Fractal mechanisms for the allosteric effects of proteins and enzymes

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ABSTRACT Investigations have been made on the reaction kinetics of proteins and enzymes by the statistical methods of random walks on fractal structures. From the point of view of networks of Trap (R) and Free (T) sites, the relationships between the Hill coefficients (h) and the fractal (d_f) as well as spectral (d_s) dimensions of protein molecules are obtained. For example, $h = (1 + 2/d_f)$ for the one-step conformational changes and $h_b = (2 + 2/d_s)$ for the multistep conformational changes, respectively. In comparison with that of the literature, the theoretical value is reasonable, thus suggesting a new mechanism for the allosteric effects of proteins and enzymes.

INTRODUCTION

For many years, it has been known that the binding of oxygen to haemoglobin follows a sigmoid curve which differs appreciably from the typical Mechaelis-Menten equation covering the same concentration range. This remarkable phenomenon has aroused much interest because it cannot be interpreted in terms of the classical theories of enzyme action. As is well known, haemoglobin is not an enzyme but plays the role of transporting oxygen. The study of the binding of oxygen to it has contributed much to the understanding of allosteric effect and cooperativity. In 1910, Hill proposed the following equation, which is now commonly known as the Hill equation, to account for the oxygen-binding curves that he and others had observed from haemoglobin

$$Y = K_h[S]^h/(1 + K_h[S]^h), \quad (1)$$

where $[S]$ is the concentration of substrate or the partial pressure of oxygen and k_h is a constant. The exponent h is now commonly known as the Hill coefficient and Y is the fractional saturation, which is defined as follows:

$$Y = \frac{\text{number of occupied binding sites}}{\text{total number of binding sites}} = \frac{N(t)}{N_0}. \quad (2)$$

Eq. 1 is purely empirical, of which the theoretical background is not clearly understood. It has been thought that the Hill coefficient measures the cooperativity of the enzyme subunits or the number of substrates needed to bind thereon, i.e., in a sense, it implies the order of the reaction. The h is widely used as an index of cooperativity and the degree of cooperativity is considered to increase, as h increases, at the upper limit h is equal to the number of binding sites (No). If $h = 1$, there is no cooperativity; if $h > 1$, there is positive cooperativity; if $h < 1$, there is negative cooperativity. The property of responding with exceptional sensitivity to changes in metabolite concentrations is commonly dubbed as cooperativity. The allosteric effect implies the phenomenon of conversion of protein conformation from one state to another. Many allosteric enzymes are also cooperative, and vice versa, though allosteric effect and cooperativity are related to the change of protein conformation, this does not mean that the two concepts are interchangeable. In fact, they describe two different properties and should be clearly distinguished. The main purpose of this paper is to discuss the allosteric effects.

In general, the Hill coefficient h is equivalent to the varieties of the distribution of liganded species. However, as Monod et al. (1) pointed out, the h is not the number of interacting sites, but an interaction coefficient. Under certain conditions, the h can be interpreted as a measure of the free energy of interaction between sites. (1*a*) To clarify the nature of allosteric effect and the physical basis of the Hill coefficient, the well-known MWC and KNF models are proposed by Monod et al. (1) and Koshland et al. (2), respectively. The former is the so-called Monod-Wyman-Changeux concerted mechanism, which assumed that the quaternary structure of the protein is always symmetrical, and the protein exists in equilibrium of two states T (tense) and R (relaxed). Wherein the T state has a lower affinity for ligands. The latter is the so-called Koshland-Nemethy-Filmer sequential model, which, instead of the assumption of symmetry, assumes that the progress from T to the ligand-bound R state is a sequential process. The conformation of each subunit changes alternatively as it binds to the ligands and there is no abrupt switch from one state to another. The MWC model uses a quaternary structural change whereas the

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KNF model uses a series of tertiary structural changes. The KNF model is at the expense of simplicity, more general, and probably a better description of some proteins than the MWC model. In return, the explanation of phenomena is often somewhat more complicated. Recently, Schweitzer-Stenner and Dreybrodt (3) brought forth an extended MWC model, the mathematical basis of which had been formulated by Herzfeld and Stanley. Such a model was used to fit oxygen and carbonmonoxide binding curves of hemoglobin trout IV measured at different pH-values between 8.0 and 6.0. It has been shown that the interaction between the quaternary T $\rightarrow$ R and tertiary t $\rightarrow$ r-transition is different from the α and β subunits.

The experimental value of h, often nonintegral, is rarely equal to the number of ligand-binding sites on each molecule of protein. In general, the values of h range from 1 to 3.2 (4). Why? The nonintegral phemonena could not be interpreted by the MWC and KNF models. The goal of science is to understand why things are the way they are. In the present paper, we wish to submit and discuss a new mechanism, fractal kinetics (5), for the interesting phenomenon in terms of fractal theory.

FRACTAL THEORY

A brief introduction to fractal theory is to be presented in this section because this theory is generally unfamiliar to biochemists and biophysicists.

Fractal theory (6) is new mathematical theory that has been developed rapidly in this decade. A fractal implies a complex pattern with self-similarity or dilation and self-affinity (7), e.g., the shape of cloud in the sky or the coastline on the map and elastic vibrations, etc. This theory provides means to extract a rule or regularity hidden in the irregular forms and complex systems. The fractal structures are described by (at least) three dimensions: d, the dimension of the embedding Euclidean space; d_f, the fractal dimension; and d_s the spectral (fracton) dimension (8). For Euclidean spaces, these three dimensions are equal. The spectral dimension d_s is defined by

$$\rho(\omega) \propto \omega^{d_s-1} \tag{3a}$$

or

$$N_t \propto t^{d_s/2}, \tag{3b}$$

where ω is the frequency, $\rho(\omega)$ is the density of states, and N_t is the number of distinct sites in the fractal visited by a random walker up to time t. The spectral dimension d_s differs in general from fractal dimension d_f because the d_s reflects the topological structure properties of the fractal, and d_f reflects the geometrical structure of the fractal. For example, let's take the Sierpinski gasket (9) in d-dimensional Euclidean space. Its fractal dimension is easily found as $d_f = \ln(d+1)/\ln 2$ and the spectral dimension $d_s = 2\ln(d+1)/\ln(d+3)$. It can be readily shown that d_s is related to d_f by

$$d_s = 2d_f/d_w, \tag{4}$$

where d_w is the exponent connecting the root-mean-square displacement R_w of the random walker on the fractal with the number of steps N_w, $R^{dw} \propto N_w$, where d_w is called the fractal dimension of the walk, similar to d_w. Furthermore many other fractal dimensions may be defined.

Currently, several lines of evidence suggest that both the structure and dynamics of proteins and enzymes are fractal. Stapleton and co-worker (10–13) found that the geometry of the carbon backbone determined from x-ray diffraction data and the vibrational dynamics of proteins as measured by Raman scattering are both fractals. Measurements on 70 proteins showed that the fractal dimension determined from the structure correlates elegantly with that determined from the dynamics. Experimental evidences indicate that each substate of the protein has in itself a large number of substates, and the potential energy function is statistically self-similar, having the same form on many different scales. Lewis and Rees (14) found the surfaces of proteins are fractals. Liebovitch and co-workers (15–17) proposed a fractal model of ion channel kinetics. The model is more consistent with the conformational dynamics of proteins. In addition, for the conformational motion of protein and the substrate-enzyme reactions based on random walks in nonintegral dimensions, many outstanding theoretical and experimental works in this respect have been done in recent years (18–22). Dewey and Datta (23) determined the fractal dimension of membrane protein aggregates using fluorescence energy transfer method. This technique provides a means of assessing the nature of protein-protein interactions in membranous systems. The purpose of this paper is to extend the kinetics of allosteric protein and enzyme to fractal and to suggest a plausible physical model for the allosteric effect. We hope that the Hill coefficient h can be theoretically calculated by using the fractal theory.

MODELS AND METHODS

To study the allosteric effects of proteins and enzymes, and the implications of the Hill coefficient, Eq. 1 is rearranged to give $Y/(1-Y) = K_h[S]^h$ and assuming $R = Y/(1-Y)$ we have

$$R = K_h[S]^h, \tag{5}$$

where R is the total reaction rate and h denotes the reaction order. As will be shown later, the exponent h is the Hill coefficient relevant to the fractal dimension of proteins and conformation.

In this paper, we attempt to propose a fractal mechanism for the allosteric effect of a protein. Before describing the model, we first define the terminology to be used as follows:

(*a*) The excited molecule: the one with sufficient energy leading to effective collision.

(*b*) The random walker: chaotic motion without limitation.

(*c*) One-step conformational change: there is no transient state from one state to another.

(*d*) Multi-step conformational change: there are subsequent middle states from one state to another.

The model is described by the following statements:

(*a*) The protein and enzyme are fractal objects.

(*b*) The protein may exist in two conformational states, T and R. In the absence of ligands, the protein exists in conformation T, and R in negligible amounts.

(*c*) The R state has a high affinity for ligands, i.e., substrate molecules S and may be viewed as the active site.

(*d*) Whether the transition from T state to R state is a one or multistep conformational change depends on the external condition and the property or variety of the attacking excited molecule.

(*e*) The annihilation of an excited molecule means the birth of an R state and the association between the R state and substrate S with rapidity and easiness.

(*f*) The R state and T state may be regarded as Trap site and Free site for the substrate S, respectively. While the R and T are randomly distributed on or in the surface of enzyme or protein, forming random fractal networks consisted of Trap and Free sites (24–25).

(*g*) The diffusion of substrate molecules on the protein may be regarded as random walker until it hits the active site.

The present model embodies the idea of the MWC and KNF models, and fractal theory, which postulates that the binding of a substrate to an enzyme may cause conformational changes that align the catalytic groups in their correct orientations. Using these assumptions, it is possible to describe the fractal mechanism of allosteric effect of protein and reveal the meanings of the Hill coefficient.

(*a*) Fractal chemical kinetics

We are now in a position to present a general theory for studying the chemical kinetics on fractal structure, with particular emphasis on the relationship between the reaction order (X) and the fractal dimension for the heterogeneous reaction. For a single-reactant bimolecular reaction,

$$A + A \rightarrow \text{products} \qquad \text{(I)}$$

as well as for a two reactant bimolecular reaction.

$$A + B \rightarrow \text{products} \qquad \text{(II)}$$

One has second-order reaction rates for the classical or homogeneous, that is, all the concentration dependence of the reaction can be expressed as follows

$$\text{Rate} = -\frac{d[A]}{dt} = -\frac{d[B]}{dt} = K[A]^2 = K[A][B], \qquad (6)$$

where $[A]$ and $[B]$ are the reactant concentrations (or density) of A and B, respectively, K is the rate constant. Note that K is independent of time. If $[A] = [B]$, the solution (the integrated rate equation) of Eq. 6 is given by

$$[A]^{-1} - [A_0]^{-1} = Kt, \qquad (7)$$

where $[A_0]$ as the initial concentration (at $t = 0$). However, for the heterogeneous chemical reaction, the reaction rate is given by

$$\text{Rate} = \frac{d[A]}{dt} = K[A]^x (x > 2), \qquad (8)$$

where the power (order) X may be much larger than 2. It seems necessary to present here a brief argument, relating X to the fractal dimension of random walks in a fractal structure. The mean number N (the number of excited molecules) of distinct sites visited by reactant molecule (random walker) is given by (9)

$$N \propto t^{ds/2} (t \rightarrow \infty). \qquad (9)$$

Obviously, the formula is the same as Eq. 3*b*. Here ds is the spectral dimension. It has been shown that the rate for the simple binary reaction I and II, is (26)

$$\text{Rate} = -\frac{d[A]}{dt} = K_0 \frac{dN}{dt} \cdot [A]^2 = K_0 t^{(ds/2-1)} [A]^2. \qquad (10)$$

This has been verified by extensive simulations (27). The integrated rate equation, obtained from Eq. 10, is

$$[A]^{-1} - [A_0]^{-1} = K_0 (2/ds) t^{ds/2}, \qquad (11)$$

where $[A_0] = [A]$ $(t \equiv 0)$. Hence

$$t = (K_0^{-1} ds/2)^{2/ds} [A]^{-2/ds} (t \rightarrow \infty). \qquad (12)$$

A simple substitution of Eq. 12 into Eq. 10 gives

$$\text{Rate} = K[A]^{(1+2/ds)} (t \rightarrow \infty), \qquad (13)$$

where $K \equiv K_0^{2/ds} (ds/2)^{(1-2/ds)}$. An elementary reaction order X is given by

$$X = 1 + (2/ds), \tag{14}$$

thus relating X to the effective spectral dimension of the medium. Obviously, for the case of $ds = 2$, the classical result $X = 2$, is regained. For the Sierpinski gasket $ds = 1.36$, then $X = 2.47$; whereas for the percolating cluster $ds = 1.33$, then $X = 2.50$. The above new approach to low-dimensional chemical kinetics might reveal a new insight to the heterogeneous reactions of technological and biological importance.

(b) One-step conformational change

In this case we suppose that an excited substrate molecule S^* is directly bound to the R-site, which is randomly distributed on the protein. The reaction processes are two-step elementary bimolecules, as follows

$$E_T + S^* \overset{k_1}{\rightleftharpoons} E_R \cdot S \tag{III}$$

$$E_R \cdot S + S^* \overset{k_2}{\rightleftharpoons} [S \cdot E_R \cdot S] \xrightarrow{k_P} P + E_T, \tag{IV}$$

where k_1, k_2, and k_P are rate constants. We must first find the survival probability $p(k; t)$ of the excited S^*, and then calculate the reaction rate. We start our considerations by assuming that the R-site and the S^* are embedded in a fractal. For a fixed protein configuration K, and a certain time t the $p(k; t)$ of the excited S^* (assumed at the origin) is exponential

$$p(k; t) = \prod_{j \in k} \exp[-t\phi(R_j)], \tag{15}$$

where $\phi(R_j)$ denotes the transfer rate to an R-site at position R_j and the product extends over all R-sites of a protein. The $\phi(R_j)$ is defined by

$$\phi(R_j) = a_m R_j^{-n}, \tag{16}$$

where a_m and n are polar interaction constants. The quantity of experimental interest is not $p(k; t)$ but its ensemble average over all possible configurations of the protein molecules distributed on the fractal, $P(t)$, that is, $P(t) = \langle p(k; t) \rangle$. If the R-sites are randomly occupied by the S^* with probability Ψ, we obtain from Eq. 15

$$P(t) = \prod_i \{1 - \Psi + \Psi \exp[-t\phi(R_j)]\}. \tag{17}$$

Here the product extends over all sites of the fractal structure with the exception of the R-sites. This means that the ensemble average reproduces the details of the structure, and in the process of the direct transfer the whole fractal structure is being sampled.

For low density of the R-sites, $\Psi \ll 1$, an approximate form to $P(t)$ can be derived from Eq. 17. Distinct from Eq. 15 this form does not depend any more on the position of the substrate molecule. In the continuous description we obtain

$$P(t) \approx \exp(-\Psi \int dR\, \rho(R)\{1 - \exp[t\phi(R)]\}), \tag{18}$$

where $\rho(R)$ is the density of the R-sites on the fractal structure. Because protein is a fractal object, we then have

$$\rho(R) = \rho_0 R^{d_f - d}, \tag{19}$$

where ρ_0 is a proportionality constant. Eq. 18 for isotropic interactions is then $\phi(R)$,

$$P(t) \approx \exp(-\Psi \rho_0 d V_d \int dR\, R^{d_f - d}\{1 - \exp[-t\phi(R)]\}), \tag{20}$$

where V_d is the volume of the d-dimensional unit sphere, inserting Eq. 16 into Eq. 20, we can obtain

$$P(t) = \exp(-\Psi A t^{d_f/n}), \tag{21}$$

where A is independent of time. Eq. 21 is an extension of a known result for Euclidean dimension d to fractal dimension d_f. Eq. 21 is directly verified by the experimental work on the closed-open transition in ion channel protein (15–17). Based on the assumptions involved in the model of the present paper, the rate of the conformational change is given by (15, 28, 29)

$$r = -[dP(t)/P(t)]/dt = -d \ln P(t)/dt$$
$$= \Psi A (d_f/n) t^{(d_f/n - 1)}. \tag{22}$$

For reactions III and IV, the total rate R is given by [26]

$$R = -d[S^*]/dt = k_0 r [S^*]^2 = k' [S]^h, \tag{23}$$

where $[S]$ is the concentration of the reactant S, k_0, and k' are the rate constants. Inserting Eqs. 21 and 22 into Eq. 23 gives the integrated rate equation as follows

$$[S]^{-1} - [S]_0^{-1} = k_0 \Psi A t^{d_f/n}, \tag{24}$$

where $[S]_0 = [S]$ $(t = 0)$. Hence

$$t \propto [S]^{-n/d_f}. \tag{25}$$

A simple substitution of Eq. 25 into Eq. 23 gives:

$$R \propto [S]^{(1 + n/d_f)} \tag{26}$$

and comparing with Eq. 5, we obtain

$$h = (1 + n/d_f). \tag{27}$$

This form indicates the relation between the Hill coefficient h and the fractal dimension d_f. In fact, $n = 6$ for the multipolar interaction, and $n = 2$ for the elementary

reaction III according to our computer simulation results. Thus, when $n = 2$, the $h = 1.66\sim3$, because $1 < d_f < 3$. In general, $d_f = 1.33\sim1.66$ for a fractal structure in two-dimensional space (7), then $h = 2.50\sim2.20$, e.g., the Sierpinski gasket ($d = 2$), $d_f = 1.585$, $h = 2.26$.

(c) Multi-step conformational change

A different mechanism for reactions III and IV is the multistep process, where the excitation of a reactant molecule migrates among the fractal sites until encountering an active site of protein. The active sites (R-sites) are randomly distributed on the protein structure with probability Ψ. The microscopic transfer rates from a site to its neighboring sites are assumed to be equal. The above processes may be regarded as random walks on fractal structure. The walker gets trapped at the first encounter of a trap.

For a particular realization of the random walk on the trap-free fractal structure, let R_n denote the number of distinct sites visited in n steps. Here the stochastic variable R_n depends both on the starting point on the fractal and the sequence of directions of steps. Let P_n denote the probability that trapping site has not been occuppied up to the nth step in the ensemble of fractal structure with traps. The measurable survival probability of an excited substrate molecule is given by

$$P_n = \langle (1 - \Psi)^{R_n - 1} \rangle. \tag{28}$$

Note, the average in Eq. 28 also includes the average over starting points, and may be viewed as a double average. Introducing $\lambda = \ln(1 - \Psi)$, Eq. 28 allows a straightforward cumulant expansion

$$P_n = e^{\lambda} \tilde{P}_n, \tag{29}$$

where

$$\tilde{P}_n = \exp\left[\sum_{j=1}^{\infty} k_{j,n}(-\lambda)^j/j!\right] \tag{30}$$

the $k_{j,n}$ are the cumulants of the distribution of R_n. As an example, the first two cumulants are

$$k_{1,n} = \langle R_n \rangle \equiv S_n \tag{31a}$$

$$k_{2,n} = \langle R_n^2 \rangle - \langle R_n \rangle^2 \equiv \sigma_n^2, \tag{31b}$$

where S_n and σ_n^2 are the mean and the variance of R_n. In general, for the first cumulant,

$$\tilde{P}_n = \exp(-\lambda S_n). \tag{32}$$

This equation has been derived in many areas (24, 30) and it corresponds to the first-passage time approximation (25) in the fractal field. Introducing the second cumulant, we can obtain from Eq. 30, of the form

$$P_n = \exp(-\lambda S_n + \lambda^2 \sigma_n^2/2). \tag{33}$$

Translating the number of steps into time, and

$$S(t) = at^{d_s/2} \tag{34}$$

$$\sigma^2(t) = bt^{ds}. \tag{35}$$

We have the following results

$$\tilde{P}(t) = \exp(-\lambda a t^{d_s/2}) \tag{36}$$

for the first-passage time limit and

$$\tilde{P}(t) = \exp[-\lambda a t^{d_s/2} + (\lambda^2/2) b t^{d_s}] \tag{37}$$

for the first correction, where a and b are constants. Clearly, the survival probability is dominated by the spectral dimension ds. For the short-time behavior, the expansions of $P(t)$ are

$$P(t) \propto \exp(-c\lambda t^{d_s/2}) \quad (d_s < 2) \tag{38}$$

and

$$P(t) \propto \exp(-c'\lambda t) \quad (d_s > 2), \tag{39}$$

where c and c' denote numerical factors. These results are valid at short time and all concentrations of traps. Taking into account the fluctuations of the trap density, it is clear that reactant molecule survival for a long time will occur only in sufficiently large trap-free regions. These regions are rare, but govern the limit of large t. The result is given by

$$P(t) \approx \exp[-a\Psi^y t^x], \tag{40}$$

where $x = ds/(ds + 2)$ and $y = 1 - x = 2/(ds + 2)$.

Eq. 40 is a generalization of the long-time survival probability Euclidean space, it reduces to the Euclidean result by replacing ds by d. Now, we propose a scaling law for the survival probability $P_n(t)$ in moderate time regime, which interpolates between the two time regimes. Using Eqs. 29, 30, 37, and an extension of Eq. 40 to all Ψ, we obtain the following expression

$$P_n = e^{\lambda} \exp[-g(z)], \tag{41}$$

where $g(z)$ is a universal function which reduces in limiting cases to

$$g(z) \sim \begin{cases} z & (z \ll 1) \quad (42a) \\ z^{2/(ds+2)} & (z \gg 1) \quad (42b) \end{cases}$$

and $z = \lambda t^{d_s/2}$, let us return to the calculation of the Hill coefficient. The relationships between spectral dimension

and Hill coefficient are derived from Eqs. 42*a* and 42*b* by the similar manner, as previously employed in deriving Eqs. 22 and 23 the results are

$$h_a = 1 + 2/d_s \quad (z \ll 1) \tag{43a}$$

$$h_b = 2 + 2/d_s \quad (z \gg 1). \tag{43b}$$

Based on the Alexander-Orbach conjecture (8) $ds \approx 4/3$, we obtain $h_a = 2.50$ and $h_b = 3.50$. Here the h_b value is probably the upper limit of the Hill coefficient.

(*d*) The generalization of rate equation basis on the fractal reaction kinetics

We are now in a position to suggest a general form of the rate equation as follows

$$\frac{d[x^*]}{dt} = kr^{\eta}[x^*]^{\theta} = k_0[x]^h, \tag{44}$$

where $[x^*]$ is the concentration of excited reactant molecules; k and k_0 are rate constants; and η and θ are the scaling exponents whose definitions and meanings can be seen from Eq. 23. Applying Eqs. 21 and 42*b*, the relationships between the Hill coefficients and other scaling exponents are derived from Eqs. 5 and 44 as follows

$$h' = \theta + \eta(1 - \theta)(d_f - n)/[n + \eta(d_f - n)] \tag{45}$$

$$h_b = \theta + 2\eta(\theta - 1)/(ds + 2 - 2\eta). \tag{46}$$

These formulae are in essence generalizations of Eqs. 27 and 43*b*. Assuming $\eta = 1$ and $\theta = 2$, the same results, as in the previous sections, are obtained from Eqs. 45 and 46.

DISCUSSION

The occurrences of any bimolecular chemical reactions all need the contact and collision among reactant molecules. The allosteric phenomena are induced by the attack of the excited substrate molecules. From the above studies, we have seen that nonintegral characters of the Hill coefficients are direct reflections of the fractal properties of proteins. Thus, the h value is a kind of fractal dimension, not the order of reaction. This is an interesting discovery in our research. If we consider the protein chain as the Brownian motion, the dw in Eq. 4 is equal to 2, and $d_s = d_f$, therefore Eqs. 27 and 43*a* are equivalent. The exponent n and η are related to the properties of protein conformations. In particular, the θ and n are measures of interactions among the protein subunits, and therefore also indices of cooperativity. However, in this paper, we have no attempt to give further discussions for the problem and will discuss it elsewhere.

Using Eq. 27 ($n = 2$) or 43*a*, we calculated the values of the Hill coefficients according to the values of fractal dimensions. The results are listed in Table 1. The calculated value of h is ~2.5, because $d_f \approx 1.35$ and is close to the experimental values (31). However, the theoretical h values of the first two proteins in Table 1, compared with the experimental results, are different from the later in numerical values. This indicates that Eq. 43*a* is not very suitable for them. If applying Eq. 43*b*, and taking $d_f = 2.15$, 2.09, then $h = 2.93$ or 2.95, and is in good agreement with the experimental results. It is shown that there are long-range interactions among subunits of the two proteins. For the Glyceraldehyde-3-phosphate dehydrogenase, the theoretical average value in Table 1 equals 2.28, being in good agreement with the 2.3 of experimental value. This indicates that Eq. 43*a* is suitable for the protein, and there are short-range interactions among subunits of the protein.

Let us now return to Eqs. 45 and 46. When $\theta = 1$, then h' and $h_b' = 1$. There is no cooperativity, and the following process is a plausible mechanism,

$$E + S^* \rightleftharpoons E \cdot S \rightarrow P + E \quad \text{and} \quad [S^*] \gg [E].$$

The enzyme molecule has no change of conformation. When $\eta = 0$, there is no cooperativity and change of conformation. The values of all Hill coefficients equal θ, and this is the case of classical chemical reaction. If $\theta < 1$, then $h < 1$. This indicates the negative cooperativity. For example, taking $\theta < 0.5$, and $\eta = -1$, then $h' = 0.68$ ($n = 2$), and $h_b' = 0.35$. The further details and Monte

TABLE 1 Allosteric constants for some proteins

Protein	Ligand	Number of binding sites	h(exp) (31)	d_f (34)	h (cal)	h (simulation)
Haemoglobin	O_2	4	2.8	1.40 ~ 2.15	2.43 ~ 1.93	2.93 ± 0.09
Pyruvate kinase	Phosphenol pyruvate	4	2.8	1.34 ~ 2.09	2.49 ~ 1.97	2.87 ± 0.07
Glyceraldehyde-3-phosphate dehydrogenase	NAD^+	4	2.3	1.34 ~ 1.87	2.49 ~ 2.07	2.49 ± 0.05

Carlo simulation of this question will be discussed elsewhere (32, 33).

It has been known that the Hill coefficient is not a constant for a given protein but depends on conditions. This fact indicates that the change of protein conformation, in one-step or multi-step processes, is dominated by the substrate molecule and circumstance because the fractal and spectral dimensions are related to the changes of protein conformations. The protein is a biological macromolecule consisting of amino acid residues whose branches can form fractals; the multi- and hierarchical structures of protein are basic premise on which the protein and enzyme are of statistical self-similarity. Furthermore, the surface of enzyme is of multifractal feature because of its rough and coarse features (34, 35). The substrate molecules randomly walk over the fractal networks of amino acid residues until they hit the active sites, that is, R states of protein. The substrate molecule could be bound to these residues by means of interaction of hydrogen bond and Van der Walls force (36).

If the substrate-enzyme reaction is diffusion limited, the Hill coefficient can also arise from diffusion to the protein and on the protein. In this case, the rate constant K is linearly proportional to the diffusion constant D (5, 37) for homogeneous reaction in three-dimensional systems. Both K and D are time independent. However, this result is not true for lower dimensions, because the K is related to the fractal dimension. Kopelman (5) has discussed this point in detail.

In summary, the allosteric effects are of relevance to changes of protein conformations, and the Hill coefficient, of characterizing the allosteric effects and cooperativity, are the reflections of the fractal properties of proteins and enzymes, not the number of binding sites on each molecule of protein.

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