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FLUORESCENCE QUENCHING IN PROTEIN-LIPID SYSTEMS: CONTRIBUTION OF DIFFUSION-CONTROLLED FACTORS

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Using the model of diffusion-controlled reactions between asymmetric species the quenching of the protein fluorescence by extrinsic quenchers has been considered. The changes in bimolecular quenching rate constant stemming from the protein immobilization on the surface of lipid vesicles have been evaluated. It has been shown that association of the proteins whose radius is less than 2 nm with lipid vesicles can be followed by the apparent decrease of the quenching efficiency due to reduced translational and rotational diffusion coefficients of the bound protein.

KEY WORDS: quenching of protein fluorescence, diffusion of lipid-bound protein, quenching rate constant

Protein-lipid interactions are known to play an essential role in determining the structural and functional properties of biological membranes [1]. Numerous studies indicate that such interactions involve conformational changes of the protein molecule [2]. To gain insight into the nature of these changes a variety of physical techniques have been employed, including, particularly, collisional quenching of the protein fluorescence [3]. Comparison of the quenching efficiencies observed for the free and lipid-bound protein states allows one to monitor the conformational changes that manifest themselves in the increased or decreased accessibility of the protein fluorophores to a quencher. However, in examining the fluorescence quenching one should bear in mind that the quenching efficiency depends not only on the fluorophore microenvironment but also on the rates of translational and rotational diffusion of the protein molecule [3-5]. More specifically, immobilization of soluble protein on the surface of lipid vesicle may be conducive to the decreased extent of quenching without any significant changes in the protein conformation. Therefore, correct interpretation of the quenching data requires knowing the magnitude of diffusion-related effects. In view of this the goal of the present study was to evaluate how the differences in the rates of translational and rotational diffusion between the free and lipid-bound protein could affect the quenching efficiency.

THEORY

The fluorescence quenching is commonly described in terms of Stern–Volmer model [3]:

$$I_o / I = 1 + k_{FQ}\tau[Q] = 1 + K_Q[Q], \quad (1)$$

where I_o and I are the fluorescence intensities in the absence and presence of a quencher, respectively, k_{FQ} is the bimolecular quenching rate constant, τ is the fluorophore lifetime in the absence of a quencher, K_Q is the Stern–Volmer quenching constant, [Q] is the quencher concentration.

For the case where the quenching efficiency γ is 1, i.e. quenching occurs on every collision, the bimolecular quenching rate constant k_{FQ} is given by Smoluchowski equation [4]:

$$k_{FQ} = \gamma k_o = 4\pi(R_F + R_Q)(D_F + D_Q), \quad (2)$$

where k_o is the diffusion-controlled bimolecular rate constant, R_F and R_Q are the molecular radii, D_F and D_Q are the translational diffusion coefficients of the fluorophore (F) and quencher (Q), respectively. This equation describes the diffusive flux of a molecule with a diffusion coefficient $D_F + D_Q$ through the surface of a sphere of radius $R_F + R_Q$. In this study we consider fluorophore and quencher as spherical molecules. In the case in which a macromolecule contains the fluorophore in its composition, i.e. the fluorophore covers only a portion of its surface, the interaction of quencher molecules with the macromolecule can be considered as diffusion-limited reaction between asymmetric molecules. The macromolecule reactive area is characterized by the cone angle $2\theta_0$. It is easy to understand that in such asymmetric reaction the rate constant (and hence the bimolecular quenching rate constant k_{MQ}) is determined by both translational and rotational diffusion coefficients of the macromolecule and θ_0. Various approximate solutions for this type of reaction were offered by different authors. Here we use the solution of Shoup et al. for the analogous reaction [5]:

$$k_{MQ} = \frac{8\pi D_{MQ} R_{MQ}^2 \kappa (1-\cos\theta_0)^2}{4D_{MQ}(1-\cos\theta_0) - \kappa R_{MQ} \sum_{n=0}^{\infty} \frac{[P_{n-1}(\cos\theta_0) - P_{n+1}(\cos\theta_0)]^2 K_{n+1/2}(\xi_n^*)}{(n+1/2)[nK_{n+1/2}(\xi_n^*) - \xi_n^* K_{n+3/2}(\xi_n^*)]}}, \quad (3)$$

where $\xi_n^* = R_{MQ}[n(n+1)D_R / D_{MQ}]^{1/2}$, D_{MQ} is a sum of D_Q and the macromolecule diffusion coefficient, D_M, R_{MQ} is a sum of the R_Q and the macromolecule radius R_M, D_R is the macromolecule rotational diffusion coefficient, κ is a parameter related to the probability of reaction upon encounter which approaches a value of infinity for a probability of 1, K is a modified spherical Bessel function of the third kind, $P_n(\cos\theta_0)$ is a nth order Legendre polynomial. The value of D_R can be obtained by the equation [6]:

$$D_R = \frac{kT}{8\pi R_M^3 \eta}, \quad (4)$$

where T is the temperature, k is the Boltzmann's constant and η is the solvent viscosity. The parameter θ_0 can be evaluated with the equation for the fraction of a sphere's surface area circumscribed by θ_0 [4]: $\cos\theta_0 = 1 - 2f_{MQ}R_F^2/(R_M^2 + f_{MQ}R_F^2)$, where f_{MQ} is the fraction of the fluorophore's surface area that is exposed on the surface of the macromolecule. f_{MQ} changes from 0 (for completely nonexposed fluorophore) to 1 (for completely exposed fluorophore) and is a measure of quencher accessibility.

RESULTS AND DISCUSSION

In addressing the problems concerning the protein structure and dynamics the quenching of tryptophan fluorescence by extrinsic quenchers appears to be most extensively employed [3]. Therefore, as a first step in approaching the goal of the study, it seemed of interest to compare the quenching efficiencies for the tryptophan free in solution and incorporated in the protein globule. The iodide was chosen as an ionic collisional quencher that is most widely used in examining the protein conformational transitions [3,4]. The J^- ions are believed to quench fluorescence of the solvent-exposed aromatic residues [3]. As seen in Fig. 1, bimolecular quenching rate constant of fully (f_{MQ}=1) or partially exposed (f_{MQ}<1) protein-bound Trp is markedly lower than that of the free Trp. The magnitude of this effect reaches its maximum and becomes virtually independent of the protein radius for R_M values exceeding 2 nm.

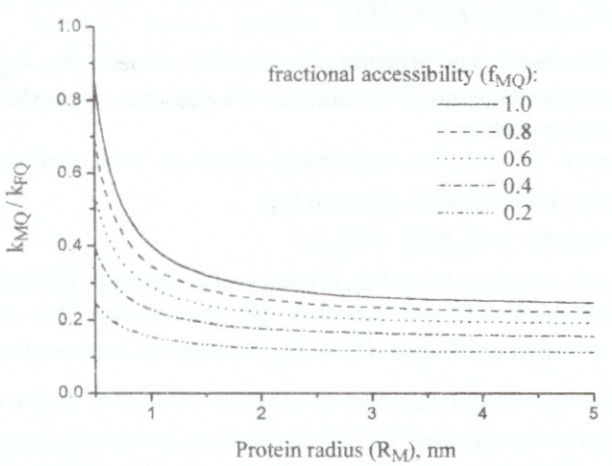

Fig. 1. The ratio of the bimolecular quenching rate constants for the iodide quenching of the protein-bound (k_{MQ}) and free Trp (k_{FQ}) as a function of protein radius and fractional fluorophore accessibility to a quencher. The value of k_{FQ} calculated from eq.(2) was found to be k_{FQ}= =1.51·10^{-11}cm^3·s^{-1}. The following parameters were used: R_F= 2.3·10^{-8} cm [3], R_Q= 2.15·10^{-8} cm [4], D_F =6.6·10^{-6} cm^2/s [3], D_Q =2.04·10^{-5} cm^2/s [7]. The value of k_{MQ} was calculated from eq.(3), where the value of D_M was obtained from equation [4]: D_M=$kT/6\pi\eta R_M$=0.23·10^{-4}/R_M (T=296 K, η = 9.4·10^{-4} kg·m^{-1}·s^{-1}), D_R was obtained from eq.(4).

Another observation noteworthy is that the extent of k_{MQ} reduction increases with decreasing fluorophore accessibility to a solvent. Next, we have compared the quenching rate constants of the protein free in solution (k_{MQ}) and bound lipid vesicles (k_{VQ}). To estimate k_{VQ}, eq.(3) was used in the form valid for the limiting case the radius of the protein-vesicle complex (R_C) is much greater than the quencher radius ($R_C >> R_Q$, $\theta_o << \pi$) [5]:

$$k_{VQ} = 4D_Q R_C \theta_o \quad (5)$$

It appeared that for the vesicle radii exceeding 20 nm and protein radii varying up to 5 nm k_{VQ} value remains practically constant at given R_M and f_{MQ}. This suggests that the growth of R_C is compensated by the decrease of the angle θ_0, which depends on the portion of vesicle surface covered by the fluorophore. Nevertheless, the ratio k_{VQ}/k_{MQ} proved to be dependent on the protein radius. As illustrated in Fig. 2, this ratio drastically increases with increasing the protein size and reaches the plateau at R_M values exceeding 2 nm. These data imply that immobilization of the protein on the surface of lipid vesicles will lead to the decrease in the quenching rate constant if the protein radius is less than 2 nm. For instance, when R_M equals 1 nm the quenching efficiency (Stern-Volmer constant) can be expected to reduce by a factor of *ca.* 1.4 (f_{MQ} =0.2) -1.7 (f_{MQ} =1) due to the slower translational and rotational diffusion of the vesicle-bound protein.

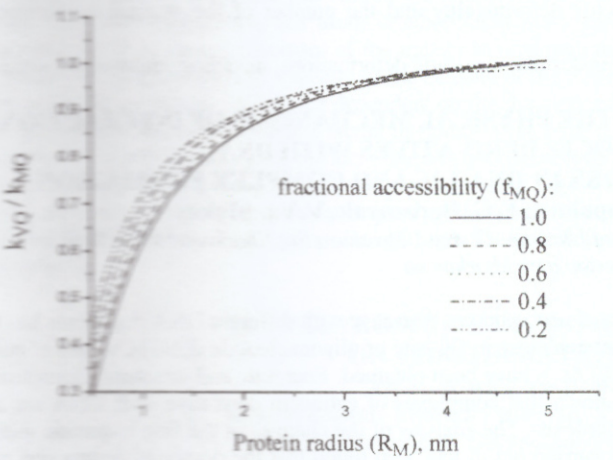

Fig.2 Changes in the quenching rate constant due to protein binding to lipid vesicles. The values of k_{MQ} and k_{VQ} were calculated using eqs. (3) and (5), respectively.

Finally, it is important to illustrate how the results presented here could be applied to analyzing the experimental data. As shown in our previous study [8] association of the cationic protein ribonuclease A with the vesicles composed of zwitterionic (lecithin) and anionic (cardiolipin) phospholipids (1:1) is followed by the decrease of Stern-Volmer constant for iodide quenching from 5.2 M^{-1} to 3.2 M^{-1} (*i.e.* by a factor of *ca.* 1.6). Taking the ribonuclease radius to be *ca.* 1.7 nm and using the plots presented in Fig. 2 one obtains that protein immobilization on the vesicle surface can result in the decrease of K_Q value by a factor of *ca.* 1.2. Since the magnitude of experimentally observed K_Q changes insignificantly exceeds the diffusion-controlled limit the quenching data must be cautiously interpreted in terms of the modified conformation of ribonuclease A or reduced iodide concentration near the surface of negatively charged lipid vesicles. In this context it is also noteworthy that the approach used in the present study allows to evaluate only the possible magnitude of immobilization effect rather than to describe rigorously very complex real situation.

CONCLUSIONS

Fluorescence quenching studies can give adequate information on the mechanisms by which protein conformation is modified on the formation of protein-lipid complexes provided that the differences in the rates of translational and rotational diffusion between the free and lipid-bound protein are taken into account. Immobilization of peptides or proteins whose radius ranges from 0.5 to 2 nm on the surface of lipid vesicles can lead to a decrease of the quenching rate constant by a factor of 1.2 – 2. This effect is slightly dependent on the fluorophore fractional accessibility to a quencher and insensitive to the vesicle size. Quantitative interpretation of the quenching data derived for the protein-lipid systems must include correction of the measured quenching constant for immobilization effect.

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