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Spatial arrangement of selected fluorescence labels in lipid bilayer

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ABSTRACT

The method for the determination the orientation factor κ^2, spatial arrangement and depth position of fluorescence labels located in hydrophilic layers of vesicles bilayer from resonance energy transfer (RET) data is presented. The method is based on the broadened Wolber and Hudson RET model in two dimensions (*Biophys J.* 1979). The vesicles were labeled with N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine (NBD-PE) as the donor and N-(Lissamine rhodamine B sulfonyl) 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine (NRh-PE) as the acceptor. It was found that in basic environment sodium dithionite quenches fluorescence of both labels located in outer leaflet of bilayer. Therefore, RET data prior to and following dithionite treatment were compared and the donor–acceptor *cis* and *trans* distances of the closest approach as well as *cis* and *trans* Förster radii R_0, and orientation factors κ^2 for *cis* RET equal to 0.61 ± 0.06 and for *trans* RET equal to 0.17 ± 0.01 were assigned. Knowing the κ^2 data, the spatial arrangement of NBD and NRh labels as dipoles in dipalmitoylphosphatidylcholine bilayer were described.

1. Introduction

Structure–function relationships in biological membranes are known to be governed by a variety of factors depending on the spatial and orientation organization of the constituent particles. Small-angle X-ray scattering (SAXS) and small-angle neutron scattering (SANS) may furnish information about the membrane thickness and electron density distribution; however, a combination of various spectroscopic method may be require to obtain data on the environmental characteristics of suitable particles in these membranes or the interactions between these particles [1,2]. The approximate locations of fluorescent molecules in membranes may be determined via microscope [3,4]. However, highly accurate information relating to the location of intrinsically fluorescing or fluorescently labeled molecules in a lipid bilayer may be derived from resonance energy transfer (RET) data. In order to describe the experimental RET data according to Förster model, a few parameters must be known. While some of these parameters are readily specified, the donor–acceptor (D–A) distance of closest approach and the orientation factor κ^2 of D and A as electric dipoles are more difficult to determine. The RET effect has been described for a few lipid structure models on the basis of the density of the fluorescent moieties and their symmetry placement [5–10]. The depth location of many of fluorescence labels has been determined in certain cases, mainly in dioleoylphosphatidylcholine bilayer, but the spatial arrangement remains undetermined to date [11–14].

The aim of the present study is to evaluate the RET effect of symmetry labeled vesicles in which donors and acceptors are located near the surfaces of vesicles (but not in hydrophobic core). In the present design, the fluorescence of both labels (D and A) from the outer surface can be quenched by an adequate quencher. The spatial arrangement of the labels, treated as dipoles, can then be determined on the basis of the broadened RET model proposed by Wolber and Hudson, by comparing the fluorescence data acquired before and after the quencher treatment [6].

2. Materials and Methods

2.1. Chemicals

1,2-Dihexadecanoyl-*sn*-glycero-3-phosphocholine (DPPC), N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine triethylammonium salt (NBD-PE), Lissamine™ rhodamine B 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine, triethylammonium salt (NRh-PE), 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-(1-pyrenesulfonyl) ammonium salt (pyS), 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-(5-dimethylamino-1-naphthalenesulfonyl) ammonium salt (DAN) were obtained from Avanti Polar Lipids (Alabaster, Alabama, USA). 4-Di-16-ASP4-(4-(dihexadecylamino)styryl)-N-methylpyridinium iodide (DiA) and 2-(3-(diphenylhexatrienyl)propanoyl)-1-hexadecanoyl-*sn*-glycero-3-phosphocholine (β-DPH HPC) were from Molecular Probes (Eugene, OR). Other reagents were fromFluka (Buchs, Switzerland) or POCH (Gliwice, Poland).

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2.2. Preparation of vesicles

The samples (each of 5 ml volume) of large unilamellar vesicles (LUVs) were prepared by the modified reverse phase evaporation method (mREV) in 0.2 M TRIZMA buffer (pH = 10.5) [15]. The concentrations of fluorescence labels in the stock solutions were determined from absorbance data, and values of the molar absorption coefficients used were received from the suppliers. The lipid quantity in the vesicle suspensions was determined in accordance with the generalized method [16], using 1 ml of sample.

2.3. Fluorescence measurements

Steady-state fluorescence measurements were performed using a Hitachi 2500 spectrofluorimeter (Japan). Suspensions (each of 4 ml) of vesicles DPPC/NBD-PE, DPPC/NRh-PE, DPPC/pyS, DPPC/DAN, DPPC/DiA, and DPPC/β-DPH HPC, all 100/1 (mol/mol) were treated with sodium dithionite in TRIZMA buffer, which was added gradually (10 μl of 50 mg/ml). The fluorescence emission intensity was registered before and after dithionite treatment and subsequently compared with the initial value. In the case of the NBD-PE and NRh-PE labeled vesicles, the fluorescence emission intensity reduced to about one-half of the initial value following dithionite treatment This observation indicated that dithionite quenches NBD-PE and NRh-PE labels from the outer leaflet only, in contrast to the other labels.

Fluorescence emission data were corrected for reabsorption and the inner filter effect using the correcting formula developed by the author (up to $A = 0.2$):

$$F_{corr} = F_m(1 + 0.152 \times A - 0.199 \times A^2) \tag{1}$$

where A is the absorbance of suspension corresponding to the emission intensity at $\lambda_{\max}$, F_m is the measured fluorescence intensity at λ_{em}, F_{corr} is the corrected fluorescence intensity. On the basis of the corrected fluorescence data, NBD-PE quantum yield (Ψ) of NBD-PE in DPPC bilayer was estimated using quinine sulfate as a standard ($\Psi = 0.55$). In the next step, DPPC/NBD-PE and DPPC/NBD-PE/NRh-PE vesicles with varying DPPC and NRh-PE content were prepared. The fluorescence emission intensity of NBD donor ($\lambda_{\text{exc}} = 463$ nm, $\lambda_{em} = 535$ nm) was registered for each sample prior to dithionite treatment (50 μl sodium dithionite, a few minutes) and several minutes after the treatment.

2.4. Theory

The experimental data obtained in this study were interpreted quantitatively in terms of the broadened Wolber and Hudson RET model and compared with data from source Wolber and Hudson RET model [6]. However, in the source model, derivation of expression Φ/Φ_0 is not complete; nevertheless, the final equation Φ/Φ_0 obtained from this model is provided below:

$$\frac{\Phi}{\Phi_0} = \frac{\int_0^\infty e^{-\frac{t}{\tau}+\pi C\left[\frac{d^2}{R_0^2}(1-e^{-x_d})-\left(\frac{t}{\tau}\right)^{1/3}\gamma\left(\frac{2}{3},x_d\right)\right]}dt}{\int_0^\infty e^{-\frac{t}{\tau}}dt} \tag{2a}$$

where Φ_0 and Φ are donor quantum yield in the absence and presence of acceptor, respectively, the expression $\Phi_0 = \int_0^\infty e^{-\frac{t}{\tau}}dt$ will be later omitted, R_0 is the Förster radius, $C = c \times R_0^2$, where c is the surface acceptor density (number of acceptors per unit area), τ is the lifetime of the excited donor in the absence of the acceptor, $x_d = (t/\tau) \times (R_0/d)^6$, γ is the lower incomplete gamma function and "d" is the D–A distance of closest approach. In fact, in this model, d is not distance of D–A closest approach but distance from the excited donor to the inner edge of acceptors annulus plane with surface acceptors density C.

Application abovementioned equation to describe the real experimental RET data of membrane systems labeled with well known fluorescence labels gives contradictory values of the D–A distances of closest approach, R_0 and orientation factor κ^2 values.

Taking this into consideration, it is postulated that Eq. (2) should be used instead:

$$\frac{\Phi}{\Phi_0} = \int_0^\infty e^{-\frac{t}{\tau}-\pi C\left[\frac{d^2}{R_0^2}(n-1)(1-e^{-x_d})+\left(\frac{t}{\tau}\right)^{1/3}\gamma\left(\frac{2}{3},x_d\right)\right]}dt \tag{2}$$

where n is a number of acceptors at the d D–A distance of closest approach to the exited donor located at the edge of acceptors annulus plane with surface acceptors density C.

Plots of the $\Phi/\Phi_0 = f(d)$ dependences, based on Eq. (2) are shown in Fig. 1.

The source Wolber and Hudson RET model [6] describes the case of the single flat surface homogenously covered by donors and acceptors with the following parameters: c, the surface density of the acceptors, d and R_0 (previously defined). The broadened model describes the case in which two donor layers are at deeper location than are the two acceptor layers with respect to the bilayer center. Because of the symmetry, only the RET from one donor can be analyzed. The system is then described using additional parameters: R_{0T} and R_{0C} the Förster radii for nonradiative *trans* and *cis* energy transfer, respectively, *l-trans* D–A distance of closest approach and ϕ, the angle (explanation below and see Fig. 2). When the upper and lower donor layers are parallel to the acceptor layers, the donor energy may be transferred either to the neighboring *cis* layer of acceptors A_{Ci}, or to the *trans* layer A_{Ti} up to opposite side of the bilayer center. The rate of *cis* or *trans* de-excitation of the donor $k_{CT,}$ is given by the following equation:

$$k_{CT} = \tau^{-1}\left[1 + \sum_{i=1}^{N1}\left(\frac{R_{0T}}{r_i}\right)^6 + \sum_{j=1}^{N2}\left(\frac{R_{0C}}{r_j}\right)^6\right] \tag{3}$$

where N_1 is the number of *trans* acceptors in a disc of radius R around the excited donor given by: $N_1 = \pi R^2 c$; N_2 is the number of *cis* acceptors in a disc of radius R around excited donor given generally by: $N_2 = \pi c[R^2 + (n - 1)\, d^2 \cos^2 \phi]$, where n is number of accep-

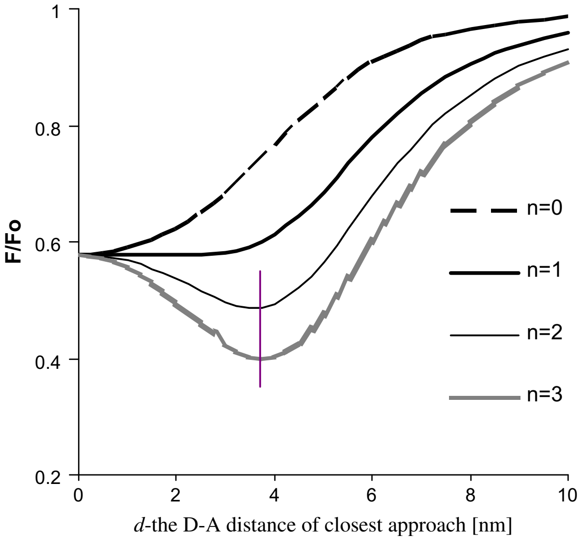

Fig. 1. Theoretical dependence between Φ/Φ_0 and d- the D–A distance of closest approach, according with source ($n = 0$, Wolber and Hudson 1979) and generalized Wolber and Hudson RET model, Eq. (2). ($R_0 = 5$ nm, $c = 0.006$ (nm^{-2})). n is the number of acceptors at the d distance of closest approach to the excited donor. Vertical line marked $d = 3.7$ nm the D–A distance of closest approach for Φ/Φ_0 equal to the experimental data.