DIFFUSION ON FRACTAL LATTICES — A STATISTICAL MODEL OF CHEMICAL REACTIONS INVOLVING PROTEINS*

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Construction of a contemporary, truly advanced statistical theory of biochemical processes will need possibly simple but realistic models of microscopic dynamics of the enzymatic proteins involved. Many experiments performed with the help of various techniques since the mid 70s have demonstrated that native proteins, apart from the usual fast vibrational dynamics, reveal also a much slower activated dynamics of conformational transitions in the whole range of relaxation times from 10^{-11} to 10^{5} s or longer. At least in the range from 10^{-11} to 10^{-7} s the relaxation time spectrum is quasi-continuous and often has an approximate self-similarity symmetry (time scaling). Diffusion on fractal lattices is a particular model of stochastic dynamics displaying this property. Application of this model in construction of some elements of a novel theory of protein involving reactions is preceded by detailed analysis of the general concepts of the stochastic theory of reaction rates. Two kinds of experiments give especially strong grounds for the model of dynamics considered: small ligand rebinding to protein after laser flash photolysis and observations, with the help of the patch clamp technique, of fluctuations of the ionic current through single protein channels. Under special conditions realized in these experiments the initial conformational substates of the protein already belong to the transition state of the reaction. A theoretical model of such reactions is proposed, assuming that the reaction transition state is reduced to a single conformational substate (the gate). Computer simulations indicate predominance of the initial stage of the reaction proceeding according to the algebraic power law, over the final exponential stage. Simple formulae are proposed for description of the whole time course of the reaction and its variation with temperature. An application to describe the steady-state stage of a complete enzymatic reaction is also considered.

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1. Introduction

The necessary basis of any statistical theory of physical processes is a certain, intentionally simplified model of phenomena underlying microscopic dynamics. The quality and the relation to reality of the models in use are, however, different in different fields of research. In biochemistry and molecular biology, until recently, most scientists acted as if following the rule stating that what was not known, simply did not exist. The transition state theory, commonly used in interpretation of biomolecular reactions [1, 2], excludes in principle the existence of any microscopic dynamics of involved enzymatic proteins more complex than negligibly fast vibrations. This picture, adapted directly from the physical chemistry of low-molecular weight compounds, has eventually proved untrue. More and more studies, performed with the help of various techniques since the mid 70s [3–8], have indicated the existence, apart from the usual vibrations, also of a rich activated dynamics of conformational transitions within the protein native state. The slow character of this dynamics propels rather essential alteration in our understanding of biochemical processes [9–11]. Consequently, the time has come to start thinking seriously about formulation of a contemporary, truly advanced statistical theory of these processes based on more realistic models of microscopic dynamics of proteins. This paper is addressed primarily to physicists-theoreticians who would like to pick up this glove.

The paper consists of three parts. In the first part the microscopic dynamics of native proteins is shortly described and pointed to comprise, in the time-scale longer than 10^{-11}s, the purely stochastic conformational transitions of a quasi-continuous, at least in the range up to 10^{-7}s, spectrum of relaxation times. Two classes of models of the conformational transition dynamics are determined, referred symbolically to as 'protein machine' and 'protein glass'. Next, the essential ideas of the theory of reaction rates basing on stochastic models of intramolecular dynamics are outlined. Special attention is paid to reactions proceeding under conditions of the initial microstate of the involved molecule confined to the reaction transition state. The mechanism of reaction of the 'fluctuating barrier' type is opposed to the mechanism of 'gating' by intramolecular dynamics. And finally, the application of a particular class of models of protein glass type represented by diffusion on fractal lattices is considered and confronted with unfortunately still very poor experimental data.

2. Internal dynamics of proteins

Proteins are linear polymers of amino acids. The fundamental structural (and probably also evolutionary) unit of protein is a domain [12] consisting, on the average, of one hundred amino acid residues, each of a dosen or so atoms, thus having about 5×10^3 internal degrees of freedom. These

are not only covalent bond lengths and angles but also dihedral angles of rotations about the bonds. It is the ability to perform such rotations (limited only to some degree by steric hindrances), combined with the possibility of hydrogen bonds break up and reformation, that makes the landscape of the configurational potential energy extremely complex. A general feature of this landscape is the presence of an astronomical number (of an order of 10^{100} per domain) of local minima separated by higher or lower energy barriers of non-covalent nature [3, 4, 12]. As in the stereochemistry of low-molecular weight organic compounds, regions of the configurational space surrounding the local minima can be referred to as protein *conformational states* (*substates* in particular contexts) or, simpler, protein *conformations.*

In a reasonable approximation, internal dynamics of protein is to be decomposed into *vibrations* within particular conformational states and *conformational transitions* [8, 10, 13, 14]. The former are more or less damped harmonic oscillations, subjected accidentally to stochastic perturbations, whereas the latter are purely stochastic activated processes. The approximation is valid when interconformational barriers are high enough to ensure equilibration of vibrational modes preceeding each transition to another conformational state. As a lower bound of the interconformational barrier heights one can assume a few units of k_BT, say 10 kJ/mol, which is a typical energy barrier height for a local rotation about a single covalent bond in the absence of any steric constraints and, simultaneously, a typical energy of a hydrogen bond. Barriers lower than 10 kJ/mol can be treated as a particular manifestation of the vibrational anharmonicity, to be taken into account on assuming a finite correlation time of stochastic forces and, accordingly, a time-dependent friction [15].

The vibrational dynamics is characterized by a spectrum of periods of vibrational normal modes of the number equal to the number of degrees of freedom ($\sim 5 \times 10^3$ per domain). Vibrational periods range from 10^{-14}s (weakly damped localized N-H or C-H stretching modes) to 10^{-11}s (overdamped collective modes involving the whole domains), hence the vibrational dynamics is too fast to influence essentially the processes of chemical reactions involving proteins. The conformational transition dynamics is characterized by a spectrum of relaxation times of the number equal to the number of conformational states ($\sim 10^{100}$ per domain). In physiological conditions, this spectrum begins at 10^{-11}s (local side chain rotations or hydrogen bond rearrangements on the protein surface, related to overcoming the just assumed lowest energy barier of the order of 10 kJ/mol) and its upper limit is the mean waiting-time for spontaneous unfolding, of a value carefully estimated to be within the range $10^3 - 10^{11}$s [12]. Beginning from the pioneer study of the low-temperature dispersive kinetics of ligand rebinding to myoglobin after a laser flash photolysis by Frauenfelder and coworkers [16] an increas-

ing number of experiments give almost every year new evidence indicating that the conformational transition dynamics is characteristic not only of the unfolded but also the native state of protein [3–8].

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 (Fig. 1).Their relaxation time spectrum seems to be practically quasi-continuous, at least in the range from 10^{-11}s to 10^{-7}s [8,11]. Because the experiments at hand cannot elucidate the nature of conformational transition dynamics in detail, the problem of modelling this dynamics is to some extent left open to speculation. In two classes of models provided in previous literature the speculative element seems to be kept within reasonable limits [8, 10]. In the first class, we refer symbolically to as 'protein machine' [11], the dynamics of conformational transitions is represented by a quasi-continuous diffusive motion in a certain effective potential along a few 'mechanical' coordinates, *e.g.* angles or distances describing mutual orientation of approximately rigid fragments of secondary structure or larger structural elements (Fig. 1). The spectrum of reciprocal relaxation times for dynamics of such a type is more or less homogeneous. Otherwise, in the second class of models the dynamics is assumed to look

Fig. 1. A schematic cross-section of the fundamental structural unit of protein, a domain. Heavily shaded are solid-like fragments of secondary structure (α-helices or β-sheets) and weakly shaded are surrounding liquid-like regions. Black is the catalytic centre localized at two neighbouring solid-like elements. In models of Protein-Machine type, the dynamics of conformational transitions is treated as a quasi-continuous diffusive motion of solid-like elements relative to each other. Alternatively, in models of Protein-Glass type this dynamics is treated as a diffusion of structural defects through the liquid-like medium. The picture can be reinterpreted on a higher structural level: solid-like elements represent then the whole domains moving in a multidomain enzymatic complex.

alike in every time scale, *i.e.*, the spectrum of reciprocal relaxation times has a self-similarity symmetry (Fig. 2(a)). The latter is considered to be a generic property of glassy materials thus we propose to refer to this second, more extensive class of models as 'protein glass'.

Time scaling is observed directly in the closed-time distribution density of certain protein ionic channels studied with the help of the patch clamp technique [17–21]. It can originate either from a hierarchy of barrier heights in the potential energy landscape (the 'fractal time'), or from a hierarchy of bottle-necks in the network joining conformations between which direct transitions take place (the 'fractal space') [22–24].

A hierarchy ('tiers') of interconformational barrier heights was proposed originally by Frauenfelder and coworkers in order to give a unitary interpretation of the results of various experiments concerning the process of ligand binding to myoglobin [5–7, 16, 25–27]. The existence of such a hierarchy can explain also the low-temperature specific heat behaviour of proteins [28, 29], the temperature dependence of the Young modulus [30], results of the specific heat spectroscopy [31] and the temperature anomalies of the Lamb–Mössbauer and the Debye–Waller factors observed in Mössbauer spectra [32, 33] and inelastic neutron scattering [34–37], respectively. A hierarchical organization of barrier heights was confirmed by numerical simulations [38–40], though not by all of them [41].

The potential energy landscape with a hierarchy of barrier heights forms what in mathematics is called the ultrametric space [42]. A particular realization of the ultrametric space, especially predisposed for the application to proteins, seem to offer various spin-glass models [43, 44] with dynamics of the Glauber or Metropolis type [45]. Most spin-glass models display a discontinuos phase transition to the spin-glass phase which is to be interpreted as the protein folding transition. The information on the relation of the primary structure to the tertiary structure of protein can be, following the Hopfield theory of associative memory [46], directly incorporporated into the spin interaction matrix [47, 48].

An alternative to the hierarchy of barrier heights in the potential energy landscape is the hierarchy of bottlenecks (the entropy barrier heights) in the network joining neighbouring conformational states. Most already mentioned experimental observations can be equally well interpreted in terms of the hierary both of the energy and the entropy barrier heights. Mathematical realization of hierarchical networks are fractal lattices being the subject of this paper. The process of diffusion on a lattice can (but does not have to) be interpreted as directly simulating structural defect motions in the liquid-like regions between solid-like fragments of secondary structure. The structural defect diffusion in 3-dimensional liquid medium was suggested for an explanation of the solvent penetration into the protein interior observed

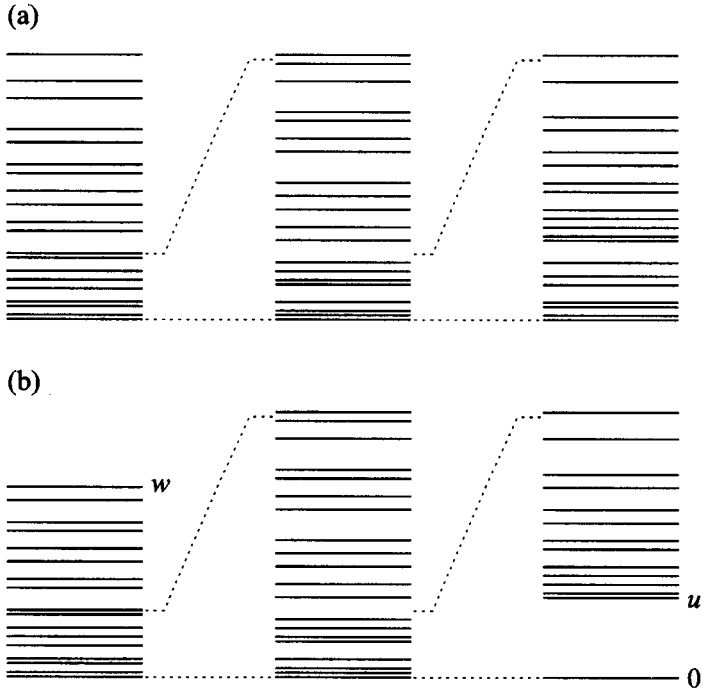

Fig. 2. A schematic spectrum of reciprocal relaxation times of the native state conformational transition dynamics in protein glass models. When changing time scale the spectrum looks approximately alike (a). This is, however, only an approximation. Because of the finite transition time between the neighbouring conformational states and the finite number of these states the spectrum has both the upper and the lower bounds (b). For many reactions involving proteins the reasonable values of these bounds are $w \approx 10^{11}\mathrm{s}^{-1}$ (by definition) and $u \approx 10^{7}\mathrm{s}^{-1}$ (slower processes of conformational relaxation probably do not affect the very reaction).

in hydrogen exchange experiments [49] as well as the time course of electric current flowing through single ionic channels [50]. But from the point of view of many physical properties, proteins can hardly be considered 3-dimensional structures [51]. In fact, the anomalous temperature dependence of spin-lattice relaxation time and the frequency density of vibrational states point to the effective dimension of proteins to be between 1 and 2 [52]. Similarly, the static conductivity [53] and the dielectric response dispersion [54] of variously hydrated protein powders can be explained in terms of percolating networks, *i.e.*, in the limit case a fractal lattice, of hydrogen bonds.

Time scaling characterizes diffusion on lattices with the spectral (or fracton) dimension between 0 and 2 [55–58]. As yet, only several models of diffusion on one-dimensional [59–61] and random one-dimensional [62, 63] chains have been proposed and only for interpretation of the closed-time

distribution density of certain protein ionic channels [18–20]. The model of diffusion on the percolation cluster, of a really fractional spectral dimension, has been studied in the effective medium approximation and also only in the application to the ionic channels [64,65]. For the purpose of interpretation of the special temperature variation of the ligand binding rate to myoglobin only the phenomenological Kohlrausch stretched-exponential law has been used [26]. We pointed to a number of experimental facts supporting models of diffusion on fractal lattices thus they seem to deserve more detail theoretical studies in the nearest future, and their application to other processes involving proteins, *e.g.* enzymatic catalysis, should also be considered.

Of course, the set of conformational states of real native protein is finite which is manifested as the presence of the upper and the lower bound in the quasi-continuous spectrum of reciprocal relaxation times (Fig. 2(b)). Consequently, any protein glass model shows unrealistic behaviour both within the limits of very short and very long times and in practice should be restricted only to a few levels of the hierarchy [5–7,25,26].

3. Stochastic modelling of reaction rates

3.1. The longest relaxation time

In formal terms, the stochastic dynamics of conformational and chemical transitions in the native state of a protein macromolecule is described by a system of master equations [66,67]

$$\dot{p}_l(t) = \sum_{l'} [w_{ll'} p_{l'}(t) - w_{l'l} p_l(t)]. \tag{1}$$

The quantity $p_l(t)$ denotes the probability of the system being in the microstate (conformation) l at time t, the dot is a derivative with respect to time and the transition probabilities per unit time $w_{ll'}$ are assumed to satisfy the detailed balance condition:

$$w_{l'l} p_l^{\mathrm{eq}} = w_{ll'} p_{l'}^{\mathrm{eq}}, \tag{2}$$

where p_l^{eq} denotes the equilibrium solution to Eq. (1). The explicit dependence of the solution $p_l(t)$ on the initial probability distribution $p_{l_0}(0)$ is to be expressed in terms of conditional probabilities (propagators):

$$p_l(t) = \sum_{l_0} p_{l|l_0}(t)\, p_{l_0}(0). \tag{3}$$

The propagators $p_{l|l_0}(t)$ are solutions to the equations analogous to (1) with the initial condition

$$p_{l|l_0}(0) = \delta_{ll_0}. \tag{4}$$

In the appropriate linear combinations of probabilities

$$Y_k(t) = \sum_l \mathcal{Y}_{kl} p_l(t) \equiv \langle \mathcal{Y}_k(t) \rangle \tag{5}$$

the system of linear equations (1) is decoupled into the system of independent equations

$$\dot{Y}_k(t) = -\tau_k^{-1} Y_k(t). \tag{6}$$

If condition (2) is satisfied, the coefficients τ_k^{-1} are real and positive, and have the meaning of reciprocal relaxation times (Fig. 3). The normal modes of relaxation (5) are written in such a way that they can be interpreted as the mean values of certain physical quatities (real functions on the set of microstates) $\mathcal{Y}_k$.

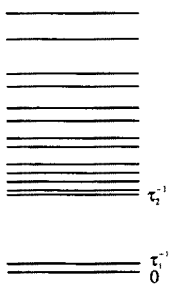

Fig. 3. Time-scale separation means the existence of a gap in the spectrum of reciprocal ralaxation times and isolation of the longest relaxation time.

The master equations like (1) or their continuous counterparts are assumed as the models of microscopic intramolecular dynamics in the stochastic theory of reaction rates. Origins of this theory go back to the Smoluchowski [68] description of the diffusion-controlled coagulation and the Kramers [69] one-dimensional theory of reactions in the overdamped limit. A general formulation of the theory is due to Montroll and Shuler [70]. A more detailed discussion of concepts involved can be found in clear papers by Widom [71] and Northrup and Hynes [72].

When speaking about a unimolecular reaction

$$\mathrm{R} \rightleftarrows \mathrm{P}$$

one assumes that the set of internal states of the considered molecule is divided into two subsets corresponding to chemical species R and P (Fig. 4(a)). The mole fractions of individual species, proportional to the molar concentrations, are the sums of probabilities:

$$C_\mathrm{R} = \sum_{l \in \mathrm{R}} p_l \equiv \langle \mathcal{C}_\mathrm{R} \rangle, \qquad C_\mathrm{P} = \sum_{l \in \mathrm{P}} p_l \equiv \langle \mathcal{C}_\mathrm{P} \rangle. \tag{7}$$

Above, $\mathcal{C}_{\mathrm{R}}$ and $\mathcal{C}_{\mathrm{P}}$ denote the characteristic functions of the subsets R and P, respectively:

$$\mathcal{C}_{\mathrm{R}l} \equiv \begin{cases} 1 & \text{if } l \in \mathrm{R} \\ 0 & \text{if } l \in \mathrm{P}, \end{cases} \qquad \mathcal{C}_{\mathrm{P}l} \equiv \begin{cases} 1 & \text{if } l \in \mathrm{P} \\ 0 & \text{if } l \in \mathrm{R}. \end{cases} \tag{8}$$

Following the normalization of probability the mole fractions (7) are normalized to unity:

$$C_{\mathrm{R}} + C_{\mathrm{P}} = 1. \tag{9}$$

The reaction is an *activated process* if, as a result of a bottleneck on the border of either the energetic or entropic origin, transitions between both subsets are not very probable. A consequence is a time-scale separation in the system (Fig. 3) which means that after the elapse of an *initial period* τ_2 the evolution of the mole fractions C_{R} and C_{R} is described by the kinetic equation

$$\dot{C}_{\mathrm{R}} = -\dot{C}_{\mathrm{P}} = \tau_1^{-1}(C_{\mathrm{R}} - C_{\mathrm{R}}^{\mathrm{eq}}) = -k_+ C_{\mathrm{R}} + k_- C_{\mathrm{P}}. \tag{10}$$

For given equilibrium values of the mole fractions $C_{\mathrm{R}}^{\mathrm{eq}}$ and $C_{\mathrm{P}}^{\mathrm{eq}}$ the longest *chemical relaxation time* τ_1 determines in a unique way the *forward* and *backward reaction rate constants* k_+ and k_-, respectively, through the equations:

$$\tau_1^{-1} = k_+ + k_-, \qquad k_+/k_- = C_{\mathrm{P}}^{\mathrm{eq}}/C_{\mathrm{R}}^{\mathrm{eq}}. \tag{11}$$

The quantities C_{R} or C_{P} do not have to coincide exactly (up to some multiplicative and additive constant) with the slowest variable of the system Y_1. If it holds, the kinetic equation (10) is valid at any time scale, also at the very beginning stage of the reaction.

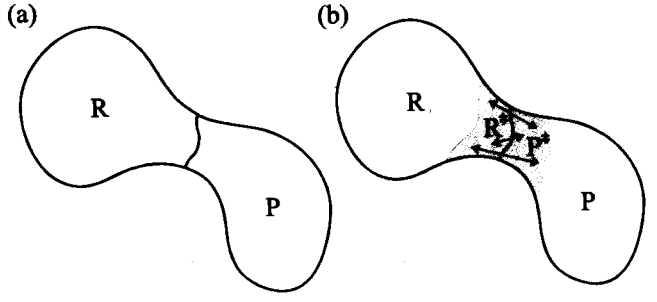

Fig. 4. A schematic partition of the set of molecule microstates (here, conformational states) into two subsets corresponding to different chemical species (a). In both subsets regions can be distinguished, referred to as the transition states, between which direct transitions take place (b).

The quantities $C_{\mathrm{R}}^{\mathrm{eq}}$ and $C_{\mathrm{P}}^{\mathrm{eq}}$, thus k_+ and k_-, depend on the choice of R and P but $\tau_1^{-1} = k_+ + k_-$ is independent of this choice; it is determined only by the equations of microscopic dynamics (1). The exact position of the R-P border is, however, not very crucial as in the case of the activated processes the bottleneck between R and P makes the equilibrium occupation of boundary microstates negligibly small.

Because of the nice properties of the characteristic functions:

$$\mathcal{C}_{\mathrm{R}}^2 = \mathcal{C}_{\mathrm{R}}, \qquad \mathcal{C}_{\mathrm{P}}^2 = \mathcal{C}_{\mathrm{P}}, \qquad \mathcal{C}_{\mathrm{R}}\mathcal{C}_{\mathrm{P}} = 0, \tag{12}$$

the thermodynamic perturbation theory for the problem discussed can be applied exactly, up to the infinite order [73], which results in the exact expression (valid arbitrarily far from the equilibrium) of the reaction rate constant k_+ in terms of the equilibrium time correlation function of fluxes:

$$k_+ = \int_0^\infty dt' \langle \dot{\mathcal{C}}_{\mathrm{R}}(t')\, \dot{\mathcal{C}}_{\mathrm{R}}(0)\rangle^{\mathrm{eq}} / \langle \mathcal{C}_{\mathrm{R}}\rangle^{\mathrm{eq}}. \tag{13}$$

A similar formula determines the backward reaction rate constant k_-. Formula (13) was derived for the first time by Yamamoto [74] with the help of the first order perturbation theory; Chandler [75] derived it simply by resorting to the Onsager's regression hypothesis.

After integration over time Eq. (13) can be formally rewritten as the limit

$$k_+ = \lim_{t\to\infty} J_+(t)/C_{\mathrm{R}}^{\mathrm{eq}} \tag{14}$$

of the *reactive flux* divided by the equilibrium occupations of species:

$$J_+(t)/C_{\mathrm{R}}^{\mathrm{eq}} \equiv \langle \mathcal{C}_{\mathrm{R}}(t)\, \dot{\mathcal{C}}_{\mathrm{R}}(0)\rangle^{\mathrm{eq}} / C_{\mathrm{R}}^{\mathrm{eq}} = \sum_{l'} \sum_{l\in \mathrm{R}^\ddagger} p_{l'|l}(t)\, v_l\, p_l^{\mathrm{eq}} / C_{\mathrm{R}}^{\mathrm{eq}}. \tag{15}$$

Similarly, k_- can be rewritten as a limit of $J_-(t)/C_{\mathrm{R}}^{\mathrm{eq}}$ given by a formula analogous to Eq. (15). To determine the reactive fluxes explicitely in terms of the dynamics described by Eqs. (1) we distinguish in both subsets R and P the *transition states* $\mathrm{R}^\ddagger$ and $\mathrm{P}^\ddagger$ of reaction, respectively, composed of the microstates from which direct transitions to the alternate chemical state take place (Fig. 4(b)) and define, *e.g.* for $l \in \mathrm{R}^\ddagger$,

$$v_l \equiv \sum_{l'\in \mathrm{P}^\ddagger} w_{l'l}. \tag{16}$$

Because of the detailed balance, direct transitions in both directions are possible only between the transition states.

The initial value of expression (15),

$$J_+(0)/C_{\mathrm{R}}^{\mathrm{eq}} = \sum_{l \in \mathrm{R}\ddagger} v_l \, p_l^{\mathrm{eq}}/C_{\mathrm{R}}^{\mathrm{eq}} \equiv k_+^{\mathrm{eq}}, \tag{17}$$

coincides with the value of the reaction rate constant provided by the *transition state theory* [76] which can be easily seen after rewritting it in the form

$$k_+^{\mathrm{eq}} = v \, C_{\mathrm{R}\ddagger}^{\mathrm{eq}}/C_{\mathrm{R}}^{\mathrm{eq}} \equiv v \exp(-\Delta G_{\mathrm{R}}^{\ddagger}/k_{\mathrm{B}}T). \tag{18}$$

Here, v has the meaning of a *mean frequency of transitions*, $C_{\mathrm{R}\ddagger}^{\mathrm{eq}}$ is the equilibrium occupation of the transition state and $\Delta G_{\mathrm{R}}^{\ddagger}$ denotes the *free energy of activation.*

The assumption of the time-scale separation corresponds to the plateau value behaviour of $J_+(t)$ and $J_-(t)$ [77] (Fig. 5). Note a possibility of faster proceeding of the reaction in the initial stage and the necessity of cutting the long-time exponential decay by the appropriate regularization factor in the integral (13) [78]. The reactive flux vanishes for $t < 0$ which denotes the necessity of careful treatment of also the lower bound of the integral (13) (the moment $t = 0$ should be the internal point of the interval of integration). The jump at $t = 0$ is related to a Dirac-delta component of the time correlation function of fluxes which thus appears to have the form of a sum [72]

$$\langle \dot{C}_{\mathrm{R}}(t) \, \dot{C}_{\mathrm{R}}(0) \rangle^{\mathrm{eq}} / \langle C_{\mathrm{R}} \rangle^{\mathrm{eq}} = k_+^{\mathrm{eq}} \delta(t) + S_+(t). \tag{19}$$

Formulas (13) and (19) state clearly that the core of the transition state theory is the assumption of the flux $\dot{C}_{\mathrm{R}}(t)$ being a delta-corelated white noise.

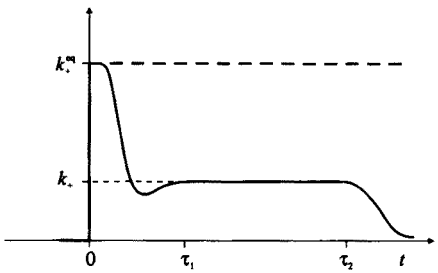

Fig. 5. A schematic variation of the reactive flux $J_+(t)$ (divided by $C_{\mathrm{R}}^{\mathrm{eq}}$) with time. For the reaction which is an activated process characteristic is the plateau value behaviuour. Transition state theory approximates the reactive flux time course by the Heaviside step function.

For determination of the transition state theory rate constant (18) no knowledge of the intramolecular dynamics is needed. It is the finite correlation-time component $S_+(t)$ in the sum (19) that results from the intramolecular dynamical processes. Quite generally, the exact reciprocal rate constant can be decomposed into three time components [72]:

$$k_+^{-1} = (k_+^{eq})^{-1} + (k_+^{R})^{-1} + (k_+^{P})^{-1} \tag{20}$$

and similarly k_-^{-1}. The first component in Eq. (20) determines the time needed to cross the boundary under the assumption that the transition state $R^{\ddagger}$ is in a local equilibrium with the rest of microstates composing the chemical state R. As a result of transition this equilibrium is, however, disturbed. The second component in Eq. (20) determines the time needed for restoring this equilibrium from the side of the R species and the third component determines the time needed for the same process but from the side of the P species (recrossing the border). From Eq. (20) it follows that k_+^{eq} is always larger than the exact rate constant k_+ (Fig. 5). If two latter components in Eq. (20) are much smaller than the first component the reaction is well described by the transition state theory, possibly with a certain transmission coefficient smaller than unity. This is, however, not the case of reactions *controlled* by processes of intramolecular dynamics when the latter terms prevail.

3.2. Reaction rate and the first-passage time problem

One should note that "the rate constants k_+ and k_- are not the probabilities per unit time of an R molecule making the R $\rightarrow$ P transition and a P molecule making the P $\rightarrow$ R transition, and k_+C_R and k_-C_P are not the separate P $\rightarrow$ R and R $\rightarrow$ P fluxes" (Widom [71]). This holds only for *imagined* irreversible reactions

$$R \rightarrow P \quad \text{or} \quad P \rightarrow R$$

with the *absorbing* boundary between the R and P subsets of microstates, which can be realized by adding an imagined totally absorbing *limbo state* (Fig. 6). The stochastic theory of such imagined or real irreversible reactions is identical to the first-passage time problem for the corresponding stochastic processes [66, 70].

Because of the finite number of conformational states the spectrum of reciprocal relaxation times characterizing dynamics in the sets R or P alone has a gap as shown in Fig. 2(b). For irreversible reactions it is the adding of the limbo state that introduces the longest, additional relaxation time into the gap as shown in Fig. 3.

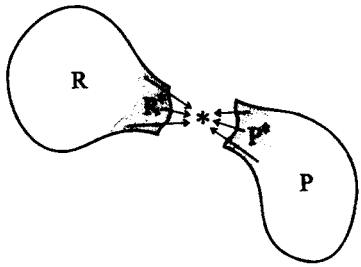

Fig. 6. Any reversible reaction can be formally divided into two irreversible reactions after introducing the imagined *limbo state* $*$.

Later on we will consider only the irreversible reaction R $\rightarrow$ P; the case of the irreversible reaction P $\rightarrow$ R is analogous. By definition, transition probabilities per unit time from the limbo state $*$ to any microstate l in the transition state $R^{\ddagger}$ vanish:

$$w_{l,*} = 0. \tag{21}$$

Consequently, the occupation probability of the limbo state tends in time to unity:

$$\lim_{t\to\infty} p_*(t) = 1. \tag{22}$$

In the presence of the limbo state the quantity

$$C(t|l_0) \equiv \sum_{l\in R} p_{l|l_0}(t) = 1 - p_{*|l_0}(t) \tag{23}$$

has the meaning of *survival probability* in R through time t (probability that at time t the system started from state l_0 is still in R). In various contexts time t in Eq. (23) is referred to as the *dwell-time* in R, the *waiting-time* for transition to P or the *first-passage time* through the boundary. The quantity $1 - C(t|l_0)$ is the cumulative probability of the first-passage time being shorter than t, thus its derivative

$$f(t|l_0) = -\dot{C}(t|l_0) \tag{24}$$

has the meaning of the *first-passage time distribution density*. Eq. (24) can be integrated to the equation

$$C(t|l_0) = 1 - \int_0^t dt' f(t'|l_0). \tag{25}$$

From the second equation (23) and Definition (16) it follows that

$$f(t|l_0) = \dot{p}_{*|l_0}(t) = \sum_{l\in R^{\ddagger}} v_l\, p_{l|l_0}(t). \tag{26}$$

Note that $p_{l|l_0}(t)$'s are in general determined by dynamics in the whole set R; in order to find them one has to solve the full set of equations (1) with the absorbing boundary conditions (21). Having known the first-passage time distribution density, one can calculate the *mean first-passage time*:

$$\tau(l_0) \equiv \int_0^\infty dt\, t\, f(t|l_0) = -\int_0^\infty t\, dt\, \frac{dC(t|l_0)}{dt} = \int_0^\infty dt\, C(t|l_0), \tag{27}$$

of course, if it is finite. In the last equality we applied integration by parts.

The mole fraction $C(t)$ of the molecules R which survived through time t is the survival probality $C(t|l_0)$ averaged over the initial distribution of states $p_{l_0}(0)$:

$$C(t) = \sum_{l_0 \in \mathrm{R}} p_{l_0}(0)\, C(t|l_0). \tag{28}$$

If the mole fraction C obeys the usual kinetic equation for the irreversible reaction

$$\dot{C} = -kC, \tag{29}$$

i.e., if it decays exponentially:

$$C(t) = e^{-kt}, \tag{30}$$

from the last equality (27) one concludes that the reciprocal rate constant equals to the mean first-passage time averaged over the initial distribution of states:

$$k^{-1} = \sum_{l_0} p_{l_0}(0)\, \tau(l_0). \tag{31}$$

For the reaction being an activated process, after a short initial period the form of equation (29) and the very rate constant k do not depend on the initial distribution of states. In such a case for a great majority of internal states in R the mean first passage time should have the same value coinciding, following Eq. (31), with the value of the reciprocal rate constant:

$$\tau(l_0) = \tau = k^{-1}. \tag{32}$$

Formula (32) is often used for calculating the rate constant k [77].

In general the average survival probability C does not obey the simple kinetic equation (29) but one can always formally determine a *time-dependent rate parameter* $k(t)$ through the equation

$$\dot{C} = -k(t)C. \tag{33}$$

Equivalently,

$$k(t) \equiv -\frac{\dot{C}}{C} = -\frac{d}{dt} \ln C = \frac{f(t)}{C(t)}. \tag{34}$$

From the latter equation it follows immediately that the rate parameter k is time-independent only if the first-passage time distribution density $f(t)$ is Poissonian:

$$f(t) = ke^{-kt} \tag{35}$$

(*c.f.* Eq. (30)).

Following Eqs. (26) and (28) the rate parameter $k(t)$ is given by the equation

$$k(t) = \sum_{l \in \mathrm{R}^{\ddagger}} v_l \, p_l(t)/C(t) \equiv J(t)/C(t). \tag{36}$$

Here $J(t)$ is in fact the separate R $\rightarrow$ P flux. If the reaction considered is the activated process $k(t)$ in Expression (36) reaches the long-lasting stationary value

$$k = \sum_{l \in \mathrm{R}^{\ddagger}} v_l \, p_l^{\mathrm{st}}/C^{\mathrm{st}} \equiv J(t)^{\mathrm{st}}/C(t)^{\mathrm{st}}. \tag{37}$$

The flux-over-population formula (37) is usually simpler in applications than the time correlation function formula (13) which needs the calculations of the full reactive flux (15). This method has been used in the pioneering papers by Smoluchowski [68] and Kramers [69]. The formula (37) includes exactly both the process of crossing the boundary on assuming the local equilibrium conditions and the process of restoring this equilibrium from the R side, but of course it neglects the process of recrossing the boundary (compare Eq. (20)). One can, however, take into account effects of the latter process on considering the backward P $\rightarrow$ R reaction and the appropriately chosen probabilities of eqilibration in both subsets of microstates [77].

3.3. Especially prepared initial state

The forward and backward reaction rate constants k_+ and k_- we have considered in Subsection 3.1. are the parameters that are measured in experiments with an *ensemble* of molecules tending to the total, chemical equilibrium. In such experiments the initial distributions of microstates in R and P are not specially prepared and usually not very different from the local equilibria. However, experiments are also presently possible in which a *single* molecule is observed, changing stochastically its state between R and P (the patch clamp technique [17–21], Fig. 7). As a result the experiments with single molecules bring the first-passage time distributions, separately for the forward and backward reaction. The reaction rate parameter determined by a certain first-passage time distribution corresponds to this given

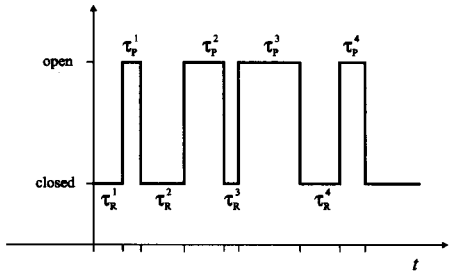

Fig. 7. A schematic 'telegraphic noise' recorded in experiments performed with the help of the patch clamp technique. The ionic current flowing through a single protein channel fluctuates between two values corresponding to two 'chemical' states of the protein. Distribution of the first-passage times $(\tau_{\mathrm{P}}^1, \tau_{\mathrm{P}}^2, \tau_{\mathrm{P}}^3, \ldots)$ from the open to the closed state as well as $(\tau_{\mathrm{R}}^1, \tau_{\mathrm{R}}^2, \tau_{\mathrm{R}}^3, \ldots)$ from the closed to the open state can be thus directly determined. Very often the time course of fluctuations recorded has a similar character in several subsequent time scales.

by Eqs. (34) and (36) rather than that given by Eq. (13). Each time after a transition, the molecule starts its microscopic evolution from a conformational substate within the transition state of the return reaction. Consequently, the initial states l_0 occurring in Eq. (28) are always those belonging to the transition state $\mathrm{R}^{\ddagger}$ (or $\mathrm{P}^{\ddagger}$ in the case of the backward reaction).

The initial state distribution confined only to the transition state $\mathrm{R}^{\ddagger}$ is realized also in experiments concerning the *ensemble* of molecules which, being initially in the thermodynamically stable P state (the reaction $\mathrm{R} \to \mathrm{P}$ has to be practically irreversible), is excited *nonthermally* to the unstable R state. An important class of such experiments are the already mentioned studies of the small ligand rebinding to protein in various conditions after the laser flash photolysis [5–7, 16, 25–27].

Both the experiments using the patch clamp technique and those with the application of the laser pulses appeared very effective for the study of protein dynamics. This is why we shall focus here on the case of reactions involving molecules in the initial microstates confined to the transition state. Special preparation of the initial state very often makes the initial stage of reaction the most important one, even if the reaction is an activated process and the initial stage is short as compared to the forthcoming exponential stage. Consequently, the generalized kinetic equation (33) is more appropriate for description of the entire course of reaction rather than the usual kinetic equation (29), valid in the exponential stage of the reaction. However, Eq. (33) is in general only a definition of the time-dependent rate parameter $k(t)$. It can be considered the proper kinetic equation provided that the parameter $k(t)$ is independent on the concentration $C(t)$. If it is not the case, the only correct description is in terms of the kinetic equation

with memory:

$$\dot{C}(t) = -\int_0^t dt'\, k'(t')\, C(t-t') = -\int_0^t dt'\, k'(t-t')\, C(t'). \tag{38}$$

Eq. (38) is too general to be worth detailed considerations. Fortunately, when the molecule initial microstates are confined to the transition state there is a simple way of making Eq. (33) a meaningful kinetic equation. It consists in the assumption that the *entire* microscopic dynamics of the molecule takes place within the transition state, *i.e.* is described by a set of master equations of the form

$$\dot{p}_l(t) = -v_l\, p_l(t) + \sum_{l'}[\bar{w}_{ll'} p_{l'}(t) - \bar{w}_{l'l} p_l(t)], \tag{39}$$

where all l and $l' \in \mathrm{R}^{\ddagger}$ and $\bar{w}_{ll'}$ are some effective rather then the bare transition probabilities per unit time (compare Eq. (1)). Reduction of the microscopic dynamics to Eq. (39) is equivalent to considering the parameter $k(t)$ in Eq. (33) a random function of time [79–81]. Several models of reactions with such a 'dynamical disorder' or a 'fluctuating barrier' were treated either exactly or to a good analytical approximation. Let us mention the continuous one-dimensional models of Agmon and Hopfield [82] and Zwanzig [83], the two-state model of Fuliński [84] and the continuous time random walk model of Zharikov and Fischer [85]. For all these models in the limit of extremely slow internal dynamics (the $\bar{w}$'s in Eq. (39) much smaller than the v's) the dynamical disorder goes over the 'static disorder', with the latter term denoting the *dispersive kinetics* [86]:

$$C(t) = \sum_{l\in \mathrm{R}\ddagger} p_l(t) = \sum_{l\in \mathrm{R}\ddagger} p_l(0) e^{-v_l t} \simeq \int_0^{\infty} dk\, g(k)\, e^{-kt} \tag{40}$$

with a continuous distribution $g(k)$ of reaction rate parameters (or the corresponding activation barrier heights). Such a kinetics was originally proposed for description of the time course of protein reaction by Frauenfelder and coworkers already in 1975 [16].

Of course, any model of dynamical disorder is only an approximation, sometimes a very rough one as the influence of intramolecular dynamics within the space of microstates outside the transition state can dominate the reaction time course. The effects of the latter dynamics can be simply distinguished from the effects of fluctuating barriers when considering models with the transition state $\mathrm{R}^{\ddagger}$ reduced to a single microstate $l = 0$. Such a

microstate has the meaning of a *gate* for the reaction which is thus referred to as the *gated reaction* [11, 87].

For the gated reactions Eqs. (24) and (26) are replaced by

$$\dot{C}(t|0) = -f(t|0) = -v_0\, p_{0|0}(t) \tag{41}$$

and Eq. (25), after averaging over the only initial microstate $l_0 = 0$, reads

$$C(t) = 1 - v_0 \int_0^t dt'\, p_{0|0}(t'). \tag{42}$$

Following Eqs. (41) and (42) the central problem of the theory is calculation of the probability $p_{0|0}(t)$ of returning to the initial point during time t. We shall consider this quantity in more detail in the next section.

A slightly more complicated is the problem of calculating of the probability $p_{0|l_0}(t)$ of transition between two *different* points l_0 and 0 during time t. It appears when modelling *several* coupled gated reactions, *e.g.* a complete enzymatic reaction composed of a few steps proceeding in the steady state conditions [11]. For such a case Eqs. (41) and (42) are replaced, respectively, by more general ones:

$$\dot{C}(t|l_0) = -f(t|l_0) = -v_0\, p_{0|l_0}(t) \tag{43}$$

and

$$C(t|l_0) = 1 - v_0 \int_0^t dt'\, p_{0|l_0}(t'). \tag{44}$$

4. Models assuming diffusion on fractal lattices

4.1. The spectral dimension

A set of points is called a *lattice* if one can define for it the notion of the nearest-neighbourhood. A stochastic process of values in a certain lattice is referred to as the *random walk* or *diffusion* if the only transitions possible are between the nearest neighbours. Otherwise we speak about the *random fly* [22].

In Fig. 8 two examples of fractal lattices with a hierchy of bottle-necks are shown: the planar Sierpiński gasket and the planar percolation cluster. The *fractals* are defined as objects of a fractional value of the fractal dimension but the hierarchical properties of lattices are related with the spectral rather than the fractal dimension. It is worth distinguishing here clearly both concepts.

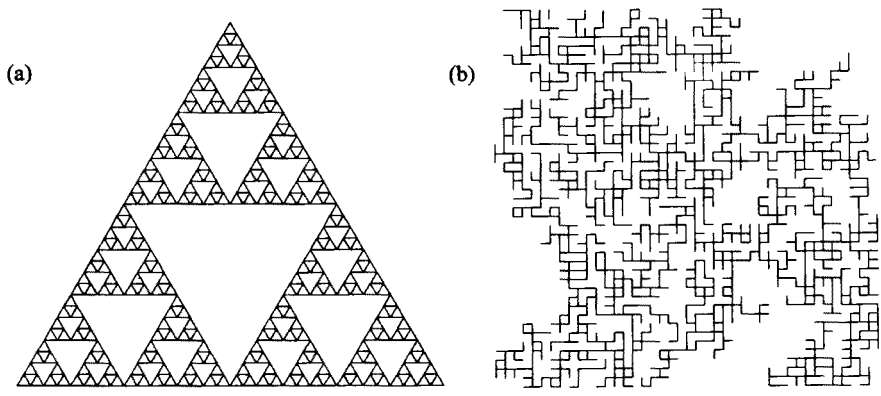

Fig. 8. Two examples of fractal lattices that can be considered a reasonable model of the network joining conformational substatates of a protein macromolecule between which direct transitions take place. (a) Sierpiński gasket. Three small equilateral triangles are combined into a larger triangle, three larger triangles into an even larger one and so on *ad infinitum*. (b) Percolation cluster. Bonds on a square lattice are realized stochastically with the probability 1/2 and then clusters which are not connected to the largest one are removed. Note the hierarchical structure of bottle-necks in both lattices resulting in time scaling: the equilibration completes first within subclusters of a lower order and only then, in a longer time-scale, within the subclusters of a higher order. Finite number of conformational substates in real proteins makes the hierarchy bounded both from below and from above.

The notion of the *fractal* (Hausdorff–Besicovitch) dimension $\bar{d}$ of a given lattice is simple [88]. It is the exponent in the power law determing how the number of sites n changes with the scale (size) s:

$$n = s^{\bar{d}}. \tag{45}$$

Consequently,

$$\bar{d} = \log n / \log s. \tag{46}$$

For instance, for the planar Sierpiński gasket shown in Fig. 8(a) the two-fold change of the scale entails a three-fold increase of the number of sites thus $\bar{d} = \log 3 / \log 2 \approx 1.585$.

The notion of the *spectral* (Rammal and Toulouse [55,56]) or the *fracton* (Alexander and Orbach [57,58]) dimension is more complex. It resorts to the functional dependence of the density of vibrational normal modes vs. frequency when a given lattice is considered to consist of massive points with an elastic coupling between the nearest-neighbours. Quite generally the Hamiltonian dynamics of a system of coupled harmonic oscillators is described by the equation

$$\dot{a} = -i\,\Omega\,a, \tag{47}$$

where a is the vector of complex numbers with the real and imaginary parts corresponding to positions and momenta, respectively, of particular harmonic oscillators:

$$a_l = \frac{1}{\sqrt{2}}(q_l + i\,p_l) \tag{48}$$

and Ω is the frequency matrix. In the coordinates of the normal modes of vibrations the frequency matrix becomes diagonal and the set of equations represented by Eq. (47) is decoupled into a set of independent equations

$$\dot{a}_k = -i\,\omega_k a_k. \tag{49}$$

If the density of vibrational modes in the spectrum of frequencies ω behaves regularly, according to a certain power law

$$\rho(\omega) \propto \omega^{\tilde{d}-1}, \tag{50}$$

the number $\tilde{d}$ is referred to as the spectral dimension of the lattice. The relation (50) can be considered a generalization of the Debye relation for acoustical phonons in crystal lattices of the integer Euclidean dimension $\tilde{d}$ [89]; the normal modes of vibration in lattices of a fractional dimension $\tilde{d}$ are referred to as the *fractons* [57,58].

Important for the present problem is that the set of master equations (1) describing diffusion on a given lattice can be rewritten in the form analogous to Eq. (47):

$$\dot{p} = -\Gamma\,(p - p^{\mathrm{eq}}) \tag{51}$$

with Γ being the matrix made of of the transition probabilities w. The corresponding set of decoupled equations for the relaxational normal modes reads (*c.f.* Eq. (6))

$$\dot{p}_k = -\gamma_k(p_k - p_k^{\mathrm{eq}}). \tag{52}$$

There are, in fact, twice as many Eqs. (49) as Eqs. (52) (a_k's are complex variables whereas p_k's are the real ones) thus, following the relation (50), the density of relaxational modes in the spectrum of reciprocal relaxation times γ should behave as

$$\rho(\gamma) \propto \gamma^{\tilde{d}/2-1}. \tag{53}$$

It is an alternative definition of the spectral dimension [55,56].

Time scaling takes place only if the density of relaxational normal modes increases with decreasing reciprocal relaxation time γ (Fig. 2(a)). Consequently, the hierarchy of bottle-necks is characteristic only for lattices with the spectral dimension smaller than 2:

$$\tilde{d} < 2. \tag{54}$$

For a Sierpiński gasket embedded in d-dimensional Euclidean space $\tilde{d} = \log(d+1)/\log(d+3)$ [55, 56], thus for the planar Sierpiński gasket ($d = 2$, Fig. 8(a)) the spectral dimension $\tilde{d} = \log 3/\log 5 \approx 1.365$. The spectral dimension of any percolation cluster (in particular that embedded in 2-dimensional Euclidean space, Fig. 8(b)) is very close to the value $\tilde{d} = 4/3$ (the Alexander–Orbach conjecture) [56–58].

The spectral dimension influences two, physically very important, quantities [55–58]. The first is the *probability to return to the original point*, which in the case of free diffusion (without any boundary conditions) behaves asymptotically in time as

$$p_{0|0}(t) \propto t^{-\tilde{d}/2}. \tag{55}$$

This equation is a generalization of the well-known result for the free diffusion in the Euclidean spaces [66]. And the second quantity is the *mean number of distinct sites visited by a random walker*, which in the case of free diffusion behaves asymptotically in time as

$$S(t) \propto \begin{cases} t^{\tilde{d}/2} & \text{if } \tilde{d} < 2 \\ t & \text{if } \tilde{d} > 2. \end{cases} \tag{56}$$

4.2. Scaling of the rate parameter and spectral dimension

In Section 3.2. we introduced the notion of the time-dependent rate parameter $k(t)$, Eqs. (33) and (34), and in Section 3.3. we pointed out that Eq. (33) can be treated a meaningful kinetic equation only if $k(t)$ is a beforahand given function of time (independent of $C(t)$). For a given $k(t)$ the general solution to Eq. (33) is

$$C(t) = C(0)e^{-\int_0^t dt' k(t')}. \tag{57}$$

Eq. (33), when applied for description of the *whole* time course of a reaction which is an activated process, needs an introduction of a rate parameter of the form given schematically in Fig. 9(a), which is time-dependent only in the initial stage of the reaction. This stage can be characterized by one or more additional time-scales (*c.f.* Fig. 3).

For reactions which are not activated processes the rate parameter k is time-dependent in the whole time domain. Of special importance is the case when the rate parameter *scales* with time as shown schematically in

Fig. 9(b). This is observed directly, *e.g.*, when recording closed-time distribution density of certain protein channels with the help of the patch clamp technique [17, 18, 21] (the 'stairs' in Fig. 9(b). mean that the ionic current recorded, shown schematically in Fig. 7, has a similar time course in several subsequent time scales). When approximated by a straight line on the log-log plot (Fig. 9(b)), the time dependence of k takes the form [17]:

$$k(t) \propto t^{\tilde{d}/2-1}. \tag{58}$$

Here $\tilde{d}$ is a certain number from the closed interval $0 \leq \tilde{d} \leq 2$.

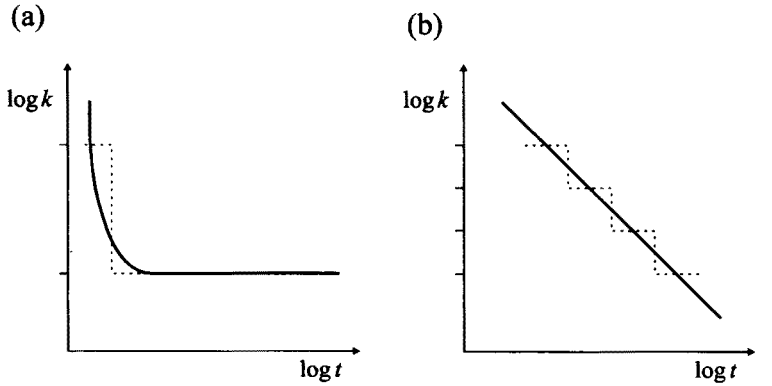

Fig. 9. A schematic time dependence of the reaction rate parameter $k(t)$ in the log-log scale. (a) The case of reaction which is an activated process. Rate parameter is time-dependent only in the initial stage. (b) The case of a non-activated reaction. Rate parameter can scale with time: in several subsequent time scales its value changes proportionally.

For the time dependence (58) with $0 < \tilde{d} \leq 2$ the time integral in Eq. (57) becomes

$$\int_0^t dt' k(t') = (t/\tau)^{\tilde{d}/2} \tag{59}$$

with τ being some constant and the time variation of the survival probability is determined by Kohlrausch–Williams–Watts *stretched exponential* law:

$$C(t) = C(0)e^{-(t/\tau)^{\tilde{d}/2}}. \tag{60}$$

For $\tilde{d} = 0$ (the critical value) we have

$$\int_0^t dt' k(t') = \alpha \ln t/\tau \tag{61}$$

with τ and α being some constants and the time variation of the survival probability shows the *power law (algebraic)* behaviour:

$$C(t) = C(\tau)(t/\tau)^{-\alpha}. \tag{62}$$

In the most applications to physical chemistry diffusion on various lattices is considered as modelling the translational motion of the molecules. For the *target* or the *trapping* problems [56, 90–92] (for the latter case only in the limit of moderate times) the survival probability

$$C(t) \approx C(0)\, e^{-cS(t)}, \tag{63}$$

where c is the concentration of annihilators diffusing towards the immobile target R molecule, or the concentration of randomly distributed immobile traps awaiting to annihilate the diffusing R molecule, and $S(t)$ is the mean number of distinct sites visited on the undoped lattice by any of two kinds of walkers during time t. On comparing Eqs. (63) and (56) with Eq. (60) we find that the number $\tilde{d}$ in the latter equation can have in fact a meaning of the spectral dimension of an appropriate lattice, which justifies the notation used.

However, our problem of gated reaction with the fractal lattice representing the set of internal states of the protein molecule differs from both the target and the trapping problem, thus given a particular time course of reaction, either of the stretched-exponential form or of the algebraic power law form, no conclusions concerning the spectral dimension of the lattice of conformational substates can be drawn. Morover, which is rather unfortunate, we cannot apply directly results of numerous studies concerning the target and the trapping problems. Only the models of random walk on a one-dimensional chain [59–61] and integer-dimensional Euclidean spaces [93] have been considered untill now in the context of gated reactions (*c.f.* also Appendix). The model of diffusion on percolating lattices considered by Doster and Schirmacher in the effective medium approximation [64, 65] concerns the trapping problem rather than that of the gated reaction. In the next subsection we present preliminary results of recent studies of diffusion on lattices of actually fractional dimension carried out in our laboratory [94, 95].

4.3. Results of computer simulations for gated reactions. A universal behaviour of survival probability in time

Krzysztof Palacz performed computer simulations of the random walk on the planar Sierpiński gasket (Fig. 8(a) [94] and the planar percolation cluster (Fig. 8(b)) [95] assuming the initial site for this walk to be simultaneously

the gate for the reaction. The studied lattices were assumed finite due to additionally imposed reflecting boundary conditions and the probability of leaving the lattice was assumed to be the same as the probability of transition between the neighbouring sites. Typical time variations obtained of the survival probability (the mole fraction) $C(t)$, Eq. (42), are shown in figures 10 and 11.

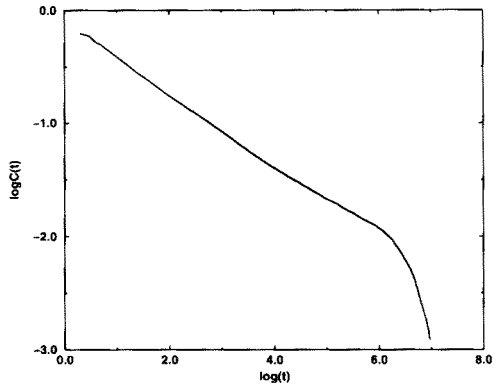

Fig. 10. Typical result of a random walk simulation on the Sierpiński gasket (Fig. 8(a)). The lattice has been limited to $3^{10}/2 \approx 3 \times 10^4$ sites. Some 10^5 walkers started at the same site which simultaneously is the only gate to exit the lattice. The probability of leaving the lattice was assumed to be the same as the probability of transition between the neighbouring sites. Time is measured in number of steps in which transitions were randomly generated. Survival probability vs time is plotted in the log-log scale. The power law and the exponential stages of a reaction are clearly distinguished.

Despite the fact that the modelled reaction is an activated processes (for the range of parameters considered the reaction rate is limited by the rate of restoring the local equilibrium at the gate rather than the very act of leaving the lattice) its pre-exponential stage appeared to be predominant for both lattices. For diffusion on the Sierpiński gasket (Fig. 10) the pre-exponential stage of reaction can be described by a simple formula

$$C(t) = \exp(\eta t)^{2\alpha} \operatorname{erfc}(\eta t)^{\alpha}, \tag{64}$$

where the symbol erfc denotes the complementary error function [96], η^{-1} is a certain unit of time and α is a certain exponent, for the case discussed taking a value $\alpha \approx 0.33$. In the limit of short times Eq. (64) represents the stretched-exponential law and in the limit of long times, the algebraic power law:

$$C(t) \approx \begin{cases} \exp[-2(\eta t)^{\alpha}/\sqrt{\pi}] & \text{for } t \ll \eta^{-1} \\ (\eta t)^{-\alpha}/\sqrt{\pi} & \text{for } t \gg \eta^{-1}. \end{cases} \tag{65}$$

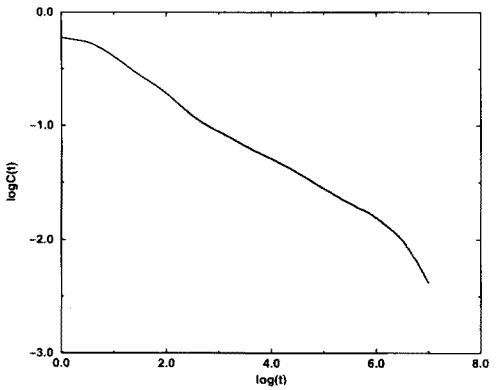

Fig. 11. Typical result of a random walk simulation on the percolation cluster (Fig. 8(b)) composed of approximately 5×10^5 sites. Some 10^5 walkers started at the same site which simultaneously is the only gate to exit the lattice. The probability of leaving the lattice was assumed to be the same as the probability of transition between the neighbouring sites. Time is measured in number of steps in which transitions were randomly generated. Survival probability vs time is plotted in the log-log scale. Because the percolation cluster is itself a random lattice and each its site has a random number of the nearest neighbours, the power law stage of the reaction is preceded by a certain initial stage strongly dependent on a particular choice of the gate.

Eq. (64) with the exponent $\alpha = 1/2$ is the exact solution of the continuous one-dimensional counterpart of the problem considered (*c.f.* Ref. [93] and Appendix A). As opposed to the case of free diffusion [56–58], *c.f.* Eq. (55), for diffusion in the presence of absorbing sites there is no direct relation between the value of the exponent α and the spectral dimension $\tilde{d}$ but, contrary to the supposition stated in Ref. [93] (*c.f.* also result in Refs. [64,65]), the exponent α does not seem to assume a universal value 1/2 independent of the lattice dimension. As mentioned, our simulations indicate the value $\alpha \approx 0.33$ for the planar Sierpiński gasket and a similar fit of the result obtained for the planar percolation cluster (Fig. 11) gives the value $\alpha \approx 0.25$.

In general, the time course of the reaction was found to consist of three stages: an introductory one (present in the case of the percolation cluster, Fig. 11), the one of the decay according to the algebraic power law (a straight line on the log-log plot) and the final one of exponential decay. In Fig. 12 all these stages are distinguished in a slightly overstressed form.

The moment of crossing over to the exponential stage of the reaction depends on the size of the lattice and the probability of leaving it relative to the probability of transition between the neighbouring sites. The smaller the lattice and the lower the probability of leaving it, the earlier the exponential stage of the reaction begins. The crossover from the power-law decay,

Eqs. (64) and (65), to the exponential decay with the chemical relaxation time κ^{-1} can be described with the help of a simple corrected formula

$$\bar{C}(t) = [(1-a)\,C(t) + a]e^{-\kappa t} \tag{66}$$

with a denoting the level (concentration) from which the exponential decay begins (Fig. 12).

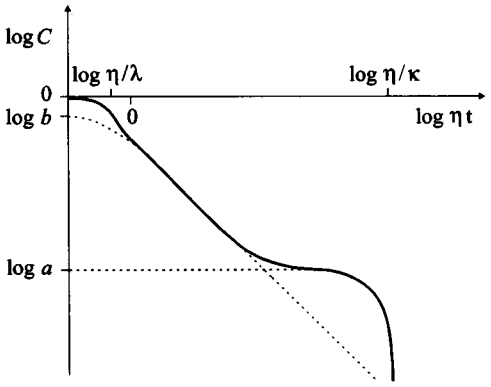

Fig. 12. A schematic form of the survival probability behaviour in time plotted in the log-log scale. Three stages are distinguished: introductory one, one of the decay according to the algebraic power law and the final one of the exponential decay. Two dashed lines correspond to the functional dependence given by Eq. (64) and the exponential long-time correction in Eq. (66), respectively. The short-time behaviour is given by Eq. (67).

For the model of diffusion on the percolation cluster the reaction starts with a certain introductory stage (Fig. 11) that cannot be described by Eq. (64). 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 neighbour sites (*c.f.* Fig. 8(b); it should be noted that as opposed to the solid state physics [97], the configurational averaging over the lattice disorder does not seem to have any meaning in application to protein macromolecules with the well-defined primary structure). The very introductory stage can be fitted to a stretched-exponential law different from that given by Eq. (65) and the crossover from this stage to the standard power-law decay, Eq. (64), can be described with the help of the formula

$$\bar{C}(t) = \left\{(1-b)\exp\left[-(\lambda t)^{\beta}\right] + b\right\} C(t) \tag{67}$$

with λ and β being certain new parameters for the stretched-exponential law and b denoting the level from which the algebraic decay begins (Fig. 12).

4.4. The mean first-passage time. Application to gated enzymatic reaction in the steady-state conditions

Simulations starting at initial sites l_0 different from the gate $l = 0$ result in time courses of the reaction similar to those shown in Figs. 10 and 11 but the curves in the log-log plots are shifted upwards (compare Eq. (A.26) in Appendix A) in such a way that the reaction starts with a stage of nondecreasing C (Palacz, unpublished result). This is quite obvious as there is now a certain period needed for the walkers to reach the gate for the first time.

In the one-dimensional continuous counterpart of the problem considered (*c.f.* Appendix A) the influence of the distance between the initial site and the gate on the time course of the reaction is determined by clear analytical formulas (A.24) to (A.27). No such formulas are, of course, obtainable for diffusion on lattices with non-integer spectral dimension. What is worse, because of the lack of translational symmetry, there is no well defined notion of diffusion distance in such cases. Nevertheless, we would like to have some *ansatz* describing dependence of the survival probability, Eq. (44), or the corresponding first-passage time distribution density, Eq. (43), on at least an *effective* diffusion distance. Let us try to construct such an ansatz using formulas for the one-dimensional case. Having this, we shall be able to calculate the mean first-passage times that determine the rate or the turnover number of complete enzymatic reaction proceeding under the steady state conditions [11,98] (Fig. 13).

In Section 2.2 we argued that in the case of the reaction being an activated process (diffusion on the finite lattice) the mean first-passage time does not depend on the position of the most initial sites l_0 and equals directly to the chemical relaxation time κ^{-1}, Eq. (32). On the other hand side, the mean first-passage time for diffusion on the unbounded lattice is divergent (this holds, *e.g.*, for survival probabilities given by formulas (A.24) to (A.27) in Appendix). To determine correction to the time κ^{-1} for the position of initial site l_0 and, simultaneously, to make convergent values of the mean first-passage time corresponding to Eqs. (A.24) to (A.27), we follow Eq. (66) and express the survival probability $\bar{C}(t|l_0)$ for the bounded, finite lattice in terms of the survival probability $C(t|l_0)$ for the infinite lattice:

$$\bar{C}(t|l_0) = [(1-a)\,C(t|l_0) + a]\,e^{-\kappa t}. \tag{68}$$

Following Eq. (43) we get the corresponding first-passage time distribution density:

$$\bar{f}(t|l_0) = (1-a)\left\{f(t|l_0) + \kappa\left[C(t|l_0) + \frac{a}{1-a}\right]\right\}e^{-\kappa t} \tag{69}$$

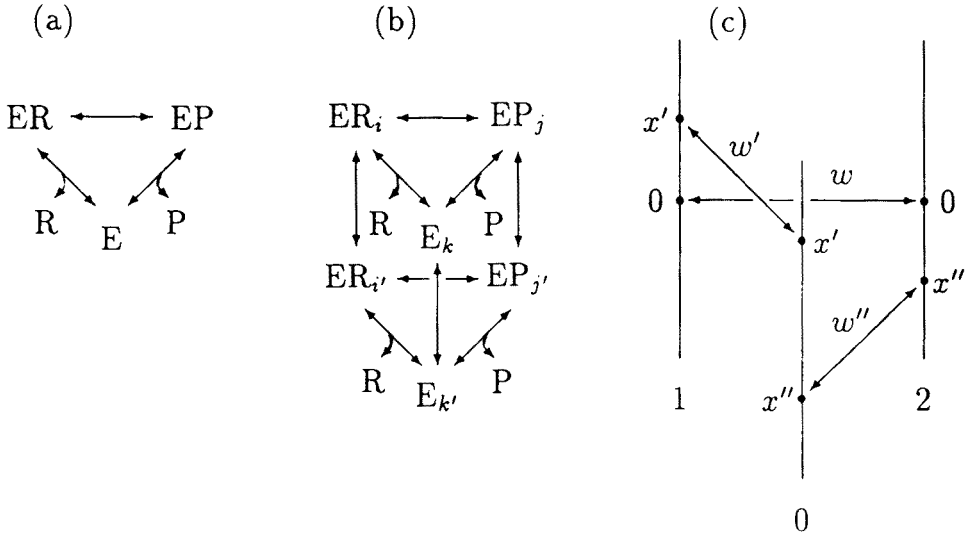

Fig. 13. Enzymatic reaction involving a single covalent transformation. (a) Conventional kinetics of Haldane [1, 2]. R and P stand for the reactant (substrate) and the product, respectively, E stands for the free enzyme, and ER and EP stand for the enzyme-reactant and enzyme-product complexes, respectively. (b) The actual kinetics involving a large number of conformational substates of the enzyme and its complexes. (c) A scheme of enzymatic reaction gated by the conformational transition dynamics. The vertical lines labelled with 0, 1 and 2 symbolize the lattices of conformational substates composing the chemical states E, ER and EP, respectively. Three chemical transitions are localized at the sites 0, x' and x''.

and following Eq. (27), the corresponding mean first-passage time:

$$\bar{\tau}(l_0) = (1-a)\int_0^\infty dt\, C(t|l_0)\, e^{-\kappa t} + a\kappa^{-1}. \tag{70}$$

The integral has the meaning of the Laplace transform of the survival probability $C(t|l_0)$; the finite value of κ secures the cutoff of its long-time tail. After integration by parts and taking into account Eq. (43) we get finally:

$$\bar{\tau}(l_0) = \kappa^{-1} - (1-a)\frac{v_0}{\kappa}\int_0^\infty dt\, e^{-\kappa t} p_{0|l_0}(t). \tag{71}$$

In general, the mean first-passage time is always smaller than the value determined by the reaction rate constant κ. This result, when applied for calculating the enzyme turnover number means that under the steady state

conditions the enzyme macromolecule can pass to the succeeding chemical states with the process of internal equilibration omitted [11].

For the continuous one-dimensional model considered in Appendix A, l_0 is replaced by x, v_0 is replaced by η, and after applying Eq. (A.21) one gets

$$\bar{\tau}(x) = \kappa^{-1} - (1-a)\,\kappa^{-1}\frac{e^{-2x\sqrt{\kappa/\eta}}}{\sqrt{\kappa/\eta}+1}. \tag{72}$$

This can be used to describe how the mean first passage time depends on the *effective*, in this context, distance x.

5. Summary

The substantial majority of biochemical processes are influenced by purely stochastic dynamics of conformational transitions in the proteins involved. We know at present that native proteins reveal such a dynamics in the whole range of time scales from 10^{-11} to 10^{5} s or more, and that at least in the range from 10^{-11}s to 10^{-7}s the corresponding spectrum of relaxation times is practically quasi-continuous. Despite the fact that the experimentally provided picture of conformational transition dynamics is still far from complete the time has come to start thinking about the formulation of a statistical theory of biochemical processes taking this dynamics into account. This paper is an attempt in this direction.

We started with an outline of general ideas of the theory of reaction rates based on stochastic models of intramolecular dynamics. Some important experimentally realized reactions involving proteins and, perhaps, most reactions *in vivo* [8, 11] take place in the conditions under which the initial conformational substate of protein already belongs to or is very close to the reaction transition state. Special attention has been paid to such conditions. Under them the initial, non-exponential stage of a reaction can be very important, even if the reaction is a usual activated process. In more detail we considered the case of gated reactions, when the transition state is reduced to a single conformational substate of the protein (the gate).

Next we considered a particular model of the conformational transition dynamics in proteins — a random walk on fractal lattices — and its application to describe gated reactions with the initial conformational substate of protein coinciding with the gate. The computer simulations performed for the planar Sierpiński gasket and the planar percolation cluster indicate in general three stages of the reaction:an introductory one, the one of the decay according to the algebraic power law and the final one of the exponential decay, present for finite fractal lattices.

We propose to describe the whole time course of the reaction in terms of three simple formulas (64), (66) and (67). Apart from the basic unit of time

η^{-1}, the proposed description introduces six parameters: two exponents α and β, two rates κ and λ, and two probabilities (molar ratios) a and b. Four of those parameters: κ, λ, a and b depend on temperature in the Arrhenius manner so it should be no problem to describe in these terms a time course of any experimentally observed reaction including its variation with temperature. We refrain here from discussion of any particular experimental data as reaching the really valuable conclusions needs, in any case, much more careful and detailed analysis.

At the end we suggest a general formula for the mean first-passage time for diffusion on finite fractal lattices depending on the effective distance between the initial state and the gate. It can be useful in description of complete enzymatic reactions proceeding under the steady state conditions.

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Appendix A

Exactly solvable continuous one-dimensional model

In the continuous limit the process of one-dimensional diffusion is described by the partial differential equation of the general form [66,67]

$$\frac{\partial}{\partial t}p + \frac{\partial}{\partial x}j = 0, \tag{A.1}$$

where t and x denote, respectively, time and position, $p(x,t)$ is the probability density and $j(x,t)$ the diffusion flux density, linearly depending on $p(x,t)$. As a complement to Eq. (A.1) we assume the general *reactive* boundary condition at $x = 0$:

$$j(0,t) = -\eta\, p(0,t), \qquad p(x,t) = 0 \text{ for } x < 0. \tag{A.2}$$

In the limit $\eta \to \infty$ the condition (A.2) determines the *absorbing* boundary:

$$p(0,t) = 0, \tag{A.3}$$

whereas in the limit $\eta \to 0$, the *reflecting* boundary:

$$j(0,t) = 0. \tag{A.4}$$

There is a jump of the flux density j at $x = 0$, thus Eq. (A.1) with the boundary condition (A.2) is equivalent to the very equation

$$\frac{\partial}{\partial t}p + \frac{\partial}{\partial x}j = -\eta\,\delta(x)\,p \tag{A.5}$$

with a delta-type sink.

A general solution to Eq. (A.5) with an arbitrary initial probability density $p(x,0)$ can be written in the form of the integral

$$p(x,t) = \int_{-\infty}^{\infty} dx'\,p(x,t|x')\,p(x',0) \tag{A.6}$$

with the kernel (Green function or the propagator) $p(x,t|x')$ being the solution to Eq. (A.5) with the delta-type initial probability distribution

$$p(x,0|x') = \delta(x-x'). \tag{A.7}$$

Our goal is to calculate in this Appendix the survival probability in the region $\mathrm{R} = [0,\infty)$,

$$C(t|x) = \int_{0}^{\infty} dx'\,p(x',t|x). \tag{A.8}$$

The continuous counterpart to Eqs. (43) and (44) are the equations

$$\dot{C}(t|x) = -f(t|x) = -\eta\,p(0,t|x) \tag{A.9}$$

and

$$C(t|x) = 1 - \eta\int_{0}^{t} dt'\,p(0,t'|x), \tag{A.10}$$

respectively. On assuming that the solution $p^0(x,t|x')$ to the equation (A.1) for the free diffusion, without any boundary condition or a sink, is known, one can find the solution $p(x,t|x')$ to the full equation (A.5) by treating the sink term formally as an external time-dependent perturbation. Following the theory of temporal Green functions [99] the particular propagator we need $p(0,t|x)$ satisfies the self-consistent integral equation

$$p(0,t|x) = p^0(0,t|x) - \eta\int_{0}^{t} dt'\,p^0(0,t'|0)\,p(0,t-t'|x). \tag{A.11}$$

This can be solved in the Laplace transforms:

$$\tilde{p}(0,s|x) = \frac{\tilde{p}^0(0,s|x)}{1+\eta\tilde{p}^0(0,s|0)}, \tag{A.12}$$

where

$$\tilde{p}(0,s|x) \equiv \int_0^\infty dt\, e^{-st} p(0,t|x). \tag{A.13}$$

The exact solution to Eq. (A.11) can be easily found in the case of the *homogeneous* one-dimensional diffusion with the reactive boundary condition, described by the equation

$$\frac{\partial}{\partial t} p - D \frac{\partial^2}{\partial x^2} p = -\alpha\, \delta(x)\, p \tag{A.14}$$

with D denoting the diffusion constant and α, the transition probability to the sink per unit time. In this problem there is a natural unit of length:

$$\xi \equiv 4D/\alpha, \tag{A.15}$$

and of time:

$$\eta^{-1} \equiv 4D/\alpha^2. \tag{A.16}$$

On passing to the dimensionless position variable

$$\xi^{-1} x \to x, \tag{A.17}$$

Eq. (A.14) reads

$$\frac{\partial}{\partial t} p - \frac{\eta}{4} \frac{\partial^2}{\partial x^2} p = -\eta\, \delta(x)\, p. \tag{A.18}$$

The free propagator, in the absence of sink, is the Gaussian:

$$p^0(0,t|x) = \frac{1}{\sqrt{\pi \eta t}}\, e^{-x^2/\eta t}, \tag{A.19}$$

and its Laplace transform [96]

$$\tilde{p}^0(0,s|x) = \eta^{-1} \frac{1}{\sqrt{s/\eta}}\, e^{-2x\sqrt{s/\eta}}. \tag{A.20}$$

Following Eq. (A.12) the Laplace transform of the full propagator

$$\tilde{p}(0,s|x) = \eta^{-1} \frac{e^{-2x\sqrt{s/\eta}}}{\sqrt{s/\eta} + 1}. \tag{A.21}$$

It can be exactly inverted [96]:

$$p(0,t|x) = \frac{1}{\sqrt{\pi \eta t}}\, e^{-x^2/\eta t} - \exp(\eta t + 2x)\, \operatorname{erfc}\left(\sqrt{\eta t} + \frac{x}{\sqrt{\eta t}}\right), \tag{A.22}$$

where the symbol erfc denotes the complementary error function,

$$\operatorname{erfc} z \equiv \frac{2}{\sqrt{\pi}} \int_z^\infty dy\, e^{-y^2} \approx \begin{cases} \frac{1}{\sqrt{\pi} z^2} e^{-z^2} & \text{for } z \gg 1 \\ e^{-2z/\sqrt{\pi}} & \text{for } 0 < z \ll 1. \end{cases} \tag{A.23}$$

After integration of the propagator (A.22) following Eq. (A.10) we get, finaly, the exact expression for the survival probability for the model considered:

$$C(t|x) = \exp(\eta t + 2x) \operatorname{erfc}\left(\sqrt{\eta t} + \frac{x}{\sqrt{\eta t}}\right) + 1 - \operatorname{erfc} \frac{x}{\sqrt{\eta t}}. \tag{A.24}$$

In the limit $\eta \to \infty$ (the absorbing boundary) Eq. (A.22) goes over the well-known *Smirnov distribution* [22]:

$$p(0, t|x) = \frac{x}{\sqrt{\pi}} (\eta t)^{-3/2} e^{-x^2/\eta t} \tag{A.25}$$

and Eq. (A.24) is reduced to

$$C(t|x) = 1 - \operatorname{erfc} \frac{x}{\sqrt{\eta t}}. \tag{A.26}$$

For η finite but large with respect to t^{-1} we find, following the asymptotic expansion of the complementary error function, Eq. (A.23), that the survival probability $C(t|x)$ decreases asymptotically in time according to the power law

$$C(t|x) \approx (1+2x)(\pi \eta t)^{-1/2}. \tag{A.27}$$

For $x = 0$ we get from Eq. (A.22) the probability density of returning to the initial state:

$$p(0, t|0) = \frac{1}{\sqrt{\pi \eta t}} - e^{\eta t} \operatorname{erfc}(\sqrt{\eta t}), \tag{A.28}$$

and from Eq. (A.24), the corresponding survival probability:

$$\begin{aligned} C(t|0) &= e^{\eta t} \operatorname{erfc}(\sqrt{\eta t}) \\ &\approx \begin{cases} \exp\left[-(4\eta t/\pi)^{1/2}\right] & \text{for } t \ll \eta^{-1} \\ (\pi \eta t)^{-1/2} & \text{for } t \gg \eta^{-1}. \end{cases} \end{aligned}$$

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