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BOJARSKI HOPPING MODEL OF MULTISTEP NONRADIATIVE ENERGY TRANSPORT. APPLICATION TO SELECTED BIOLOGICAL SYSTEMS

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The applications of Bojarski hopping mulistep nonradiative energy transport model to the investigations of energy transfer between biological molecules (for example flavin mononucleotide, FMN) are described. These investigations concern the following problems: (1) the processes of multistep energy migration between biological molecules followed by energy transfer to acceptors, (2) the directional energy transfer in rigid solutions, (3) the manifestations of energy transfer in the system of fluorescent dimers and monomers in rigid matrices containing FMN molecules, (4) the manifestation of reverse energy transport.

INTRODUCTION

Förster resonance energy transfer plays an important role in numerous photochemical processes: photosynthesis, information transfer in DNA, vision process and other photoreception phenomena. During last three decades a lot of papers devoted to resonance energy transfer between biological molecules have been published (Eftink, 1991; Eftink & Ghiron, 1981; Frąckowiak, Cegielski & Abdurakhmanov, 1991 Frąckowiak *et al*., 1988, 1994; Gobets *et al*., 2003; Laible *et al*., 2003; Malicka *et al*., 2003; Young *et al*., 2003). Resonance energy transfer has been discovered by Perrin over 80 years ago (Perrin, 1925a; 1925b) and it is still a subject of extensive theoretical and experimental works. In 1949 Förster elaborated a quantum-mechanical theory of single step nonradiative energy transfer from an excited molecule – donor D* to an unexcited acceptor A based on the long range dipole-dipole interaction mechanism (Förster, 1948; 1949). In this case resonance energy transfer can be schematically presented as:

$$D^* + A \xrightarrow{k_{DA}} D + A^* \qquad (1)$$

Förster has shown that the probability of energy transfer per time unit can be connected with the transition probability in emission and absorption of interacting molecules. He obtained the expression for the rate constant for nonradiative energy transfer between two transition dipole moments D* and A (Förster, 1948):

$$k^{dd} = \frac{1}{\tau_{0D}} \left(\frac{R_{0DA}}{R} \right)^6 \qquad (2)$$

where τ_{0D} is the real averaged lifetime of D* in its excited state when $C_A \to 0$, R is the distance between D and A, and R_{0DA} is the critical distance for energy transfer (so called Förster radius). R_{0DA} can be determined from the spectroscopic data according to the formula:

$$R_{0DA} = \left(\frac{9 < \kappa^2 > (\ln 10) \eta_{0D} I_{DA}}{128 \pi^5 n^4 N'} \right)^{\frac{1}{6}} \qquad (3)$$

where η_{0D} is the donor fluorescence quantum yield for $C_A \to 0$ is the refractive index of the medium, N' – is the number of molecules in mM, and I_{DA} – the overlap integral:

$$I_{DA} = \int_0^\infty F_D(\bar{\nu}) \varepsilon_A(\nu) \bar{\nu}^{-4} d\bar{\nu} \qquad (4)$$

where $\varepsilon_A(\bar{\nu})$ is the molar decimal acceptor extinction coefficient, $F_D(\bar{\nu})$ – is the spectral donor fluorescence distribution expressed in the number of quanta and normalized to unity $\int_0^\infty F_D(\bar{\nu}) d\bar{\nu} = 1$, and $\bar{\nu}$ – the wave number (overlap of these spectra is the necessary condition for nonradiative energy transfer). κ^2 in formula (3) is the orientation factor, dependent on mutual orientation of transition dipole moments of interacting D* and A molecules:

$$\kappa^2 = (\cos\theta_{DA} - 3\cos\theta_D \cos\theta_A)^2 \qquad (5)$$

where θ_{DA} is the angle between two transition moments, θ_D and θ_A are angles between the respective transition moment and the vector connecting D and A molecules.

At the same time other resonance energy transfer theories have also been developed by Dexter, Jabłoński. Galanin, Ore, Knox, Maksimow and Rozman and others. These theories have been limited to the case of low donor concentration relative to the acceptor concentration ($C_D << C_A$).

BOJARSKI HOPPING MODEL OF MULTISTEP NONRADIATIVE ENERGY TRANSPORT

In 1971 Bojarski & Domsta (1971) extended the Förster model to arbitrary dye concentration and for the first time took into account the multistep energy migration effect within the donors set on excitation trapping by acceptors. This process can be presented according to the following scheme:

$$\begin{aligned} &D^* + D + \ldots + D + A \xrightarrow{k_{DD}} \\ &D + D^* + \ldots + D + A \xrightarrow{k_{DD}} \\ &D + D + \ldots + D^* + A \xrightarrow{k_{DA}} \\ &D + D + \ldots + D + A^* \end{aligned} \qquad (6)$$

Excitation energy migrates (EM – energy migration) visiting different donor molecules before it is transferred to any acceptor. This is the base for so called hopping forward energy transport model (FET-HM) (Bojarski & Domsta, 1971). This model has then been extended to reverse nonradiative energy transfer (RET-HM) (Bojarski, 1984) from the acceptors set to the donors set. This process takes place if acceptor fluorescence band overlaps partly with the donor absorption band. Then, energy may return to donors according to the scheme:

$$A^* + D \xrightarrow{k_{AD}} A + D^* \qquad (7)$$

The nonradiative energy transfer studies require usually the measurements of concentration depolarization and concentration quenching of fluorescence. Bojarski (1984) within the RET-HM obtained the following expression for relative donor quantum yield and emission anisotropy:

$$\frac{\eta_D}{\eta_{0D}} = \frac{1 - f(\gamma)}{1 - \alpha\, f(\gamma)} \times \frac{1}{1 - B} \qquad (8)$$

$$\frac{r_D}{r_{0D}} = (1 - \alpha\, f(\gamma))(1 - B) \qquad (9)$$

where $B = \dfrac{(1-\alpha) f(\gamma)}{1 - \alpha\, f(\gamma)} \times \dfrac{(1-\alpha') f(\gamma')}{1 - \alpha'\, f(\gamma')}$ (10)

$$\gamma = \frac{\gamma_{DD}}{\sqrt{2}} + \gamma_{DA} = \frac{\sqrt{\pi}}{2}\left(\frac{C_D}{\sqrt{2}C_{0DD}} + \frac{C_{DA}}{C_{0DA}}\right), \qquad (11)$$

$$\alpha = \frac{\gamma_{DD}}{\gamma}$$

$$\gamma' = \frac{\gamma_{AA}}{\sqrt{2}} + \gamma_{DA} = \frac{\sqrt{\pi}}{2}\left(\frac{C_A}{\sqrt{2}C_{0AA}} + \frac{C_D}{C_{0AD}}\right), \qquad (12)$$

$$\alpha' = \frac{\gamma_{AA}}{\gamma'}$$

where γ is total reduced concentration in the case of energy migration between donors and nonradiative forward energy transfer from donor to acceptor (FET), γ' – is total reduced concentration for energy migration between acceptors and nonradiative reverse energy transfer from acceptor to donor (RET). Critical concentrations C_{0DD}, C_{0DA}, C_{0AD} and C_{0AA} are given by the formula:

$$C_{0XY} = 4.23 \times 10^{-10} n^2 \left(<\kappa^2> \eta_{0X} I_{XY}\right)^{-\frac{1}{2}}, \qquad (13)$$
$$X, Y \in \{D, A\}$$

where I_{XY} is expressed by formula (4). The function $f(\mu)$, where $\mu \in \{\gamma, \gamma'\}$, is connected with the error function $erf(\mu)$ by:

$$f(\mu) = \sqrt{\pi}\, \exp(\mu^2)[1 - erf(\mu)] \qquad (14)$$

FET-HM and RET-HM are particularly valuable for studies of energy transfer between biological molecules. In these models energy transfer from donors to acceptors preceded by energy migration between donors can be studied, which takes place in photoreception systems or photosynthesis phenomena. Fluorescence concentration quenching (FCQ) model elaborated by Bojarski can also be used to study energy transport if the role of acceptors is played by dimers (Bojarski & Kawski, 1986; Bojarski & Sienicki, 1990).

APPLICATION OF BOJARSKI MODEL TO BIOLOGICAL MOLECULES

In rigid systems (PVA films or biological membranes) the directional energy transfer can take place (Bojarski & Sienicki, 1990; Grajek, 2007; Grajek *et al.*, 1998; Żurkowska, Grajek & Bojarski, 1996) due to inhomogeneous orientation broadening of energy levels (Rubinov, Tomin & Bushuk, 1982; Yuzhakov, Grachev & Ponomarev, 1990). This effect leads to the formation of energetic continous distribution of fluorescent centers with different energies of 0–0 transitions. Additionally, if dimers appear in the system, then the energy from excited monomer centers flows to energetically lower dimeric centers (Grajek, 2007). In photosynthetic systems such a directional energy transfer is important, since in reaction centers, in which the photochemical reaction takes place, dimers appear and monomers being the antenas transfer the absorbed energy to such centers. Moreover, in rigid systems dimers exhibit weak fluorescence and are able to return energy to monomers (Bojarski *et al*., 2003; Grajek, 2007).

In this work based on Bojarski model all processes of energy transport between fluorescent flavin mononucleotide (FMN) monomers (donors) and dimers (acceptors) in PVA film can be analyzed.

FMN is a biologically important molecule acting as coenzyme and photoreceptor in living organizms. FMN acts as a photoreceptor in many plants (Kottke *et al*., 2003; Okajima *et al*., 2006). Recent studies have shown the existence of flavins dimers in biological systems (Bieger, Essen & Oesterhelt, 2003; Müralidhara & Wittung-Stafshede, 2003) as well as in photoreception systems (Nakasako *et al*., 2005; Salomon, Lempert & Rüdiger, 2004). Salomon, Lempert & Rüdiger (2004) have shown that the fragments of phototropin – photoreceptor containing LOV domains with FMN act as dimers. Detailed knowledge of the system studied is important for understanding the processes occurring in biological systems containing FMN.

The dimerization of FMN in water (Grajek *et al*., 1984, 2001), glycerol-water solutions (Grajek *et al*., 1984) and solid PVA matrices (Grajek, 2003; 2007; Grajek *et al*., 1998) has been studied in detail. Investigations performed in (Grajek, 2007; Grajek *et al*., 1986) have shown the existence of dimer electronic level splitting to H and J levels (Kasha, Rawls & El-Bayoumi, 1965). It has been shown that both levels can be active in nonradiative energy transfer in rigid systems (Grajek, 2007).

FMN is a very interesting and exceptional model system for study of energy transfer because it practically does not form aggregates of higher order than dimers (Grajek, 2003; 2007; Grajek *et al*., 1986; 2001, 2007) and FMN dimers in rigid solution have also a unique feature – they are fluorescent (Grajek, 2007; Grajek *et al*., 1998, 2007) and form imperfect traps for excitation energy (Bojarski *et al*., 2003; Grajek, 2007).

Fluorescence (Grajek, 2007; Grajek *et al*., 1998; Żurkowska, Grajek & Bojarski, 1996) and fluorescence anisotropy (Grajek, 2007) measurements of FMN in PVA versus concentration at different excitation and observation wavelengths allowed for directional energy migration from "blue" to "red" centers to be observed. These observations were possible both for monomers and dimers (Grajek, 2007). Also directional energy trasfer from monomers to dimers, reverse energy transfer from dimers to monomers and remigration to primarily excited monomers could be analyzed (Grajek, 2007). The results obtained (Grajek, 2007) have shown that the excitation wavelength is the most important factor determining the nonradiative energy transport processes and allowing for their control.

Measurements of fluorescence or fluorescence anisotropy spectra upon different excitation wavelength enable to observe energy migration tracking between donors (monomers) which took place more or less effectively depending on the excitation wavelength. Excitation wavelength controlled the number of excitation transfer acts and the whole process of multistep energy transfer within the set of FMN monomers and dimers (Grajek, 2007), and allowed for the observation of energy transfer with the contribution of dimeric H and J levels (Grajek, 2007). For example, at $\lambda_{ex} = 445$ nm (maximum of monomer emission and maximum of H dimer absorption) numerous processes of possible energy transport acts are possible in the set of FMN monomers and dimers in PVA ($M^* \to M$, $M^* \to D$, $D^* \to M$, $D^* \to D$) in which H and J dimer levels can participate. Hence, low emission anisotropy values were obtained for such conditions (Jabłoński, 1970; 1972). However, upon the excitation $\lambda_{ex} = 510$ nm (dimers are excited to the J level) only energy migration was observed between dimers.

It should be mentioned that depending on the selected observation wavelength more or less depolarized FMN fluorescence was also observed due to stronger or weaker sink of multistep energy migration. Investigations at different observation wavelengths enable tracking of particular stages of multistep nonradiative energy transport by observation of light originating from given centers starting from the "blue" ones and ending at the "red" ones (Grajek, 2007). For example, emission anisotropy measurements carried out for extremely concentrated samples, C = 0.684 M and C = 1 M, at $\lambda_{obs} = 510$ nm allowed to observe fluorescence originating from short wavelength dimeric centers and primarily excited molecules which resulted in high emission anisotropy values (Grajek, 2007), whereas at $\lambda_{obs} = 600$ nm depolarized fluorescence was observed due to energy transfer to long wavelength centers.

Also the quantitative studies of resonance energy transfer with the use of FET-HM and RET-HM (Eqs. 8 and 9) as well as Monte-Carlo simulations were performed for the concentration quenching and depolarization of FMN fluorescence (Grajek, 2007). These studies were carried out using different models too (Bojarski *et al*., 2003). The obtained agreement between the theoretical and experimental data confirmed the correctness of the analysis of energy transport processes in the system of interest. Theoretical data (Grajek, 2007) confirmed: (1) the existence of fluorescent FMN dimers and their contribution to the resonance energy transfer (2) the appearance of multistep nonradiative energy transport, (3) existence of reverse energy transfer from D* to M, (4) high role of energy migration in the monomer and dimer sets. The calculated values of overlap integrals for nonradiative energy transport I_{XY} from formula (4), where $X,Y \in \{D,A\}$, have shown that the probability of energy migration between dimers is higher than that from dimers to monomers of FMN in PVA. In rigid systems, where fluorescent dimers are formed the return of the excitation energy from D* to M is possible and the energy transport is well described by the RET-HM. In the case of high temperatures where the FMN dimers in PVA become perfect traps the energy transport is well described by FET-HM. FET-HM has been successfully applied to describe multistep nonradiative energy transport from excited monomers M* to dimers – energy traps in the case of glycerol – aqueous FMN solutions (Grajek *et al*., 1992). However, in the case of aqueous solutions the FET-HM takes into account the effect of diffusion on resonance energy transfer (Grajek, Żurkowska & Kuśba, 2005).

The models mentioned are widely used for studies of different donor-acceptor systems, both fluid and viscous or rigid (Bojarski, 1997a; Bojarski & Kułak, 1997; 1998a) or in the case of rhodamine dimers (Bojarski, 1997b; Bojarski & Kułak, 1998b; Bojarski *et al*., 1996) as well as in partly ordered systems (Bojarski *et al*., 2003, 2004; Bojarski, Kułak & Kamińska, 2002; Sadownik *et al*., 2007).

Taking account of the diversity of energy transport processes (Grajek, 2007) the question arises: Do living organisms select certain energy quanta out of the solar spectrum to excite photoreceptors which in turn activate only specific processes of energy transfer?

Abbreviations: EM – energy migration, FET-HM – forward energy transfer hopping model, RET-HM – reverse energy transfer hopping model, FET – forward energy transfer, RET – reverse energy transfer, FCQ – fluorescence concentration quenching

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