to pairs of nearest neighbor sites where one site is of class 1 and the other of class 2 [29]. Again, these conservation relations allow the effects of all possible interactions between a catalytic site and a nearest neighbor modifier site to be expressed in terms of one apparent interaction energy $w_{12}$ for the pair when both sites are occupied. In other words, interactions between only occupied nearest neighbor sites is sufficient to describe all possible interactions in our nearest neighbor model.

In principle, we would now develop the time evolution equations for the various configurations of the lattice and from them derive the stationary state properties. However, to do this, we need expressions for the number of ways in which a given configuration may evolve to another. A related task is to determine the degeneracies of the configurations. Unfortunately, this has not yet been accomplished completely even for the classical Ising model. We therefore turn immediately to approximations, of which we present two. These are extensions of the well known Bragg–Williams [23] and quasi-chemical [24,25] approximations.

We should point out that the canonical ensemble approach implicit in these approximations is valid only for large numbers of sites, while there are only relatively few sites of a given class in many biological problems. However, large systems present the greater theoretical difficulties, whereas, for small numbers of sites, we can at least resort to a grand canonical approach and a procedure analogous to counting degeneracies if all else fails. Furthermore, according to Thompson [30], the error incurred on assuming a large number of sites for the one-dimensional classical Ising model becomes negligible if there are more than four. Thus, the limit of a large number of sites may actually obtain for very few. Of course, these comments do not apply to phase transitions. There are also biological processes involving large numbers of sites, such as the activation of muscle contraction mentioned above.

## 3. The Bragg–Williams approximation

The environment with which a given site interacts and which therefore influences the rate at which the site undergoes transitions between its two states consists of the states of its nearest neighbors; in general each possible environment must be considered. However, in the approximation originally developed for the classical Ising model by Bragg and Williams [23] the sites in their various states are assumed to be distributed at random throughout the lattice, so that only an "average" site interacting with an "average" environment need be considered. It is then relatively simple to develop the time evolution equations for the average state of each class of sites, as Hill [14] has done for the case of identical subunits. The assumption of a random distribution is of course incorrect, but the Bragg–Williams approximation (BWA) yields qualitatively reasonable results for the classical Ising model (with the exception of some aspects of phase transitions). We would expect the results here to be as good.

We define $\Theta_I$ as the average fraction of class I sites which are occupied. The average energy of interaction of an occupied catalytic site with its nearest neighbors is then

$$\bar{w}_c = c_1 w_1 \Theta_1 + c_{12} w_{12} \Theta_2 \; . \tag{3.1}$$

The corresponding time evolution equation becomes

$$\mathrm{d}\Theta_1/\mathrm{d}t = (\bar{k}_s z_s + \bar{k}_{-p} z_p)(1-\Theta_1) - (\bar{k}_p + \bar{k}_{-s})\Theta_1 \, , \tag{3.2}$$

where the average rate constants are obtained from (2.4) with (3.1); e.g.,

$$\bar{k}_s = \exp\left[-f_s(c_1 w_1 \Theta_1 + c_{12} w_{12} \Theta_2)\right] k_s^0 \; . \tag{3.3}$$

The stationary state "adsorption" isotherm for the catalytic sites is then

$$\frac{\exp\left[(1-f_s-f_p)(c_1 w_1 \Theta_1 + c_{12} w_{12} \Theta_2)\right] k_s^0 z_s + k_{-p}^0 z_p}{\exp\left[(1-f_s-f_p)(c_1 w_1 \Theta_1 + c_{12} w_{12} \Theta_2)\right] k_{-s}^0 + k_p^0}$$

$$= \frac{\Theta_1}{1-\Theta_1} \exp(c_1 w_1 \Theta_1 + c_{12} w_{12} \Theta_2) \; . \tag{3.4}$$

Similarly, the stationary state adsorption isotherm for the modifier sites is

$$q_2 z_2 = \frac{\Theta_2}{1-\Theta_2} \exp(c_2 w_2 \Theta_2 + c_{21} w_{12} \Theta_1) \, , \tag{3.5}$$

where $z_2$ is the activity of the modifier m and

$$q_2 \equiv k_m^0 / k_{-m}^0 \, , \tag{3.6}$$

is the partition function for a single occupied site.

Note that, as mentioned above, if $f_s + f_p = 1$, then (3.4) takes an equilibrium form

$$q_1 z_1 \equiv \frac{k_s^0 z_s + k_{-p}^0 z_p}{k_p^0 + k_{-s}^0}$$

$$= \frac{\Theta_1}{1-\Theta_1} \exp(c_1 w_1 \Theta_1 + c_{12} w_{12} \Theta_2), \tag{3.7}$$

analogous to that for the modifier sites. It is potentially misleading that (3.5) always has an equilibrium form. Since (3.5) is coupled to (3.4) for the catalytic sites, $\Theta_2$ will not have an equilibrium relation to $z_2$ unless the catalytic sites are operating in the quasi-equilibrium regime (3.7).

For a lattice with an arbitrary number of classes of sites, there would be an adsorption isotherm for each catalytic class of the form (3.4) or (3.7) (quasi-equilibrium) and one for each modifier class of the form (3.5), but with additional terms in the average interaction energy of an occupied site of a given class with its nearest neighbors; i.e., for the Ith class, (3.1) would become

$$\bar{w}_I = c_I w_I \Theta_I + \sum_{J \neq I} c_{IJ} w_{IJ} \Theta_J \; . \tag{3.8}$$

If two modifier classes would adsorb the same species, then $z_I = z_J$. If two classes would catalyze the same reaction, then $z_{sI} = z_{sJ}$, $z_{pI} = z_{pJ}$, and the rate constants would have to be such that $k_p^0 k_s^0 / k_{-s}^0 k_{-p}^0$ were the same for both classes.

Returning to the lattice with two classes of sites, one catalytic and one modifier, we would expect an exceedingly rich array of possible behaviors, as Hill and coworkers have demonstrated for a lattice of identical interacting two-state enzyme molecules [26,32–34]. We will therefore restrict further discussion in this initial paper to the quasi-equilibrium case, where (3.5) and (3.7) are the adsorption isotherms for the modifier and catalytic sites, respectively. As a byproduct, we will also be discussing the population properties of the corresponding equilibrium problem of adsorption of two species of interacting ligands with activities $z_1$ and $z_2$.

The adsorption isotherms (3.5) and (3.7) possess interesting symmetry properties, which can best be illustrated by defining

$$\begin{aligned} y_1 &\equiv \exp(-c_1 w_1/2)\exp(-c_{12} w_{12}/2) q_1 z_1 \, , \\ y_2 &\equiv \exp(-c_2 w_2/2)\exp(-c_{21} w_{12}/2) q_2 z_2 \, , \end{aligned} \tag{3.9}$$

which represent "activities" normalized to the highest symmetry point of the lattice:

$$\Theta_1 = \Theta_2 = 1/2 \Rightarrow y_1 = y_2 = 1 \; . \tag{3.10}$$

One symmetry can be expressed in general as

$$\begin{aligned} &y_1(\Theta_1, \Theta_2) y_1(1-\Theta_1, 1-\Theta_2) \\ &\quad = y_2(\Theta_1, \Theta_2) y_2(1-\Theta_1, 1-\Theta_2) = 1 \; ; \end{aligned} \tag{3.11}$$

if one of the $y$'s, say $y_1$, is 1, this symmetry becomes

$$y_2(\Theta_2) y_2(1-\Theta_2) = 1 \, , \quad \Theta_1(\Theta_2) + \Theta_1(1-\Theta_2) = 1 \; ; \tag{3.12}$$

or

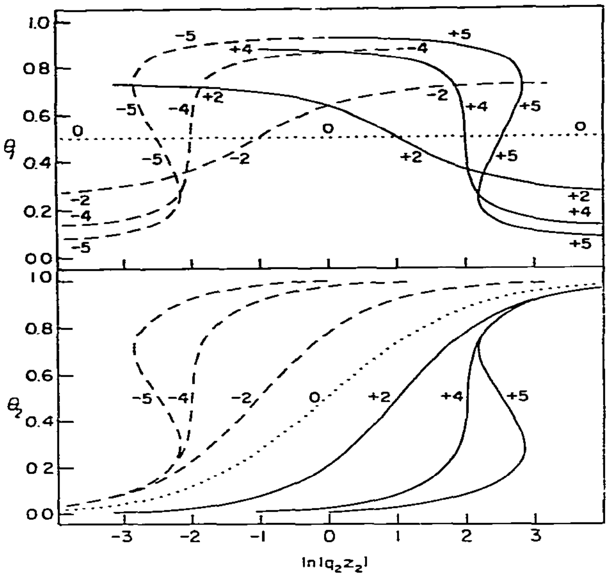

Fig. 3. The effect of $w_{12}$ on the catalytic ($\Theta_1$) and modifier ($\Theta_2$) adsorption isotherms versus $\ln(q_2z_2)$ for the quasi-equilibrium BWA. Numbers by curves are $c_{12}w_{12}$. Other conditions: $w_1 = w_2 = 0$; $c_{12} = c_{21}$, $\ln(q_1z_1) = c_{12}w_{12}/2$.

$$y_2(\Theta_1)y_2(1-\Theta_1) = 1\,, \quad \Theta_2(\Theta_1) + \Theta_2(1-\Theta_1) = 1.$$

However, if $y_1 \neq 1$, then in general, $y_2(\Theta_2)y_2(1-\Theta_2) \neq 1$, and the symmetry is lost.

There is a second symmetry property with respect to $w_{12}$:

$$\Theta_2(y_1, w_{12}, \Theta_1) = \Theta_2(1/y_1, -w_{12}, 1-\Theta_1)$$

$$\Rightarrow y_2(\Theta_1, \Theta_2, w_{12}) = y_2(1-\Theta_1, \Theta_2, -w_{12}) \quad (3.13)$$

or

$$y_2(y_1, \Theta_2, w_{12}) = y_2(1/y_1, \Theta_2, -w_{12})\,.$$

In other words, if $w_{12} \to -w_{12}$, $y_1 \to 1/y_1$, then the adsorption isotherm $\Theta_2$ versus $\ln y_2$ is unchanged, but $\Theta_1$ versus $\ln y_2$ is reflected about the line $\Theta_1 = 1/2$.

In addition to these symmetry properties, we see immediately from (3.5) and (3.7) that generally an attractive interaction energy (any $w < 0$) decreases the activities necessary for given levels of adsorption and a repulsive interaction ($w > 0$) does the opposite. Furthermore, we see that the adsorptions to the catalytic and modifier sites are mutual activators if $w_{12} < 0$ and mutual inhibitors if $w_{12} > 0$, as would be expected. Of course, if $w_{12} = 0$, the two adsorptions are independent of each other.

Several examples of the adsorption isotherms (3.5) and (3.7) at various values of the interaction energies are shown in figs. 3–5 for $c_{12} = c_{21}$. We have chosen to illustrate only cases where the activities of the chemical species s and p participating in the catalyzed reaction are constant and the activity of the adsorbed ligand $z_2$ is varied. Because we are considering the quasi-equilibrium regime, the adsorption isotherms $\Theta_1$ and $\Theta_2$ versus $\ln(q_1z_1)$ with $z_2$ constant can be obtained simply by interchanging subscripts 1 and 2. We have also taken advantage of properties (3.11) and (3.12) by setting $y_1 = 1$ (3.9) so that all examples are symmetric about the point $\Theta_1 = \Theta_2 = 1/2$.

In fig. 3 only the effects of the interaction between catalytic and modifier sites are shown ($w_1 = w_2 = 0$). As intimated above, an attractive 1–2 interaction ($w_{12} < 0$) shifts the adsorption isotherms to lower values of $z_2$, and a repulsive one, to higher values. Also illustrated here is the activation of catalytic adsorption by adsorption to the class 2 sites if $w_{12} < 0$ and the inhibition if $w_{12} > 0$. Note that the larger the magnitude of $w_{12}$, the larger the magnitude of the total effect of adsorption to the modifier sites on catalytic adsorption. By total effect, we mean the difference between catalytic adsorption when all class 2 sites are occupied and that when none are occupied. For the conditions of fig. 3, it is simply

$$\Theta_1(\Theta_2 = 1) - \Theta_1(\Theta_2 = 0) = \tanh(-c_{12}w_{12}/4)\,. \quad (3.14)$$

The slopes of the adsorption isotherms are also of interest. From (3.5) and (3.7), they are given by

$$\left(\frac{\partial\Theta_2}{\partial \ln z_2}\right)_{q_1z_1} = \Theta_2(1-\Theta_2)[1+\Theta_1(1-\Theta_1)c_1w_1]/D\,;$$

$$\left(\frac{\partial\Theta_1}{\partial \ln z_2}\right)_{q_1z_1} = -\Theta_1(1-\Theta_1)\Theta_2(1-\Theta_2)c_{12}w_{12}/D\,;$$

$$D \equiv [1+\Theta_1(1-\Theta_1)c_1w_1][1+\Theta_2(1-\Theta_2)c_2w_2] - \Theta_1(1-\Theta_1)\Theta_2(1-\Theta_2)c_{12}c_{21}w_{12}^2\,. \quad (3.15)$$

Delaying a discussion of phase transitions for the moment, we require $D > 0$. We see from (3.15) that the slope of the class 2 adsorption isotherm and the magnitude of the slope of the catalytic adsorption isotherm

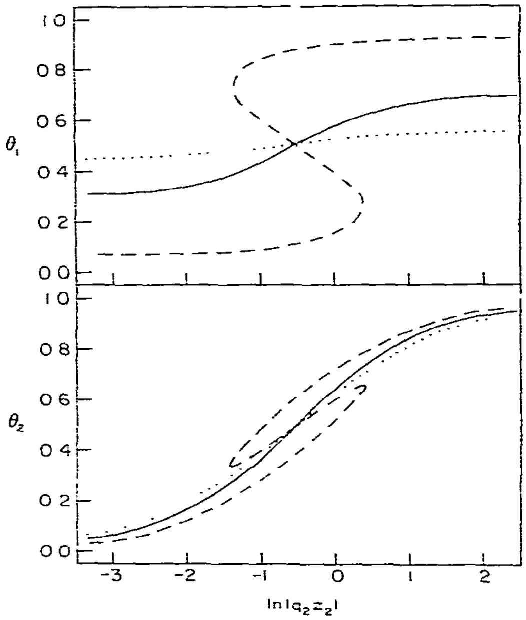

Fig. 4. The effect of $w_1$ on the catalytic ($\Theta_1$) and modifier ($\Theta_2$) adsorption isotherms versus $\ln(q_2z_2)$ for the quasi-equilibrium BWA. (...) $c_1w_1 = 4.0$; (——) $c_1w_1 = -2.0$; (– – –) $c_1w_1 = -5.0$. Other conditions: $w_2 = 0$; $c_{12}w_{12} = c_{21}w_{12} = -1.0$; $\ln(q_1z_1) = (c_1w_1 + c_{12}w_{12})/2$.

increase with the magnitude of $w_{12}$ for given values of $\Theta_1$ and $\Theta_2$ whether the interaction is attractive or repulsive. For fig. 3, in fact, at the one-half saturation point for adsorption to both classes of site, $\Theta_1 = \Theta_2 = 1/2$, we have

$$\left(\frac{\partial\Theta_2}{\partial \ln z_2}\right)_{q_1z_1} = 4/(16 - c_{12}c_{21}w_{12}^2)\,;$$

$$\left|\left(\frac{\partial\Theta_1}{\partial \ln z_2}\right)_{q_1z_1}\right| = c_{12}|w_{12}|/(16 - c_{12}c_{21}w_{12}^2)\,; \tag{3.16}$$

so that we have another symmetry property with respect to $w_{12}$: two different values, of equal magnitude but opposite sign, yield the same values for the magnitudes of the slopes. Also note that since $w_1 = w_2 = 0$, we have observed an apparent "cooperativity" in the class 2 adsorption due to its interaction with the catalytic adsorption.

In fig. 4, the effects of $w_1$ are illustrated for the case of an attractive 1–2 interaction. If $w_{12} > 0$, $\Theta_1$ would decrease with increasing $z_2$, of course. There is no shift of the adsorption isotherms to the left or the right because of our choice of $y_1 = 1$. In general, an increase in $w_1$ would shift the point $\Theta_2 = 1/2$ to higher values of $z_2$ when $w_{12}$ and $\ln y_1$ are of opposite sign, and to lower values when $w_{12}$ and $\ln y_1$ are of the same sign. We see that $w_1$ affects the total effect of modifier adsorption on catalytic adsorption, as would be expected: an attractive interaction increases the total effect, whereas a repulsive one decreases it. We also note that attractive interactions increase the slopes of the adsorption isotherms and repulsive interactions make them less steep (3.15).

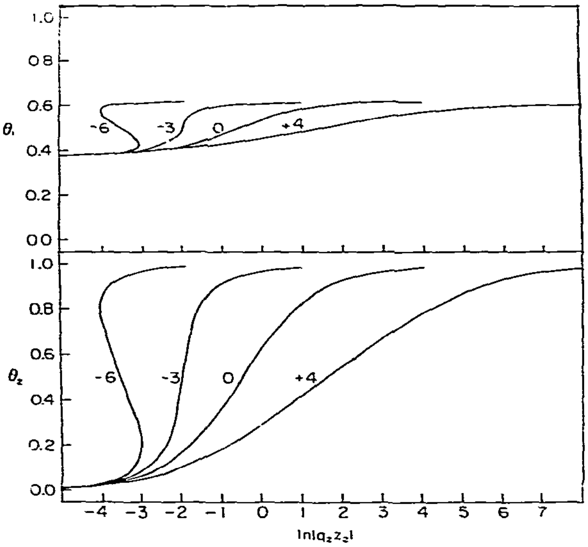

Fig. 5. The effect of $w_2$ on the catalytic ($\Theta_1$) and modifier ($\Theta_2$) adsorption isotherms versus $\ln(q_2z_2)$ for the quasi-equilibrium BWA. Numbers by curves are $c_2w_2$. Other conditions: $w_1 = 0$; $c_{12}w_{12} = c_{21}w_{12} = -1.0$; $\ln(q_1z_1) = c_{12}w_{12}/2$.

The effects of the remaining interaction energy $w_2$ are shown in fig. 5, again for $w_{12} < 0$. As would be expected, $w_2$ does not affect the total effect of the class 2 adsorption on catalytic adsorption. There is a shift along the $\ln z_2$ axis, $w_2 < 0$ causing a leftward shift and $w_2 > 0$, one to the right. Like $w_1$, $w_2$ increases the slopes of the adsorption isotherms if $w_2 < 0$, and decreases them if $w_2 > 0$.

The terms "positive" and "negative cooperativity" are often used (see, for example, ref. [31]) to refer to the slopes of adsorption isotherms, the former corresponding to the case of a slope steeper than that of noncooperative (Langmuir) adsorption and the latter to a less steep slope. However, we have seen here that there is not a one to one correspondence between this terminology and the fundamental parameters of the interactions, the energies. For the interactions between catalytic sites ($w_1$) or those between the modifier sites ($w_2$), there is no problem: an attractive energy increases the magnitudes of the slopes and a repulsive one decreases them. However, with the interaction between catalytic and modifier sites, the magnitudes of the slopes are increased regardless of the sign of $w_{12}$. How this comes about with a repulsive 1–2 interaction is easily seen. With $d(q_1 z_1) = 0$, as $z_2$ increases, $\Theta_2$ increases; but because the catalytic and modifier adsorptions are antagonistic, the increase in $\Theta_2$ causes a decrease in $\Theta_1$, which in turn makes further modifier adsorption more favorable. Because of the lack of correspondence between the terms positive and negative cooperativity and the nature of the interactions, it would be perhaps appropriate if the former terms were used only purely phenomenologically.

One further aspect of the adsorption isotherms (3.5) and (3.7) deserves mention, although a complete discussion is beyond the scope of this work. In each of figs. 4 and 5, a loop corresponding to a phase transition is shown, and in fig. 3, there are two loops. From (3.15) we find that the general condition for a phase transition with $d(q_1 z_1) = 0$ is $D \leqslant 0$. We see that a phase transition may be caused by any of the three different interactions, and that phase transitions are predicted in both isotherms $\Theta_1$ versus $\ln z_2$ and $\Theta_2$ versus $\ln z_2$ simultaneously. However, note from the form of $D$ that a phase transition is predicted only for sufficiently attractive interactions between catalytic sites or between modifier sites, but that a 1–2 interaction of sufficient magnitude can cause a phase transition, whether attractive or repulsive. In fact, with $w_1 = w_2 = 0$, the critical value for the highest order symmetry point, $\Theta_1 = \Theta_2 = 1/2$, is given by

$$(c_{12} c_{21})^{1/2} |w_{12}| = 4 \,. \tag{3.17}$$

This is reminiscent of the critical point for the BWA to the classical Ising model, $cw = -4$, except that $w_{12}$ may be negative or positive.

One further comment about these phase transitions is in order. The loops corresponding to phase transitions due to $w_2$ or $w_{12}$ (figs. 3 and 5) are of the usual van der Waals sort; i.e., the slope of the middle branch is negative. However, in a phase transition due to $w_1$, part of the middle branch of the modifier adsorption isotherm may have a positive slope. This results since there is a van der Waals type loop in a plot of $\Theta_1$ versus $\Theta_2$ in this case. Since the middle branch of this loop has a negative slope, as does that of $\Theta_1$ versus $\ln z_2$, the middle branch of $\Theta_2$ versus $\ln z_2$ has a positive slope. It is interesting to note that the critical point for the $\Theta_1$–$\Theta_2$ loop is $c_1 w_1 = -4$, identical to that for the classical Ising model. This is not surprising since it can be seen from (3.7) that, with $d(q_1 z_1) = 0$, $\Theta_2$ plays the role of a chemical potential for the catalytic adsorption in the BWA.

For systems with nonequilibrium stationary states, rates of processes are often of even greater interest than population properties, the adsorption isotherms in our case. Since our lattice has only a single class of catalytic sites, one phenomenological flux, the rate of the catalyzed reaction per catalytic site, is adequate to describe these rates at the stationary state(s). In the direction s → p for the BWA, it is given simply by

$$J = \bar{k}_{\mathrm{p}} \Theta_1 - \bar{k}_{-\mathrm{p}} z_{\mathrm{p}} (1 - \Theta_1) = \bar{k}_{\mathrm{s}} z_{\mathrm{s}} (1 - \Theta_1) - \bar{k}_{-\mathrm{s}} \Theta_1 \,. \tag{3.18}$$

In the limit $k^0_{-\mathrm{p}} z_{\mathrm{p}} \to 0$, a frequent condition for enzyme kinetic studies, (3.18) becomes

$$J = k^0_{\mathrm{p}} \exp\,[f_{\mathrm{p}}(c_1 w_1 \Theta_1 + c_{12} w_{12} \Theta_2)]\, \Theta_1 \,. \tag{3.19}$$

If $f_{\mathrm{p}} > 0$, we see that attractive interactions acting directly on the catalytic sites ($w_1$ or $w_{12} < 0$) decrease the flux relative to its value in the absence of interactions, $J = k^0_{\mathrm{p}} \Theta_1$, whereas repulsive interactions increase it relative to $k^0_{\mathrm{p}} \Theta_1$. Thus, these interactions may have competing effects on the flux. From (3.7), it is obvious that attractive interactions, for example, increase $\Theta_1$ for given values of $q_1 z_1$. This tends to increase $J$ through the factor $\Theta_1$. On the other hand, the attractive interactions tend to decrease the flux through the factor $\exp\,[f_{\mathrm{p}}(c_1 w_1 \Theta_1 + c_{12} w_{12} \Theta_2)]$.

The results of these competing effects are illustrated in fig. 6. Here we have shown a variety of typical responses of the flux to changes in modifier activity, along with the corresponding catalytic adsorption isotherms. The curves were calculated for the quasi-equilibrium regime with the conditions $k^0_{-\mathrm{p}} z_{\mathrm{p}} \to 0$, $w_{12} < 0$ and the symmetric choice $f_{\mathrm{p}} = 1/2$. The flux may either increase or decrease (fig. 6a) monotonically with an increase in $z_2$ while the adsorption is monotonically increasing. The flux may also show a biphasic response while that of the adsorption remains monotonic (fig. 6b). Finally, we have the possibility of

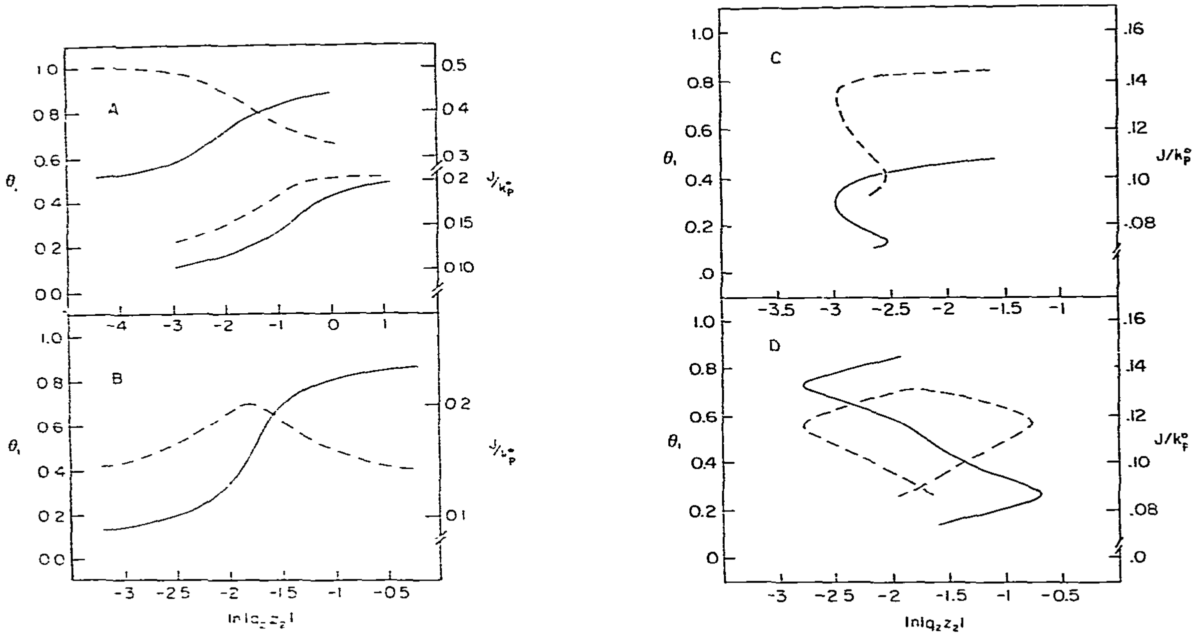

Fig. 6. Some typical responses of the catalytic adsorption $\Theta_1$ and catalytic flux $J/k_p^0$ to changes in modifier activity $z_2$ for the quasi-equilibrium BWA. $\Theta_1$ is shown by the solid lines and the flux by the dashed lines. Note that the scales for the flux and $\ln(q_2z_2)$ are not the same for all curves. For all flux calculations $f_p = 1/2$, $k_{-p}^0z_p = 0$ (3.19). (A) Monotonic responses of the flux. Upper curves: $q_1z_1 = 1.0; c_{12}w_{12} = c_{21}w_{12} = -2.197; c_1w_1 = c_2w_2 = 0$. Lower curves: $q_1z_1 = 0.1658; c_{12}w_{12} = c_{21}w_{12} = -3.797$, $c_1w_1 = 4.0; c_2w_2 = 0$. (B) Biphasic response of the flux: $q_1z_1 = 0.1658; c_{12}w_{12} = c_{21}w_{12} = -7.594; c_1w_1 = c_1w_2 = 4.0$. (C) Phase transition with "normal" loop for the flux: $q_1z_1 = 0.08231; c_{12}w_{12} = c_{21}w_{12} = -0.997; c_1w_1 = -3.0; c_2w_2 = -5.0$. (D) Phase transition with biphasic loop for the flux: $q_1z_1 = 0.06739; c_{12}w_{12} = c_{21}w_{12} = -0.394; c_1w_1 = -5.0; c_2w_2 = -3.0$.

phase transitions. If the flux exhibits a monotonic relationship to catalytic adsorption (as in fig. 6a), then the loop in the flux has the same general shape as that in the adsorption (fig. 6c). If the flux is related biphasically to adsorption (as in fig. 6b), however, then the flux loop may be similar to what Hill [14,15] calls a "bow tie" loop (fig. 6d).

We note also that the terms positive and negative cooperativity have even less meaning for the flux than they do for the adsorptions. Because of the competing effects of the interactions on the flux, it may show the oppositive cooperativity as the catalytic adsorption isotherm. In fact, the flux may even change in the opposite direction as the catalytic adsorption. Moreover, it is not possible in general to classify the modifier as an activator or inhibitor of the catalyzed reaction based on the sign of $w_{12}$. It may act as either depending on the values of the other parameters.

## 4. The quasi-chemical approximation

The predictions of the BWA presented in the previous section are qualitatively reasonable. It would thus be appropriate for a first investigation of a system about which little is known or for a study of possible behaviors of a system. However, the BWA does have a serious shortcoming, the lack of topological information: in the equations of the previous section, the numbers of nearest neighbors $c$ appear only as scale factors for the interaction energies. One manifestation of this shortcoming is the prediction of phase transitions for any lattice, whereas it is known that the one-dimensional classical Ising model, for example, does not show such behavior.

The more elaborate quasi-chemical approximation (QCA), developed independently by Bethe [24] and Guggenheim [25] for the classical Ising model, does retain some topological information in so far as it places correct constraints concerning the numbers of nearest neighbors on the conditions for phase transitions. The QCA has two further advantages over the BWA: it is quantitatively more accurate for the classical Ising model, and we would expect a similar improvement here; moreover, we will see that the QCA is exact for several simple lattices with two classes of sites. In the BWA we neglected the difference in the possible environments for a given site (as specified by the numbers of occupied nearest neighbors of each class) and used an average environment. In the QCA we assume that pairs of nearest neighbor sites are occupied independently. This assumption does not hold in general, of course, but it does allow us to take each environment into account explicitly. Herein lies the improvement over the BWA.

There are at least two ways of utilizing the quasi-chemical assumption which are equivalent at equilibrium [28]. One uses the independence of nearest neighbor pairs to develop an expression for the degeneracy of each configuration of the lattice, which allows one to write down the partition function. The other begins by noting the so-called quasi-chemical relations

$$({}^{x}_{\mathrm{I}}N^{x}_{\mathrm{J}})({}^{0}_{\mathrm{I}}N^{0}_{\mathrm{J}})/[({}^{x}_{\mathrm{I}}N^{0}_{\mathrm{J}})({}^{0}_{\mathrm{I}}N^{x}_{\mathrm{J}})] = \mathrm{e}^{-w}\,, \tag{4.1}$$

where ${}^{\mathrm{A}}_{\mathrm{I}}N^{\mathrm{B}}_{\mathrm{J}}$ is the number of nearest neighbor pairs consisting of a class I site and a class J site, with the class I site in state A and the class J site in state B; A, B = x, 0; x indicating that a site is occupied and 0, that it is unoccupied; and $w$ is the appropriate interaction energy. (If I = J, a factor of 1/4 must be included on the right hand side, since ${}^{x}_{\mathrm{I}}N^{0}_{\mathrm{I}} = {}^{0}_{\mathrm{I}}N^{x}_{\mathrm{I}}$.)

Eq. (4.1) implies a detailed balance among nearest neighbor pairs in the stationary state, however, and therefore would be valid only in the quasi-equilibrium regime for our problem [35]. On the other hand, it might be possible to use the assumption of independence of nearest neighbor pairs to develop an expression for the number of ways that a configuration specified by the numbers of occupied sites and the numbers of nearest neighbor pairs in the various states could undergo a transition to another configuration. This would allow the time evolution equations for the configurations to be written, and from these, the stationary state properties would follow. It is not clear that included in these properties would be the detailed balance relations (4.1). Thus, it might be possible to develop a non-quasi-equilibrium QCA. However, we have not been able up to the present to carry out this approach; so we are forced to restrict our QCA to the quasi-equilibrium regime. We follow closely the development of Hill [15].

Consider a class I site. It has a total of $(c_{\mathrm{I}}+1)(c_{\mathrm{IJ}}+1)$ possible environments; $i = 0, 1, \ldots c_{\mathrm{I}}$ nearest neighbor class I sites occupied and $j = 0, 1, \ldots c_{\mathrm{IJ}}$ nearest neighbor class J ≠ I sites occupied. If a class I site is occupied, the probability that a nearest neighbor class J site is occupied is

$$\beta = {}^{x}_{\mathrm{I}}N^{x}_{\mathrm{J}}/({}^{x}_{\mathrm{I}}N^{x}_{\mathrm{J}} + {}^{x}_{\mathrm{I}}N^{0}_{\mathrm{J}})\,, \tag{4.2}$$

and the probability that a nearest neighbor class J site is unoccupied is $(1-\beta)$. Thus, the probability that an occupied class I site has $j$ occupied class J nearest neighbors is

$$P^{x}_{\mathrm{I}}(j) = \frac{c_{\mathrm{IJ}}!}{j!(c_{\mathrm{IJ}}-j)!}\,\beta^{j}(1-\beta)^{(c_{\mathrm{IJ}}-j)}\,. \tag{4.3}$$

Similarly, the probability that an unoccupied class I site has $j$ occupied class J nearest neighbors is

$$P^{0}_{\mathrm{I}}(j) = \frac{c_{\mathrm{IJ}}!}{j!(c_{\mathrm{IJ}}-j)!}\,(\beta')^{j}(1-\beta')^{(c_{\mathrm{IJ}}-j)}\,, \tag{4.4}$$

where $\beta' \equiv {}^{0}_{\mathrm{I}}N^{x}_{\mathrm{J}}/({}^{0}_{\mathrm{I}}N^{0}_{\mathrm{J}} + {}^{0}_{\mathrm{I}}N^{x}_{\mathrm{J}})$. However, because of the conservation relations [29] among the ${}^{\mathrm{A}}_{\mathrm{I}}N^{\mathrm{B}}_{\mathrm{J}}$ mentioned in section 2,

$$\beta' = (\Theta_{\mathrm{J}} - \Theta_{\mathrm{I}}\beta)/(1-\Theta_{\mathrm{I}})\,, \tag{4.5}$$

where $\Theta_{\mathrm{I}}(\Theta_{\mathrm{J}})$ is, as before, the average fractional occupation of the class I(J) sites.

Similarly [15], the probability that an occupied class I site has $i$ occupied class I nearest neighbors is

$$R^{x}_{\mathrm{I}}(i) = \frac{c_{\mathrm{I}}!}{i!(c_{\mathrm{I}}-i)!}\,\alpha^{i}(1-\alpha)^{(c_{\mathrm{I}}-i)}\,, \tag{4.6}$$

and the probability that an unoccupied class I site has $i$ occupied class I nearest neighbors is

$$R^{0}_{\mathrm{I}}(i) = \frac{c_{\mathrm{I}}!}{i!(c_{\mathrm{I}}-i)!}\left[\left(\frac{\Theta_{\mathrm{I}}}{1-\Theta_{\mathrm{I}}}\right)(1-\alpha)\right]^{i} \times \left[1-\left(\frac{\Theta_{\mathrm{I}}}{1-\Theta_{\mathrm{I}}}\right)(1-\alpha)\right]^{(c_{\mathrm{I}}-i)}\,, \tag{4.7}$$

where $\alpha \equiv {}^{x}_{\mathrm{I}}N^{x}_{\mathrm{J}}/({}^{x}_{\mathrm{I}}N^{x}_{\mathrm{I}} + {}^{x}_{\mathrm{I}}N^{0}_{\mathrm{I}})$. (Hill's notation differs from ours.)

Since the probability that a class I site is occupied is $\Theta_I$ and since we are assuming independence of nearest neighbor pairs, the probability that a class I site is occupied and has $i$ occupied class I nearest neighbors and $j$ occupied class J nearest neighbors is simply

$$\Theta_I^x(i,j) = \Theta_I R_I^x(i) P_I^x(j) , \qquad (4.8)$$

and the probability that a class I site is unoccupied and has the same number of occupied nearest neighbors is

$$\Theta_I^0(i,j) = (1 - \Theta_I) R_I^0(i) P_I^0(j) . \qquad (4.9)$$

Now, let the class I sites be catalytic ones (I = 1) and the class J sites be the modifier sites (J = 2). Since we are dealing with the quasi-equilibrium regime, the simulated detailed balance requires

$$\begin{aligned}&[k_s(i,j)z_s + k_{-p}(i,j)z_p]\,\Theta_1^0(i,j)\\ &\quad = [k_p(i,j) + k_{-s}(i,j)]\,\Theta_1^x(i,j) , \qquad (4.10)\end{aligned}$$

where the rate constants are for catalytic sites with $i$ occupied nearest neighbor class 1 sites and $j$ occupied nearest neighbor class 2 sites. The interaction energy of an occupied catalytic site with this environment is

$$w_c(i,j) = iw_1 + jw_{12} , \qquad (4.11)$$

so that from (2.4)

$$\begin{aligned}&k_s(i,j)/k_s^0 = k_{-p}(i,j)/k_{-p}^0\\ &\quad = \exp\,[-(1-f)(iw_1 + jw_{12})] ,\\ &k_p(i,j)/k_p^0 = k_{-s}(i,j)/k_{-s}^0 \qquad (4.12)\\ &\quad = \exp\,[f(iw_1 + jw_{12})] ,\end{aligned}$$

where $f \equiv f_p = 1 - f_s$.

If we use (4.12) and (4.3)–(4.9) in (4.10), and then divide the resultant equation evaluated at $i = j = 0$ by that evaluated at $i = 0, j = c_{12}$, we eventually find

$$\beta = \frac{(e^{-w_{12}} - 1)(\Theta_1 + \Theta_2) + 1 - \gamma_{12}}{2(e^{-w_{12}} - 1)\Theta_1} ,$$

$$\begin{aligned}\gamma_{12} \equiv \{&[(e^{-w_{12}} - 1)(\Theta_1 + \Theta_2) + 1]^2\\ &- 4e^{-w_{12}}(e^{-w_{12}} - 1)\Theta_1\Theta_2\}^{1/2} . \qquad (4.13)\end{aligned}$$

If we divide the resultant equation for $i = j = 0$ by that for $i = c_1, j = 0$, we confirm Hill's result

$$\begin{aligned}&\alpha = (\gamma_1 - 1 + 2\Theta_1)/(\gamma_1 + 1) ;\\ &\gamma_1 \equiv [1 - 4\Theta_1(1 - \Theta_1)(1 - e^{-w_1})]^{1/2} . \qquad (4.14)\end{aligned}$$

Putting (4.13) and (4.14) back into (4.10), we find the stationary state adsorption isotherm for the catalytic sites:

$$\begin{aligned}q_1z_1 = &\left(\frac{\Theta_1}{1-\Theta_1}\right)\left[\frac{(1-\Theta_1)(\gamma_1 - 1 + 2\Theta_1)}{e^{-w_1}\Theta_1(\gamma_1 + 1 - 2\Theta_1)}\right]^{c_1/2} \qquad (4.15\\ &\times\left\{\left[\frac{(e^{-w_{12}} + 1)\Theta_1 - (e^{-w_{12}} - 1)\Theta_2 - 1 + \gamma_{12}}{2e^{-w_{12}}\Theta_1}\right]^{c_{12}}\right\} ,\end{aligned}$$

where $q_1z_1 \equiv (k_s^0z_s + k_{-p}^0z_p)/(k_p^0 + k_{-s}^0)$ as before.

If the arguments following (4.9) are repeated with the roles of the catalytic and modifier classes of sites reversed, an adsorption isotherm analogous to (4.15) but with subscripts 1 and 2 interchanged is found for the class 2 sites ($\gamma_{21} = \gamma_{12}$). Of course, since these are quasi-equilibrium adsorption isotherms, they also apply to the analogous equilibrium case. If there would be additional classes of site, each would contribute a factor analogous to that enclosed in braces in (4.15) to the right hand sides of the adsorption isotherms.

The net stationary state flux of the catalysed reaction for a given environment of $i$ occupied class 1 nearest neighbors and $j$ occupied class 2 nearest neighbors is

$$J(i,j) = k_p(i,j)\Theta_1^x(i,j) - k_{-p}(i,j)z_p\Theta_1^0(i,j) . \qquad (4.16)$$

The total flux (per catalytic site) is just the sum over all environments:

$$\begin{aligned}J = &\sum_{i=0}^{c_1}\sum_{j=0}^{c_{12}} J(i,j)\\ &= k_p^0[1 - \alpha(1 - e^{fw_1})]^{c_1}[1 - \beta(1 - e^{fw_{12}})]^{c_{12}}\Theta_1\\ &- \left\{k_{-p}^0z_p\left[1 - \left(\frac{\Theta_1}{1-\Theta_1}\right)(1-\alpha)(1 - e^{(f-1)w_1})\right]^{c_1}\right.\\ &\times\left.\left[1 - \left(\frac{\Theta_2 - \Theta_1\beta}{1 - \Theta_1}\right)(1 - e^{(f-1)w_{12}})\right]^{c_{12}}(1-\Theta_1)\right\} .\end{aligned}$$

(4.17)

The behavior of the QCA adsorption isotherms, (4.15) and its counterpart for the class 2 sites, and catalytic flux (4.17) is, for the most part, qualitatively similar to that of the corresponding quasi-equilibrium

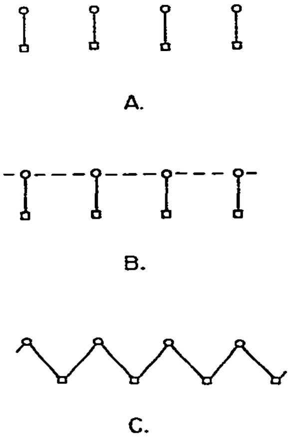

Fig. 7. Lattices for which the QCA is exact. Circles are class 1 or catalytic sites, and squares, class 2 or modifier sites. (A) $w_1 = w_2 = 0; c_{12} = c_{21} = 1$. (b) $w_2 = 0; c_1 = 2; c_{12} = c_{21} = 1$. (C) $w_1 = w_2 = 0; c_{12} = c_{21} = 2$.

BWA equations. For $f > 0$, for example, the interactions may have competing effects on the flux. However, notice that the lattice topology, as specified by the numbers of nearest neighbors $c_1, c_2$, and $c_{12}$, is manifest in the QCA in contrast to the BWA. Striking effects of the topology are seen in the critical points for the QCA. With $d(q_1 z_1) = 0$, we find that the critical point in the $\Theta_1$–$\Theta_2$ plane is

$$\frac{w_1}{2} = \ln \left| \frac{c_1 - 2}{c_1} \right| . \tag{4.18}$$

If $w_1 = w_2 = 0$, the highest order critical point ($\Theta_1 = \Theta_2 = 1/2$) in the $\Theta_1$–$\ln z_2$ or $\Theta_2$–$\ln z_2$ plane is

$$\frac{w_{12}}{2} = \pm \ln \left| \frac{[(c_{12} - 1)(c_{21} - 1)]^{1/2} + 1}{[(c_{12} - 1)(c_{21} - 1)]^{1/2} - 1} \right| . \tag{4.19}$$

(4.18) requires $c_1 > 2$, and (4.19) requires $c_{12} \geqslant 2$, $c_{21} \geqslant 2$ and $c_{12}$ or $c_{21} > 2$. Thus, the QCA places topological restrictions, minimum numbers of nearest neighbors, on the conditions for phase transitions, whereas the BWA allows a phase transition for any lattice. Since the topological restrictions predicted by the QCA for the classical Ising model are known to be correct, we expect that they are also correct here. In fact, (4.18) is identical to the result for the classical Ising model.

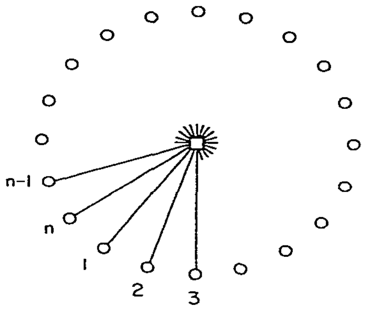

Fig. 8. The "concerted" lattice. $w_1 = w_2 = 0, c_{12} = 1; c_{21} = n$. The $n$ circles represent catalytic sites, and the square represents the conformation.

$$\frac{w}{2} = \ln \left| \frac{c - 2}{c} \right| , \quad c > 2 , \tag{4.20}$$

and (4.19) is similar. Also note that either an attractive or a repulsive 1–2 interaction may lead to a phase transition, as in the BWA.

The QCA is also exact for several special cases. Of course, if $w_{12} = 0$, then the adsorption isotherms for both the catalytic sites (4.15) and the modifier sites reduce to the result for the classical Ising model, which is exact for the one-dimensional case, $c = 2$. If $w_1 = w_2 = 0$ and $c_{12} = c_{21} = 1$ (fig. 7a), the QCA is also exact, as it must be since nearest neighbor pairs are occupied independently. Two further cases for which the QCA is exact are $w_2 = 0, c_1 = 2, c_{12} = c_{21} = 1$ (fig. 7b) and $w_1 = w_2 = 0, c_{12} = c_{21} = 2$ (fig. 7c). Thompson [3,30] has derived the adsorption isotherms for these two lattices using the equilibrium approach of the matrix method.

A final most important case for which the QCA is exact is $w_1 = w_2 = 0, c_{12} = 1, c_{21} = n$ (fig. 8). Here the adsorption isotherms and catalytic flux may be rearranged to yield

$$\begin{aligned} \Theta_1 &= [q_1 z_1 (1 + q_1 z_1)^{(n-1)} \\ &\quad + r \mathrm{e}^{-w_{12}} q_1 z_1 (1 + \mathrm{e}^{-w_{12}} q_1 z_1)^{(n-1)}]/\Xi , \\ \Theta_2 &= r(1 + \mathrm{e}^{-w_{12}} q_1 z_1)^n / \Xi , \\ J &= \left( \frac{k_\mathrm{p}^0 k_\mathrm{s}^0 z_\mathrm{s} - k_{-\mathrm{s}}^0 k_{-\mathrm{p}}^0 z_\mathrm{p}}{k_\mathrm{p}^0 + k_{-\mathrm{s}}^0} \right) [(1 + q_1 z_1)^{(n-1)} \\ &\quad + r \mathrm{e}^{(f-1) w_{12}} (1 + \mathrm{e}^{-w_{12}} q_1 z_1)^{(n-1)}]/\Xi , \\ \Xi &\equiv (1 + q_1 z_1)^n + r(1 + \mathrm{e}^{-w_{12}} q_1 z_1)^n , \quad r \equiv q_2 z_2 . \end{aligned} \tag{4.21}$$

We now make a slight reinterpretation of the model. We picture the lattice of fig. 8 as a protein with $n$ catalytic sites, and allow the protein to be in two conformational states, which are represented by the states of the central class two site. As before, there is an interaction energy $w_{12}$ when a catalytic site is occupied and the protein is in conformation x, but not when it is in conformation 0. $q_2 z_2$ is then replaced by $r \equiv q_2^{\mathrm{x}} / q_2^0$, where $q_2^{\mathrm{x}}(q_2^0)$ is the partition function for the protein in conformation x(0) when all catalytic sites are unoccupied. $\Theta_2$ is the fraction of protein molecules in conformation x.

This special case as reinterpreted is identical to the concerted model [7]. That the concerted model is just a special case of a nearest neighbor lattice model with two classes of sites is particularly significant. Although it has been realized that the concerted model and the sequential model [4] are both special cases of a more general scheme, it has also been thought that they differed in important ways (see, for example, ref. [31]). However, as mentioned in the introduction, the sequential model is identical to the classical Ising model, which is obviously a special case of the work here. Thus, both the concerted and sequential models are special cases of our nearest neighbor lattice model with two classes of sites, the difference being that the latter considers only the catalytic sites whereas the former considers both classes, but only the interaction between the catalytic and modifier (conformational) sites.

There is also an explanation here for why the sequential model may predict either "positive" or "negative cooperativity" in the catalytic adsorption isotherm, but the concerted model may predict only "positive cooperativity". (The latter statement is not true for the flux [36].) The concerted model considers only the 1–2 interaction, which, as pointed out in our discussion of the BWA, always increases the slopes of the adsorption isotherms whether the interaction energy is attractive or repulsive. However, an interaction between identical sites, the only one considered in the sequential model, can increase the slope of the adsorption isotherm if it is attractive or decrease the slope if it is repulsive. Thus, this difference between the two models is due to the fundamental difference between the effects of the interactions between like sites and those between unlike sites.

## 5. Discussion

In the absence of interactions both the BWA and the QCA are exact, and the catalytic adsorption is given by

$$\Theta_1 = q_1 z_1 / (1 + q_1 z_1) ; \tag{5.1}$$

$$q_1 z_1 \equiv (k_{\mathrm{s}}^0 z_{\mathrm{s}} + k_{-\mathrm{p}}^0 z_{\mathrm{p}}) / (k_{\mathrm{p}}^0 + k_{-\mathrm{s}}^0) . \tag{5.2}$$

(5.1) has just the form of the Langmuir isotherm for the equilibrium adsorption of a ligand of activity $z_1$ to independent sites with single site partition functions $q_1$. In the limit $k_{-\mathrm{p}}^0 z_{\mathrm{p}} \to 0$, we can make the further identifications

$$z_1 = z_{\mathrm{s}} ; \qquad q_1 = k_{\mathrm{s}}^0 / (k_{\mathrm{p}}^0 + k_{-\mathrm{s}}^0) . \tag{5.3}$$

Moreover, in this situation, the catalytic flux becomes

$$J / k_{\mathrm{p}}^0 = \Theta_1 . \tag{5.4}$$

Although many authors have suggested that it might not be adequate, the classical approach carries these analogies between stationary state catalysis and equilibrium ligand adsorption over to situations where there are interactions. That is, the catalytic adsorption isotherm is taken to have exactly the form of that for the corresponding ligand adsorption if the identifications (5.2) or (5.3) are made, and the flux is taken to be proportional to the catalytic adsorption. However, the validity of the approach requires four assumptions [13]. For the catalytic adsorption isotherm to be obtainable from the corresponding equilibrium expression simply by noting (5.2) or (5.3) requires (1) that the catalytic cycle have only two states and (2) the quasi-equilibrium condition, $f_{\mathrm{s}} + f_{\mathrm{p}} = 1$. (There are other situations where there is a formal resemblance between the nonequilibrium and the equilibrium stationary state adsorption isotherms, but in these cases replacements (5.2) or (5.3) do not render them identical [32].) Given (1) and (2), we must further assume (3) the standard condition $k_{-\mathrm{p}}^0 z_{\mathrm{p}} \to 0$ and (4) that $k_{\mathrm{p}}$ is not affected by the interactions ($f_{\mathrm{p}} = 0$), if the flux is to be proportional to the catalytic adsorption.

In this paper we have presented a nearest neighbor lattice model of the effect of modifiers on enzyme catalysis. We have restricted the model to two state catalytic cycles [assumption (1)] and, other than in the derivation of the BWA, we have considered only the quasi-equilibrium regime [assumption (2)]. Even with assumptions (1) and (2), we found a fundamental difference between the effects of interactions between identical sites and those between sites of the two different classes. Whereas the former increase the magnitudes of the slopes of the adsorption isotherms if attractive and decrease them if repulsive, the latter in general increase the magnitude of the slopes whether attractive or repulsive. Thus, the concerted model [7], which considers only interactions between modifier and catalytic sites, displays only "positive cooperativity" while the sequential model [4], which considers only interactions between catalytic sites, may exhibit "positive" or "negative cooperativity". This difference between the two types of interaction also implies that the interaction between catalytic and modifier sites may lead to a phase transition if of sufficient magnitude, whether attractive or repulsive, but interactions between identical sites can cause a phase transition only if sufficiently attractive.

Of most importance for studies of enzyme catalysis, of course, is the rate of catalyzed reaction. In this regard, we have relaxed both assumptions (3) and (4) and not assumed a proportionality between the flux and catalytic adsorption. Thus, we have developed both the BWA and the QCA for the complete quasi-equilibrium regime, and we also have exact results in this range for those cases for which the QCA is exact, including the concerted model and those of Thompson [3,30]. Phase transitions are excluded for these cases, since they do not have the minimum numbers of nearest neighbors required by the QCA for such behavior.

We have seen that the modifier cannot be classified as an activator or inhibitor of the catalyzed reaction simply on the basis of the sign of the interaction energy between modifier and catalytic sites because of the possibility of competing effects of the interactions on the flux. An elegantly simple example is the case $c_{12} = c_{21} = 1$, $w_1 = w_2 = 0$, for which the QCA is exact (in the quasi-equilibrium regime). The flux is given by

$$J = \left(\frac{k_\mathrm{p}^0 k_\mathrm{s}^0 z_\mathrm{s} - k_{-\mathrm{s}}^0 k_{-\mathrm{p}}^0 z_\mathrm{p}}{k_\mathrm{p}^0 + k_{-\mathrm{s}}^0}\right) \left[1 + \mathrm{e}^{(f_\mathrm{p}-1)w_{12}} q_2 z_2\right]$$

$$\times \left(1 + q_1 z_1 + q_2 z_2 + \mathrm{e}^{-w_{12}} q_1 z_1 q_2 z_2\right)^{-1} . \quad (5.5)$$

Even in the limit $k_{-\mathrm{p}}^0 z_\mathrm{p} \to 0$, we find

$$\operatorname{sgn}\left[(\partial J/\partial z_2)_{z_1}\right]$$

$$= \left\{\begin{matrix} -\operatorname{sgn}(f w_{12}) \\ 0 \\ \operatorname{sgn}(f w_{12}) \end{matrix}\right\} \text{ if } q_1 z_1 \left\{\begin{matrix} < \\ = \\ > \end{matrix}\right\} (q_1 z_1)^\dagger ; \quad (5.6)$$

$$(q_1 z_1)^\dagger = \left[\mathrm{e}^{(f_\mathrm{p}-1)w_{12}} - 1\right] / \left[\mathrm{e}^{-w_{12}} - \mathrm{e}^{(f_\mathrm{p}-1)w_{12}}\right] . \quad (5.7)$$

This is very different from the result of the classical approach, where $J = k_\mathrm{p}^0 \Theta_1$, and

$$\operatorname{sgn}\left[(\partial \Theta_1/\partial z_2)_{z_1}\right] = -\operatorname{sgn}(w_{12}) . \quad (5.8)$$

In addition to the possibilities that the modifier activates or inhibits the flux for a given value of $w_{12}$, we have also seen in the BWA that the flux may respond biphasically to the modifier. Such behavior is also possible in the QCA for cases slightly more complicated than the one above.

One further matter deserves some mention. Just as the flux may show a biphasic response to the modifier, it may also respond biphasically to $z_\mathrm{s}$ or $z_\mathrm{p}$. Since at the stationary state $J$ = net rate of disappearance of s = net rate of production of p, if $\partial J/\partial z_\mathrm{s} < 0$ or $\partial J/\partial z_\mathrm{p} > 0$, the catalytic process is unstable. If the catalysis is then coupled appropriately to transport processes, the possibility of multiple stationary states or spatio—temporal patterns is raised. As noted in the introduction, both of these behaviors have been noted for the concerted model [8,9]. The multiple stationary states are particularly interesting since the concerted model does not allow a phase transition without transport processes.

It is also interesting that in both of these cases one of the assumptions (3) and (4) was relaxed. Gō and Anan [9] kept $k_{-\mathrm{p}}^0 z_\mathrm{p}$ nonzero to obtain the multiple stationary states, and in the model of glycolytic oscillations [8], the interactions were allowed to affect $k_\mathrm{p}(f_\mathrm{p} \neq 0)$. However, additional considerations were also made in both cases. Gō and Anan let $w_{12} \to \infty$. Since we have normalized the interaction energies to temperature, the prediction of multiple stationary states when $w_{12} \to \infty$ seems equivalent to that of a phase transition for the one-dimensional classical Ising model at zero temperature. For the glycolytic oscillations, sites which adsorb p but do not catalyze the reaction s → p were added to the concerted model. These sites function as modifier sites which activate the reaction to create the instability $\partial J/\partial z_\mathrm{p} > 0$. On the other hand, if one considers the complete quasi-equilibrium concerted model (4.21), it is straightforward to show that instabilities may exist without taking the limit $w_{12} \to \infty$ or adding extra sites. Thus, multiple stationary states are possible for the concerted model plus transport processes for nonzero temperatures. Although we have not carried out a complete analysis, it also seems likely that the concerted model without additional sites for adsorption of p could produce oscillations if the other necessary conditions [37] are met.

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## References

[1] E. Ising, Z. Physik 31 (1925) 253.
[2] W. Weidlich, Collective Phenomena 1 (1972) 51.
[3] C.J. Thompson, Biopolymers 6 (1968) 1101.
[4] D.E. Koshland, G. Némethy and D. Filmer, Biochemistry 5 (1966) 365.
[5] A.V. Hill, Biochem. J. 7 (1913) 471.
[6] J.F. Nagle and J.C. Bonner, Ann. Rev. Phys. Chem. 27 (1976) 291.
[7] J. Monod, J. Wyman and J.-P. Changeux, J. Mol. Biol. 12 (1965) 88.
[8] A. Goldbeter and R. Lefever, Biophys. J. 12 (1972) 1302.
[9] N. Gō and Y. Anan, J. Theor. Biol. 66 (1977) 475.
[10] R.J. Glauber, J. Math. Phys. 4 (1963) 294.
[11] R.T. Bush and C.J. Thompson, Biopolymers 10 (1971) 961.
[12] J.C. Kimball, J. Stat. Phys. 21 (1979) 289.
[13] T.L. Hill, Proc. Natl. Acad. Sci. (USA) 74 (1977) 3632.
[14] T.L. Hill, Proc. Natl. Acad. Sci. (USA) 74 (1977) 4111.
[15] T.L. Hill, Proc. Natl. Acad. Sci. (USA) 74 (1977) 5227.
[16] T.L. Hill, Free energy transduction in biology (Academic Press, New York, 1977).
[17] T.L. Hill and L. Stein, Proc. Natl. Acad. Sci. (USA) 76 (1979) 1014.
[18] T.L. Hill and L. Stein, Proc. Natl. Acad. Sci. (USA) 77 (1980) 693.
[19] T.L. Hill, Proc. Natl. Acad. Sci. (USA) 75 (1978) 1101.
[20] T.L. Hill and Y. Chen, Proc. Natl. Acad. Sci. (USA) 66 (1970) 607.
[21] T.L. Hill and Y. Chen, Biophys. J. 11 (1971) 685.
[22] T.L. Hill, Proc. Natl. Acad. Sci. (USA) 76 (1979) 714.
[23] W.L. Bragg and E.J. Williams, Proc. Roy. Soc. A 145 (1934) 699.
[24] H.A. Bethe, Proc. Roy. Soc. A 150 (1935) 552.
[25] E.A. Guggenheim, Proc. Roy. Soc. A 148 (1935) 304.
[26] T.L. Hill and L. Stein, J. Chem. Phys. 69 (1978) 1139.
[27] J. Schnakenberg, Rev. Mod. Phys. 48 (1976) 571.
[28] E.A. Guggenheim, Proc. Roy. Soc. A 169 (1938) 134.
[29] J.S. Shiner, Ph. D. Thesis, Medical College of Virginia (1978).
[30] C.J. Thompson, Mathematical statistical mechanics (MacMillan, New York, 1972).
[31] G. Némethy, in: Subunits in biological systems, part C, eds. S.N. Timasheff and G.D. Fasman (Marcel Dekker, New York, 1975) p. 1.
[32] T.L. Hill, J. Theor. Biol. 75 (1978) 391.
[33] T.L. Hill and Y. Chen, J. Chem. Phys. 69 (1978) 1126.
[34] T.L. Hill and Y. Chen, Proc. Natl. Acad. Sci. (USA) 75 (1978) 5260.
[35] T.L. Hill, personal communication.
[36] A. Goldbeter, J. Mol. Biol. 90 (1974) 185.
[37] B. Hess and T. Plesser, Annals New York Acad. Sci. 316 (1979) 203.