Time course of reactions controlled and gated by intramolecular dynamics of proteins: Predictions of the model of random walk on fractal lattices

(conformational transition dynamics/reaction rate theory/ligand rebinding to proteins/single ion-channel activity)

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ABSTRACT Computer simulations of random walk on the Sierpiński gasket and percolation clusters demonstrate that the short, initial condition-dependent stage of protein involving reactions can dominate the progress of the reaction over the main stage described by the standard kinetics. This phenomenon takes place if the intramolecular conformational transition dynamics modeled by the stochastic process is slow enough and the initial conformational substate of the protein already belongs to the transition state of the reaction. Both conditions are realized in two kinds of experiments: small ligand rebinding to protein after laser flash photolysis and direct recording of single protein channel activity. The model considered suggests simple analytical formulae that can explain the time behavior of the processes observed and its variation with temperature. The initial condition-dependent stage, and not the stage described by the standard kinetics, is expected as responsible for the coupling of component reactions in the complete catalytic cycles and more complex processes of biological free energy transduction.

Formulation of an advanced statistical theory of biochemical processes needs simple but realistic models of phenomena underlying microscopic dynamics of proteins. Many experiments performed since the mid-70s have indicated the existence of a slow activated dynamics of conformational transitions in the protein native phase apart from the usual much faster vibrations (1–6). The spectrum of relaxation times characterizing the conformational transition dynamics spreads over many orders of magnitude from $10^{-11}$ s (local side chain rotations or hydrogen bond rearrangements on the protein surface) to hours or even years (the mean waiting time for protein spontaneous unfolding in physiological conditions). Because of the slow character of intramolecular dynamics, the hitherto assumed description of most biochemical processes involving proteins, based as a rule on the classical transition state theory of reaction rates, needs radical changes (6–8).

Conformational transitions do not take place in the entire bulk of native proteins but are limited to liquid-like regions surrounding solid-like fragments of secondary structure. At least in the range from $10^{-11}$ to $10^{-7}$ s the relaxation time spectrum of conformational transition dynamics looks practically like a quasi-continuous one (6). There are two classes of models provided hitherto by literature, which display this phenomenon. One of them is the so-called “protein glass” (10), the density of the spectrum is assumed to vary according to a power law, which causes the dynamics to be approximately alike in many time scales. The latter is considered a generic property of glassy materials, thus the name.

Two kinds of experiments give especially strong grounds for the protein glass type models: studies of small ligand rebinding to heme proteins in various conditions after a laser flash photolysis (3–5, 11) and direct observations, with the help of the patch clamp technique, of fluctuations of the ionic current flowing through single protein channels (12–14). More detailed references to the experimental work are given in the review (6). Time scaling can originate either from a hierarchy of barrier heights in the conformational potential energy landscape (the “fractal time”), or from a hierarchy of bottleneck energies (the entropy barrier heights) in the network joining conformations between which direct transitions take place (the “fractal space”) (15). A hierarchy of energy barrier heights was proposed originally by Frauenfelder and coworkers (3–5) to give a unitary interpretation of the results of a variety of rebinding experiments and a particular mathematical realization of such a hierarchy, especially predisposed for the application to proteins, seems to offer various spin-glass models (16).

Most experimental observations supporting the protein glass picture of dynamics can be, however, equally well interpreted in terms of the hierarchy of both the energy and the entropy barrier heights (6). Mathematical realizations of hierarchical networks are lattices with the spectral dimension smaller than 2 (17–19). Except the one-dimensional chain, all of the remaining lattices with such a property are fractals (20). Fig. 1 shows two examples of fractal lattices: the planar Sierpiński gasket of the spectral dimension $d_\text{spectral} = 2\log_3/\log_5 \approx 1.365$ and the planar percolation cluster of the spectral dimension $d_\text{spectral} = 4/3$. The notion of the spectral or fractional dimension should not be confused with the notion of the fractal dimension; the latter can be much larger than 2 in the case of hierarchical lattices. A simple explanation of both concepts in the context of protein dynamics is given in ref. 6.

Our study clearly indicates the importance of the initial condition-dependent stage of biochemical reactions. It is this stage that is observed in the two kinds of experiments mentioned above. Unfortunately, the initial stage of the reaction is indescribable in terms of the usual rate constants. A more abstract notion of the first-passage time distribution density is necessary, and this notion is the main topic of the first, conceptual part of our paper.

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11685
Reaction Rate and the First-Passage Time Problem. In formal terms, the stochastic dynamics of conformational transitions within a given chemical state $R$ of a protein macromolecule is described by a system of differential master equations (21)

$$\frac{d}{dt} p_{l \rightarrow l_0}(t) = \sum_{l'} [w_{l'l} p_{l' \rightarrow l}(t) - w_{l'} p_{l \rightarrow l_{0}}(t)].$$\[1]\]

The quantity $p_{l \rightarrow l_0}(t)$ denotes the probability of the macromolecule being in a conformational state $l$ at time $t$ if initially it was in the conformational state $l_0$ and the coefficients $w_{l'l}$ are transition probabilities per unit time from $l'$ to $l$. A set of points is called a 'lattice' if one can identify in it pairs of nearest neighbors. A stochastic process described by Eq. 1 is referred to as a random walk or diffusion if the only nonvanishing transition probabilities are those between the nearest neighbors.

If no reaction is in progress the sum of probabilities of all the conformational substates composing the chemical state $R$

$$C_{l_0} = \sum_{l} p_{l \rightarrow l_{0}}(t)$$\[2]\]

is constant and equals to unity for each $l_0$. The progress of an irreversible reaction

$$R \rightarrow P$$

means that there is a subset $R^f$ distinguished in $R$, referred to as the transition state of the reaction, composed of conformational substates for which Eq. 1 is to be replaced by

$$\frac{d}{dt} p_{l \rightarrow l_0}(t) = \sum_{l'} [w_{l'l} p_{l' \rightarrow l}(t) - w_{l'} p_{l \rightarrow l_{0}}(t)] - v_l p_{l \rightarrow l_{0}}(t),$$\[3]\]

with additional coefficients $v_l$ describing the probabilities per unit time of leaving the set $R$. Any reversible reaction can be formally decomposed into two independent irreversible reactions (6).

In the presence of the reaction the quantity $C_{l_0}$, Eq. 2, is no longer constant and decreases in time. It has the meaning of survival probability in $R$ through time $t$ (probability that at time $t$ the molecule which started from the conformational state $l_0$ is still in $R$). The quantity $1 - C_{l_0}(t)$ is the cumulative probability of the first-passage time to $P$ being shorter than $t$, thus its derivative

$$f_{l_0}(t) = -\frac{d}{dt} C_{l_0}(t) = \sum_{l'} v_l p_{l' \rightarrow l}(t)$$\[4]\]

has the meaning of the first-passage time distribution density. The mole fraction $C(t)$ of protein molecules being at time $t$ in the chemical state $R$, proportional to the concentration $[R]$, is the survival probability $p_{l \rightarrow l_0}(t)$ averaged over the initial distribution of conformational substates $p_{l \rightarrow l_0}(0)$:

$$C(t) = \sum_{l} p_{l \rightarrow l_{0}}(0) C_{l_0}(t).$$\[5]\]

Following Eq. 4, the most general equation determining the time course of $C(t)$ is of the form

$$\frac{d}{dt} C(t) = -f(t),$$\[6]\]

with $f$ being a given function of time determined by the particular initial distribution of conformational substates. If the reaction is an activated process, i.e., if the events of a molecule leaving the state $R$ are very rare, when compared with the time of interconformational equilibration, then, after a short initial period Eq. 6 is simplified to the usual kinetic equation

$$\frac{d}{dt} C(t) = -\kappa C(t)$$\[7]\]

of the solution tending exponentially to zero with the relaxation time equal to the reciprocal reaction rate constant $\kappa^{-1}$. The question is whether or not the initial period of the reaction can be neglected.

In standard kinetic experiments with an ensemble of molecules, the initial distribution of microstates is not especially prepared and usually not much different from the local equilibrium distribution, which results practically in the absence of the pre-exponential stage of the reaction, even if the reaction rate is controlled by the intramolecular dynamics. However, in the experiments using the patch clamp technique mentioned at the beginning of this paper, a single protein molecule can be observed changing stochastically its state between $R$ and $P$. As a result, the experiments with single molecules bring the first-passage time distribution densities, separately for the forward and backward reaction, each treated formally as irreversible. Each time after a transition the molecule starts its microscopic evolution from a conformational state within the transition state of the return reaction. The initial distribution of conformational substates confined only to the transition state $R^f$ is realized also in the second group of experiments mentioned at the beginning, where an ensemble of molecules being initially in the thermo-
dynamically stable state P is nonthermally excited to the unstable state R.

Special preparation of the initial conditions can make the initial stage of a reaction the most important one, despite the fact that the reaction is an activated process and this initial stage is short when compared with the forthcoming exponential stage. Consequently, it is not the reaction rate constant \( \kappa \) occurring in Eq. 7, that is important in description of such reactions, but the whole first-passage time distribution density \( f(t) \) occurring in Eq. 6.

The dynamical model described by Eqs. 1 and 3 is quite general and in practical applications has to be complemented by additional, seriously simplifying assumptions. Those provided by the so far available literature seem to develop toward two opposite limits. In the one extreme, all the microscopic dynamics of the molecule is assumed to take place within the transition state \( (R^s = R) \). This enables one to apply the kinetic Eq. 7 in the whole time domain on identifying the rate parameter \( \kappa \) with the transition probability \( v \) in Eq. 3 considered, because of the dependence on \( l \), a random function of time [the picture of "fluctuating barrier" (3–5) or "dynamical disorder" (22)]. The opposite extreme is based on the assumption that the transition state \( R^s \) is reduced to a single conformational substate \( l = 0 \) being a "gate" for the reaction, which is thus referred to as the "gated reaction" (1, 9). For gated reactions with the initial conformational state confined to the transition state (coinciding with the gate), Eq. 6 is specified to

\[
\frac{d}{dt} C(t) = -v_0 p_{00}(t). \quad [8]
\]

Only the models that assume gating, when applied in description of the complete enzymatic cycles proceeding under the steady-state conditions, lead to the reconstruction of the commonly observed Michaelis–Menten kinetics (9, 23). This is a strong argument for a supposition that just the models with gating and not those of fluctuating barriers are usually much better approximation of the actual situation. The literature devoted to the former models is much more modest, however. We hope to complement somewhat this scarceness, considering in the second, detailed part of the paper the time course of reactions gated by intramolecular dynamics represented by a random walk on fractal lattices.

Random Walk on Fractal Lattices in the Presence of a Gate.

Following Eq. 8, the central problem of the theory of gated reactions is a calculation of the probability \( p_{00}(t) \) of returning to the initial point during time \( t \). It should be stressed that, in the presence of a gate, the time dependence of this quantity differs essentially from that in the case of the free diffusion (17–19, 24). Also, it should be noted that the problem of reactions gated by intramolecular dynamics has to be distinguished from the much more extensively studied target and trapping problems (19, 25). Only the models of random walk on a one-dimensional chain (26–28), random one-dimensional chains (29, 30), and integer-dimensional lattices (31) or spaces (32, 33) have been considered until now in the context of gated reactions. The model of diffusion on a percolation cluster, considered in the application to proteins by Doster and coworkers (34), concerns the trapping problem rather than that of the gated reaction.

It is a difficult task to find a reasonably approximated solution to the system of coupled master Eqs. 1 and 3 in the case of diffusion on lattices of actually fractional dimension. An alternative to solving equations is a direct computer simulation of the corresponding stochastic process for a sufficiently large statistical sample. We performed computer simulations of the random walk both on the percolating cluster and the Sierpiński gasket, assuming the initial site for this walk to be simultaneously the gate for the reaction. The studied lattices were made finite due to additionally imposed periodic boundary conditions (Fig. 1). Samples of \( 10^3 \) to \( 10^6 \) walkers were considered. Preliminary results of computer simulations of the reactions gated by diffusion on the percolation cluster are given in Ref. 35. Here, we present more complete analysis for diffusion on the Sierpiński gasket.

There are two parameters of the model: the ratio

\[
q = v/w
\]

of the probability of leaving the lattice to the probability of transition between the neighboring sites (cf. Eqs. 1 and 3), and the order \( r \) of the cluster, which we impose the boundary conditions on, determining the number \( N \) of sites in the lattice:

\[
N = (3^r + 3)/2.
\]

Fig. 2 shows the lin–log plots of the survival probability \( C \) vs. time \( t \) obtained for rather small cluster of the 6th order \( (N = 366) \) in a wide range of values of the ratio \( q \). The exactly exponential long-time decays are apparent with the rate constant \( \kappa \) varying from the value close to \( qN^{-1} \) for \( q = 0.01 \) (such a value is provided, for the model considered, by the transition state theory assuming the gate to be in the local equilibrium with the rest of the lattice; ref. 6) to the limit value independent of \( q \), reached practically for \( q = 10 \) (the regime of reaction controlled by the process of restoring the equilibrium occupation of the gate; ref. 6). Also, it is seen clearly that the slower the intersite diffusion compared with the very crossing the gate, the more dominant the initial, nonexponential stage of the reaction. These stages are better exposed in the log–log plots shown in Fig. 3. The results of simulations for larger clusters of the 9th and 12th order are given there as well.

Study of clusters of different size allowed us to determine a relation \( \kappa \propto L^{-2.25} \) between the rate constant \( \kappa \) in the regime controlled by the intramolecular dynamics and the linear size \( L = 2^r \) of the cluster.

The pre-exponential stages of the simulated reactions turned out to be well described by a formula

\[
C_{ini}(t) = \exp(\eta t)^{2q} \text{erfc}(\eta t)^q. \quad [11]
\]

![Fig. 2. Lin–log plot of the time courses of the survival probability obtained in computer simulations of a random walk on the Sierpiński gasket with all walkers starting at the same site which simultaneously is the only gate to exit the lattice. Time is measured by the number of steps in which transitions were randomly generated. The lattice has been limited to the cluster of the 6th order, and the ratio \( q \) of the probability of leaving the lattice to the probability of transition between the neighboring sites was assumed to vary from 0.01 (practically no initial, pre-exponential stage of the reaction) to 100 (more than 99% of the reaction progress proceeds in the pre-exponential stage).](image-url)
where the symbol erfc denotes the complementary error function, $\eta^{-1}$ is a certain unit of time and $\alpha$ is an exponent of a value $a \approx 0.32$. Eq. 11 with the exponent $\alpha = 1/2$ is the exact solution of the continuous one-dimensional counterpart of the problem considered (10, 33) (cf. also the exact results for discrete one-dimensional chains; refs. 26 and 27). In the limit of short times, Eq. 11 represents the stretched-exponential law and in the limit of long times, the algebraic power law (6, 10):

$$C_{\text{ini}}(t) \sim \left\{ \begin{array}{ll}
\exp \left[ -2(\eta t)^y / \sqrt{\pi} \right] & \text{for } t \ll \eta^{-1} \\
(\eta t)^{-\alpha} / \sqrt{\pi} & \text{for } t \gg \eta^{-1}.
\end{array} \right. \quad [12]
$$

The simulations clearly indicate that, contrary to the supposition stated in refs. 32 and 34, the exponent $\alpha$ does not have to assume a universal value 1/2. In fact, approximate renormalization of Eqs. 1 and 3, similar to that performed exactly for the case of the free diffusion in ref. 36, strongly suggests a relation between the value of the exponent $\alpha$ and the spectral dimension of the lattice $d$:

$$\alpha = 1 - d/2, \quad [13]$$

for $d < 2$ (following the Polya theorem (24), for $d \geq 2$ the walker can escape to infinity from the point gate). The value $\alpha \approx 0.32$, found in our simulations of the random walk on the Sierpiński gasket (with $d' = 1.365$), is in the very good agreement with Eq. 13 and also the fits of the power-law decays observed in simulations of the random walk on the percolating cluster (35) give the values of $\alpha$, which do not contradict this relation.

The crossover from the nonexponential decay, Eqs. 11 or 12, to the exponential decay with the “chemical” relaxation time $\kappa^{-1}$ can be described with the help of a simple formula

$$C(t) = [(1 - a) C_{\text{ini}}(t) + a]e^{-\kappa t}, \quad [14]$$

with $a$ denoting the level (concentration) from which the exponential decay begins. The approximation of the results of simulations with the help of the combined analytical Eqs. 11 and 14 is very good. Its quality is directly evident from Fig. 4.

Also the results of the simulations performed on the percolation cluster (35) can be fitted to an equation like Eq. 14. In this case, however, both the time constant and the exponent in the introductory stretched-exponential stage of the reaction differ from those characterizing the power-law decay stage and Eq. 11 is not sufficiently general to fit the simulation data (6, 10). The reason for this is that the percolation cluster is a random lattice and the choice of the initial site is crucial— it can have from one up to four nearest neighbor sites (cf. Fig. 1b). It is the local properties of the initial site that influence the initial stretched-exponential stage of the reaction and not the global properties of the whole lattice.

A similar effect is to be obtained for diffusion on the Sierpiński gasket on introducing a somewhat extended gate comprising a certain number of sites. Fig. 5 shows the results of simulations for a cluster of the 9th order and a gate composed of $3^3 = 27$ sites, each at the beginning occupied with the same probability $1/2$. If the set of $3^3$ sites composing the gate is chosen in such a way that the threefold rescaling of the lattice replaces it exactly by a single supersite, the long-time result of the simulation for a cluster of the 9th order in the presence of such an extended gate is, as it should be after an appropriate change of the time scale, identical to that obtained for a cluster of the 6th order and in the presence of the single point gate (curve a in Fig. 5). The short-time course of the reaction is, however, quite different. It also can be approximated by the stretched exponential with a larger value of the exponent $\alpha$ and a much shorter time unit $\eta^{-1}$. It should be noted that the long-time rescaling of the gate geometry that breaks the self-similarity symmetry of the lattice. For instance, if the chosen gate of $3^3$ sites is extended to the maximum (colnearly), also the power-law stage of the reaction is characterized by a larger value of the exponent $\alpha$ (curve b in Fig. 5). The opposite effect takes place in the case of the gate of $3^3$ points chosen in the most compact way (not shown in Fig. 5).

Of course, like the models of fluctuating barriers on the one hand, also the models with the point gates, on the other hand, are only approximations of the reality. The models with extended gates are somewhere in between, and thus the allowance for certain variations in the exponent $\alpha$ and the
The necessary condition for the presence of a certain initial condition-dependent stage of protein involving reaction is not only the slow character of the conformational transition dynamics but also the confinement of the initial conformational substrates of the protein to the transition state of the reaction. The latter condition is realized, however, only in the special kinds of experiments. Usually, the initial distribution of conformational substrates is not very different from the local equilibrium. It is thus not a surprise that no initial condition-dependent stages have been observed in the time course of the majority of biochemical reactions proceeding in standard conditions.

Nevertheless, the initial condition-dependent stages of reactions can be dominant also in standard conditions, provided that a steady-state is realized. These stages, and not the following ones described by the standard kinetics, appear to be responsible for the coupling of component reactions in complete catalytic cycles, which was proven specifically for the particular protein machine model of intramolecular dynamics (9). The same also is expected for more complex processes of biological free energy transduction (6). Importance of the latter statement, if it is actually true, hardly can be overestimated.

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