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Protein machine model of enzymatic reactions gated by enzyme internal dynamics

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Abstract

The slow character of conformational transition dynamics in native proteins, recently becoming more and more apparent, makes conventional theories of chemical reactions inapplicable for the description of enzymatic reactions. Any contemporary statistical theory of biochemical processes has to be based on a possibly simple but realistic model of microscopic dynamics of participating biomolecules. In a model considered in this paper the dynamics of enzymatic protein is approximated by a quasi-continuous diffusive motion of its solid-like structural elements relative to each other. The enzymatic reaction is assumed to involve three steps (a covalent tranformation preceded and followed by association–dissociation processes with the substrate and the product), each step being gated by conformational diffusion. In general, the reaction proceeds in three stages: initial, transient and steady-state. Carefully approximated analytical formulae describing the kinetics in each stage are derived. In the limit of the fast internal dynamics of the enzyme, when compared to the local chemical transformations, the initial stage of reaction, dependent on the initial distribution of enzyme conformations, is absent and all the formulae describing the remaining two stages simplify to those provided by the classical theory of Haldane. However, following recent studies, the rule seems to be that it is the conformational dynamics of the enzyme, and not the details of chemical mechanism, that affects the rate of enzymatic reaction. Apart from the possibility of the initial inhomogeneous kinetics, the important result obtained in the limit of slow conformational dynamics is that the kinetic mechanisms of a reaction differ in general between the transient and steady-state stages. Possibilities of carrying out an *experimentum crucis* directly discrediting the conventional approach are considered.

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1. Introduction

Great progress in studies of protein dynamics accomplished in recent years [1–6] propels an essential alteration in our understanding of enzymatic reactions. Many experiments performed with the help of various techniques have demonstrated that native proteins, in particular enzymes, apart from the usual vibrational dynamics, reveal also a rich activated dynamics of conformational transitions in the whole range of time scales from 10^{-11} to 10^{5} s or longer (Fig. 1). The slow character of this dynamics makes

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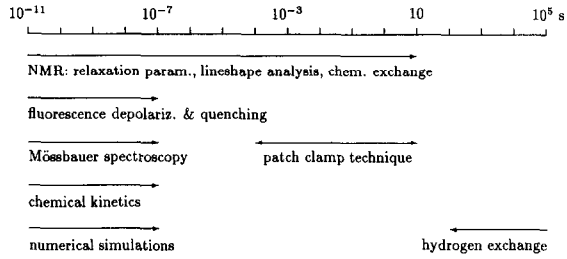

Fig. 1. Time scale of conformational transitions within the protein native state observed with the help of various experimental techniques. Time period 10^{-11} s at one edge of the scale characterizes localized conformational transitions on the protein surface, related to overcoming the energy barrier of 10 kJ mol^{-1} assumed in this paper to be the lower bound of the interconformational barrier heights. Time period 10^{5} s at the other edge is a (rather underestimated) value of the waiting time for spontaneous unfolding of the protein in physiological conditions. Note that the typical reciprocal turnover number of enzymatic reactions, 10^{-3} s, is exactly in the middle of the scale. The time scale of conformational transitions observed in the chemical kinetics experiments at low temperatures is extrapolated to physiological temperature. The longest time period marked for numerical simulations concerns only Brownian dynamics; molecular dynamics simulations are at present two orders of magnitude shorter. References to representative papers can be found in the reviews [1–6].

conventional theories of chemical reactions inapplicable for the description of enzymatic reactions [7,8]. The simple classical statement offered by the transition state theory, commonly used in interpretation of the enzymatic catalysis phenomenon [9,10], "enzymes accelerate reactions by decreasing the free energy of activation", appears to represent only half the truth. Enzymatic reactions actually proceed through 'gates' of relatively low free energy, but it is probably not the process of gate crossing that usually limits the reaction rate but the process of gate opening, controlled by internal dynamics of the enzyme. Any contemporary statistical theory of enzymatic reactions has to take this dynamics into account.

The internal degrees of freedom of protein macromolecules are the atomic covalent bond lengths and angles as well as the dihedral angles of rotation about the bonds. It is the ability to perform such rotations (limited only to some degree by steric hindrances), combined with the possibility of break-up and reformation of hydrogen bonds, that makes the landscape of the internal 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 consisting, on average, of 100 amino acid residues) of local minima separated by higher or lower energy barriers of non-covalent nature [1,2,11]. 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* or, more simply, as protein *conformations*. In a reasonable approximation, the internal dynamics of protein is to be decomposed into *vibrations* within particular conformational states and *conformational transitions* [6]. The former are more or less damped harmonic oscillations, subjected accidentally to stochastic perturbations, whereas the latter are purely stochastic activated processes. This 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 $k_B T$, 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 vibrational anharmonicity, to be taken into account on assuming a finite correlation time of stochastic forces and, accordingly, a time-dependent friction [12].

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 whole domains), hence the vibrational dynamics is too fast to influence essentially the processes of enzymatic reactions. 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 lowest energy barrier of the order of 10 kJ/mol assumed above) and its upper limit is the mean waiting time for spontaneous

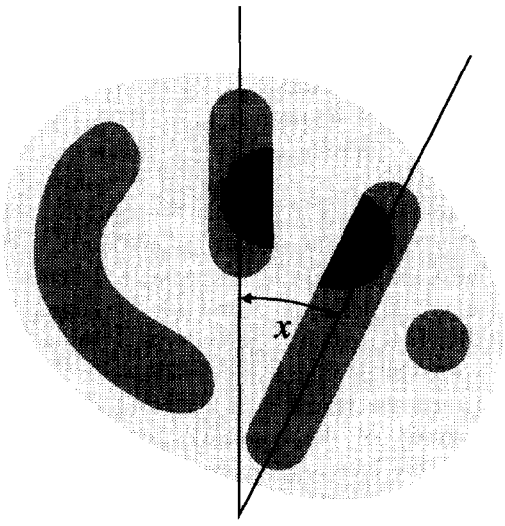

Fig. 2. Schematic cross-section of the fundamental structural unit of protein, a domain. Heavily shaded are solid-like fragments of secondary structure (α-helices or β-sheets) and lightly shaded are surrounding liquid-like regions. Black is the catalytic centre localized at two neighbouring solid-like elements. In models of the protein glass type, the dynamics of conformational transitions is treated as a diffusion of structural defects through the liquid-like medium. Alternatively, in models of the protein machine type this dynamics is treated as a relative motion of solid-like elements, also of a diffusional nature, along a mechanical coordinate, identified here with the angle x. The picture can be reinterpreted on a higher structural level: solid-like elements then represent whole domains moving in a multidomain enzymatic complex.

unfolding, of a value carefully estimated to be within the range 10^3–10^{11} s [11]. Beginning with the pioneer study of the low-temperature dispersive kinetics of ligand rebinding to myoglobin by Frauenfelder and coworkers [13], an increasing number of experiments give almost every year new evidence that conformational transition dynamics is, as we have already stated, characteristic of not only the unfolded but also the native state of protein [1–6].

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. 2). The corresponding relaxation time spectrum seems to be practically quasi-continuous, at least in the range from 10^{-11} to 10^{-7} s (Fig. 1). Two classes of theoretical models of conformational transition dynamics provided in previous literature display this property [6,8]. In the first, 'protein glass' class of models, the dynamics is assumed to look alike in every time scale. Time scaling can originate either from a hierarchy of potential barrier heights or from a hierarchy of bottlenecks encountered by structural defects diffusing across the liquid-like region of the protein. By the very definition, protein glass models show unrealistic behaviour within the limits of both very short and very long times, and in practice should be restricted to only a few levels of the hierarchy [4,5]. An alternative, free of such disadvantages, is the second, 'protein machine' class of models, in which the dynamics of conformational transitions is represented by a quasi-continuous motion along a few 'mechanical' coordinates, e.g. angles describing mutual orientation of approximately rigid fragments of secondary structure or larger structural elements (Fig. 2).

In the present paper a simple version of the protein machine model is studied and applied to construct a statistical theory of a complete enzymatic reaction involving a single covalent transformation. Preliminary and partial results, unfortunately not free from certain inaccuracies which we shall correct here, have already been published elsewhere [8,14].

2. Formulation and experimental justification of the protein machine model

The concept (and the name) 'protein machine' was proposed by Chernavsky *et al.* in 1967 [15] but a similar picture of protein dynamics, rather speculative at that time, has been considered independently also by McClare [16], Blumenfeld [17], Williams [18] and Gavish [19] (for a review see Kováč [20]). All these authors use a similar notion of the machine as "a device which uses energized motion to bring about transformation" [18], "a structure which displays high mobility in certain directions and rigidity in others" [19] or "a device with mechanically constrained parts predetermined to give some effects by restricting motion along one or several degrees of freedom" [20]. According to Kováč [20], after Blumenfeld [17], "any machine exhibits a particular degree of freedom which, when excited by an input of energy, exchanges its energy with other degrees of freedom very slowly; in other words, its rate of relaxation is low". The latter statement coincides,

however, with a definition of any macroscopic system [21]. For the protein machine concept it is important that the distinguished variable is intensive rather than extensive and that the system is not macroscopic but mesoscopic, not with a deterministic but a stochastic dynamics.

A motion of fragments of secondary structure relative to each other is often observed when two or more different crystalline structures of the same protein are compared [22]. Also, a relative motion of entire domains has been the subject of numerous structural investigations [23,24]. Naturally, in comparative structural studies only a few, usually two, conformations are visible, and in terms of these few *discrete* conformational states the conformational dynamics is described, if necessary, by conventional biochemistry [9,10]. An important experimental contribution leading to a change in this picture of protein dynamics comes from the paper by Haran *et al.* [25] who found a *quasi-continuous* distribution of interdomain distances, thus protein conformational states, when studying the fluorescence energy transfer between donor and acceptor centres located on different domains.

A quasi-continuum of short-lived conformational states within the native state of protein has been observed directly in numerical simulations [26–28]. A careful analysis [27,29] indicated that numerically studied conformational transition dynamics actually has the character of a relative motion of the secondary structure elements.

Also, the normal modes of low-frequency collective vibrations of proteins take the form of mutual motions of relatively rigid fragments of secondary structure [30,31]. Mainly because of the presence of the surrounding water, these motions are overdamped, i.e. have a nature of a limited random walk rather than harmonic vibration. One should, however, be careful in identifying the mechanical variables directly with the normal mode coordinates. The reason is the short relaxation time of the overdamped normal modes, only twice as long as their period, i.e. 10^{-11} s at the most [32,33]. A better counterpart seems to be the *molecule optimal dynamical coordinates* [28] of the *essential modes* of motion [34] along directions diagonalizing not the force matrix (which the normal mode directions do) but the covariance matrix of atomic displacements. In almost nanosecond molecular dynamics simulations no equilibration has been observed of trajectories projected onto at least one of these coordinates [28,34].

Moreover, the above-mentioned study of fluorescence energy transfer [25] indicates that the motion of domains relative to each other is "slow on the nanosecond time scale". In fact, the quoted value of the intramolecular diffusion constant and that of the equilibrium dispersion of interdomain distance give the value of the upper limit of quasi-continuous relaxation time spectrum to be of the order of 10^{-7} s. It is worthwhile noting that the same order of magnitude is also the value, extrapolated to physiological temperatures, of the relaxation time between the lowest in the hierarchy of conformational substates observed by Frauenfelder and coworkers in experiments of ligand binding to myoglobin [4] (Fig. 1).

In the unfolded state of protein the protein machine picture of conformational transition dynamics is identical with the Karplus and Weaver diffusion–collision model of folding [35,36]. In general, the mechanical variables do not have to be necessarily related to the orientation of structural, 'mechanical' elements of the protein. The distance, considered by Frauenfelder and coworkers [37], between particular atoms composing a physical gate to ligands diffusing through the protein matrix, the displacement of the iron from the heme plane, considered by Agmon and Hopfield [47], and the local electric dipole moment at the active centre or the amount of water bound to the enzyme surface, considered by Careri and coworkers [38], are, in fact, also mechanical variables. All these quantities have relaxation times of the order 10^{-8}–10^{-7} s.

The first, though implicit, practical employment of the protein machine picture of dynamics was the application of Kramer's theory of reaction rates in the spatial diffusion limit [39,40] for interpretation of two particular protein reactions that were studied by Gavish and Werber [41] and Frauenfelder with coworkers [37] in solvents of various viscosities. Also, the description of Mössbauer nuclei displacements in the protein interior as diffusion in a potential which is the envelope to the actual multi-well potential, proposed first by Frauenfelder *et al.* [42] and next realized by Knapp *et al.* [43] and by Nadler and Schulten [44,45], was in the spirit of the protein machine model.

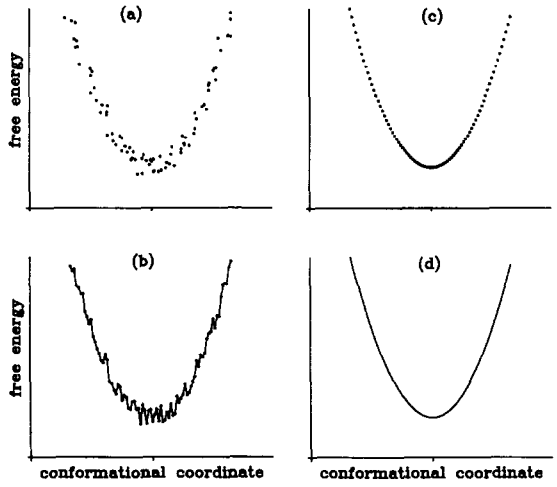

Fig. 3. Formulation of the protein machine model. (a) Conformational states (free energy levels) are labelled with the help of a conformational (mechanical) coordinate, e.g. the angle describing mutual orientation of solid-like structural elements. (b) Transitions are assumed to be possible only between neighbouring conformational states. (c) Smoothing of the conformational potential. (d) Transition to the limit of continuous diffusion.

Explicitly, the protein machine model was formalized in terms of diffusion in an effective potential along the mechanical coordinate by Shaitan and Rubin in 1982 [46]. This kind of dynamics with the simplest, parabolic potential has been applied to the description of a single irreversible protein reaction by Agmon and coworkers [47,48], and of two coupled irreversible reactions by Cartling [49]. A general mathematical analysis of the model with parabolic potential applied to a single reversible reaction was performed by the present author [14]. The latter paper gives references to works on other applications of the model.

Assumptions which have to be made in order to approximate the dynamics of conformational transitions by the process of limited diffusion along a mechanical coordinate are schematically presented in Fig. 3. By definition, any conformational state of the protein is characterized by a definite value of free energy; the thermodynamic averaging is taken over equilibrated vibrational degrees of freedom. One can always label a set of all 'free energy levels' of the protein with the help of one or a few 'conformational' coordinates (Fig. 3a). What distinguishes the *mechanical* coordinates from the others is that direct transitions take place only between the nearest neighbour conformational states along them (Fig. 3b). Variation of, e.g., the angle between two structural elements of a protein molecule (Fig. 2) goes through a series of well defined successive conformational transitions involving breakup and reformation of hydrogen bonds in the liquid-like interior of the protein as well as the surrounding water. Smoothing of the free energy (Fig. 3c) and transition to the continuous limit (Fig. 3d) result in replacement of a chain of conformational jumps by diffusion in an effective, approximately parabolic conformational potential.

Diffusion in a parabolic potential along the coordinate x is described by the partial differential equation for the probability density $p(x,t)$ with t denoting time [50–52]:

$$\frac{\partial}{\partial t}p(x,t) = \frac{\partial}{\partial x}D\left[\frac{\partial}{\partial x} + \beta B(x - x_0)\right]p(x,t). \tag{2.1}$$

The parameter B determines the stiffness of the potential, x_0 is the position of the minimum, D denotes the diffusion constant and β is the reciprocal temperature, $\beta \equiv (k_B T)^{-1}$. Historically, Eq. (2.1) was introduced in order to describe the position of a harmonic oscillator in the overdamped (Smoluchowski) limit, or the velocity of a Brownian particle driven by a friction force (the Ornstein–Uhlenbeck process). Here, we give yet another interpretation of that equation.

The form of the equilibrium solution to Eq. (2.1),

$$p^{eq}(x) \propto e^{-\beta B(x-x_0)^2/2}, \tag{2.2}$$

indicates that the quantity $(\beta B)^{-1}$ stands for the equilibrium dispersion of the mechanical variable. For the sake of convenience we shall pass to a dimensionless mechanical variable (of dispersion $1/2$) by the replacement

$$\sqrt{\beta B/2}\, x \to x. \tag{2.3}$$

In terms of the new variable, Eq. (2.1) reads

$$\frac{\partial}{\partial t}p(x,t) = \frac{1}{2}\gamma\frac{\partial}{\partial x}\left[\frac{\partial}{\partial x} + \frac{\partial G}{\partial x}\right]p(x,t), \tag{2.4}$$

where G is our parabolic potential in $k_B T$ units:

$$G(x) = (x - x_0)^2. \tag{2.5}$$

The only parameter

$$\gamma \equiv \beta BD \tag{2.6}$$

has the meaning of the reciprocal relaxation time of the mean value of the mechanical variable

$$X(t) \equiv \int_{-\infty}^{\infty} \mathrm{d}x\, x p(x,t). \tag{2.7}$$

Indeed, substitution of Eq. (2.4), with G given by Eq. (2.5), into Eq. (2.7) results in an ordinary differential equation of the form

$$\dot{X} = -\gamma(X - x_0) \tag{2.8}$$

(the dot means derivation with respect to time).

Eq. (2.4), with the parabolic potential (2.5), is equivalent to the Schrödinger equation with imaginary time for a quantum-mechanical harmonic oscillator [14,52] and, in general, has an equidistant spectrum of reciprocal relaxation times:

$$\tau_n^{-1} = n\gamma,\ n = 0,1,2,... \tag{2.9}$$

The infinite relaxation time τ_0 corresponds to the preserved normalization of probability, whereas τ_n with $n \geq 2$ describe increasingly faster relaxation of higher moments of the probability density p.

In the approximation of a chain of discrete conformational transitions by a diffusion process (Fig. 3) the diffusion constant D replaces the product ka^2, k being the rate of transitions between the neighbouring conformations and a the distance between them along the diffusion coordinate x [50]. On taking this into account and recalling that $(\beta B)^{-1}$ determines the equilibrium dispersion of conformational states along this coordinate, one can estimate the value of γ, Eq. (2.6), with the help of a more directly interpretable relation

$$\gamma = k/N^2. \tag{2.10}$$

Here, $N \equiv (\beta B)^{-1/2}/a$ denotes the mean square root number of thermally populated conformational states of the protein and (we cannot forget it!) its closest environment. For $k^{-1} = 10^{-11}$ s, a value typical for a transition consisting of breakup and reformation of a single hydrogen bond, we obtain $\gamma^{-1} = 10^{-7}$ s, the earlier estimated upper limit of the quasi-continuum of relaxation time spectrum, for $N = 10^2$, which seems to be quite a reasonable number.

Relation (2.10) also enables an estimation of the mean square root number of conformational states, divided by potential barriers of the assumed height of at least 10 kJ/mol (which corresponds to $k^{-1} = 10^{-11}$ s), visited by the system during the period of time shorter than γ^{-1}. For periods of 10^{-10} to 10^{-9} s (a hundred picoseconds to a nanosecond) one obtains $N = 3$ to 10, values which can be compared with the presently available results of molecular dynamics simulations [26,28].

It is worthwhile noting that Eqs. (2.4) and (2.5), when completed by a source term, can also describe dynamical processes much slower than the longest time of conformational relaxation $\gamma^{-1} \approx 10^{-7}$ s. Such processes are those of the first passage, considered in Appendix A. They last several orders of magnitude longer than γ^{-1}, say 10^{-3} s, which is a typical time of enzymatic reactions.

Of course, we do not claim that the description of protein dynamics in terms of diffusion in a parabolic potential represents a comprehensive version of the one-dimensional protein machine model. Firstly, the effective potential can differ considerably from the parabolic one. This is, however, not a problem as various approximation techniques well known from quantum mechanics can then be applied in order to solve the diffusion equation. Secondly, the smoothing procedure (transition from diagram (b) to (c) in Fig. 3) can appear too rough an approximation. In that case the model of "random walk in a random potential" [53–55] is more appropriate. Thirdly, the restriction to transitions only between the nearest neighbours on the conformational coordinate, diagram (b) in Fig. 3, can appear insufficient. This is, technically, the most difficult problem. When the insufficiency arises from too small a number of the considered degrees of freedom, one can try to replace the neglected degrees of freedom by memory effects [12]. And when, despite of this, transitions between next neighbours are still important, one can try, following Kramers–Moyal expansion [50], to introduce higher derivatives with respect to the mechanical coordinate. There are many ways to generalize the described version of the protein machine model, and some of them are certainly worth detailed studies in future.

3. Generalized Haldane's kinetics

It is not the aim of this paper to consider particular chemical mechanisms of enzymatic catalysis, so only the simplest reaction involving a single covalent transformation

$$R \leftrightarrow P$$

will be considered. Usually, such a reaction is modelled by the three-step kinetics of Haldane [9,10], with the addition of only two association–dissociation reactions of the substrate or the product to the enzyme (Fig. 4a).

In conventional biochemistry [9,10] the assumption of a particular kinetic mechanism automatically implies that the elementary component reactions are well separated on the time scale from other processes. But we know that this cannot be true: conformational dynamics within the native state of enzyme is as slow, if not slower, as the overall reaction. Certainly, some conformational transitions are independent of the reaction but others must affect the latter to some extent. Consequently, to describe the actual kinetic mechanism of enzymatic reaction one has to treat conformational (non-covalent) transitions on an equal footing with chemical (covalent or binding) transformations. The actual kinetic scheme of the enzymatic reaction involving a single covalent step, given in Fig. 4b, appears far more complex even than the 'oscillating enzyme' scheme of Jencks [9].

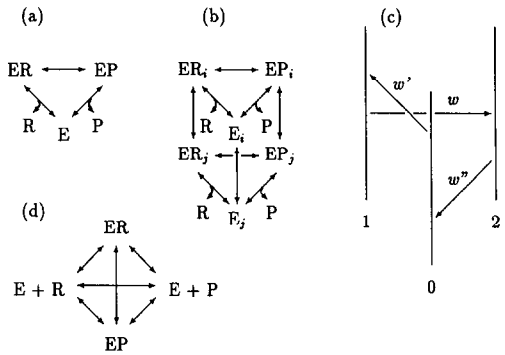

Fig. 4. Enzymatic reaction involving a single covalent transformation. (a) Conventional kinetics of Haldane. R and P stand for the reactant and the product respectively, E stands for the free enzyme, and ER and EP stand for the enzyme–reactant and enzyme–product complexes respectively. (b) The actual kinetics, involving a large number of conformational states of the enzyme or its complexes labelled with indices i and j. (c) Protein machine model. Dynamics of conformational transitions within each of the three chemical species E, ER and EP is approximated by diffusion along a mechanical coordinate. Perpendicular chemical transitions are, in general, reversible; the arrows indicate only the assumed signs of local reaction rates. (d) Effective kinetic scheme (see Section 6): direct reactions are possible between each pair of distinguished chemical species.

Obviously, a scheme like that in Fig. 4b can hardly be recognized as a proper phenomenological description of the reaction [7]. As in the conventional approach, the process should be described phenomenologically in terms of concentrations of a few observable species, in the simplest case only the *chemical* ones: E, ER and EP. Formally, one can write down standard kinetic equations for the concentrations [E], [ER] and [EP]; however, the rate parameters occurring in them will be functions (in general, stochastic) of time. The problem of a reasonable approximation to those equations, acceptable by experimentalists, is highly non-trivial [56,57] and its satisfactory solution crucially depends on the assumed model of protein dynamics.

In the protein machine model considered, the dynamics of conformational transitions within each of the three distinguished chemical enzyme species E, ER and EP is approximated by diffusion along the mechanical coordinate x in a certain effective parabolic potential. Chemical transformations are to be interpreted as transitions perpendicular to the mechanical coordinate; thus the dynamics of the enzyme together with the reaction is described by a set of three coupled diffusion–reaction equations for probability densities $p_i(x, t)$ ($i = 0, 1, 2$ for E, ER and EP, respectively):

$$\frac{\partial}{\partial t} p_i = -\frac{\partial}{\partial x} j_i + w_i, \tag{3.1}$$

with the diffusion fluxes

$$j_i = -\gamma \left[\frac{1}{2} \frac{\partial}{\partial x} + (x - x_i) \right] p_i \tag{3.2}$$

(we assume that the conformational potential within each of the chemical states i of the enzyme has the same stiffness and differs only in the position of the minimum, x_i) and the local reaction rates

$$w_0 = -w' + w'', \; w_1 = -w + w', \; w_2 = w - w'' \tag{3.3}$$

(see Fig. 4c).

The reaction rates w, w' and w'' have to satisfy the detailed balance condition; i.e., they have to vanish in the equilibrium at each point x. Consequently, we shall write them down in the form

$$w = \kappa C_1^{eq} C_2^{eq} W(x)\left(p_1/p_1^{eq} - p_2/p_2^{eq}\right),$$
$$w' = \kappa' C_0^{eq} C_1^{eq} W'(x)\left([R]p_0/[R]^{eq} p_0^{eq} - p_1/p_1^{eq}\right),$$
$$w'' = \kappa'' C_2^{eq} C_0^{eq} W''(x)\left(p_2/p_2^{eq} - [P]p_0/[P]^{eq} p_0^{eq}\right). \tag{3.4}$$

The coefficients κ, κ' and κ'' are reciprocal time units of a meaning which will be explained further on, W, W' and W'' are certain functions of the conformational coordinate x, normalized to unity:

$$\int_{-\infty}^{\infty} dx\, W(x) = \int_{-\infty}^{\infty} dx\, W'(x) = \int_{-\infty}^{\infty} dx\, W''(x) = 1, \tag{3.5}$$

and [R] and [P] denote the molar concentrations of the reactant and product, respectively (the superscript 'eq' distinguishes the corresponding equilibrium values). If the detailed balance condition is satisfied, Eqs. (3.1) have the equilibrium solution

$$p_i^{eq}(x) = C_i^{eq} \pi^{-1/2} e^{-(x-x_i)^2}. \tag{3.6}$$

The coefficients C_i^{eq} in Eqs. (3.6) and (3.4) denote the equilibrium values of the mole fractions of species 0, 1 and 2 of the enzyme, defined as

$$C_i(t) \equiv \int_{-\infty}^{\infty} dx\, p_i(x,t) \tag{3.7}$$

and normalized to unity:

$$C_0 + C_1 + C_2 = 1. \tag{3.8}$$

The quantities C_i are directly related to the molar concentrations of E, ER and EP:

$$C_0 = [E]/[E]_0,\ C_1 = [ER]/[E]_0,\ C_2 = [EP]/[E]_0, \tag{3.9}$$

with

$$[E]_0 \equiv [E] + [ER] + [EP] \tag{3.10}$$

denoting the total molar concentration of enzyme.

Because diffusion fluxes j_i vanish in infinity, the mole fractions C_i vary in time according to the equations

$$\dot{C}_i = \int_{-\infty}^{\infty} dx\, w_i(x) \tag{3.11}$$

and, from Eqs. (3.4), it follows that

$$[\dot{R}]/[E]_0 = -\int_{-\infty}^{\infty} dx\, w',\ [\dot{P}]/[E]_0 = \int_{-\infty}^{\infty} dx\, w'' \tag{3.12}$$

(the dot means derivation with respect to time). Note that in general the right-hand sides of these equations depend not only on the mole fractions C_i but also on higher moments of probability densities p_i. Only when reactions are *activated* processes, i.e. when equilibration of microstates within individual chemical species proceeds much faster than equilibration between the species [58], do Eqs. (3.11) and (3.12) take the form of closed kinetic equations, possibly after a short initial period.

In particular, when internal equilibration within the species proceeds infinitely fast, one can approximate the instantaneous values of probability densities $p_i(x, t)$ by

$$p_i(x,t) = \left(C_i(t)/C_i^{eq}\right) p_i^{eq}(x) \tag{3.13}$$

to get

$$\int_{-\infty}^{\infty} dx\, w = \left(\kappa C_2^{eq}\right) C_1 - \left(\kappa C_1^{eq}\right) C_2,$$
$$\int_{-\infty}^{\infty} dx\, w' = \left(\kappa' C_1^{eq}/[R]^{eq}\right)[R]C_0 - \left(\kappa' C_0^{eq}\right) C_1,$$
$$\int_{-\infty}^{\infty} dx\, w'' = \left(\kappa'' C_0^{eq}\right) C_2 - \left(\kappa'' C_2^{eq}/[P]^{eq}\right)[P]C_0. \tag{3.14}$$

Expressions in brackets have the meaning of rate constants for the conventional kinetic scheme given in Fig. 4a. The assumption of partial equilibrium, Eq. (3.13), is the core of the transition state theory [14]. Consequently, the three parameters κ, κ' and κ'' are to be interpreted as chemical relaxation times provided for the three component reactions by the transition state theory.

Of course, general requirements for a reaction to be an activated process are much weaker than those assumed by the transition state theory [58] and the assumption of fast internal equilibration does not contradict the possibility that intramolecular dynamics can essentially affect the effective reaction rates.

4. Covalent transformation alone. The assumptions of activation and gating

A general study of the complete enzymatic reaction described by the system of Eqs. (3.1) to (3.4) is mathematically complex and physically rather abstract so, from the very beginning, we are forced to make certain additional assumptions. To understand in more detail the way they function let us consider first the covalent transformation of the enzyme alone:

$$\mathrm{ER} \leftrightarrow \mathrm{EP}$$

in the scheme given in Fig. 4a. This transformation, together with the dynamics of conformational transitions of the enzyme, is described by a system of two coupled equations [14]:

$$\begin{aligned}\frac{\partial}{\partial t}p_1 &= \gamma\frac{\partial}{\partial x}\left[\frac{1}{2}\frac{\partial}{\partial x}+(x-x_1)\right]p_1 \\ &\quad -\kappa C_1^{\mathrm{eq}}C_2^{\mathrm{eq}}W(x)\left[p_1/p_1^{\mathrm{eq}}-p_2/p_2^{\mathrm{eq}}\right], \\ \frac{\partial}{\partial t}p_2 &= \gamma\frac{\partial}{\partial x}\left[\frac{1}{2}\frac{\partial}{\partial x}+(x-x_2)\right]p_2 \\ &\quad +\kappa C_1^{\mathrm{eq}}C_2^{\mathrm{eq}}W(x)\left[p_1/p_1^{\mathrm{eq}}-p_2/p_2^{\mathrm{eq}}\right].\end{aligned} \tag{4.1}$$

The mole fractions of species 1 and 2, Eq. (3.7), normalized for the present to unity,

$$C_1 + C_2 = 1, \tag{4.2}$$

vary in time according to the equation

$$\begin{aligned}\dot{C}_1 &= -\dot{C}_2 \\ &= -\kappa C_2^{\mathrm{eq}}\int_{-\infty}^{\infty}\mathrm{d}x\,C_1^{\mathrm{eq}}W(x)\,p_1(x,t)/p_1^{\mathrm{eq}}(x) \\ &\quad +\kappa C_1^{\mathrm{eq}}\int_{-\infty}^{\infty}\mathrm{d}x\,C_2^{\mathrm{eq}}W(x)\,p_2(x,t)/p_2^{\mathrm{eq}}(x).\end{aligned} \tag{4.3}$$

The first assumption we made is that the reaction is an *activated process*. In such a case, after a short initial period of time which will be considered in the next section, Eqs. (4.3) take the form of the linear kinetic equations

$$\dot{C}_1 = -\dot{C}_2 = -k_1C_1 + k_2C_2. \tag{4.4}$$

Following Relation (4.2) the forward, k_1, and backward, k_2, reaction rate constants determine the longest chemical relaxation time:

$$\tau^{-1} = k_1 + k_2, \tag{4.5}$$

and vice versa; the relaxation time τ determines both reaction rate constants:

$$k_1 = C_2^{\mathrm{eq}}\tau^{-1},\quad k_2 = C_1^{\mathrm{eq}}\tau^{-1}. \tag{4.6}$$

The general way of calculating the longest relaxation time, Eq. (4.5), is the variational method [14,59]. Substitution of

$$p_i(x,t) - p_i^{\mathrm{eq}}(x) = \mathrm{e}^{-t/\tau}f_i(x)\,p_i^{\mathrm{eq}}(x) \tag{4.7}$$

into Eqs. (4.1) results in a set of two coupled ordinary differential equations for the new functions f_i:

$$\begin{aligned}&-\frac{1}{2}f_1'' + (x-x_1)f_1' \\ &\quad +\frac{\kappa}{\gamma}\sqrt{\pi}\left(C_1^{\mathrm{eq}}C_2^{\mathrm{eq}}/p_1^{\mathrm{eq}}(x)\right)W(x)(f_1-f_2) \\ &\quad =\frac{\tau^{-1}}{\gamma}f_1, \\ &-\frac{1}{2}f_2'' + (x-x_2)f_2' \\ &\quad +\frac{\kappa}{\gamma}\sqrt{\pi}\left(C_1^{\mathrm{eq}}C_2^{\mathrm{eq}}/p_2^{\mathrm{eq}}(x)\right)W(x)(f_2-f_1) \\ &\quad =\frac{\tau^{-1}}{\gamma}f_2,\end{aligned} \tag{4.8}$$

posing the eigenvalue problem for the relaxation time τ. Here, the prime denotes a derivative with respect to the conformational coordinate x. Functions f_i should satisfy the boundary conditions

$$f_i(\pm\infty) = \mathrm{const}, \tag{4.9}$$

ensuring that the diffusion fluxes

$$j_i = -\gamma\left[\frac{1}{2}\frac{\partial}{\partial x}+(x-x_i)\right]p_i = -\frac{1}{2}\gamma p_i^{\mathrm{eq}}f_i' \tag{4.10}$$

vanish in infinity. Condition (4.2) is satisfied if the functions f_1 and f_2 are related by the equation

$$\int_{-\infty}^{\infty}\mathrm{d}x\left(p_1^{\mathrm{eq}}f_1 + p_2^{\mathrm{eq}}f_2\right) = 0. \tag{4.11}$$

Eq. (4.7) can be considered a generalization of Eq. (3.13).

On multiplying Eqs. (4.8) by $p_i^{eq} f_i$, integrating by parts, and adding by sides we obtain the general formula for the eigenvalue τ^{-1}:

$$\tau^{-1} = \frac{1}{2}\left[\int_{-\infty}^{\infty} dx p_1^{eq}(f_1')^2 + \int_{-\infty}^{\infty} dx p_2^{eq}(f_2')^2\right] + \kappa C_1^{eq} C_2^{eq} \int_{-\infty}^{\infty} dx W(x)(f_1 - f_2)^2. \quad (4.12)$$

In deriving Eq. (4.12) we assumed that the functions f_i are normalized in such a way that the condition

$$\int_{-\infty}^{\infty} dx\left(p_1^{eq} f_1^2 + p_2^{eq} f_2^2\right) = 1 \quad (4.13)$$

is satisfied. When interpreting Eqs. (4.1) in terms of the Schrödinger equation with imaginary time [14], Eqs. (4.13) and (4.11) have, respectively, the meaning of normalization and orthogonalization of the excited state with respect to the ground state (one should not confuse the normalization of 'states', Eq. (4.13), with the normalization of the probability densities, Condition (4.2); the latter is, in fact, equivalent to Eq. (4.11)).

The second assumption we made is that the transition probability W is strongly localized in a narrow region of values of the mechanical variable:

$$W(x) = \delta(x) \quad (4.14)$$

(δ stands for Dirac's delta), i.e., the reaction is *gated* by conformational dynamics (Fig. 5a); the reaction with a weak dependence of the transition probability W on the mechanical variable x was considered in another paper by the author [14]. The reaction is the activated process if the chemical relaxation time τ is much longer than the internal equilibration time γ^{-1},

$$\tau \gg \gamma^{-1}. \quad (4.15)$$

For the case of a gated reaction, Eq. (4.14), this holds when the point of transition $x = 0$ is distant enough from the minima of both potentials:

$$|x_1|, |x_2| \gg 1 \quad (4.16)$$

(see Fig. 5a); for the irreversible reaction and small $|x_1|$, the case of delta-function type localization of the transition probability has been discussed elsewhere [60–62].

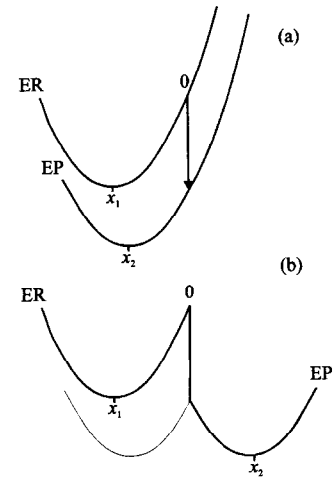

Fig. 5. Protein machine model of a separate chemical transformation gated by the enzyme conformational dynamics. (a) Two conformational potentials with minima at x_1 and x_2, corresponding to two individual chemical species of the enzyme. The gate is located at the point $x = 0$. The arrow indicates the assumed sign of the reaction flux. Positions (on the right, left, or on both sides) of points x_1 and x_2 with respect to the gate are arbitrary. Forward and backward transition rates through the gate obey the detailed balance condition, thus they depend on the equilibrium concentrations C_i^{eq} of both species. The corresponding differences of the equilibrium free energies are shown as relative vertical displacements of the parabolas. (b) Effective potential for the separate gated reaction. The region with the minimum at x_2 is set opposite the region with the minimum at x_1.

Assumptions (4.14) and (4.15) lead to a simpler system of equations:

$$-\frac{1}{2}f_1'' + (x - x_1)f_1' + \frac{\kappa}{\gamma}\sqrt{\pi} C_2^{eq} e^{x_1^2}\delta(x)(f_1 - f_2) = 0$$

$$-\frac{1}{2}f_2'' + (x - x_2)f_2' + \frac{\kappa}{\gamma}\sqrt{\pi} C_1^{eq} e^{x_2^2}\delta(x)(f_2 - f_1) = 0. \quad (4.17)$$

Eqs. (4.17) have an exact solution in terms of parabolic cylinder functions [63]; however, it has been shown that in order to get the Kramers' approximation [40] for the chemical relaxation time it is sufficient to know only the asymptotic solution in the vicinity of the transition point [59]. For $|x| \to 0$, one can neglect x in comparison with x_i in the

second terms of Eqs. (4.17) to obtain such an asymptotic solution:

$$f_i(x) = \begin{cases} B_i e^{-2x_i x} + A_i & \text{for } x \text{ of the same sign as } x_i \\ B_i + A_i & \text{otherwise,} \end{cases} \tag{4.18}$$

with constants B_1 and B_2 related to constants A_1 and A_2 through the equations

$$B_1 = \frac{C_2^{eq} e^{x_1^2}}{|x_1|}\left(\frac{\gamma}{\kappa\sqrt{\pi}} + \frac{C_2^{eq} e^{x_1^2}}{|x_1|} + \frac{C_1^{eq} e^{x_2^2}}{|x_2|}\right)^{-1} \times (A_1 - A_2) \tag{4.19}$$

and

$$B_1 C_1^{eq} |x_1| e^{-x_1^2} + B_2 C_2^{eq} |x_2| e^{-x_2^2} = 0. \tag{4.20}$$

The latter equation has the meaning of the continuity condition of the diffusion flux at the transition point:

$$j_1(0) + j_2(0) = 0. \tag{4.21}$$

The solution (4.18) is continuous, satisfies boundary conditions (4.9) and assumes that the diffusion fluxes above the transition point vanish.

The very constants A_i can be determined from the conditions of orthogonality, Eq. (4.11), and normalization, Eq. (4.13). To do this let us note that, because of Inequality (4.16), in the vicinity of x_i, where values of the functions p_i^{eq} are large, the functions f_i are practically constant and equal to A_i. As a consequence, Eqs. (4.11) and (4.13) read, to a good approximation,

$$C_1^{eq} A_1 + C_2^{eq} A_2 = 0 \tag{4.22}$$

and

$$C_1^{eq} A_1^2 + C_2^{eq} A_2^2 = 1, \tag{4.23}$$

respectively, from which we get

$$A_1 = -\sqrt{C_2^{eq}/C_1^{eq}}, \quad A_2 = \sqrt{C_1^{eq}/C_2^{eq}}. \tag{4.24}$$

After calculating the integrals in Eq. (4.12):

$$\begin{aligned} &\int_{-\infty}^{\infty} dx\, p_i^{eq} (f_i')^2 \\ &= 4\pi^{-1/2} C_i^{eq} B_i^2 x_i^2 \int_{xx_i \text{ positive}} dx\, e^{-(x-x_i)^2} e^{-4x_i x} \\ &= 4\pi^{-1/2} C_i^{eq} B_i^2 x_i^2 \int_{-\infty}^{-|x_i|} dx\, e^{-x^2} \\ &\approx 2\pi^{-1/2} C_i^{eq} B_i^2 |x_i| e^{-x_i^2} \end{aligned} \tag{4.25}$$

(the last, approximate equality results from the asymptotic expansion of the error function in the limit (4.16), cf. Eq. (A.5) in Appendix A) we get the expression for the chemical relaxation time:

$$\tau = \kappa^{-1} + \gamma^{-1}\sqrt{\pi}\left(\frac{C_2^{eq}}{|x_1|} e^{x_1^2} + \frac{C_1^{eq}}{|x_2|} e^{x_2^2}\right). \tag{4.26}$$

The forward and backward reaction rate constants are related to the relaxation time (4.26) through Eqs. (4.6). In particular,

$$k_1^{-1} = (\kappa C_2^{eq})^{-1} + \gamma^{-1}\sqrt{\pi}|x_1|^{-1} e^{x_1^2} + K^{-1}\gamma^{-1}\sqrt{\pi}|x_2|^{-1} e^{x_2^2}, \tag{4.27}$$

where

$$K \equiv C_2^{eq}/C_1^{eq} \tag{4.28}$$

stands for the chemical equilibrium constant for the unimolecular reaction considered.

Eq. (4.27) has the form of a 'rate limiting step' formula [64,65]. The first component on the right-hand side represents the time needed for crossing the border between species 1 and 2 on assuming the equilibrium distribution of microstates within species 1, including the border. The second component represents the diffusion time from the minimum of the conformational potential at $x = x_1$ to the transition point at $x = 0$ (cf. Eq. (A.6) in Appendix A) and describes the process of restoration of the equilibrium distribution of microstates at the border, disturbed by the transition. And the third component describes a similar process of restoration of the equilibrium distribution at the border, but from the side of the product.

Following this interpretation, the chemical relaxation time, Eq. (4.26), can be rewritten as a sum

$$\tau = \kappa^{-1} + \bar{\tau}_{up}, \tag{4.29}$$

where $\bar{\tau}_{up}$ is the time of diffusion up the conformational potential from the equilibrium position to the transition point, Eq. (A.6), averaged over two chemical species (cf. a comment to Eq. (A.19) in Appendix A). If the very process of crossing the border is the rate limiting step

$$\kappa^{-1} \gg \tau_{up}, \tag{4.30}$$

Eq. (4.26) is reduced to that provided by the transition state theory:

$$\tau = \kappa^{-1} \tag{4.31}$$

(cf. the first of Eqs. (3.14)). If, on the other hand, the process of diffusion up the conformational potential is the rate limiting step

$$\tau_{up} \gg \kappa^{-1}, \tag{4.32}$$

we have

$$\tau \gg \kappa^{-1} \tag{4.33}$$

in addition to Inequality (4.15), and Eq. (4.26) is reduced to

$$\tau = \gamma^{-1}\sqrt{\pi}\left(\frac{C_2^{eq}}{|x_1|}e^{x_1^2} + \frac{C_1^{eq}}{|x_2|}e^{x_2^2}\right). \tag{4.34}$$

We say that the reaction is now *controlled* by the internal dynamics of the molecule. In connection with Eq. (4.34) we would like to point out the inaccuracies in Eqs. (6.12) to (6.15) and Fig. 5 in Ref. [14].

It is worth clarifying a little a question of terminology. Very often the concept of reaction, which is the *activated* process, is opposed to that of reaction *controlled* by processes of internal dynamics. Indeed, the present author did not avoid such an oversimplification [14,58]. However, the fast equilibration of microstates within a given chemical species, assumed in the definition of an activated process [58], does not automatically imply fast internal dynamics. In fact, in our case, Condition (4.15) of fast conformational relaxation is independent of Condition (4.33) which demonstrates the slow character of the process of restoring conformational equilibrium at the border. There are activated processes with fast internal dynamics, quite well described by the transition state theory, possibly with a transmission coefficient smaller than unity, and there are reactions controlled by slow internal dynamics which are not the activated processes, and where concentrations are fast thermodynamic variables adiabatically following certain non-chemical slow variables [14,58]. But there are also reactions which are *both* activated and controlled processes. In fact, the well-known Kramers model of reaction in the spatial diffusion limit [40], a generalization of which is the model considered here, represents such a case.

The form of Solution (4.18) suggests that the two Eqs. (4.17) in the limit of inequality, (4.32), represent in fact a single equation describing diffusion in the effective potential shown schematically in Fig. 5b. Indeed, Eq. (4.34) coincides exactly with Eq. (A.19) in Appendix A, calculated for such a potential ($\bar{x} = 0$) with the help of the mean first-passage time, and provided by the Kramers theory of reaction rates in the spatial diffusion limit [40].

Instead of solving Eqs. (4.17), one could simply

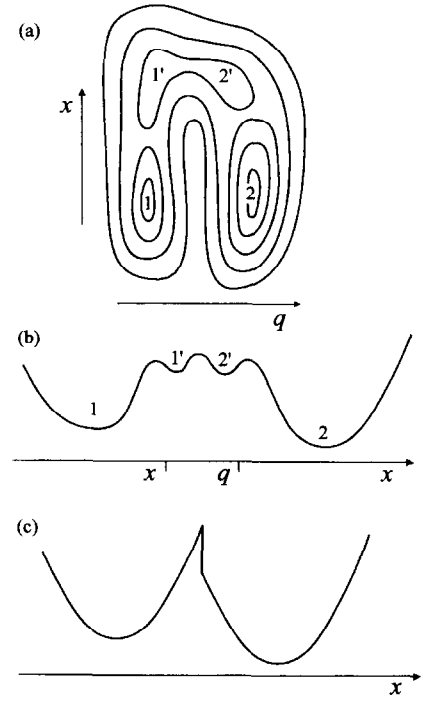

Fig. 6. (a) Reaction gated by the transition between two discrete conformational states of the enzyme (after Ref. [66]). The potential, represented with the help of contour lines, is assumed to be a function of the 'reaction coordinate' q and the 'conformational coordinate' x. Along the reaction coordinate, two minima 1 and 2 are divided by a very high potential barrier. Transitions are, however, possible, first along the conformational coordinate from 1 to 1′ (opening the gate), next through the much lower barrier from 1′ to 2′ (the gate) and, finally, along the conformational coordinate from 2′ to 2 (closing the gate). (b) One-dimensional profile of the reaction. The path along the valley in the potential consists in part of the conformational coordinate and in part of the reaction coordinate. The conformational transitions are assumed to be the limiting steps of the overall reaction. (c) Reduction of the reaction coordinate component to the minimum and replacement of the very chemical step by a certain finite transition probability through the border leads to the effective potential profile of the reaction identical with the one considered in the present paper (cf. Fig. 5b).

assume that the functions f_i have the form (4.18) with parameters x_i to be determined by the variation (hence the name for the method) of the functional Eq. (4.12) with W given by Eq. (4.14), restricted to additional constraints (4.11), (4.13) and (4.21). A result of the variational procedure would be the identity of the variational parameters x_i with those occurring in Eqs. (4.17).

A few words should be said about the relation of the mechanism of gating considered in the present paper to that introduced for the first time by Gavish and Werber, and by Frauenfelder and coworkers, in order to explain the inverse proportionality of reaction rate constants to the solvent viscosity, observed in enzymatic hydrolysis of a specific peptide bond by carboxypeptidase A [41] and the CO molecule translocation to the heme pocket (the bimolecular process) within myoglobin [37]. If proteins were rigid, the solvent viscosity would not affect their interior; thus the reactions observed would have to be coupled in some way with the protein's conformational transitions. In the original formulation of the gating mechanism two possible discrete conformational states were considered [37,66] (Fig. 6a) and the corresponding conformational transitions were assumed to be the limiting steps of the overall reaction (Fig. 6b). To relate the rates of these steps with the solvent viscosity, both groups of authors applied Kramers' theory of reaction rates in the spatial diffusion limit [39]. On comparing Fig. 6b with Fig. 6c, it is seen that the theory of gated chemical transformation just described differs from the original only by a replacement of the very chemical step by a certain finite transition probability through the border point.

5. The initial condition effects: inhomogeneous kinetics

The model considered in the previous section is more general than the Kramers model of a single chemical reaction, not only because of additional limiting transition probabilities through the border point, but also because it provides a possibility of some non-trivial initial stage kinetics.

In the previous discussion we have assumed that the diffusion fluxes above the gate vanish. However, this assumption can appear invalid at the very beginning of the reaction when, due to a specific preparation of the initial state, there are nonzero populations of highly energetic enzyme conformations above the gate in one or both chemical states. During the process of equilibration, diffusion down the conformational potential ends with a jump, with a certain probability, through the gate to the other state. Macroscopically, it is manifested as some initial stage of reaction [64], dependent on the initial distribution of enzyme conformations.

For simplicity, we shall assume that higly energetic conformations above the gate are present only in one chemical state of the enzyme; thus the reaction proceeds initially as if it were *irreversible*. In that case Eqs. (4.1) with the localization condition, Eq. (4.14), reduce to a single equation

$$\frac{\partial}{\partial t}p - \gamma\frac{\partial}{\partial x}\left[\frac{1}{2}\frac{\partial}{\partial x} + (x - x_0)\right]p = -\eta\delta(x)\,p \tag{5.1}$$

with the transition probability through the gate per unit time

$$\eta = \kappa\sqrt{\pi}\,\mathrm{e}^{x_0^2} \tag{5.2}$$

(in this section we omit the subscripts 1 or 2 and assume, as in Eqs. (2.4) and (2.5) in Section 2, that the minimum of the conformational potential is at the point x_0, whereas the gate is localized at the point $x = 0$). In terms of the diffusion time up the conformational potential from its minimum to the gate,

$$\tau_{\mathrm{up}} \equiv \sqrt{\pi}\,\gamma^{-1}\frac{\mathrm{e}^{x_0^2}}{|x_0|} \tag{5.3}$$

(cf. Eqs. (4.34) and (A.6) in Appendix A), the coefficient η can be rewritten in the form

$$\eta = \gamma|x_0|\kappa\tau_{\mathrm{up}}, \tag{5.4}$$

which will be convenient in further discussion.

We shall look for a solution to Eq. (5.1), with the help of the Green function method [67], by resorting to the solution of the equation without a sink (Eq. (2.4) with the potential (2.5)). In terms of the Green function $p(x,t|y)$, obeying the initial condition

$$p(x,0|y) = \delta(x - y), \tag{5.5}$$

a general solution to Eq. (5.1) with an arbitrary initial distribution $p(x,0)$ reads

$$p(x,t) = \int_{-\infty}^{\infty} \mathrm{d}y\, p(x,t|y)\, p(y,0). \tag{5.6}$$

The Green function for the equation without a sink ($\eta = 0$) is [51]

$$p^0(x,t|y) = \pi^{-1/2}(1-e^{-2\gamma t})^{-1/2} \times \exp\left\{ -\frac{\left[(x-x_0)-(y-x_0)e^{-\gamma t}\right]^2}{1-e^{-2\gamma t}} \right\}. \tag{5.7}$$

It represents the Gaussian distribution evolving from the Dirac delta $\delta(x-y)$ at $t=0$ to the equilibrium distribution

$$p^{\mathrm{eq}}(x) = \pi^{-1/2} e^{-(x-x_0)^2} \tag{5.8}$$

at $t \to \infty$; the mean position moves from y to x_0 with the relaxation time γ^{-1}, and the dispersion varies from 0 to 1/2 with the relaxation time $(2\gamma)^{-1}$ (cf. Eq. (2.9)). Following the general theory of temporal Green functions [67], the Green function for the complete Eq. (5.1) satisfies the equation

$$p(x,t|y) = p^0(x,t|y) - \eta \int_0^t \mathrm{d}t' p^0(x,t'|0)\, p(0,t-t'|y). \tag{5.9}$$

The first summand on the right-hand side is the solution to the equation without the sink (the homogeneous or 'unperturbed' equation), and the second summand is the reaction to the sink term treated formally as an external perturbation.

The occupation of the initial chemical state,

$$C(t) = \int_{-\infty}^{\infty} \mathrm{d}x\, p(x,t), \tag{5.10}$$

varies according to the equation

$$\dot{C} = -\eta p(0,t). \tag{5.11}$$

A solution to this equation, expressed in terms of the Green function solution (5.6) to Eq. (5.1), has the form

$$C(t) = \int_{-\infty}^{\infty} \mathrm{d}x \left[1 - \eta \int_0^t \mathrm{d}t' p(0,t'|x) \right] p(x,0). \tag{5.12}$$

For the calculation of the integral over time in Eq. (5.12), the Green function $p(0,t|x)$ with only one variable spatial argument x is sufficient. The corresponding unperturbed Green function can be written as

$$p^0(0,t|x) = \pi^{-1/2}(1-e^{-2\gamma t})^{-1/2} \times \exp\left\{ -x_0^2 \frac{\left[1-e^{-\gamma(t-\tau(x))}\right]^2}{1-e^{-2\gamma t}} \right\} \tag{5.13}$$

with

$$\tau(x) \equiv \gamma^{-1} \ln \frac{|x_0 - x|}{|x_0|}. \tag{5.14}$$

The Green function $p(0,t|x)$ satisfies the integral equation

$$p(0,t|x) = p^0(0,t|x) - \int_0^t \mathrm{d}t' p^0(0,t'|0)\, p(0,t-t'|x). \tag{5.15}$$

The latter can be solved on introducing the Laplace transform

$$\tilde{p}(0,s|x) \equiv \int_0^{\infty} \mathrm{d}t\, e^{-st} p(0,t|x). \tag{5.16}$$

In terms of Laplace transforms, Eq. (5.15) reads

$$\tilde{p}(0,s|x) = \tilde{p}^0(0,s|x) - \eta \tilde{p}^0(0,s|0)\, \tilde{p}(0,s|x), \tag{5.17}$$

thus

$$\tilde{p}(0,s|x) = \frac{\tilde{p}^0(0,s|x)}{1+\eta \tilde{p}^0(0,s|0)}. \tag{5.18}$$

The general solution to Eq. (5.15) with the help of Eq. (5.18), for arbitrary t and x, is rather complex, and in order to obtain clear analytical formulae certain approximations are necessary, well justified only in limiting cases. In the previous section we have considered, in fact, this solution in the limit of large $|x_0|$ and large t (as compared to γ^{-1}), and of x on the same side of the gate as the equilibrium

position x_0. Here, we are still interested in the case of the activated process

$$|x_0| \gg 1, \tag{5.19}$$

but of the initial position x on the other side of the gate from where x_0 is, i.e. for

$$xx_0 < 0. \tag{5.20}$$

In such conditions the function $\tau(x)$, Eq. (5.14), is positive and has the meaning of the diffusion time down the conformational potential from x to the gate at 0 (cf. Eq. (A.8) in Appendix A with an appropriately changed reference point). For $t = \tau(x)$ the exponential factor in the unperturbed Green function (5.13) reaches a maximum and, when Inequality (5.19) is satisfied, the latter can be well approximated by the Gaussian distribution in time around $\tau(x)$:

$$p^0(0,t|x) = \left[2\pi\alpha(x)^2\right]^{-1/2} \frac{1}{\gamma|x_0|} e^{-[t-\tau(x)-\epsilon]^2/2\alpha(x)^2}, \tag{5.21}$$

where

$$\alpha(x)^2 \equiv \frac{1-e^{-2\gamma\tau(x)}}{2\gamma^2 x_0^2} \tag{5.22}$$

and a small positive number ϵ (to be put equal to zero at the end of the calculations) secures $p^0(0,t|x)$ to vanish for $t \to 0$ in the limit (5.19). Note that, as the function (5.21) does not tend to the value (5.8) for $t \to \infty$, this approximation is valid only for times shorter than or comparable with γ^{-1} (the initial period of reaction). For inital states not very distant from the transition point,

$$|x| \ll |x_0|, \tag{5.23}$$

the functions $\tau(x)$ and $\alpha(x)$ can be approximated, respectively, by

$$\tau(x) = \gamma^{-1} \frac{|x|}{|x_0|} \tag{5.24}$$

and

$$\alpha(x) = \gamma^{-1}|x_0|^{-1} \sqrt{\frac{|x|}{|x_0|}}. \tag{5.25}$$

The Laplace transform of the function (5.21) can be calculated exactly [68]:

$$\tilde{p}^0(0,s|x) = \frac{e^{-(\tau(x)+\epsilon)s} e^{\alpha(x)^2 s^2/2}}{2\gamma|x_0|} \times \operatorname{erfc}\left\{\left[\alpha(x)s - \alpha(x)^{-1}(\tau(x)+\epsilon)\right]/\sqrt{2}\right\}, \tag{5.26}$$

where the symbol erfc denotes the complementary error function (Eq. (A.5) in Appendix A). For $x \to 0$ the function (5.26) reduces to

$$\tilde{p}^0(0,s|0) = \frac{e^{-\epsilon s}}{\gamma|x_0|}. \tag{5.27}$$

The Laplace transform of the complete Green function (5.18) is

$$\tilde{p}(0,s|x) = \frac{e^{-(\tau(x)+\epsilon)s} e^{\alpha(x)^2 s^2/2}}{2(\gamma|x_0|+\eta)} \times \operatorname{erfc}\left\{\left[\alpha(x)s - \alpha(x)^{-1}(\tau(x)+\epsilon)\right]/\sqrt{2}\right\}, \tag{5.28}$$

and the exact inverse Laplace transform of the function (5.28) is again a Gaussian distribution, but with a corrected coefficient:

$$p(0,t|x) = \left[2\pi\alpha(x)^2\right]^{-1/2} \times \frac{1}{\gamma|x_0|+\eta} e^{-[t-\tau(x)-\epsilon]^2/2\alpha(x)^2}. \tag{5.29}$$

For the Green function (5.29), Eq. (5.12) reads

$$C_{\text{init}}(t) = \int_0^{\pm\infty} dx \left[1 - \frac{\eta}{\gamma|x_0|+\eta} g(x,t)\right] p(x,0) \tag{5.30}$$

(the sign of the upper limit of the integral depends on the sign of x), where

$$g(x,t) = \frac{1}{2}\left\{\operatorname{erf}\left[(t-\tau(x)-\epsilon)/\sqrt{2}\,\alpha(x)\right] - \operatorname{erf}\left[-(\tau(x)+\epsilon)/\sqrt{2}\,\alpha(x)\right]\right\} \tag{5.31}$$

with the symbol erf denoting the error function (Eq. (A.5) in Appendix A). The function (5.31) can be considered the 'softened' Heaviside theta function representing a jump from zero to unity at $t = \tau(x)$ (the mean time needed for reaching the gate from the point x), this jump being diffused with the dispersion $\alpha(x)^2$. Here, the presence of the small positive number ϵ secures that the limit $x \to 0$ is to be made properly (only after that can we put $\epsilon \to 0$). Because the approximation (5.21) is valid only for times not very much longer than γ^{-1}, we denote explicitly that Eq. (5.30) applies only for the initial stage of reaction and for the initial distribution of conformations above the gate.

The fraction before the function $g(x,t)$ in Eq. (5.30) represents the *branching probability* of the diffusion flux down the conformational potential into the part that crosses the gate, and the rest that tends toward the equilibrium position x_0. For the case of fast conformational diffusion, Inequality (4.30), following Eq. (5.4) we have

$$\eta \ll |x_0|\gamma, \tag{5.32}$$

and the branching ratio in Eq. (5.30) is negligibly small. This means that practically the whole initial distribution of conformations reaches first the local equililibrium and only after that does the reaction begin. In other words, there are no initial condition effects and no specific initial stage of reaction. On the contrary, when the reaction is controlled by the process of internal equilibration, Inequality (4.32), following Eq. (5.4) we have

$$\eta \gg |x_0|\gamma, \tag{5.33}$$

and the branching ratio in Eq. (5.30) can be approximated by unity. In this case, on neglecting diffusion of the jump in the function $g(x,t)$, Eq. (5.31), i.e., on assuming

$$g(x,t) \approx \theta[t - \tau(x)] \tag{5.34}$$

(which is an instructive but not a very good approximation), one can rewrite Eq. (5.30), for $\tau(x)$ given by Eq. (5.24), in an especially simple form:

$$C_{\text{init}}(t) \approx \int_{|x_0|\gamma t}^{\infty} \mathrm{d}x p(x,0) \tag{5.35}$$

(the signs of the integration limits are for negative x_0; for positive x_0 they should be reversed).

On performing the appropriate change of the integration variable $x \mapsto k = k(x)$ (which, however, canot be done analytically in our case) one can always rewrite equations such as (5.30) or (5.35) in the form of an inhomogeneous combination of a continuum of exponentials [69]:

$$C_{\text{init}}(t) = \int_0^{\infty} \mathrm{d}k f(k) \mathrm{e}^{-kt} \tag{5.36}$$

(the distribution function of reaction rates $f(k)$ is the inverse Laplace transform of $C_{\text{init}}(t)$). We have thus shown that our protein machine model in the limit of slow conformational diffusion provides, for the appropriate initial state preparations, a possibility of inhomogeneous kinetics.

The initial stage inhomogeneous kinetics is by the factor exp x_0^2 faster than the main stage kinetics studied in the previous section. Consequently, it should become important at low (liquid nitrogen) temperatures, where the main stage kinetics is too slow to be observed. In fact, it was the observation in 1975 by Frauenfelder and coworkers [13] (see also reviews [3–5]) of such a low-temperature inhomogeneous kinetics of ligand rebinding to myoglobin, after a laser flash photolysis, that gave for the first time direct evidence of slow conformational transition dynamics in native proteins. With increasing temperature, the main, exponential stage of the reaction becomes increasingly dominant. This fact leads to an interesting phenomenon: as the reaction in the initial stage is by assumption faster, the rate of the reaction can happen to *decrease* with growing temperature. Such a phenomenon was actually observed by Frauenfelder and coworkers [13,70] and, later on, by Doster and coworkers [71].

Various forms and mechanisms of the inhomogeneous kinetics have been considered by a number of authors [14,72–76] but there are still controversies over the interpretation of the whole of the experimental material available over a wide range of temperature [3,70,71]. Of particular interest is the transition, with growing temperature, from 'static' to 'dynamic disorder' [56] in the observed kinetics. To verify the usefulness of the present model for interpreting experimental data, a systematic study of the solution to Eq. (5.15) for arbitrary time t and arbitrary initial state x is necessary. Unfortunately, this

can be done in its entirety only by numerical methods and will be a subject of a separate paper.

6. The complete enzymatic reaction

We are now ready to consider our protein machine model for the complete enzymatic reaction

$$E + R \leftrightarrow ER \leftrightarrow EP \leftrightarrow E + P,$$

described by Eqs. (3.1) to (3.5). The equilibrium concentrations of all the distinguished chemical species are related to each other by the three laws of mass action:

$$[ER]^{eq}/[E]^{eq}[R]^{eq} = C_1^{eq}/C_0^{eq}[R]^{eq} = K',$$

$$[EP]^{eq}/[ER]^{eq} = C_2^{eq}/C_1^{eq} = K,$$

$$[E]^{eq}[P]^{eq}/[EP]^{eq} = C_0^{eq}[P]^{eq}/C_2^{eq} = K''. \quad (6.1)$$

We assume that *all* the three chemical transitions (3.4) are gated by conformational dynamics:

$$W(x) = \delta(x),\ W'(x) = \delta(x - x'),$$
$$W''(x) = \delta(x - x'') \quad (6.2)$$

(Fig. 7). Besides a formal simplicity it is not an unrealistic assumption. For instance, for a certain value of the angle x (cf. Fig. 2), a free space large enough to allow substrate motions inside the enzyme can appear. For another value of the angle x all the catalytic groups of the active centre can simultaneously be properly oriented. Yet another value of x can favour product desorption.

Let us first try to answer the question whether it is sufficient to describe the complete enzymatic reaction considered in terms of a certain set of rate constants. For *separate* bimolecular reactions

$$E + R \leftrightarrow ER \text{ or } EP \leftrightarrow E + P,$$

proceeding in the *closed* reactor under the constraints

$$[R] + [ER] = \text{const or } [P] + [EP] = \text{const} \quad (6.3)$$

and in a large excess of the reactant or product with respect to the enzyme,

$$[R] \gg [ER] \text{ or } [P] \gg [EP], \quad (6.4)$$

we can approximate the instantaneous values of the

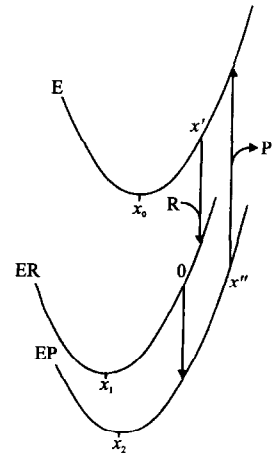

Fig. 7. Protein machine model of the complete enzymatic reaction gated by the enzyme conformational dynamics. Three different conformational potentials with minima at x_0, x_1 and x_2 correspond to individual chemical species of the enzyme: E, ER and EP respectively. Three chemical transitions are localized at the points 0, x' and x''. The arrows indicate the assumed signs of the reaction fluxes. Forward and backward rates of each transition obey the detailed balance condition, thus they depend on the equilibrium concentrations C_i^{eq} of the species. The corresponding differences of the equilibrium free energies are shown as relative vertical displacements of the parabolas.

reactant or product concentrations by the corresponding equilibrium values:

$$[R] \approx [R]^{eq} \text{ or } [P] \approx [P]^{eq}, \quad (6.5)$$

and thus treat the former as practically time independent. There is no formal difference between the bimolecular reactions in such conditions and the unimolecular reaction considered in Section 4, and we immediately (cf. Eq. (4.26)) obtain the expressions

$$\tau' = (\kappa')^{-1} + \gamma^{-1}\sqrt{\pi}\left[\frac{C_1^{eq}}{|x' - x_0|}e^{(x'-x_0)^2} + \frac{C_0^{eq}}{|x' - x_1|}e^{(x'-x_1)^2}\right]$$

or

$$\tau'' = (\kappa'')^{-1} + \gamma^{-1}\sqrt{\pi}\left[\frac{C_0^{eq}}{|x'' - x_2|}e^{(x''-x_2)^2} + \frac{C_2^{eq}}{|x'' - x_0|}e^{(x''-x_0)^2}\right] \quad (6.6)$$

for the chemical relaxation times of the reactions involving R or P, respectively. Following the laws of mass action (6.1) the concentrations of R or P affect, under Approximation (6.5), only the ratios of the equilibrium concentrations of enzyme species that occur in those expressions.

The laws of mass action (6.1), determining the equilibrium constants K', K and K'', are the universal equlibrium property, completely independent of kinetics. There are, however, no universal kinetic laws and, in order to define the rate parameters, we have to assume a particular form of kinetic equations. Let this form be identical to that of the conventional kinetic equations:

$$\dot{C}_0 = -\dot{C}_1 = -k_0'[\mathrm{R}]C_0 + k_1'C_1$$

and

$$\dot{C}_2 = -\dot{C}_0 = -k_2''C_2 + k_0''[\mathrm{P}]C_0 \qquad (6.7)$$

for the reaction involving, respectively, R and P. On replacing, following Approximation (6.5), the concentrations [R] and [P] by the corresponding equilibrium values, we can relate, for the reactions proceeding separately, the rate parameters occurring in Eqs. (6.7) with the relaxation times (6.6). In this way we obtain

$$(k_1')^{-1} = K'(k_0')^{-1} = \tau'(C_0^{\mathrm{eq}})^{-1} = (\kappa' C_0^{\mathrm{eq}})^{-1} + \gamma^{-1}\sqrt{\pi}\left[\frac{\mathrm{e}^{(x'-x_1)^2}}{|x'-x_1|} + [\mathrm{R}]^{\mathrm{eq}}K'\frac{\mathrm{e}^{(x'-x_0)^2}}{|x'-x_0|}\right]$$

for the reaction involving R, and

$$(k_2'')^{-1} = (K''k_0'')^{-1} = \tau''(C_0^{\mathrm{eq}})^{-1} = (\kappa'' C_0^{\mathrm{eq}})^{-1} + \gamma^{-1}\sqrt{\pi}\left[\frac{\mathrm{e}^{(x''-x_2)^2}}{|x''-x_2|} + [\mathrm{P}]^{\mathrm{eq}}(K'')^{-1}\frac{\mathrm{e}^{(x''-x_0)^2}}{|x''-x_0|}\right] \qquad (6.8)$$

for the reaction involving P. By definition, the purely chemical contributions $\kappa' C_0^{\mathrm{eq}}$ and $\kappa'' C_0^{\mathrm{eq}}$ to the rate parameters (6.8) do not depend on the concentrations $[\mathrm{R}]^{\mathrm{eq}}$ or $[\mathrm{P}]^{\mathrm{eq}}$. However, this cannot be said about the contributions originating in the internal dynamics of the enzyme. Consequently, the rate parameters determined by the full expressions (6.8) can hardly be recognized as the 'rate constants'.

The situation becomes yet more complex if we realize that we do not deal with separate chemical reactions. All the reactions considered are *coupled* to each other because, when they proceed simultaneously, the conservation laws, Eqs. (6.3), are no longer valid and, instead, in the closed reactor we have

$$[\mathrm{R}] + [\mathrm{P}] + [\mathrm{ER}] + [\mathrm{EP}] = [\mathrm{R}]_0 = \mathrm{const.} \qquad (6.9)$$

Consequently, [R] and [P] vary in time and the reaction rate parameters calculated in the way described above become time dependent. Moreover, the dynamics above the transition points, discussed in Section 5 as the initial stage dynamics, leads in the case of coupled reactions to a novel effect. On examining Fig. 7 we find that, after the transition at the point x' from the state E + R to ER, the enzyme can either equilibrate within ER or, if crossing the gate is not the rate limiting step, pass directly to the next state EP, or even E + P, with the process of equilibration within the intermediates omitted. As a consequence, direct component reactions between *each*, in general, pair of kinetic states E, ER and EP are possible [7]. The kinetic scheme in Fig. 4d illustrates this fact in a symbolic manner.

The schematic potentials, resulting from Fig. 7, for all the six component reactions are displayed in Fig. 8. We can apply the formalism described in Appendix A to calculate diffusional contributions to the corresponding rate parameters, but we have to remember that the positions of particular local minima are determined by time-varying concentrations of R and P, so that such rate 'constants' have no direct interpretation. Only close to the equilibrium (in the case of substrate concentration small with respect to the enzyme) or the stationary state (substrate concentration high with respect to the enzyme) can one approximate concentrations [R] and [P] by the appropriate constant values, and the scheme in Fig. 4d, as well as the corresponding rate parameters, becomes meaningful. In such circumstances a description in terms of rate parameters is, however, redundant as the values of the two relaxation times are sufficient (only two of the three concentrations C_i are independent).

To conclude, the complete enzymatic reaction controlled by the enzyme internal dynamics cannot be described in terms of a set of rate constants corresponding to a certain effective kinetic scheme.

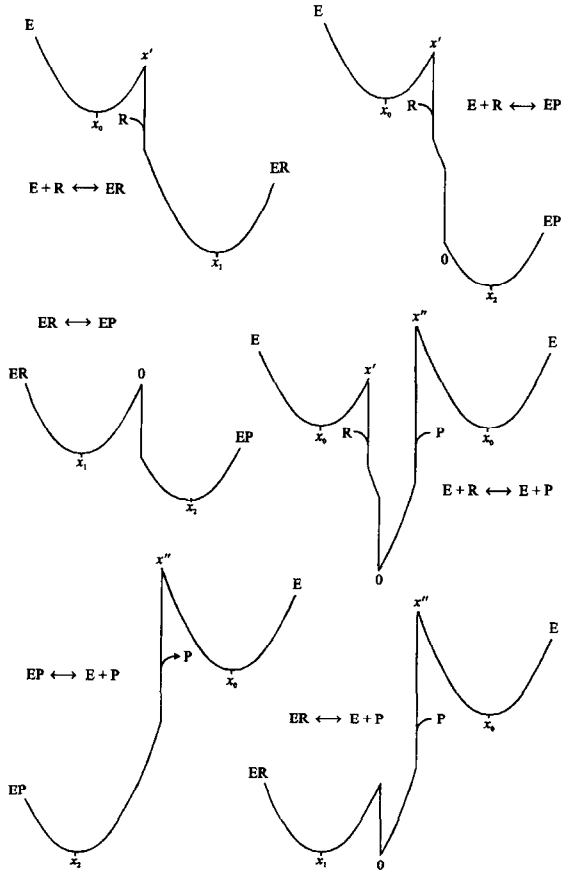

Fig. 8. Effective potentials for all six direct reactions in the scheme given in Fig. 4d as resulting from Fig. 7. In general, relative vertical displacements of the parabolas are determined by instantaneous values of concentrations [R] and [P].

Another form of phenomenological description has to be searched for, if only, of course, it exists. Fortunately, in the conditions of a large excess of the substrate with respect to the enzyme,

$$[\mathrm{R}]_0 \gg [\mathrm{E}]_0, \tag{6.10}$$

which is the rule both *in vivo* and in laboratory experiments, the concentrations of distinguished enzyme species [E], [ER] and [EP] will sooner or later reach a steady state in which they follow adiabatically the much slower varying concentrations [R] and [P]. At the phenomenological level the steady-state kinetics is comprehensively described by an effective kinetic equation for the single variable [R]; in the closed reactor, when Inequality (6.10) holds, the concentration [P] is related to [R] by the simple equation

$$[\mathrm{P}] = [\mathrm{R}]_0 - [\mathrm{R}]. \tag{6.11}$$

The form and parameters of the equation describing the steady-state kinetics are to be determined after finding the steady-state solution to Eqs. (3.1). This will be done in the next section. Here, let us consider only the transient (pre-stationary) stage of the reaction. As we have already noted, its kinetics is characterized by two relaxation times.

To calculate these two relaxation times for the three mutually dependent, effectively unimolecular reactions, composing the complete enzymatic reaction in, e.g., the conditions

$$[\mathrm{R}] = [\mathrm{R}]_0,\ [\mathrm{P}] = 0, \tag{6.12}$$

we can follow the variational method applied in Section 4 for its single, separate step. However, the general expressions obtained in such a way are very complex, which should not be a surprise if we mention an analogous problem for the conventional Haldane's kinetics [78]. A reasonable approximation follows from the fact that for the substrate at a much higher concentration than the enzyme, Inequality (6.10), practically all the enzyme molecules reach the bound state, thus

$$C_0^{\mathrm{eq}} \ll C_1^{\mathrm{eq}}, C_2^{\mathrm{eq}}. \tag{6.13}$$

In the conditions (6.12) the reaction involving P is then to be neglected and the two remaining reactions are practically independent. Consequently, for the evaluation of the two relaxation times considered, we can apply the first of Eqs. (6.6) and Eq. (4.26) in Section 4, thus obtaining

$$\tau_{\mathrm{f}} = (\kappa')^{-1} + \gamma^{-1}\sqrt{\pi}\,\frac{\mathrm{e}^{(x'-x_0)^2}}{|x'-x_0|} \tag{6.14}$$

and

$$\tau_{\mathrm{b}} = \kappa^{-1} + \gamma^{-1}\sqrt{\pi}\left(\frac{C_2^{\mathrm{eq}}}{|x_1|}\mathrm{e}^{x_1^2} + \frac{C_1^{\mathrm{eq}}}{|x_2|}\mathrm{e}^{x_2^2}\right). \tag{6.15}$$

The first time describes the relaxation of the concentration C_0 of free enzyme E to its equilibrium, a practically vanishing value, and the second time describes the equilibration between concentrations C_1 and C_2 of two bound enzyme states ER and EP.

Note the erroneously determined relaxation time in Eq. 6 of Ref. [8].

In the limit of fast conformational diffusion the relaxation times (6.14) and (6.15) are simplified, of course, to those provided, in the assumed approximation, by conventional theory [77,78]. It is worth noting that the component κ^{-1} is independent of $[\mathrm{R}]_0$, whereas the component $(\kappa')^{-1}$ is inversely proportional to this concentration.

7. Steady-state kinetics

Both at equilibrium and in the steady state, Eqs. (3.1) read

$$\frac{\partial}{\partial x} j_i = w_i, \tag{7.1}$$

but in the latter case the diffusion fluxes, which are the solutions to (7.1):

$$j_i(x) = \int_{-\infty}^{x} \mathrm{d}z\, w_i(z), \tag{7.2}$$

do not vanish for all values of x. Though

$$j_i(\infty) = \int_{-\infty}^{\infty} \mathrm{d}z\, w_i(z) = \dot{C}_i = 0, \tag{7.3}$$

the component reaction fluxes in infinity are not zero and, following Eqs. (3.3) and (3.12), determine the constant production of P at the expense of R, or the reverse:

$$[\dot{\mathrm{P}}]/[\mathrm{E}]_0 = -[\dot{\mathrm{R}}]/[\mathrm{E}]_0 = \int_{-\infty}^{\infty} \mathrm{d}z\, w(z) = \int_{-\infty}^{\infty} \mathrm{d}z\, w'(z) = \int_{-\infty}^{\infty} \mathrm{d}z\, w''(z). \tag{7.4}$$

For the localized chemical transitions, Eqs. (3.4) and (6.2), the diffusion fluxes (7.2) are related to, e.g., $[\dot{\mathrm{P}}]$ by the simple equations:

$$j_0(x) = [\theta(x - x'') - \theta(x - x')][\dot{\mathrm{P}}]/[\mathrm{E}]_0,$$

$$j_1(x) = [\theta(x - x') - \theta(x)][\dot{\mathrm{P}}]/[\mathrm{E}]_0,$$

$$j_2(x) = [\theta(x) - \theta(x - x'')][\dot{\mathrm{P}}]/[\mathrm{E}]_0, \tag{7.5}$$

where θ stands for the Heaviside step function, and Eqs. (7.4) take the form

$$\begin{aligned}
&[\dot{\mathrm{P}}]/[\mathrm{E}]_0 \\
&\quad = \kappa C_1^{\mathrm{eq}} C_2^{\mathrm{eq}} \left[p_1^{\mathrm{st}}(0)/p_1^{\mathrm{eq}}(0) - p_2^{\mathrm{st}}(0)/p_2^{\mathrm{eq}}(0) \right] \\
&\quad = \kappa' C_0^{\mathrm{eq}} C_1^{\mathrm{eq}} \left[[\mathrm{R}]\, p_0^{\mathrm{st}}(x')/[\mathrm{R}]^{\mathrm{eq}}\, p_0^{\mathrm{eq}}(x') \right. \\
&\qquad \left. - p_1^{\mathrm{st}}(x')/p_1^{\mathrm{eq}}(x') \right] \\
&\quad = \kappa'' C_2^{\mathrm{eq}} C_0^{\mathrm{eq}} \left[p_2^{\mathrm{st}}(x'')/p_2^{\mathrm{eq}}(x'') \right. \\
&\qquad \left. - [\mathrm{P}]\, p_0^{\mathrm{st}}(x'')/[\mathrm{P}]^{\mathrm{eq}}\, p_0^{\mathrm{eq}}(x'') \right].
\end{aligned} \tag{7.6}$$

The stationary values of probability densities p_i^{st}, occurring in Eqs. (7.6), are to be determined from the equations defining the diffusion fluxes

$$\left[\frac{1}{2} \frac{\partial}{\partial x} + (x - x_i) \right] p_i^{st} = -\gamma^{-1} j_i(x) \tag{7.7}$$

with $j_i(x)$ given by Eqs. (7.5). The general solutions to linear inhomogeneous Eqs. (7.7) can be written as

$$p_i^{\mathrm{st}}(x) = \frac{p_i^{\mathrm{eq}}(x)}{p_i^{\mathrm{eq}}(y)} \left[p_i^{\mathrm{st}}(y) + 2\sqrt{\pi}\, \gamma^{-1} \mathrm{e}^{-(y - x_i)^2} \int_x^y \mathrm{d}z\, \mathrm{e}^{(z - x_i)^2} j_i(z) \right]. \tag{7.8}$$

They relate the values of p_i^{st} at the point x to the values at the other point y. On integrating these solutions over y (and changing the order of integration over y and z) one can replace the latter values by the stationary concentrations of three enzyme species,

$$C_i^{\mathrm{st}} \equiv \int_{-\infty}^{\infty} \mathrm{d}x\, p_i^{\mathrm{st}}(x), \tag{7.9}$$

and obtain

$$\begin{aligned}
&p_i^{\mathrm{st}}(x) \\
&\quad = \frac{p_i^{\mathrm{eq}}(x)}{C_i^{\mathrm{eq}}} \left[C_i^{\mathrm{st}} \right. \\
&\qquad - 2\gamma^{-1} \int_{-\infty}^{x} \mathrm{d}y\, \mathrm{e}^{(y - x_i)^2} j_i(y) \int_{-\infty}^{y} \mathrm{d}z\, \mathrm{e}^{-(z - x_i)^2} \\
&\qquad \left. + 2\gamma^{-1} \int_{x}^{\infty} \mathrm{d}y\, \mathrm{e}^{(y - x_i)^2} j_i(y) \int_{y}^{\infty} \mathrm{d}z\, \mathrm{e}^{-(z - x_i)^2} \right].
\end{aligned} \tag{7.10}$$

Substitution of the particular values of p_i^{st} in Eqs. (7.6) gives a system of three independent equations for three unknown quantities: $[\dot{P}]$ and two stationary concentrations C_i^{st} (the third is related to the remaining ones by the conservation law (3.8)).

The solution for $[\dot{P}]$ has a form identical to the conventional equation of steady-state kinetics [77,78]:

$$[\dot{P}] = -[\dot{R}] = \frac{k_R K_R^{-1}[R] - k_P K_P^{-1}[P]}{1 + K_R^{-1}[R] + K_P^{-1}[P]}[E]_0, \tag{7.11}$$

which, for $[P] = 0$ (early stage of the steady state in the closed reactor or the constant total removal of the product in the open system), is reduced to the well-known Michaelis–Menten equation [10]:

$$[\dot{P}] = \frac{k_R[R]}{K_R + [R]}[E]_0. \tag{7.12}$$

For the forward reaction, the reciprocal turnover number k_R^{-1} and the apparent dissociation constant K_R are given by the formulae

$$\begin{aligned} k_R^{-1} = {} & \tau_1(x' \to 0) + \tau_2(0 \to x'') + \tau_0(x'' \to x') \\ & + (\kappa C_2^{eq})^{-1} + (\kappa'' C_0^{eq})^{-1} \\ & + K^{-1}\left[\tau_2(0 \to x'') + \tau_2(x'' \to 0) \right. \\ & \left. + (\kappa'' C_0^{eq})^{-1}\right] \end{aligned} \tag{7.13}$$

and

$$\begin{aligned} K_R = {} & k_R (K')^{-1}\left\{\tau_1(x'' \to 0) + \tau_1(0 \to x') \right. \\ & + (\kappa C_2^{eq})^{-1} + (\kappa' C_0^{eq})^{-1} \\ & + K^{-1}\left[\tau_2(0 \to x'') + \tau_2(x'' \to 0) \right. \\ & \left.\left. + (\kappa'' C_0^{eq})^{-1}\right]\right\}, \end{aligned} \tag{7.14}$$

respectively. In Eqs. (7.13) and (7.14) K and K' are the equilibrium constants (6.1) and τ_i denote diffusion times in the potentials of the ith species with the minimum at x_i (see Eq. (A.3) and (A.4) in Appendix A for the potential with the minimum at 0; in order to get formulae for the potential with the minimum at other points a simple change of variables is sufficient).

Similarly, for the backward reaction, the reciprocal turnover number k_P^{-1} and the apparent dissociation constant K_P are given by the formulae

$$\begin{aligned} k_P^{-1} = {} & \tau_2(x'' \to 0) + \tau_1(0 \to x') + \tau_0(x' \to x'') \\ & + (\kappa C_1^{eq})^{-1} + (\kappa' C_0^{eq})^{-1} \\ & + K\left[\tau_1(0 \to x') + \tau_1(x' \to 0) + (\kappa' C_0^{eq})^{-1}\right] \end{aligned} \tag{7.15}$$

and

$$\begin{aligned} K_P = {} & k_P K''\left\{\tau_2(x'' \to 0) + \tau_2(0 \to x'') + (\kappa C_1^{eq})^{-1} \right. \\ & + (\kappa'' C_0^{eq})^{-1} + K\left[\tau_1(0 \to x') + \tau_1(x' \to 0) \right. \\ & \left.\left. + (\kappa' C_0^{eq})^{-1}\right]\right\}, \end{aligned} \tag{7.16}$$

respectively. The parameters (7.13) to (7.16) of the steady-state kinetics satisfy the Haldane equation [77,78]:

$$\frac{k_R}{K_R}\frac{K_P}{k_P} = KK'K'' = \frac{[P]^{eq}}{[R]^{eq}}. \tag{7.17}$$

For a diffusion process that is fast when compared to the local chemical transformations, Eqs. (7.13) and (7.14) are simplified to the form

$$k_R^{-1} = (\kappa C_2^{eq})^{-1} + (\kappa'' C_0^{eq})^{-1} + (\kappa'' C_0^{eq} K)^{-1} \tag{7.18}$$

and

$$K_R = k_R (K')^{-1}\left[(\kappa C_2^{eq})^{-1} + (\kappa' C_0^{eq})^{-1} + (\kappa'' C_0^{eq} K)^{-1}\right], \tag{7.19}$$

identical to those well known from conventional theory [10,77,78] (the times κ^{-1}, $(\kappa')^{-1}$ and $(\kappa'')^{-1}$ and the equilibrium concentrations C_i^{eq} are simply related to the rate constants of the conventional scheme, Fig. 4a, cf. Eqs. (4.27) and (6.8)). On the other hand, for local chemical transformations not limiting the value of the enzyme turnover number,

$$\kappa, \kappa', \kappa'' \gg k_R, \tag{7.20}$$

Eqs. (7.13) and (7.14) are simplified to

$$\begin{aligned} k_R^{-1} = {} & \tau_1(x' \to 0) + \tau_2(0 \to x'') + \tau_0(x'' \to x') \\ & + K^{-1}\left[\tau_2(0 \to x'') + \tau_2(x'' \to 0)\right] \end{aligned} \tag{7.21}$$

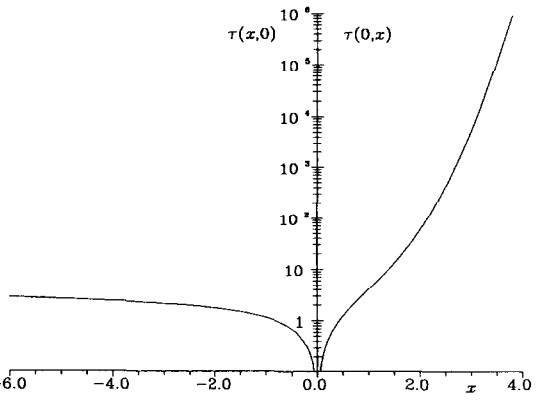

Fig. 9. Diffusion time up, $\tau(0 \to x)$, and down, $\tau(x \to 0)$, the potential x^2 following Eq. (A.6) and Eq. (A.8) in Appendix A. Time is counted in characteristic diffusion time units γ^{-1}. The dimensionless coordinate x is inversely proportional to $\sqrt{k_B T}$.

and

$$K_R = k_R (K')^{-1} \{\tau_1(x' \to 0) + \tau_1(0 \to x') + K^{-1}[\tau_2(0 \to x'') + \tau_2(x'' \to 0)]\}. \quad (7.22)$$

In Eqs. (7.21) and (7.22) one can neglect terms describing the diffusion down the conformational potential which are several orders of magnitude smaller than the terms describing the diffusion uphill (cf. Eq. (A.9) and (A.10) in Appendix A, and Fig. 9). After applying the approximation (A.10) for the diffusion time up the potential, which is valid if the transition points $x = 0$, x' and x'' do not lie very far from each other along the mechanical coordinate, this leads to the simple formulae

$$k_R^{-1} = 2\sqrt{\pi}\,\gamma^{-1}\left[K^{-1}|\Delta x_2| e^{(\bar{x}_2 - x_2)^2} + \sum_{up} |\Delta x_i| e^{(\bar{x}_i - x_i)^2}\right] \quad (7.23)$$

and

$$K_R = 2\sqrt{\pi}\,\gamma^{-1} k_R \left[(K')^{-1}|\Delta x_1| e^{(\bar{x}_1 - x_1)^2} + (KK')^{-1}|\Delta x_2| e^{(\bar{x}_2 - x_2)^2}\right], \quad (7.24)$$

where $\bar{x}_i$ denote the position of the lower transition point whereas Δx_i denote the distance between the lower and the upper transition point within the ith species. In Eq. (7.23) the summation is taken over those enzyme species (one, two, three, or none) within which conformational diffusion takes place up the conformational potential (e.g., in the situation shown in Fig. 7 it is only for the species EP).

A similar analysis can be made for the parameters k_P and K_P of the backward reaction. It should be noted that the Haldane equation (7.17) holds only when the forward and backward reactions proceed along the same mechanical coordinate x. If there are several mechanical coordinates essential for the reaction, the optimum conditions for the forward reaction can happen to be accomplished along one coordinate while those for the backward reaction, along another.

In the closed reactor, when the conservation law (6.11) is satisfied, the solution to Eq. (7.11) varies in time according to Henri's equation [77]. Close to total equilibrium, Eq. (7.11) can be linearized, which enables one to determine the third chemical relaxation time in the model considered:

$$\tau_s = \{[R]_0[E]_0\}^{-1}\{[R]^{eq}[P]^{eq}(k_R^{-1} + k_P^{-1}) + [P]^{eq} K_R k_R^{-1}\}. \quad (7.25)$$

In the limit of fast conformational diffusion, formula (7.25) is also simplified to that provided by conventional theory [77,78]. It is worth mentioning that in the conditions (6.10) the purely chemical component of the relaxation time (7.25) is directly proportional to the concentration $[R]_0$.

8. Summary

We have studied the influence of the internal enzyme dynamics of the protein machine type on the simplest, three-step enzymatic reaction involving only a single covalent transformation besides two association–dissociation steps (Fig. 4). In the model assumed, a quasi-continuous series of conformational transitions of the enzyme is approximated by diffusion in a parabolic potential along a certain mechanical variable (Fig. 3). The conformational transition dynamics and the chemical transformations are jointly formally described by a set of three coupled partial differential equations (Eqs. (3.1) to (3.5)). We have assumed that the reaction is gated by the enzyme

dynamics, i.e. all three chemical transitions are localized around the well-defined values of the mechanical variable x describing the conformational state of the enzyme (Eq. (6.2) and Fig. 7).

In general, the reaction proceeds in three stages.

(a) Initial stage. This depends essentially on the initial distribution of the enzyme conformational states. The time decay of highly energetic states excited above the gates is described by an inhomogeneous combination of a continuum of exponentials (Eqs. (5.30), (5.31), (5.35) and (5.36)).

(b) Transient stage. Concentrations of the three distinguished chemical states of the enzyme tend to their stationary values determined by the much slower varying concentrations of the reactant and the product of reaction. To a good approximation the process is described by the two relaxation times (6.14) and (6.15).

(c) Stationary stage. The rate of reaction depends only on the concentrations of the reactant and the product. The form of this dependence is the same as in the classical theory (Eq. (7.11)), and in the absence of the product (early stage of the steady state in the closed reactor or the constant total removal of the product in the open system) it is reduced to the Michaelis–Menten equation, Eq. (7.12). Close to total equilibrium the concentration of the reactant tends to its equilibrium value with the third chemical relaxation time characterizing the model (Eq. (7.25)).

In the limit of the fast internal dynamics of the enzyme, when compared to the local chemical transformations, the initial, inhomogeneous kinetics stage vanishes and all the formulae describing the (b) and (c) stages of the reaction simplify to those provided by the classical theory of Haldane [10,77,78]. However, extensive studies in two recent decades [1–7], with mention of which we have begun this paper, suggest that the conformational transition dynamics of the enzyme is as slow as, if no slower than, the overall reaction. Consequently, it is this dynamics rather than the details of chemical mechanism that should determine the effective reaction rate. In other words, quite contrary to the conventional assumption, the limit of the slow intramolecular dynamics, when compared to the local chemical transformations, is usually realized. We focused our attention mainly on this limit.

Perhaps the most important result of the theory presented is that the turnover numbers, Eqs. (7.13) or (7.15), and the apparent dissociation constants, Eqs. (7.14) or (7.16), of the enzyme in steady-state conditions are formally independent of the reaction rate constants determined by the relaxation times near equilibrium or in the pre-stationary stage, Eqs. (6.14) and (6.15). In general, the kinetic mechanisms of the reaction far from and close to equilibrium differ. This was already suggested more than twenty years ago by Blumenfeld [17] and the content of this paper is, in fact, nothing else than a more precise formulation of his ideas.

Close to equilibrium (in the case of small concentration of the substrate with respect to the enzyme), or in the pre-stationary stage (in the case of large concentration of the substrate with respect to the enzyme), the chemical transformations are preceded by conformational transitions in the vicinity of minima of the conformational potentials in individual chemical species of the enzyme, mainly *below* the lower transition points (see Fig. 7). If, however, the enzymatic reaction proceeds in steady-state conditions far from equilibrium, no partial equilibration within any chemical species is necessary and, as a continuous supply of a substrate forces the diffusion between the lower and the upper transition points, the conformational dynamics takes place mainly *above* the lower transition points. Because of the slow character of conformational dynamics, the parameters of enzymatic reaction both close to and far from equilibrium are determined essentially by the process of conformational diffusion and not by details of the chemical mechanism of the reaction.

Our theory, taking into account present-day knowledge of the internal dynamics of proteins, obviously contradicts the conventional theory. However, in physiological temperatures, when initial condition effects can be neglected, it leaves the conventional phenomenology of the enzymatic reaction essentially unaltered and changes only the interpretation of parameters.

Moreover, all the rate parameters found display Arrhenius temperature dependence, as in the conventional approach. The exponents in Eqs. (6.14), (6.15), (7.23) and (7.24), equal to the squared mechanical coordinate, are, following Replacement (2.3), inversely proportional to $k_B T$. Neglecting factors not much different from unity, both the reciprocal relax-

ation times and the enzyme turnover numbers are determined by a few terms of the form

$$\gamma e^{-\Delta G_c^{\ddagger}/k_B T}, \tag{8.1}$$

where $\Delta G_c^{\ddagger}$ is a certain free energy barrier. This form cannot be directly related to that of the reaction rate constants predicted by the transition state theory [9,10]:

$$\nu e^{-\Delta G^{\ddagger}/k_B T}, \tag{8.2}$$

as the diffusion rate constant γ, estimated to be of the order $10^7\,s^{-1}$, is six orders of magnitude smaller than the mean frequency of thermal vibrations $\nu \approx 10^{13}\,s^{-1}$. However, because the diffusion itself is an activated process, the factor γ itself depends on temperature in the Arrhenius manner and, following Eq. (2.10),

$$\gamma = N^{-2}k = N^{-2}\nu e^{-\Delta G_e^{\ddagger}/k_B T}. \tag{8.3}$$

In Eq. (8.3) we assumed that the rate constant k of a single transition between neighbouring conformations along the mechanical coordinate is interpretable in terms of the transition state theory and determined by an activation free energy $\Delta G_e^{\ddagger}$. On taking into account Equality (8.3) we find that the Kramers-type form (8.1) of the rate parameters is identical to that predicted by the transition state theory, Eq. (8.2). One has only to treat the free energy of activation $\Delta G^{\ddagger}$ as an *effective* parameter composed of the free energy of activation for diffusion up the conformational potential $\Delta G_c^{\ddagger}$, the free energy of activation for a single, 'elementary' conformational transition $\Delta G_e^{\ddagger}$, and an entropy contribution from the root mean square number of thermally populated conformations N. For $\Delta G_e^{\ddagger} \approx 10\,kJ\,mol^{-1}$ and $N \approx 10^2$ (see discussion in Section 2) we have to assume $\Delta G_c^{\ddagger} \approx 20\,kJ\,mol^{-1}$ in order to obtain the rate of enzymatic reaction as $10^3\,s^{-1}$.

That the new theory provides almost the same phenomenology as the old one testifies in favour of the new theory—this phenomenology has been successfuly used for interpretation of experimental data for over sixty years. But the lack of essential changes of the phenomenology simultaneously implies problems with carrying out an *experimentum crucis* directly discrediting the conventional approach to enzymatic reactions. The only unquestionable results are the observations, initiated by Frauenfelder and coworkers [3–5,13], of inhomogeneous kinetics at low temperatures. In Section 6 we have proven that the model considered provides such a kinetics as a result of a specific initial state preparation and only for the reaction controlled by slow intramolecular dynamics; for the internal dynamics of the enzyme, fast when compared to the local chemical transformations, no inhomogeneous kinetics can occur. A possible *experimentum crucis* in physiological temperatures could be a careful demonstration of the lack of a direct relation between the values of phenomenological parameters describing the reaction in the transient and steady-state stages, or a study of the dependence of the relaxation times in the transient and final stages of the reaction on the initial concentration of substrate $[R]_0$. Unfortunately, experiments of this type can hardly be crucial as the particular predictions provided by both the conventional and the novel theory depend on many factors that we have not discussed here in detail. Experimentalists are welcome to put forward other proposals.

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Appendix A. Mean first-passage time and reaction rate constants

For a process described by Eq. (2.4) with an arbitrary potential G (in $k_B T$ units), the time of diffusion from the point x' to x'' or, more precisely, the mean first-passage time through the point x'' for a trajectory starting at the point x' in the presence of a reflecting boundary at the point r, is given by the equation [40,51]

$$\tau(x' \to x'') = 2\gamma^{-1}\int_{x'}^{x''} dy\, e^{G(y)} \int_{r}^{y} dz\, e^{-G(z)}. \tag{A.1}$$

In particular, for the parabolic potential with a minimum at $x = 0$,

$$G(x) = x^2, \tag{A.2}$$

the time of diffusion from x' to x'' for $x' < x''$ (the reflecting point at $-\infty$) is given by the equation

$$\tau(x' \to x'') = 2\gamma^{-1} \int_{x'}^{x''} \mathrm{d}y\, \mathrm{e}^{y^2} \int_{-\infty}^{y} \mathrm{d}z\, \mathrm{e}^{-z^2}, \tag{A.3}$$

and, for $x' > x''$ (the reflecting point at ∞), by the equation

$$\tau(x' \to x'') = 2\gamma^{-1} \int_{x''}^{x'} \mathrm{d}y\, \mathrm{e}^{y^2} \int_{y}^{\infty} \mathrm{d}z\, \mathrm{e}^{-z^2}, \tag{A.4}$$

In Fig. 9 (Section 7), diffusion times *up*, $\tau(0 \to x)$, and *down*, $\tau(x \to 0)$, the parabolic potential x^2, determined by these equations, are plotted vs the distance x in the logarithmic scale. It is seen that diffusion down the potential can be several orders of magnitude faster than diffusion up it. We approximate the corresponding formulae using the asymptotic expansions of the integral that describes the error or complementary error functions [68]:

$$\begin{aligned} \int_{-\infty}^{y} \mathrm{d}z\, \mathrm{e}^{-z^2} &\equiv \frac{\sqrt{\pi}}{2}(1 + \operatorname{erf} y) \equiv \frac{\sqrt{\pi}}{2}(2 - \operatorname{erfc} y) \\ &= \begin{cases} \sqrt{\pi} - \ldots & \text{for } y \to +\infty \\ \dfrac{1}{2|y|}\mathrm{e}^{-y^2} + \ldots & \text{for } y \to -\infty. \end{cases} \end{aligned} \tag{A.5}$$

Following the expansion for $y \to \infty$, the time of diffusion up the parabolic potential x^2 for $x \gg 1$ can be expressed as

$$\begin{aligned} \tau_{\mathrm{up}}(0 \to x) &= 2\gamma^{-1} \int_0^x \mathrm{d}y\, \mathrm{e}^{y^2} \int_{-\infty}^{y} \mathrm{d}z\, \mathrm{e}^{-z^2} \\ &\approx 2\sqrt{\pi}\,\gamma^{-1} \int_0^x \mathrm{d}y\, \mathrm{e}^{y^2} \\ &\approx \sqrt{\pi}\,\gamma^{-1} x^{-1} \exp x^2. \end{aligned} \tag{A.6}$$

The first approximate equality results from the fact that the main contribution to the integral over y comes from large ys and the second approximate equality results from the asymptotic expansion of the Dawson integral [68]:

$$\int_0^x \mathrm{d}y\, \mathrm{e}^{y^2} = \frac{1}{2x}\mathrm{e}^{x^2} - \ldots \text{ for } x \to +\infty. \tag{A.7}$$

Conversely, following the expansion (A.5) of the error function for $y \to -\infty$, the time of diffusion down the parabolic potential x^2, from x to x' for $x < x' \ll -1$, can be expressed as

$$\begin{aligned} \tau_{\mathrm{dn}}(x \to x') &= 2\gamma^{-1} \int_x^{x'} \mathrm{d}y\, \mathrm{e}^{y^2} \int_{-\infty}^{y} \mathrm{d}z\, \mathrm{e}^{-z^2} \\ &\approx \gamma^{-1} \int_x^{x'} \mathrm{d}y\, \frac{1}{|y|} = \gamma^{-1} \ln \frac{x}{x'}. \end{aligned} \tag{A.8}$$

For x and x' close to each other and distanced by Δx, one can expand the logarithm in the vicinity of unity to get

$$\tau_{\mathrm{dn}}(x \to x + \Delta x) \approx \gamma^{-1} \frac{|\Delta x|}{|x|}. \tag{A.9}$$

The analogous expression for the diffusion time up the potential is, following Eq. (A.6),

$$\tau_{\mathrm{up}}(x \to x - \Delta x) \approx 2\sqrt{\pi}\,\gamma^{-1} |\Delta x| \mathrm{e}^{x^2}. \tag{A.10}$$

As expected, this time is approximately longer by the factor e^{x^2} (cf. Fig. 9).

The concept of the mean first-passage time is very useful in calculating the reaction rate constants [40]. There is, however, some controversy over the way of taking into account a possible recrossing of the energy barrier top, so we would like to clarify this question in more detail here. We shall consider a reversible unimolecular reaction of the kinetic Eq. (4.4), whose microscopic dynamics is described by diffusion in a double-well potential of the form

$$G(x) = \begin{cases} (x - x_1)^2 & \text{if } x < \bar{x} \\ (x - x_2)^2 - \ln K & \text{if } x > \bar{x} \end{cases} \tag{A.11}$$

with

$$K \equiv C_2^{\mathrm{eq}} / C_1^{\mathrm{eq}} \tag{A.12}$$

standing for the chemical equilibrium constant. In general, we do not assume the point $\bar{x}$ to be related to x_is and the equilibrium constant K, so the potential (A.11) at $\bar{x}$ can have a jump (Fig. 10).

To make sure that complete final equilibration has

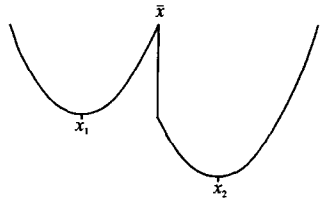

Fig. 10. General shape of the potential described by Eq. (A.11).

been reached, we identify the reciprocal forward reaction rate constant with the time of diffusion from the first potential minimum at x_1 to the second potential minimum at x_2:

$$k_1^{-1} = \tau(x_1 \to x_2) = 2\gamma^{-1}\int_{x_1}^{\bar{x}} \mathrm{d}y\, \mathrm{e}^{(y-x_1)^2}\int_{-\infty}^{y} \mathrm{d}z\, \mathrm{e}^{-G(z)} + 2\gamma^{-1}K^{-1}\int_{\bar{x}}^{x_2} \mathrm{d}y\, \mathrm{e}^{(y-x_2)^2}\int_{-\infty}^{y} \mathrm{d}z\, \mathrm{e}^{-G(z)}. \tag{A.13}$$

The main contribution to both integrals over y comes from ys in the vicinity of the transition point $\bar{x}$ (the top of the potential). In this region, for

$$|x_1 - \bar{x}| \gg 1 \text{ and } |x_2 - \bar{x}| \gg 1 \tag{A.14}$$

(the activated process), the integrals over z can be approximated by $\sqrt{\pi}$,

$$\int_{-\infty}^{y} \mathrm{d}z\, \mathrm{e}^{-G(z)} \approx \sqrt{\pi} \tag{A.15}$$

(cf. Eq. (A.5)), and, after taking into account the asymptotic expansion of the Dawson integral, Eq. (A.7), we get the Kramers-type expression

$$k_1^{-1} = \tau(x_1 \to x_2) = \frac{\sqrt{\pi}}{\gamma}\left[\frac{1}{|x_1 - \bar{x}|}\mathrm{e}^{(x_1-\bar{x})^2} + K^{-1}\frac{1}{|x_2 - \bar{x}|}\mathrm{e}^{(x_2-\bar{x})^2}\right]. \tag{A.16}$$

In a similar way, we obtain the reciprocal backward reaction rate constant:

$$k_2^{-1} = \tau(x_2 \to x_1) = \frac{\sqrt{\pi}}{\gamma}\left[\frac{1}{|x_2 - \bar{x}|}\mathrm{e}^{(x_2-\bar{x})^2} + K\frac{1}{|x_1 - \bar{x}|}\mathrm{e}^{(x_1-\bar{x})^2}\right]. \tag{A.17}$$

That Eq. (A.16) and (A.17) represent the properly defined, actually observable macroscopic rate constants [79] follows from the fact that they satisfy the detailed balance condition:

$$k_1/k_2 = K. \tag{A.18}$$

According to Eq. (4.5), the chemical relaxation time is given by

$$\tau = (k_1 + k_2)^{-1} = \frac{\sqrt{\pi}}{\gamma}\left[\frac{C_2^{\mathrm{eq}}}{|x_1 - \bar{x}|}\mathrm{e}^{(x_1-\bar{x})^2} + \frac{C_1^{\mathrm{eq}}}{|x_2 - \bar{x}|}\mathrm{e}^{(x_2-\bar{x})^2}\right]. \tag{A.19}$$

Eq. (A.19) represents the diffusion time from the bottom of the well to the top of the barrier (cf. Eq. (A.6)) averaged over both chemical species; C_2^{eq} is the probability of equilibration of the trajectory outside well 1 and, conversely, C_1^{eq} is the probability of equilibration of the trajectory outside well 2. This interpretation suggests an alternative definition of reaction rate constants in terms of the reciprocal time of diffusion *only* to the barrier top:

$$k_i = P_i\frac{\gamma}{\sqrt{\pi}}|x_i - \bar{x}|\mathrm{e}^{-(x_i-\bar{x})^2} \tag{A.20}$$

($i = 1, 2$) with the probabilities of equilibration P_i normalized to unity,

$$P_1 + P_2 = 1, \tag{A.21}$$

and chosen in such a way that the detailed balance condition (A.18) is satisfied. Indeed, calculation of P_is leads to the reconstruction of Eq. (A.16) and (A.17).

The condition of continuity of the potential (A.11) at the point $\bar{x}$,

$$(x_1 - \bar{x})^2 - \ln C_1^{\mathrm{eq}} = (x_2 - \bar{x})^2 - C_2^{\mathrm{eq}}, \tag{A.22}$$

implies that Eq. (A.19) is replaced by

$$\tau^{-1} = \frac{\gamma}{\sqrt{\pi}}\frac{|x_1 - \bar{x}||x_2 - \bar{x}|}{|x_1 - \bar{x}| + |x_2 - \bar{x}|}\times\left[\mathrm{e}^{-(x_1-\bar{x})^2} + \mathrm{e}^{-(x_2-\bar{x})^2}\right]. \tag{A.23}$$

For a symmetric reversible reaction,

$$C_1^{\mathrm{eq}} = C_2^{\mathrm{eq}} = \frac{1}{2},\ |x_1 - \bar{x}| = |x_2 - \bar{x}|, \tag{A.24}$$

after assuming $\bar{x} = 0$, we obtain the equation known in literature [63]:

$$\tau^{-1} = \frac{\gamma}{\sqrt{\pi}} |x_1| e^{-x_1^2} = k_1 + k_2 . \qquad (A.25)$$

The same chemical relaxation time,

$$\tau^{-1} = \frac{\gamma}{\sqrt{\pi}} |x_1| e^{-x_1^2} = k_1 , \qquad (A.26)$$

identical to the time of diffusion only to the top of the barrier, Eq. (A.6), we obtain for the completely irreversible, asymmetric reaction (putting $C_1^{eq} = 0$, $C_2^{eq} = 1$ and $\bar{x} = 0$ into Eq. (A.19)). Eq. (A.26) differs by a factor of two from that quoted in literature [40]. We suppose the divergence is due to the fact that in the cited paper the recrossing of the highly asymmetric cusp-shaped barrier has been superfluously assumed.

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