Abstract. Formulation of a truly advanced statistical theory of biochemical processes needs simple but realistic models of phenomena underlying microscopic dynamics of proteins. Many experiments performed in the 1980s have demonstrated that within the protein native state, apart from usual vibrational dynamics, a rich interconformational (activated) dynamics exists. The slowness of this dynamics makes any conventional theory of chemical reactions inapplicable for description of enzymatic reactions. It is presumably a rule that it is the process of conformational relaxation, and not the details of chemical mechanism, that affects their rate. In a simple model of Protein-Machine type, applied in constructing a novel theory of enzymatic reaction, conformational dynamics is treated as a realative quasi-continuous motion of solid-like structural elements of protein. Simple and tractable formulas for the chemical relaxation time and the enzyme turnover number in the steady state conditions are found. The important result obtained is that the kinetic mechanisms close to and far from the equilibrium can differ.

Keywords: Protein dynamics; Reaction rate theory; Enzymatic catalysis; Kinetic mechanisms.
1. INTRODUCTION

Any statistical theory of physical processes has to refer to simple models of phenomena underlying microscopic dynamics. The transition state theory, commonly used in interpretation of biochemical reactions [1], assumes that biomolecules, in particular enzymatic proteins, perform only fast vibrations about a single well-defined equilibrium conformation. This picture, adapted directly from chemistry of low-molecular weight compounds, has proved, however, untrue. Many experiments performed in the 1980s using various techniques have demonstrated that within the protein native state, apart from usual vibrational dynamics, a rich interconformational (activated) dynamics exists in the whole range of time scales from $10^{-11}$s to $10^5$s or longer [2, 3]. The slowness of this dynamics makes any conventional theory of chemical reactions inapplicable for description of biomolecular reactions [4]. A consequence is a challenge to physicists-theoreticians to construct a truly advanced statistical theory of biochemical processes based on still simple, but realistic models of microscopic dynamics of proteins.

Conformational transitions within the protein native state take place not in the whole bulk of protein but are limited to liquid-like regions surrounding solid-like fragments of secondary structure (Figure 1). It is not easy to infer the actual nature of conformational dynamics from experimental data so that the problem of formulating appropriate models is to some extent still left open to speculation. In two classes of models provided hitherto by literature the speculative element seems to be kept within reasonable limits. We refer to them symbolically as Protein-Glass and Protein-Machine [3].

The relaxation time spectrum of conformational dynamics of protein looks practically like quasicontinuous. The simplest way to tackle such problems is to assume that the dynamics of a system is in every time scale alike, i.e., the spectrum of relaxation times has a self-similarity symmetry. This assumption is the core of any Protein-Glass model. Time scaling can originate either from a hierarchy of interconformational barrier heights or from a hierarchy of bottlenecks met by structural defects diffusing across the liquid-like region of protein [2, 3].

The Protein-Glass models behave unrealistically both in the limit of very short and very long times, and in practice should be restricted only to a few levels of the hierarchy [2]. An alternative free of such disadvantages is the Protein-Machine class of models in which the variety of conformations composing the native state is assumed to be labelled with a few “mechanical” variables, e.g. angles describing mutual orientation of rigid fragments of secondary structure or larger structural elements (Figure 1). The Protein-Machine models not only display correct asymptotic behaviour but can also bring simple and tractable formulas for phenomenological parameters. It is the aim of this letter to present a few such formulas for a single enzymatic reaction. A formal derivation of equations will be given in a more detailed paper now in preparation.
The concept (and the name) “Protein-Machine” was proposed for the first time by Chernavsky, Khurgin and Shnol in 1967 [5] but similar picture of protein dynamics has been considered independently also by several other authors [6-10]. In 1982, the model was formalized by Shaitan and Rubin [11] in terms of diffusion in an effective potential along the mechanical coordinate. This kind of dynamics with the simplest, parabolic potential has been applied for interpretation of particular protein reactions by Agmon and Hopfield [12] and Cartling [13]. A detailed mathematical analysis of the model applied to a general reversible reaction was performed by the author of this letter [14].

For the sake of convenience we will assume that the considered mechanical variable, \( x \) (compare Figure 1 for more concreteness), is dimensionless (proportional to the square root of Boltzmann’s constant times the temperature, \( \sqrt{kT} \)) and normalized in such a way that its equilibrium dispersion is \( 1/2 \). Then, the process of diffusion in parabolic potential with the minimum at \( x_0 \) is described by the partial differential equation [14]

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

for probability density \( p \). Because of original applications, Equation (1) is named after Smoluchowski or Ornstein and Uhlenbeck. The second parameter, \( \gamma \), has the meaning of the inverse relaxation time of the mean value of the conformational variable,

\[
\dot{X}(t) \equiv \int_{-\infty}^{\infty} dx \ x p(x, t).
\] (2)

Indeed, substitution of Eq. (1) into Eq. (2) results in the ordinary differential equation:

\[
\dot{X} = -\gamma \ (X - x_0)
\] (3)

(the dot means derivation with respect to time).

A motion of fragments of secondary structure relative to each other is often being observed when two or more different crystalline structures of the same protein are compared [15]. Also a relative motion of the whole domains has been the subject of numerous structural investigations [16, 17]. As is quite natural, in comparative structural studies only a few, usually two, conformations are apparent. In the same terms of a few, at the most, discrete conformational states, the conformational dynamics is described by conventional biochemistry [1]. The recent paper by Haran, Haas, Szpikowska and Mas [18] is therefore important showing clearly, in the study of fluorescence energy transfer between donor and acceptor centres located on different domains, a quasi-continuous distribution of interdomain distances.

A quasi-continuum of short-lived conformational states within the native state of protein has been observed directly in numerical simulations [19, 20].
A careful analysis [20, 21] indicated that numerically studied dynamics of interconformational transitions actually has a character of relative motion of the secondary structure elements.

It should be noted that the mathematical equation identical to (1) also describes overdamped collective vibrations of domains, moreover, numerical analysis indicated that they also take the form of mutual motions of relatively rigid fragments of secondary structure [22]. In fact, the cross-over between sequences of conformational transitions along the mechanical coordinates and overdamped low-frequency collective vibrations is more or less a matter of convention as one can introduce effective normal modes corresponding to the envelope of the ragged (with many local minima) potential along chosen directions [23]. Protein-Machine models of dynamics are universal also in another sense displaying, possibly, a hierarchy typical for Protein-Glass models. Thus, the mechanical elements can be identified not only with the fragments of secondary structure, but also with the larger domains and, on the other hand side, smaller side chains [24].

A controversion exists as far as the time scale of mechanical motions is concerned. Direct projection of molecular dynamics trajectories onto the subspace spanned by the principal axes of the low-frequency normal modes gives relaxation times of these modes only twice as long as their period, i.e. $10^{-11}$ s at the most [25]. This result is in agreement with the estimations made almost twenty years ago on the basis of a simple hydrodynamic model [26]. However, in the longest up to date 900 ps ($\sim 10^{-9}$ s) simulation by Amadei, Linssen and Berendsen [27] no equilibration has been observed of the trajectories projected onto the subspace spanned by the principal axes of the “essential” modes of motion. Also the already mentioned study of the fluorescence energy transfer [18] indicates that the motion of domains relative to each other is “slow on the nanosecond time scale” (in fact, the value of the intramolecular diffusion constant and that of the equilibrium dispersion of interdomain distance from Ref. 18 give the value of the relaxation time greater than 1 $\mu$s).

From what was said above it follows that the mechanical variables, slow by the definition, cannot be directly identified with the low-frequency normal modes, but rather with the essential modes introduced in Ref. 27 (the principal axes of the normal modes are defined as those in which the force matrix is diagonal, whereas the principal axes of the essential modes, as those in which the covariance matrix of the atomic displacements is diagonal). Two quoted examples seem to point to a rule that the value of relaxation time estimated in a given experiment (as such we treat also numerical simulations) coincides with the value of the longest time accessible for observation with the help of a given technique. Anyway, our somewhat hypothetical assumption about the conformational dynamics of proteins observed in the time scale of enzymatic reactions ($\sim 10^{-3}$ s) [3] having the character of a quasi-continuous mechanical motion does not contradict the experimental settlements.
3. GENERALIZED HALDANE’S KINETICS

In conventional biochemistry [1] no distinction is made, in general, between the kinetic and the chemical mechanism of reaction, and the reaction involving a single covalent step

\[ R \leftrightarrow P \]

is usually modelled by the three-step kinetics of Haldane with the only two association-dissociation steps of the substrate or the product to enzyme added (Figure 2(a)). However, conformational (non-covalent) transitions are as slow, if not slower, as the very covalent or binding steps, thus have to be treated on an equal footing with them. The actual kinetic scheme of a single enzymatic reaction appears, consequently, infinitely more complex than the Haldane’s scheme. It is shown in Figure 2(b) but it can hardly be considered a proper phenomenological description of the reaction [4].

In the Protein-Machine model considered, 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 \( x \). Chemical transitions are perpendicular to this coordinate, thus the dynamics of the enzyme as well as the reaction is described by a set of three coupled balance equations for probability densities \( p_i(x,t) (i = 0, 1, 2 \) for E, ER and EP, respectively):

\[
\frac{\partial}{\partial t} p_i = -\frac{\partial}{\partial x} j_i + w_i,
\]

with diffusion fluxes

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

and local reaction rates

\[
w_0 = -w' + w'', \quad w_1 = -w + w', \quad w_2 = w - w''
\]

(see Figure 2(c)).

We assume that the chemical transitions are localized in narrow regions of the values of mechanical variable:

\[
w(x) \propto \kappa \delta(x), \quad w'(x) \propto \kappa' \delta(x - x'), \quad w''(x) \propto \kappa'' \delta(x - x''),
\]

where \( \delta \) stands for Dirac’s delta (the reaction is gated by conformational dynamics [14]). In more concrete terms, for one value of the angle \( x \) (Figure 1) free space large enough for the substrate motions inside the enzyme can, for instance, appear. For another, sharply defined, 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 the substrate desorption.

We assume also that local chemical transitions are much faster than conformational diffusion:

\[
\kappa, \kappa', \kappa'' \gg \gamma.
\]
This assumption is quite natural if we recall that conformational relaxation within the native state of enzyme is as fast as the very reaction so that it is this relaxation that determines the resultant reaction rate and not the details of the chemical mechanism. The important consequence of this assumption is in general the difference in the kinetic mechanism of the reaction close to and far from the equilibrium.

4. Reaction Close To The Equilibrium

Close to the total chemical equilibrium three concentrations (molar fractions) of the free enzyme and its two complexes

\[ C_i(t) \equiv \int_{-\infty}^{\infty} dx p_i(x,t) \]  

\( (C_0 = [E], C_1 = [ER], C_2 = [EP]) \) evolve towards their equilibrium values, \( C_i^{eq} \), with a single relaxation time, \( \tau_{eq} \), given by the formula:

\[
\tau_{eq}^{-1} = \frac{\gamma}{2\sqrt{\pi}} \sum_{i=0}^{2} (1 - C_i^{eq}) \sqrt{\Delta G_i^\dagger/kT} \exp(-\Delta G_i^\dagger/kT). 
\]  

(10)

Quantities \( \Delta G_i^\dagger \) are values of the free energy that has to be surmounted within particular species in order to reach the nearest gate from the equilibrium conformation \( x_i \) (Figure 3). Formula (10) is to be derived from Eqs. (4-7) with the condition (8) and for \( \Delta G_i^\dagger \gg kT \) using the variational method [14].

Equation (10) has the meaning of the average reciprocal time of diffusion uphill the conformational potential from its minimum. Quite generally, the time of diffusion from \( x' \) to \( x'' \) in the potential \( x^2 \) (in \( kT \) units) is given by the formula

\[ \tau(x' \rightarrow x'') = 2\gamma^{-1} \int_{x'}^{x''} dy e^{y^2} \int_{-\infty}^{y} dx e^{-x^2}. \]  

(11)

In Figure 4, diffusion times uphill and downhill the potential are plotted vs the distance \( x \) in the logarithmic scale. It is seen that diffusion uphill can be several orders of magnitude slower than diffusion downhill, taking place in the time of the order of \( \gamma^{-1} \).

On examining Figure 3 we find that after transition in the point \( x' \) from the state \( E + R \) to \( ER \), the enzyme can either equilibrate within \( ER \) or 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 [4]. The rate constant, \( k_{ij} \), of the reaction between species \( i \) and \( j \) is related to the single relaxation time (10) by the simple formula [28]:

\[ k_{ij} = \tau_{eq}^{-1} C_j^{eq} \]  

(12)

(note independence of \( k_{ij} \) of the initial species \( i \)).
5. STEADY-STATE KINETICS

No partial equilibration within any kinetic species is necessary if the enzymatic reaction proceeds far from the chemical equilibrium. In steady state conditions with the concentration of reactant kept constant, \([R] = \text{const}\), and with the constant removal of product, \([P] = 0\), the rate of the reaction is described, as in the conventional approach, by the Michaelis-Menten law:

\[
\dot{P} = \frac{k_c[R]}{K_m + [R]}
\]

The reciprocal turnover number:

\[
k_c^{-1} = \tau_1(x'\rightarrow 0) + \tau_2(0 \rightarrow x'') + \tau_0(x''\rightarrow x') + \left(\frac{C_{eq}^1}{C_{eq}^2}\right)\left[\tau_2(0 \rightarrow x'') + \tau_2(x''\rightarrow 0)\right],
\]

and the apparent dissociation constant:

\[
K_m = k_c[R]^{eq} \left\{ \left(\frac{C_{eq}^0}{C_{eq}^1}\right) \left[\tau_1(x'\rightarrow 0) + \tau_1(0 \rightarrow x')\right] + \left(\frac{C_{eq}^0}{C_{eq}^2}\right) \left[\tau_2(0 \rightarrow x'') + \tau_2(x''\rightarrow 0)\right] \right\}
\]

\((\tau_i\) denoting diffusion time in the potential of \(i\)-th species with the minimum at \(x_i\)) have, however, quite unconventional interpretation.

The theory presented gives a rule for the optimum action of enzyme: the turnover number \(k_c\) is maximum, if conformational diffusion within free enzyme E is downhill, and the transition points \(x = 0, x'\) and \(x''\) lie not very far from each other. In that case \(k_c \simeq \gamma \gg \tau_{eq}^{-1}\). Of course the backward reaction along the same path in steady state conditions should be much slower, but the latter reaction, in Protein-Machine model, can proceed along another path it may find more convenient.

6. CONCLUDING REMARKS

Perhaps the most important result of the presented theory is that the turnover number \(k_c\), Equation (14), of the enzyme in the steady-state conditions is formally independent of the reaction rate constants \(k_{ij}\), Equations (12) and (10). In general, the kinetic mechanisms of the reaction close to and far from the equilibrium can differ. This was suggested already more that twenty years ago by Blumenfeld [7] and the content of present paper is, in fact, nothing else than a more precise formulation of his ideas.

An essential feature of the new approach is that it leaves the classical phenomenology of the enzymatic reaction essentially unaltered and changes only interpretation of phenomenological parameters. As a consequence, it will be, probably, not simple to carry out experimentum crucis directly proving the conventional theory wrong. Two general predictions made by the novel theory: (a) the independence of the enzyme turnover number of a particular chemical mechanism, and (b) a distinct difference in values of the turnover...
number for the reaction proceeding in the forward and backward direction along the same pathway, do provide the explanation of two undoubtedly specific properties of enzymatic reactions: (a) a relatively small dispersion of values of the turnover number about the almost universal value $10^{3}$ s$^{-1}$, and (b) a distinct irreversibility of most reactions in the steady-state conditions. The reversible reactions observed in these conditions can be explained by the Protein-Machine theory in terms of relaxation pathways along two different mechanical coordinates for the forward and the backward directions.

Only close to the equilibrium can enzymatic reactions be thermodynamically described in terms of concentrations of kinetically distinguishable chemical species. In steady state conditions far from the equilibrium the proper thermodynamic variables are rather quantities characterizing conformational nonequilibrium of the enzyme, that is the mean values of mechanical variables in the case of the model considered. The conformational nonequilibrium should play an extremely important role in processes of coupling of several reactions taking place at the same multienzyme complex, the presumably universal statistically independent unit of biochemical processes. In the conventional mechanism of chemical coupling the complex is needed only for keeping the appropriately high concentration of intermediates. The conformational nonequilibrium implies the possibility of different, mechanical coupling of reactions, upon which energy released in the center of one reaction is directly transferred to the center of another reaction. This fascinating possibility seems to be worth further studies.

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FIGURE CAPTIONS

Figure 1. 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-pleated sheets) and weakly shaded are surrounding liquid-like regions. Black is the catalytic centre localized at two neighbouring solid-like elements. Models of Protein-Glass type treat the dynamics of conformational transitions within the native state of protein as a quasicontinuous diffusion of structural defects through the liquid-like medium. Alternatively, models of Protein-Machine type treat this dynamics as a relative motion of solid-like elements, also of the nature of a quasicontinuous diffusion, along a mechanical coordinate, identified in this picture with the angle \( x \). The main assumption of the present paper is that the slow diffusion dynamics controls the reaction. The picture can be reinterpreted on a higher level of the hierarchy: solid-like structural elements represent then the whole domains moving in a multidomain enzymatic macromolecule.

Figure 2. Single enzymatic reaction. (a) Conventional kinetics. (b) Actual kinetics involving an astronomical number of conformations 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.

Figure 3. Protein-Machine model of a single enzymatic reaction. Three different conformational potentials with minima at \( x_0, x_1 \) and \( x_2 \) correspond to individual chemical states E, ER and EP, respectively, of the enzyme. Three chemical transitions are localized at the points \( 0, x', \) and \( x'' \).

Figure 4. Diffusion time uphill, \( \tau(0 \to x) \), and downhill, \( \tau(x \to 0) \), the potential \( x^2 \). Time is counted in characteristic diffusion time units \( \gamma^{-1} \). Dimensionless coordinate \( x \) is proportional to \( \sqrt{kT} \).