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The role of conformational energetic disorder in the catalytic activity of immobilized enzymes

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Abstract

We analyze cooperative behavior in a system of immobilized enzymes which incorporates the notion of heterogeneity or disorder in the interactions. In addition to equilibrium phase changes, this system exhibits vitrification or glass-like transitions in which the overall catalytic activity freezes into one of many possible states. It is shown that these long-lived metastable phases can be produced by a combination of disorder, systematic surface and intermolecular interactions, and chemical association effects such as ligand binding. Biophysical consequences of this frozen state include greatly diminished sensitivity of enzymatic activity to thermal and chemical perturbations. This effect coincides with the appearance of a multitude of possible macroscopic catalytic states rather than a single equilibrium state. Our analysis also suggests that high surface coverages will tend to be catalytically inactive if they are fully equilibrated; rather, high activity with high surface coverage is more likely to be associated with vitrified states of surface immobilization and deep or abrupt chemical quenches.

Keywords: Immobilized enzymes; Disorder; Catalytic activity

1. Introduction

Consider a monolayer of surface-immobilized enzymes in which each molecule is assumed to exist in a conformation which may be designated as either catalytically active or inactive $(+,-)$. Ascribe to each molecule a surface interaction energy which depends on its catalytic activity and introduce a nearest neighbor coupling which tends to stabilize 'like' $(+,+)$, $(-,-)$ as opposed to unlike $(+,-)$ configurations. We also allow for the binding of one solvated ligand to each enzyme, where the binding energy also depends on the catalytic state. Such a model was proposed and analyzed by Hill many years ago. Hill called attention to the close similarities between the effects of an external field, ferromagnetic coupling, and ligand binding. He noted that the ligand binding isotherm of this system exhibits a

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critical point and that external field and ligand association effects can trigger phase transitions which dramatically alter the relative populations of active and inactive enzymes [1]. Thus, Hill's analysis may be regarded as a mean-field description of the influence of external fields and chemical association on cooperative phenomena in biological membranes and surfaces [2–4].

An interesting proposition arises if one attempts to reconcile the conventional assumption of a single nearest neighbor coupling constant and a single surface interaction energy (for a given catalytic state) with the complexity of a system of immobilized biological molecules. It seems reasonable to imagine that a combination of surface immobilization and intermolecular interactions in a system whose subunits possess many internal degrees of freedom which relax on many time scales may lead to a situation in which (for conceptual and computational purposes) a portion of the coupling must be extracted from some appropriately constructed quenched random distribution. The general idea is that covalent linkages, hydrogen bonding, surface heterogeneity, as well as hydrophobic and charge-based interactions can lead to scenarios in which the degree of enzymatic activity [taken here to be $(+,-)$] is to be associated with several, and perhaps many, energetically distinct molecular conformations which do not readily interconvert. Formally speaking, the validity of this assumption depends on the coarse graining procedure used to infer enzymatic activity from a library of conformations. From a practical point of view, one should require that the catalytic conversion rate in the active state be much faster than both the active–inactive interconversion rate and the slowest, energetically significant conformational changes. Furthermore, these 'quasi-frozen' conformational states should possess lifetimes which are longer than the duration of a typical experimental probe.

Suppose one accepts the notion of intermolecular and surface interactions having a quenched or frozen random component in addition to annealed or fully equilibrated components. A question which naturally arises is whether these random interactions can manifest themselves at the macroscopic level; i.e. whether large scale cooperative phenomena such as abrupt changes in overall surface enzymatic activity, membrane permeability, surface potential, etc., can be influenced by sluggish internal dynamics at the macromolecular level. It turns out that a natural and efficient approach to this problem consists in establishing connections to the considerable body of literature on spin glasses. For instance, Wolynes et al. [5,6] have adopted such models to describe the statics and dynamics of protein folding, where a single protein molecule constitutes a thermodynamic system and the fundamental subunits are amino acid residues. Our approach follows that of Wolynes et al. in that we utilize an approximation to a binary interaction mean field theory which was proposed by Derrida [7,8]; i.e. the random energy model of disordered systems. Unlike mean field theories encountered in equilibrium statistical mechanics, mean field theories of spin glasses tend to be quite difficult from both computational and conceptual viewpoints [9]. The random energy model possesses the virtues of being exactly solvable and relatively conceptually simple while exhibiting many of the features found in the more complex systems [10–12]. A brief description of Derrida's model is given in the following section.

2. Frozen conformational disorder and the random energy model

Imagine that we have prepared a system which contains a given number of enzymes immobilized on a given surface area, where each molecule may reside in a catalytically active $(+)$, inactive $(-)$, or neutral state. Furthermore, this surface is in contact with a solution containing ligands whose binding to a neutral enzyme produces either $(+)$ or $(-)$ activity, depending on which of two possible sites is occupied. Associate a definite energy E with this thermodynamic system.

Now consider an ensemble of such systems in which the surface and intermolecular interactions contain random components such that the probability distribution for the entire ensemble

to have the set of system energies E_1, E_2, E_3... can be factorized as $\mathbf{P}(E_1, E_2, E_3,...) = \mathbf{P}(E_1)\mathbf{P}(E_2)\mathbf{P}(E_3)$.... The properties of a Hamiltonian which gives rise to this probability factorization have been discussed by Derrida. Broadly speaking, this is a random-coupling, many-body Hamiltonian with infinite range interactions in which the coupling constants are selected from a Gaussian distribution. The variance of this distribution characterizes the degree of quenched disorder at the molecular level. The main result of this construction is that the density of energy states for a given system $n(E)$ can be equated with its ensemble average $\langle n(E)\rangle$ provided E lies within certain bounds; i.e. $|E| < E_o$. Moreover, it can be shown that within this region $\langle n(E)\rangle$ is a Gaussian whose variance characterizes the degree of energetic disorder for the entire ensemble. If the system energy lies outside these bounds, the density of states is low (of order unity), $n(E)$ is no longer Gaussian or self-averaging, and each system collapses into a distinct configuration having vanishing entropy. This set of frozen metastable configurations is associated with a glassy phase of matter.

The conditions for which Derrida's many-body Hamiltonian is a reasonable approximation for a full or partial monolayer of immobilized enzymes deserves a few comments. For one, the phase diagram of this random energy model has the same qualitative features of an infinite range spin glass with only binary interactions, which in turn is believed to possess several key features of a spin glass restricted to short-range interactions. Also consider that even the binary interaction between neighboring enzymes will generally be affected by a surface conformation which may vary from one molecule to another. By treating the surface as a separate 'body', we are naturally led to consider effective three and four body interactions. Moreover, the many body nature and long range character associated with charge-based interactions such as ion association effects supports the notion that the random energy approximation provides a reasonable framework for a qualitative description of cooperative behavior in these complex systems.

3. Catalytic models with surface conformational disorder

For the purpose of notational simplicity the direct interaction between enzyme and surface is subsumed into the ligand binding energy. We will show how to re-establish this connection later in this section. Thus we consider an enzyme with two possible states for a bound ligand $(+,-)$ and a catalytic activity which depends on which state is occupied. This idealization of the catalytic activity follows from the notion that the active site of a typical immobilized enzyme may be readily accessible to the substrate or it may be sterically shielded because of orientational constraints at the surface or the proximity of other molecules. Occupation of one state precludes occupation of the other. In addition there exists a state having no ligand association to which we may ascribe any degree of catalytic activity. A membrane or surface is covered with a complete monolayer of such enzymes which interact with both ferromagnetic and random couplings. Furthermore, the surface is immersed in a solution containing dissolved ligand at a specified activity or concentration. We refer to this model as Case A.

A second model is introduced (Case B) which is identical to Case A except that the ferromagnetic and random interactions now occur between the partially adsorbed species so that the surface is now only partially covered by enzyme molecules. Thus the adsorbate is identified with an enzyme whose degree of surface catalytic activity $(+,-)$ and fractional coverage depends on the energetics of its surface immobilization. Cases A and B become formally identical as the fractional coverage approaches unity. Case B is schematically depicted in Fig. 1, in which surface conformational disorder is assumed to be the source of disorder in surface and intermolecular interactions. Within the framework of the random energy model, it will be shown that adsorption isotherms can saturate at a glass transition and that protein surface conformations can cooperatively freeze. One then associates these long-lived metastable states with varying degrees of frozen overall catalytic activity.

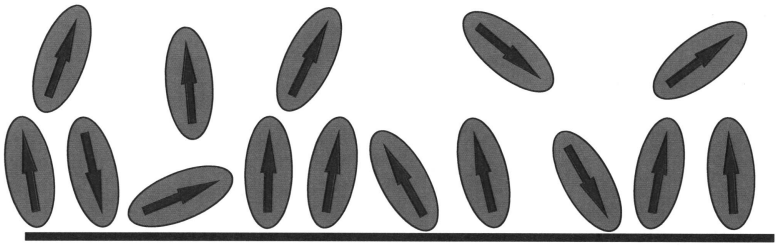

Fig. 1. A conformationally disordered array of surface-immobilized enzymes is depicted in equilibrium with solvated enzyme. The associated energetic disorder may be sufficient to drive the system into a 'glassy' state in which further adsorption from solution and surface conformational changes become impervious to thermal and chemical perturbations. Under these conditions the overall catalytic activity 'freezes' into a state which depends on the quench process.

It should be noted that there is considerable flexibility in the physical interpretation of these models. For instance, Case A could also correspond to a scenario in which the ligand is replaced by an enzyme which may be immobilized in one of two energetically distinct catalytic states, whereas the underlying systematic and quenched random interactions may be ascribed to a bare surface which contains defects, an underlying layer of a grafted biopolymer, or some other sufficiently complex surface-immobilized species. In fact, this interpretation may be of more physical relevance than our three-state catalytic activity model for a complete monolayer of enzymes. In this alternative picture one allows for the exchange of enzyme between bulk solution and some energetically heterogeneous surface which is not precisely characterized, and there is now no direct interaction between adsorbate molecules; rather, the binding and conformational ordering processes themselves can drive equilibrium and glassy phase transitions by perturbing the overall surface entropy and free energy.

4. Conformational energetic disorder and entropy collapse

The preceding discussion implies that in the self-averaging or equilibrium regime, the ensemble averaged density of energy states assumes the form

$$\langle n(E)\rangle = (\pi NJ^2)^{-1/2}\binom{N}{L}\binom{L}{L_+} \exp-\left(\frac{[E-\mu_+^o L_+ - \mu_-^o (L-L_+)]^2}{NJ^2}\right) \tag{4.1}$$

Here N is the total number of immobilized enzymes or surface sites, L is the total number of bound ligands, L_+ is the number of ligands bound to those enzymatic sites which promote catalytic activity, while μ_+^o and μ_-^o are the binding energies associated with active and inactive states, respectively. The width of this distribution, given by NJ^2, is the mean square fluctuation in the energy, and so the parameter J provides a measure of energetic disorder at the microscopic level. For clarity in presentation we omit for now a binary ferromagnetic coupling between enzymes. This interaction is included in a later section.

The characteristic thermodynamic function for this microcanonical ensemble [4] (each member possesses a definite energy E) is the entropy per site which in the thermodynamic limit may be written as

$$\frac{S}{N} = \frac{1}{N}\ln\langle n(E)\rangle = -(1-l)\ln(1-l) - (l-l_+)\ln(l-l_+) - l_+\ln l_+ - \frac{J^2}{4T^2} \tag{4.2}$$

with $l = L/N$, $l_+ = L_+/N$, the Boltzmann con-

stant is set equal to unity for notational simplicity, and where the temperature T is defined by

$$\frac{1}{T} = \left(\frac{\partial S}{\partial E}\right)_{N,l,l_+} \tag{4.3}$$

Analogous to the temperature, the ligand chemical potential is defined by

$$-\frac{\mu}{T} = \frac{1}{N}\left(\frac{\partial S}{\partial l}\right)_{E,l_+} = \ln\left[\frac{1-l}{l_-}\right] - \frac{\mu_-^o}{T}$$
$$= \ln\left[\frac{1-l}{l_+}\right] - \frac{\mu_+^o}{T} \quad l = l_+ + l_- \tag{4.4}$$

Also, in terms of l, l_+, and μ_+^o, μ_-^o, and the ligand chemical potential μ

$$\frac{S}{N} = -\ln(1-l) + \frac{\mu_-^o}{T}(l - l_+) + \frac{\mu_+^o l_+}{T}$$
$$- \frac{\mu l}{T} - \frac{J^2}{4T^2} \tag{4.5}$$

Note that the introduction of a set of frozen or quenched coupling constants is analogous to imposing constraints on the Hamiltonian of the system since in both instances the entropy is reduced. Nevertheless, it turns out that the overall free energy is also reduced and so the system is actually stabilized by quenched disorder. We speculate that this stabilization mechanism, which requires the presence of sufficient complexity along with certain very slow relaxation processes, may be a common feature of biophysical systems. It is worth noting here that very similar conjectures have been proposed by others with regard to such topics as neural network modelling, prebiotic evolution, the folding of proteins, and biomolecular recognition processes in general [6,12,13]. Eq. (4.4) immediately leads to an expression for the catalytic ratio

$$\frac{l_-}{l_+} = \exp\left[\frac{\mu_+^o - \mu_-^o}{T}\right] \tag{4.6}$$

In addition, the ligand binding isotherm takes the ideal form

$$\frac{l}{1-l} = \frac{\lambda(\lambda_+^o + \lambda_-^o)}{\lambda_+^o \lambda_-^o} \tag{4.7}$$

$$\lambda = \exp\frac{\mu}{T}, \quad \lambda_+^o = \exp\frac{\mu_+^o}{T}, \quad \lambda_-^o = \exp\frac{\mu_-^o}{T}$$

or equivalently

$$l = \frac{\lambda(\lambda_+^o + \lambda_-^o)}{\lambda_+^o \lambda_-^o + \lambda(\lambda_+^o + \lambda_-^o)} \tag{4.8}$$

So far the thermodynamic properties of this system are trivial. A non-trivial feature is introduced by the observation that the entropy, given by Eq. (4.2), vanishes at a critical temperature T_c:

$$T_c = \frac{J}{2}[-(1-l)\ln(1-l)$$
$$-(l-l_+)\ln(l-l_+) - l_+ \ln l_+]^{-1/2} \tag{4.9}$$

where l and l_+ are determined by Eqs. (4.4), (4.7) and (4.8).

T_c can also be regarded as a function of J, μ_+^o and μ_-^o. For instance, if the direct interactions μ_+^o and μ_-^o vanish, then T_c may be implicitly expressed in terms of the ligand chemical potential as

$$T_c = -\left[\frac{\mu l + [\mu^2 l^2 - J^2 \ln(1-l)]^{+1/2}}{2\ln(1-l)}\right] \tag{4.10}$$

$$l = \frac{2\lambda_c}{(1+2\lambda_c)}, \quad \lambda_c = \exp\left(\frac{\mu}{T_c}\right)$$

Therefore, this system possesses a critical or glass transition temperature for a macroscopic catalytic activity which depends on the ligand thermodynamic activity or concentration in the bulk solution. Note that in this weak surface interaction limit the critical temperature vanishes for a surface coverage above $l = 2/3$ provided the ligand chemical potential is positive (repulsive ligand–ligand interactions in the bulk). Under these conditions a glass transition becomes impossible and the surface can always be saturated with adsorbate; however, the absence of an energetic dif-

ference between the two binding states implies equal numbers of both surface conformers. Suppose we consider the parameter J to be a measure of the energetic heterogeneity per subunit. Since $T_c = \alpha J$ where α is of order unity, this implies that this system of immobilized enzymes will be near the glass transition temperature if only a few degrees of freedom per molecule are frozen. Furthermore, since enzyme folding energies are believed to be of the order T per residue [5,6], this implies that randomly quenching the native or non-native conformation of a single specific residue per molecule (the precise identities of these residues will vary from molecule to molecule and will generally depend on surface conformation) is sufficient to drive the entire macroscopic system into a glassy phase of catalytic activity. Biophysical consequences of this frozen state include greatly diminished sensitivity of enzymatic activity to thermal and chemical perturbations along with the appearance of a multitude of possible macroscopic catalytic states rather than a single equilibrium state.

Above T_c the Helmholtz free energy per site is given by

$$\frac{F}{N} = -\frac{J^2}{4T} + \mu_+^o l_+ + \mu_-^o (l - l_+) - T[-(1-l)\ln(1-l) - (l-l_+)\ln(l-l_+) - l_+ \ln l_+] \quad T > T_c \tag{4.11}$$

while below T_c the free energy reverts to the ground state energy

$$\frac{F}{N} = \frac{E_o}{N} = -\frac{J^2}{2T_c} + \mu_+^o l_{+,m} + \mu_-^o (l_m - l_{+,m}) \tag{4.12}$$

$$T < T_c, \quad l_m = l(\mu_m, T_c), \quad l_{+,m} = l_+(\mu_m, T_c)$$

Here μ_m is the chemical potential of bound ligand in the metastable phase. For a gradual quench, which might be achieved by the slow addtition of ligand to solution, μ_m may be equated to the chemical potential of adsorbate in solution at the critical temperature; i.e. $\mu_m = \mu_c$. Note that energetic disorder results in an overall increase in system stability through lowering the free energy per site by an amount of order J^2/T. At any rate, the jump in the heat capacity at T_c contains the term $J^2/2T_c^2$ as well as contributions from the temperature dependences of l and l_+ at T_c.

Below T_c the ligand binding isotherm and surface catalytic activity are frozen in a metastable state which depends on system history; i.e.

$$\frac{l_-}{l_+} = \exp\left[\frac{\mu_+^o - \mu_-^o}{T_c}\right] \tag{4.13}$$

$$\frac{l_m}{1-l_m} = \frac{\lambda_m(\lambda_{+,c}^o + \lambda_{-,c}^o)}{\lambda_{+,c}^o \lambda_{-,c}^o} \tag{4.14}$$

$$T < T_c, \quad \lambda_m = \exp\frac{\mu_m}{T_c}, \quad \lambda_{+,c}^o = \exp\frac{\mu_+^o}{T_c}, \quad \lambda_{-,c}^o = \exp\frac{\mu_-^o}{T_c}$$

One can think of this vitrified catalytic state as having been induced by ligand binding, the application of an external (electric) field, or some combination of these effects. This connection can be made explicit by restricting binding to a single site having a single binding energy, which may be regarded as a limit in which the catalytically active fraction is negligible compared to the inactive fraction. The energy per site now takes the form

$$\frac{E}{N} = \mu^o l - \frac{J^2}{2T}, \quad T > T_c \tag{4.15}$$

Define a polarization $m = 2l - 1$, a field strength $H = -\mu^o/2$ and a background energy $\varepsilon_o = \mu^o/2$. Eq. (4.15) can then be re-written as

$$\frac{E}{N} = -mH - \frac{J^2}{2T} + \varepsilon_o, \quad T > T_c \tag{4.16}$$

which essentially reproduces Derrida's [7,8] result for the random energy model of a spin glass in an external field.

Generally speaking, an increase in the energetic bias for ligand binding $|\mu_+^o - \mu_-^o|$ or an increase in the activity (concentration) of dissolved ligand tends to weakly increase T_c. This follows from a consideration of Eqs. (4.6), (4.7) and (4.9); the details of this analysis will appear in a future publication. This effect is analogous to the isothermal application of a magnetic field to a system of spins, which reduces the entropy via field-induced ordering [4]. In our case the configurational surface entropy associated with bound ligand molecules can be compensated by the entropy decrease associated with quenched energetic disorder in the intermolecular interactions between enzymes, which results in the overall collapse into a metastable catalytic state.

It should be noted that the behavior of a spin glass in an external magnetic field is a subtle issue which is not entirely resolved, at least from conceptual and computational viewpoints [11,12]. For instance, the preceding 'entropy collapse' arguement can be countered by the notion that an external field destroys the isotropy of the system, and that this symmetry reduction results in an equilibrium ferromagnetic phase rather than a glass. For the time being we adopt the view that the frozen dynamics associated with entropy collapse nullifies the symmetry breaking argument: the system is trapped in a metastable state and so it cannot escape into the equilibrium phase.

We now consider Case B in which the energetic disorder is associated with interactions among the adsorbed species at sub-monolayer coverages, where the adsorbate resides in one of two states of catalytic activity. The density of states now takes the form

$$\langle n(E)\rangle = (\pi L J^2)^{-1/2}\binom{N}{L}\binom{L}{L_+} \exp - \left(\frac{[E - \mu_+^o L_+ - \mu_-^o (L - L_+)]^2}{LJ^2}\right) \tag{4.17}$$

which simply amounts to replacing the variance NJ^2 in Eq. (4.1) by LJ^2. This should be a reasonable model for coverages that are not too low. The entropy per site has the same form as in Eq. (4.2) except that the term $J^2/4T^2$ is replaced by $lJ^2/4T^2$ $(0 < l < 1)$. The chemical potential assumes the form

$$-\frac{\mu}{T} = \ln\left[\frac{1-l}{l_-}\right] - \frac{\mu_-^o}{T} + \frac{J^2}{4T^2} = \ln\left[\frac{1-l}{l_+}\right] - \frac{\mu_+^o}{T} + \frac{J^2}{4T^2}, \quad T > T_c \tag{4.18}$$

and the adsorption isotherm may be written as

$$\frac{l}{1-l} = \frac{\lambda_J(\lambda_+^o + \lambda_-^o)}{\lambda_+^o \lambda_-^o} \tag{4.19}$$

$$\lambda_J = \exp\left[\frac{\mu}{T} + \frac{J^2}{4T^2}\right], \quad T > T_c \tag{4.20}$$

Note that, unlike Case A, the introduction of energetic disorder now results in enhanced surface adsorption. In addition, the critical temperatures for the two models are related by

$$T_c(\text{Case B}) = l^{1/2} T_c(\text{Case A}) \tag{4.21}$$

where T_c (Case A) is given by Eq. (4.9). Thus, other factors being equal, the glass transition temperature for this model is lower than that for Case A.

5. Disorder with biased intermolecular coupling

We now return to Case A and introduce a net ferromagnetic coupling [4,8,11] between enzymes which contain a bound ligand. Catalytically similar pairs $(+,+)$, $(-,-)$ are therefore energetically favored over dissimilar pairs $(+,-)$. Loosely speaking, this may be thought of as a ligand binding-induced interaction which tends to stabilize homo-dimers. The density of states is

now given by

$$\langle n(E)\rangle = (\pi NJ^2)^{-1/2}\binom{N}{L}\binom{L}{L_+}$$
$$\exp -\left(\frac{\left[E-\mu_+^o L_+ - \mu_-^o(L-L_+) + \frac{\gamma_+ L_+^2}{2N} + \frac{\gamma_-(L-L_+)^2}{2N}\right]^2}{NJ^2}\right) \tag{5.1}$$

where the coupling constants γ_+ and γ_- are to be considered as positive. The entropy has the same form as in Eq. (4.2), but ligand binding is now described by

$$\frac{l_-}{1-l} = \exp\left[\frac{\mu-\mu_-^o+\gamma_- l_-}{T}\right] \tag{5.2}$$

$$\frac{l_+}{1-l} = \exp\left[\frac{\mu-\mu_+^o+\gamma_+ l_+}{T}\right] \tag{5.3}$$

$$\frac{l_-}{l_+} = \exp\left[\frac{\mu_+^o-\mu_-^o+\gamma_- l_- - \gamma_+ l_+}{T}\right] \quad T > T_c \tag{5.4}$$

For a catalytic ratio of unity, the binding fraction l is given by the ratio of the direct interaction bias to coupling bias:

$$l = 2\left[\frac{\mu_-^o-\mu_+^o}{\gamma_- - \gamma_+}\right] \tag{5.5}$$

In Table 1 we exhibit the relation between binding fraction and critical temperature for the previously described Cases A and B with the constraint of unit catalytic ratio [see Eqs. (4.9) and (4.21)]. For Case A, T_c passes through a shallow minimum at $l = 2/3$ and diverges very weakly as l vanishes. For Case B, T_c/J decreases by a factor of 3 as the system passes from a full monolayer to 1% coverage.

Table 1
The relation between total surface coverage and glass transition temperature for a catalytic ratio of unity is shown[a]

l	T_c/J (Case A)	T_c/J (Case B)
1	0.60	0.60
2/3	0.48	0.39
1/2	0.49	0.35
1/3	0.54	0.31
0.1	0.80	0.25
0.05	1.04	0.23
0.01	1.99	0.20

[a] In Case A the energetic disorder may be associated with an enzyme monolayer at full coverage which can bind ligands, while for Case B the disorder is ascribed to a sub-monolayer of adsorbing enzymes.

In general we expect that equilibration dynamics; i.e. relaxation times, will be rather sensitive to the proximity of the system to T_c. Thus for Case A, equilibration, assuming perfect mixing of ligand in the bulk solution, should be fastest for intermediate coverages while for Case B relaxation rates should increase with decreasing coverage.

It is instructive to construct a phase diagram for this ferromagnetic coupling model which displays equilibrium regimes in addition to the glassy states. In particular, restriction to a single species and a vanishing surface interaction energy (this may be incorporated into the chemical potential) allows us to construct lines of critical points in the $T-\gamma$ plane, where γ is the intermolecular coupling parameter. Moreover, one of these lines is the locus of a set of equilibrium second-order phase transitions which is partially obscured by a glass transition, and it seems desirable to present a simple model which exhibits critical behavior for both equilibrium and metastable surface adsorption.

In order to deduce the equilibrium critical behavior we derive the surface pressure p from Eq. (5.1) with $L_+ = L$, $\gamma_- = 0$; μ_+^o, $\mu_-^o = 0$,

$$\frac{P}{T} = \left.\frac{\partial \ln\langle n(E)\rangle}{\partial N}\right|_{E,L} = -\ln(1-l) - \frac{\gamma l^2}{2T} + \frac{J^2}{4T^2} \tag{5.6}$$

The equilibrium critical line for this system is then determined by the divergence of the surface compressibility [4], or equivalently, by

$$\text{(a)}\quad \left(\frac{\partial P/T}{\partial l}\right)_T = \left[\frac{1}{1-l} - \frac{\gamma l}{T}\right]_{l=1/2} = 0 \qquad (5.7)$$

$$\text{(b)}\quad N\left(\frac{\partial P}{\partial N}\right)_{L,T} = \left[T - \frac{T}{1-l} + \gamma l^2\right]_{l=1/2} = 0$$

which gives $T_c(\text{eq.}) = \gamma/4$.

The transition temperature to the 'glassy disordered phase' GD is obtained from Eq. (4.9) with $l_+ = l = 1/2$; i.e. $T_c = J/[2(\ln 2)^{1/2}]$.

Also from Eqs. (5.2), (5.3) and (5.4) with $l_+ = l$ we have T_c implicitly determined by

$$\frac{l}{1-l} = \exp\left[\frac{\mu + \gamma l}{T_c}\right] \qquad (5.8)$$

In order to obtain the boundary separating the equilibrium ordered phase (EO, with $l > 1/2$) from the glassy ordered phase (GO), one must solve Eq. (5.8) for l, substitute the result into Eq. (4.9) with $l_+ = l$, and then solve the resulting equation for $T_c(\gamma,\mu)$. The outcome is that T_c increases very slowly with increasing γ, provided that $\mu > -\gamma/2$. The phase diagram for this adsorption model is shown in Fig. 2. Note that there are two equilibrium phases and two glassy phases. Below the line $T_c(\text{eq.}) = \gamma/4$, the system can undergo a first order equilibrium transition with a jump discontinuity in the surface coverage l. At still lower temperatures the surface coverage freezes into a metastable state, the adsorption isotherm becomes flat (apparent surface saturation), and the system exhibits hysteresis. The sluggish dynamics associated with the proximity of the equilibrium critical state to the glassy state implies that much of the EO phase in Fig. 2 may not be dynamically accessible; i.e. the system appears to be trapped in the vicinity of its critical point. This implies that near-monolayer equilibrium coverages on energetically disordered surfaces can occur only with relatively large values of γ. Insofar as strong surface-induced interactions are generally associated with a degradation in enzymatic activity, one can infer that high surface coverages will tend to be inactive if they are fully equilibrated. Our analysis suggests that high ac-

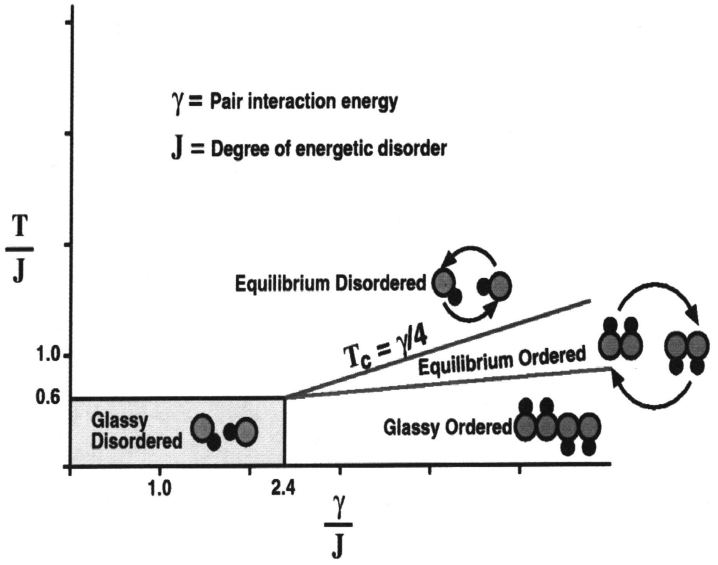

Fig. 2. This phase diagram describes various states that proteins from solution can assume at surfaces. Associations between molecules at surfaces can limit their conformational degrees of freedom. These effects can give rise to highly-ordered or disordered molecular ensembles that exist either in equilibrium or frozen in a metastable or 'glassy' condition. Glassy ensembles can arise from a thermal or chemical quench; they evolve so slowly that they appear to be static.

tivity with high surface coverage is more likely to be associated with vitrified states of surface immobilization and deep or abrupt chemical quenches; in addition, this activity should vary from sample-to-sample.

An interesting property of this ferromagnetic coupling model is that two chemical susceptibilities may be defined for the catalytic ratio l_-/l_+. Accordingly we write Eq. (5.4) as

$$\frac{l_-}{l_+} = \exp\left[\frac{\Delta\mu^o + \gamma(l_- - l_+)}{T}\right] \tag{5.9}$$

$$\Delta\mu^o = \mu^o_+ - \mu^o_-, \quad \gamma_- = \gamma_+ = \gamma$$

The derivative of this ratio with respect to the direct interaction bias yields the linear susceptibility

$$\chi^o = \lim_{\substack{\Delta\mu^o \to 0 \\ \gamma \to 0}} \frac{\partial(l_-/l_+)}{\partial\Delta\mu^o} = \frac{1}{T}, \quad T > T_c \tag{5.10}$$

$$\chi^o = \frac{1}{T_c}, \quad T < T_c$$

whereas the derivative with respect to the intermolecular coupling produces

$$\xi^o = \lim_{\substack{\gamma \to 0 \\ \Delta\mu^o \to 0}} \frac{\partial(l_-/l_+)}{\partial\gamma} = \frac{l_- - l_+}{T}, \quad T > T_c \tag{5.11}$$

$$\xi^o = \frac{l_- - l_+}{T_c} = \frac{l_{-,c} - l_{+,c}}{T_c}, \quad T < T_c \tag{5.12}$$

χ^o is the chemical analogue of the magnetic susceptibility of a spin glass. Note that ξ^o is sensitive to the degree of equilibrated surface conformational ordering of adsorbate above T_c and depends on the extent of frozen conformational order at the glass transition below T_c. In both cases the susceptibility in the frozen state is smaller than the hypothetical equilibrium susceptibility, which assumes the well-known Curie–Weiss form [4] for this random-energy model. Also, these chemical susceptibilities are not self-averaging below the glass transition; there can be significant variations among macroscopic samples of chemically identical systems.

6. Discussion

So far we have restricted ourselves to the static, quasi-thermodynamic behavior of immobilized enzymes. Although a scrupulous discussion of relaxation dynamics is outside the scope of this presentation, it seems appropriate to offer a few comments which are directly related to the previous development. If we adopt the so-called Adams–Gibbs theory [14] of glassy relaxation in polymeric liquids, then our calculation of the configurational entropy given by Eq. (4.2) provides us with a measure of the average timescale associated with the equilibration of surface catalytic activity. This average relaxation time may be expressed as

$$\tau = \tau_o \exp\left[\frac{a}{T(S/N)}\right] \tag{6.1}$$

where the parameter 'a' is proportional to the height of the potential energy barrier hindering rearrangement and τ_o is a constant. It turns out that the temperature dependence of τ has a form similar to the famous Vogel–Fulcher equation, which is widely used to correlate relaxation data for a variety of glass-forming liquids [14,15].

Another point is that the surface entropy density is equal to the negative of the variation of surface tension with respect to temperature; i.e. [16]

$$\left(\frac{\partial S}{\partial N}\right)_{T,V,L} = -\left(\frac{\partial\sigma}{\partial T}\right)_{V,N,L} \tag{6.2}$$

Here V is the volume of the bulk system in contact with a surface consisting of N lattice sites of unit area and L represents the number of various species of ligands. In our model the configurational surface entropy vanishes below the glass transition so that a portion of the interfacial tension becomes insensitive to temperature for these partially vitrified surfaces.

Note that Case A obeys Fermi–Dirac (FD) statistics since it allows for the binding of only one ligand per enzyme. For the opposite case of multi-valent or non-specific chemical association, one might ideally suppose that no restrictions are imposed on the number of 'ligands' which interact with an immobilized enzyme. Not surprisingly, this Bose–Einstein (BE) binding model differs significantly from FD statistics [4]. If we restrict ourselves to the ideal example of one species with a single direct interaction energy μ_o, then the binding isotherms for the two cases may be succinctly written as

$$l = \frac{Y}{1 \pm Y}, \quad Y = \exp\left[\frac{\mu - \mu^o}{T}\right] \tag{6.3}$$

Plus Sign FD $0 < l < 1$

Minus Sign BE $0 < l < \infty$

$T > T_c$

When $Y > 1$ (at sufficiently high bulk concentration) the BE system exhibits a binding instability, and it might be worthwhile to investigate the effects of ligand–ligand interactions along with a possible glass transition on this instability.

A legitimate question at this point is whether it is possible to use this random energy description as a basis for the systematic construction of more realistic spin glass models. While Derrida et al. [10] have reported some progress in introducing correlations between energy states, it appears that dramatic improvements to the original model have yet to be realized. From a formal viewpoint, a deeper exploration of the effects of energetic correlations would be most welcome.

At this point we note that any 'average molecular field' description raises the question of which features are realistic and which are artifacts of the implicit assumption of infinite range interactions or infinite system dimensionality. For example, mean field theories of Ising-like models are reliable for many purposes; however, they are known to predict artificial thermodynamic discontinuities in the presence of an external field. Furthermore, domain size and structure cannot be reliably deduced from such models. The question of whether abrupt spin glass transitions persist in the presence of ferromagnetic coupling or an external field has apparantly not been fully resolved. Nevertheless, we anticipate that these mean field predictions of the conditions necessary for the onset of sluggish dynamics (i.e. vanishing of the configurational entropy) and the breaking of ergodicity are at least qualitatively valid.

As a final point, it should be noted that spin glass models in general may have relevance to some long-standing mysteries of protein crystallization. Indeed, it is tempting to speculate that a glassy protein 'crystal' with a high degree of intermolecular conformational ordering (glassy ordered phase) diffracts in much the same way as its equilibrium crystalline counterpart. We expect that quenched conformational disorder among certain residues will manifest itself as anomalously large apparent Debye–Waller factors which are relatively insensitive to temperature and chemical perturbations. It might prove worthwhile to attempt to construct analogies between the phase diagrams of multi-component spin glass models and real protein 'crystal' systems.

7. Conclusion

We have demonstrated how a relatively simple model of a spin glass system may be adapted to describe the catalytic activity of a monolayer of surface-immobilized enzymes. The key idea is that quenched or frozen energetic disorder in the surface and intermolecular interactions can, under rather general and easily realized physico-chemical conditions, lead to a situation in which surface conformation transition dynamics is completely suppressed; i.e. individual molecules are unable to switch back and forth from catalytically active to inactive configurations because of entropic frustration. A particularly appealing feature of this model is that it is couched in the language of equilibrium statistical mechanics along with a single disorder parameter, and this should facilitate comparisons with the behavior of real systems. A novel prediction which has no

counterpart in the spin glass literature is the abrupt onset of sluggish metastable partitioning of a solute between bulk solution and a sufficiently disordered surface, which results in frozen adsorption isotherms. Another interesting feature is the appearance of a glassy though conformationally ordered phase which may have implications for the crystallization of proteins and for molecular self-assembly in general.

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