Novel mechanism for temperature-independent transitions in flexible molecules: role of thermodynamic fluctuations

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Novel physical mechanism is proposed for explanation of temperature-independent transition reactions in molecular systems. The mechanism becomes effective in the case of conformation transitions between quasi-isoenergetic molecular states. It is shown that at room temperatures, stochastic broadening of molecular energy levels predominates the energy of low frequency vibrations accompanying the transition. This leads to a cancellation of temperature dependence in the stochastically averaged rate constants. As an example, physical interpretation of temperature-independent onset of P2X3 receptor desensitization in neuronal membranes is provided.

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Introduction.– Biomacromolecules (proteins and nucleic acids) operate by maintaining the set of conformations and making transitions between them with proper reaction rates which are, as a rule, temperature controlled. Description of temperature-independent effects requires dissection of multi-type reactions arising within the complexity of molecular structures. In biology, one can generally indicate three types of reactions. The first type is generally associated with photoinduced oxidation-reduction processes. Basic mechanism of these reactions is an electron tunneling between rigid redox centers like metal containing porphyrin rings and quinones [1]. The fact of tunneling is supported by the absence of temperature dependence for the transfer rates in wide temperature regions including the room temperatures [2, 3]. The second type of reactions is associated with enzyme kinetics [4] where the rate constants are calculated using the concept of activated complex. Motion of reactants along the reaction coordinates is accompanied by overcoming the activation barriers so that the rate constants experience exponential temperature dependence. However, in the framework of Marcus’s theory, a barrierless behavior becomes also possible if only reorganization energy and driving force of reaction are equal thus smoothing over temperature dependence. Such situation is probably realized during the binding of NO molecule to the protoheme in the region of 200-290 K [2]. The third type of reactions is associated with conformation transformations. Some of these reactions do not exhibit temperature dependence even in the range of room temperatures. For instance, it has been demonstrated that the closing rate for both the single-stranded DNA and RNA hairpin-loop fluctuations and the rate for the cyclic β-hairpin peptide folding are weakly dependent on temperature in the interval from 10° to 60°C showing zero or even slightly negative enthalpies [6, 7]. Another example of temperature independence in the gating kinetics of membrane proteins has been recently provided for the desensitization onset of P2X3 purinoreceptors [8].

While physical mechanisms explaining both temperature-independent tunnel electron transfer and barrierless ligand binding are more or less clear for two noted types of reactions, it is not the case for the third one. But, we have to note here a recent paper of Pouthier [2] devoted to the study of exciton diffusion in a lattice of H-bonded peptide units. As it follows from the paper, a transition rate can become almost temperature independent in the strong anharmonic limit. Thus, the anharmonic nature of the phonons can be thought as one of possible mechanisms forming the specific temperature behavior of transition rates.

In the present communication, we propose a novel physical mechanism explaining the existence of quasi-isoenergetic temperature-independent reactions in biosystems at room temperatures. It is assumed that for such type of reactions, the transitions between molecular states are accompanied by low-frequency molecular vibrations, while molecular energies are alternated by thermodynamic fluctuations.

Model and master equation.– Model is based on the experimentally supported fact that protein-structural motions create electric field fluctuations accompanying the movements of polar residues [9]. Such structure fluctuations cause the molecular energies to be stochastically time dependent and, thus, the transitions between molecular states occur on the background of random time-dependent energy shifts. Associating the molecule with an open quantum system S, the respective molecular Hamiltonian can be represented as $H(t) = H_0(t) + V$ where $H_0(t) = \sum_{n} E_n(t)|n\rangle\langle n|$ is the main part of Hamiltonian with $E_n(t) = E_n + \Delta E_n(t)$ being the state energy varied owing to the stochastic addition $\Delta E_n(t)$. Transitions between molecular states $|n\rangle$ and $|m\rangle$ are achieved by nonadiabatic operator $V$. To analyze the experimental results one have to know an evolution of observable occupancy $P_n(t)$ for each molecular state involved into a given transition process. In our case, this occupancy appears as an average over realizations of random energy shifts. We denote such averaging via the symbol $\langle\langle...\rangle\rangle$ so that $P_n(t) = \langle\langle P_n(t)\rangle\rangle$. In turn, non-averaged occupancy

$$P_n(t) = \langle
\sum\sum E_n(t)|n\rangle\langle n|\langle
W(t)|m\rangle\langle m|e^{-\beta E_m(t)}|n\rangle\langle n|e^{-\beta E_n(t)}|m\rangle\langle m|W(t)^\dagger
\rangle,$$

where $W(t)$ is the density matrix of a closed system $S$, Hamiltonian $H(t)$ is the total Hamiltonian of the system coupled to the environment $H_S + H_E$.
$P_n(t)$ can be found from exact relation $P_n(t) = \langle n|\rho(t)|n\rangle$ where molecular density matrix $\rho(t) = tr_B P_{S+B}(t)$ is determined as a trace (over the thermal states) on density matrix $\rho_{S+B}(t)$ of an entire quantum system including a thermal bath with respective bath Hamiltonian $H_B$. Evolution of the $\rho_{S+B}(t)$ is governed by Liouville equation $\dot{\rho}_{S+B}(t) = (1/\hbar)[H_0(t) + V + H_B, \rho_{S+B}(t)]$. Using this equation one can derive an exact master equation just for the $\rho(t)$. For our purposes, however, it is quite enough to have a master equation for only a diagonal part of molecular density matrix, $\rho_d(t) = \sum_n \langle n|\rho(t)|n\rangle|n\rangle\langle n| \equiv D\rho(t)$. In what follows, we associate nuclear vibrations with a thermal bath. It allows one to use a well known factorization $\rho_{S+B}(t) = \rho(t)\rho_B$ where $\rho_B = \exp(-H_B/k_BT)\exp(-H_B/k_BT) = \text{the bath equilibrium density matrix}$. Using now a method of projection operators [12] and generalizing this method for the case of time-dependent Hamiltonian, we achieve the following master equation

$$\dot{\rho}_d(t) = -\int_0^t d\tau \times tr_B \{D[V,U(t,t-\tau)][V,\rho_d(t-\tau)\rho_B]U^+(t,t-\tau)]\} \quad (1)$$

where the evolution operator reads $U(t,t-\tau) = \hat{T} \exp[-i\hat{H}_0\tau] + H_B + (1 - D)\hat{V}$ (T is the Dayson’s chronological operator). Eq. (1) is the basic coarse-grained stochastic equation which can be applied for a rigorous description of transitions between molecular states on the time scale $\Delta t \gg \tau_{\text{vib}}$ where $\tau_{\text{vib}}$ is the characteristic time of establishment of a thermal equilibrium.

Averaged transition rate constant.-- In what follows we concentrate our attention on the transition processes caused by a weak coupling of molecular states to the vibrations. As it follows from a theory [11,13], if energy of a single phonon is able to cover a difference between energies of molecular states involved in the transfer process, the main contribution in transfer rate follows from single phonon-transitions while a role of multi-phonon processes becomes minor. Just such situation is assumed to be valid for low-energetic transitions in flexible molecules.

In this integro-differential equation, $\Omega_{nm} = (E_n - E_m)/\hbar$ is the transition frequency, $F_{nm}(t,t-\tau) = \exp\{i\hbar\int_{t-\tau}^t d\tau\prime (\Delta E_n(\tau') - \Delta E_m(\tau'))\}$ is the stochastic functional, and $R_\lambda(\tau) = N(\omega_\lambda)\exp(i\omega_\lambda\tau) + [1 + N(\omega_\lambda)]\exp(-i\omega_\lambda\tau)$ is the regular single-phonon factor with $N(\omega_\lambda) = \text{normalization factor}$. Substituting the

$$\Omega_{nm}^2 = \sum_\lambda |\kappa_\lambda^m|^2 \int_0^t d\tau R_\lambda(\tau) \left[ e^{i\Omega_{nm}\tau} F_{nm}(t,t-\tau) P_n(t-\tau) - e^{-i\Omega_{nm}\tau} F_{nm}(t,t-\tau) P_m(t-\tau) \right]$$

(2)

with the averaged transition rate constants

$$K_{nm} = \frac{2\text{Re} \sum_\lambda |\kappa_\lambda^m|^2 \int_0^\infty d\tau e^{i\Omega_{nm}\tau} R_\lambda(\tau) F_{nm}(\tau)}{\hbar^2}$$

(3)

Now we consider an important random process which allows for an exact calculation of the $F_{nm}(\tau)$. Let stochastically caused alternation of molecular energy differences be characterized by the only random quantity $\alpha_m(t)$ so that $\Delta E_n(\tau) - \Delta E_m(\tau) = 2\hbar\alpha_m(t)$. For the sake of definiteness, let random quantity fluctuate between two equiprobable values $+\sigma$ and $-\sigma$ with mean frequency $\nu$ (symmetric dichotomous process, DP) so that $\alpha_m(t) = \alpha(t) = \pm\sigma$. Such random variation of energy shifts yields $F_{nm}(\tau) = F(\tau) = \langle\langle X(\tau)\rangle\rangle$ where $X(\tau) = \exp[+i\int_0^\tau d\tau'\alpha(\tau')]$ is the stochastic functional. Bearing in mind exact properties of the DP so as $\alpha^2(\tau) = \sigma^2$, $\langle\langle \alpha(\tau)\rangle\rangle = 0$, and $\langle\langle \alpha(\tau)\alpha(t-\tau)\rangle\rangle = \sigma^2\delta^2$, one obtains $F(\tau) = \langle k_1 e^{-k_2\tau} - k_2 e^{-k_1\tau}\rangle/(k_1 - k_2)$. Here, quantities $k_1, k_2 = \nu/2 \pm (\nu/2)^2 - \sigma^2$ determine the above noted characteristic stochastic times $\tau_{\text{stoch}}^{(1,2)} = (Re k_{1,2})^{-1}$. Substituting the $F_{nm}(\tau) = F(\tau)$ in Eq. (4) and calculating the respective integral one achieves the following expression...
for an averaged transition rate constant

\[ K_{nm} = \frac{2\pi}{\hbar} \sum_\lambda |\kappa_{nm}^\lambda|^2 \left[ N(\omega_\lambda) L(\gamma, \nu; \Omega_{nm} + \omega_\lambda) + (N(\omega_\lambda) + 1) L(\gamma, \nu; \Omega_{nm} - \omega_\lambda) \right]. \] (5)

Here, the influence of stochastic field on the \( n \to m \) transition is concentrated in Lorentzian like function \( L(\gamma, \nu; \Omega) = \pi^{-1} \gamma / (\gamma^2 + \Omega^2) \) via parameters \( \nu \) and \( \gamma \equiv \sigma^2 / \nu \).

**Temperature independence of rate constant.** Rate constant [3] includes a stochastic field in a non-perturbation manner. This circumstance allows us to analyze various regimes of transition processes in molecular systems depending on value of field parameters \( \nu \) and \( \gamma \). Here, bearing in mind an application of theory to explanation of conformational quasi-isooenergetic transitions \((\Omega_{nm} \approx 0)\) in flexible biomolecules at room temperatures, we restrict ourself to the analysis of transitions which are accompanied by low frequency vibration modes so that \( |\Omega_{nm} \pm \omega_\lambda| \ll \nu \). This reduces function \( L(\gamma, \nu; \Omega) \) to a standard Lorentzian \( L(\gamma, \Omega) \approx \pi^{-1} \gamma / (\gamma^2 + \Omega^2) \) where parameter \( \nu \gamma \) can be treated as the broadening of molecular energy levels. At \( \gamma \to 0 \), i.e. in the case of extremely high frequency stochastic field \( (\nu \to \infty) \), Lorentzian is converted into the Dirac’s delta-function \( \delta(\Omega) \) and, thus, rate constant \( \mathbb{E} \) appears in a conventional form valid for Born approximation over a nonadiabatic perturbation. In the case of moderately high frequency stochastic field under consideration, an another limit is realized where \( \gamma \gg \Omega \). It yields \( L(\gamma, \Omega) = (\pi \nu)^{-1} \). Note now that room temperatures correspond to energies of the order 0.025 eV, identical to \( 6 \cdot 10^{-12} \) s\(^{-1}\) or 200 cm\(^{-1}\). Therefore, if transitions are accompanied by the vibrations of the order 60 cm\(^{-1}\) and lower, one can set \( N(\omega_\lambda) \approx k_B T / \hbar \omega_\lambda \). This reduces averaged rate constant [6] to the form

\[ K_{nm} = \frac{4k_B T}{\hbar^2} \gamma \sum_\lambda |\kappa_{nm}^\lambda|^2 / \hbar \omega_\lambda. \] (6)

Physical origin of stochastic parameter \( \gamma \) is dictated by random shifts of molecular energy levels. We propose a phenomenological model where mean positive and mean negative shifts \((+\sigma/2 \text{ and } -\sigma/2, \text{ respectively})\) result from thermodynamic fluctuations of an energy so that \( \sigma^2 / 2 \approx \delta E^2 / (2\pi \hbar)^2 \). In line with a general theory of thermodynamic fluctuations in canonical ensembles, the average of square of energy fluctuations is calculated as \( \delta E^2 = k_B T^2 \langle \delta E / \delta T \rangle \) with \( E \) being the mean energy of a system. At the same time, the average frequency of fluctuations is associated with a linear frequency \( \nu = E / 2\pi \hbar \). Thus, a stochastic parameter can be estimated with relation \( \gamma = (2k_B T^2 / \pi \hbar) \langle \delta \ln E / \delta T \rangle \). In classical limit under consideration, a mean energy per a separate degree of freedom, is exactly equal to thermal energy \( k_B T \). Therefore, independently of precise molecular system, a total mean energy \( E \) is proportional to a bath temperature. It yields \( \gamma = 2k_B T / \pi \hbar \). Consequently,

\[ K_{nm} = \frac{2\pi}{\hbar} R_{nm}. \] (7)

In Eq. (7), \( R_{nm} = (2\pi)^{-1} \int_{-\infty}^{+\infty} (d\omega / \omega) J_{nm}(\omega) \) is the temperature-independent factor which is expressed via spectral function \( J_{nm}(\omega) = 2\pi \sum_\lambda |\kappa_{nm}^\lambda|^2 \delta(\omega_\lambda - \omega) \). The latter is widely used in a spin-boson model for description of transport processes accompanied by vibration motions [22]. In a given case, function \( J_{nm}(\omega) \) includes an information on both a vibrational structure of flexible molecule and a character of nonadiabatic coupling to molecular vibrations.

**Experimental evidence.** Eq. (6) contains a fundamentally important result indicating that the rates of quasi-isooenergetic transition processes in molecules can be temperature-independent even at room temperatures. As an example, we consider the desensitization onset of P2X\(_3\) receptors in neuronal membranes [8]. For our knowledge, this is the first quantitative observation of temperature-independent gating in biological membranes. Fig. 1 manifests the decrease of ion current \( I(t) \) through the receptor channels. Desensitization probability of the channels, \( P_d(t) = 1 - I(t) / I(0) \) is well described by a two-exponential kinetics [8],

\[ P_d(t) = 1 - A_1 e^{-t / \tau_1} - A_2 e^{-t / \tau_2} \] (8)

where \( \tau_1 \approx 14.7 \) ms and \( \tau_2 \approx 231 \) ms are the temperature-independent characteristic times of desensitization while \( A_1 \approx 0.968 \) and \( A_2 \approx 0.032 \) are the pre-exponential weights. Since a particular molecular content of the P2X\(_3\) receptor gates is yet unknown, we interpret the noted result in the framework of simplest model supposing the

\[ [I(0) - I(t)] / I(0) = A_3 [1 - \exp(-t / \tau_3)] \] (9)

\( \tau_3 \approx 1 \) ms, and

\[ [I(0) - I(t)] / I(0) = A_4 [1 - \exp(-t / \tau_4)] \] (10)

\( \tau_4 \approx 10 \) ms. For the first case, the mean value of \( \Delta I = 0.25 \) nA, the pulling force \( F = 20 \) nN, and the capture time \( t_{cap} \) is 200 ms. For the second case, the mean value of \( \Delta I = 0.25 \) nA, the pulling force \( F = 20 \) nN, and the capture time \( t_{cap} \) is 200 ms.
formation of ion current as the sum of two partial currents. Let \( i_j \) be the current through a separate open channel of the type \( j = 1, 2 \). Let \( N_j \) be the number of respective ion channels, then a partial current reads as \( I_j(t) = N_j i_j P_o^{(j)}(t) \) where \( P_o^{(j)}(t) \) is the probability for the channel to be in the open state "o". Desensitization of each channel occurs independently of one another manifesting the process of conformational transition from the open (conductive) state to the closed (nonconductive) state so that \( P_o^{(j)}(t) = \exp(-t/\tau_j) \). Introducing the quantities \( I(0) \equiv \sum_{j=1}^{2} N_j i_j \) and \( A_j = \xi/(1 + \xi) \) and \( A_2 = 1/(1 + \xi) \) where \( \xi = N_1 i_1/N_2 i_2 \), one expresses an ion current in the form \( I(t) = I(0) P_o(t) \) where \( P_o(t) = \sum_{j=1,2} A_j P_o^{(j)}(t) \) is the apparent (statistically averaged) probability of an ion channel to be in the open state. Bearing in mind that \( P_d(t) = 1 - P_o(t) \), one arrives at the Eq. \( 3 \).

Physical explanation of temperature independency of desensitization process can be given on the base of above proposed model of quasi-isoe energetic transitions in flexible molecules. Denoting via \( n = joo \) and \( m = jdo \) the conformational isoe energetic states participating in the open-close transition, we introduce the integral occupancies of states \( o \) and \( d \) as \( P_o^{(j)}(t) = \sum_{n} P_{joo}^{(j)}(t) \) and \( P_d^{(j)}(t) = \sum_{m} P_{jdo}^{(j)}(t) = 1 - P_o^{(j)}(t) \) where \( \mu_{joo} \) and \( \mu_{jdo} \) are the number of respective degenerated substates for the \( j \)-th type of channel. Eq. \( 3 \) reads now as \( \dot{P}_o^{(j)}(t) = -[K_{o\to d}^{(j)} P_o^{(j)}(t) - K_{d\to o}^{(j)} P_d^{(j)}(t)] \) where \( K_{o\to d}^{(j)} = \xi_j/\mu_{joo} \) and \( K_{d\to o}^{(j)} = \xi_j/\mu_{jdo} \) are the integral rate constants with \( \xi_j \equiv (2\pi/\hbar) \sum_{n} \sum_{m} R_{joo,jdo}^{(j)} \).

Since the disordering increases the degeneracy of molecular state, then \( \mu_{jdo} \gg \mu_{joo} \). This reduces the kinetics of isoe energetic transitions to the noncurrent single-exponential kinetics with the characteristic time \( \tau_j \simeq (K_{o\to d}^{(j)})^{-1} \) for the \( j \)-th type of channel. Thus, physically, the receptor desensitization appears as a temperature-independent open→close transition process between quasi-isoe energetic molecular conformations of ion channel.

**Conclusion.** In this communication, a novel physical mechanism is proposed to explain the formation of temperature-independent transition processes in molecular systems. In contrast with well established mechanism describing quantum phononless site-to-site particle tunneling at low temperatures or tunneling at room temperatures that is accompanied by an emission of high frequency phonons (when \( \omega_\lambda \gg k_B T/\hbar \)), the proposed mechanism works in a classic region of temperatures with participation of low frequency phonons (when \( \omega_\lambda \ll k_B T/\hbar \)). The lack of temperature dependence in rate constant \( 3 \) occurs owing to the thermodynamical stochastic variation of energy levels participating in the transitions. Frequency of this variation, \( \nu \) is assumed to be fast in comparison to the transition frequencies \( \Omega_{nm} \sim \omega_\lambda \). This reduces an averaged rate constant \( 3 \) to a more simple form \( 6 \) which, in turn, is simplified to the temperature-independent form \( 7 \). Of course, a given mechanism is not unique and can work independently of or in parallel with other possible mechanisms, especially with anharmonic mechanism noted in ref. \( 8 \). But, for understanding the physics of quasi-isoe energetic transitions in biological macromolecules, the reference of fast molecular motions to the motions responsible for the creation of high frequency stochastic fields, is assumed to be quite fruitfull from a semi-phenomenological physical point of view.

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