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The transmembrane gradient of the dielectric constant influences the DPH lifetime distribution

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Abstract The fluorescence lifetime distribution of 1,6-diphenyl-1,3,5-hexatriene (DPH) and 1-[4-(trimethylamino)phenyl]-6-phenyl-1,3,5-hexatriene (TMA-DPH) in egg-phosphatidylcholine liposomes was measured in normal and heavy water. The lower dielectric constant (by ~12%) of heavy water compared with normal water was employed to provide direct evidence that the drop of the dielectric constant along the membrane normal shifts the centers of the distribution of both DPH and TMA-DPH to higher values and sharpens the widths of the distribution. The profile of the dielectric constant along the membrane normal was not found to be a linear gradient (in contrast to [1]) but a more complex function. Presence of cholesterol in liposomes further shifted the center of the distributions to higher value and sharpened them. In addition, it resulted in a more gradient-like profile of the dielectric constant (i.e. linearization) along the normal of the membrane. The effect of the change of dielectric constant on the membrane proteins is discussed.

Key words: Dielectric constant; Fluorescence lifetime distribution; Heavy water; Lipid–protein interaction

1. Introduction

The properties of membrane lipids have been studied extensively during the past decades with a large number of fluorescence probes among which DPH and its derivatives, e.g. TMA-DPH and DPH-PC (DPH attached to the *sn*-2 chain of phosphatidylcholine) are the most popular [2]. While DPH is distributed predominantly near the center of the membrane [3], TMA, as its cationic derivative, is located in the polar headgroup region [4,5]. The fluorescence lifetime of DPH and its derivatives is the parameter of primary importance as it is sensitive to the membrane lipid environment and to the heterogeneity of the membrane physical properties such as the dielectric constant [6].

The varying degree of water penetration into a membrane sets up a dielectric constant (properly, relative permittivity) gradient from the membrane surface, where the value is ~70, to ~5 at the bilayer center [7]. The steepness of this gradient depends on the degree and depth of water penetration. Disturbances in cooperative van der Waals interactions between the acyl residues of phospholipids locally disorder the membrane, favoring the penetration of water molecules.

With the aim of understanding the origin of the variation of DPH decay parameters on the gradient of the dielectric costant, membrane oxidative damage induced by ionizing radiation has been studied [8]. However, in previous work, changes in the gradient of the dielectric constant were postulated on the basis of the changed membrane properties, like membrane lipids phase state, lipid unsaturation, cholesterol content, etc. In our work, we tried to extend these indirect indications to prove directly that lower gradient of the dielectric constant sharpens the lifetime distribution of DPH and its derivatives and shifts the center of the distribution of the main component. This was done by replacing water (relative permittivity 87.74) with deuterium oxide (78.06) which has chemical properties very similar to water. The lifetime distributions in these two environments were compared.

2. Materials and methods

2.1. Materials

DPH and TMA-DPH were obtained from Molecular Probes (Eugene, OR). Egg phosphatidylcholine, cholesterol and deuterium oxide were purchased from Sigma (St. Louis, MO).

2.2. Preparation of vesicles

Large unilamellar vesicles (LUVs) from egg phosphatidylcholine with different cholesterol concentrations (500 μM lipid + cholesterol in 100 mM Tris-HCl buffer prepared either from H_2O or from D_2O) were prepared from multilamellar hand-shaken vesicles [9] using a Lipofast apparatus (Avestin, Canada) with pore diameter of 400 nm. LUVs were labeled with DPH (in tetrahydrofuran) or TMA-DPH (in ethanol) to give a lipid/probe molar ratio of 1:500 as described previously [10].

2.3. Fluorescence measurements and data analysis

Lifetime measurements were performed with a multifrequency phase fluorometer (ISS GREG 200) interfaced with a PC486 computer for data collection and analysis. The excitation wavelength was 360 nm (xenon arc lamp), 10 modulation frequencies between 5 and 200 were used. Measurements and data analysis were done according to [11]. The temperature of the samples was maintained at 37°C with an external bath circulator (Haake F3).

3. Results and discussion

3.1. Effect of the gradient of dielectric constant on DPH lifetime distribution

The main aim of the present study was to provide evidence whether or not the decrease of the dielectric constant along the membrane normal results in a shift-up of the center of the

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Abbreviations: DPH, 1,6-diphenyl-1,3,5-hexatriene; TMA-DPH, 1-[4-(trimethylamino)phenyl]-6-phenyl-1,3,5-hexatriene, FWHM, full width at half maximum; LUV, large unilamellar vesicle.

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distribution of DPH and its derivative TMA-DPH and is accompanied by a narrowing of the distribution. Unilamellar liposomes from egg phosphatidylicholine were labeled with DPH and TMA-DPH in normal and heavy water and fluorescence decay was determined. It should be noted that the possible contribution of the polar part of TMA-DPH to the membrane surface charge is negligible as only one molecule of TMA-DPH per 500 molecules of phospholipids is present. A two-component Lorentzian continuous distribution (Table 1) was used for the data analysis [11,12]. This analysis includes a minor component which is typical of DPH type fluorophores. At present, this component is not believed to yield useful information about the lipid bilayer [7] but it is still included in the table, using designations τ_2, w_2 and f_2 for it.

Replacement of water with D_2O resulted in an increase of the lifetime of the major component of DPH from 7.85 to 8.11 NS and of TMA-DPH from 3.25 to 3.55 NS (Table 1). The full width at half maximum (FWHM) was reduced in heavy water in both cases, viz. from 1.11 to 0.12 NS for DPH and from 0.36 to 0.12 NS for TMA-DPH.

While earlier experiments provided only indirect evidence for the effect of the dielectric constant on the lifetime distribution of DPH [8], the present results are a first direct proof of the influence of the dielectric constant on the DPH lifetime distribution in the membrane bilayer. The smaller change of the dielectric constant in heavy water resulted in a narrower distribution width (FWHM) and in a higher value at the center of the major component.

3.2. Profile of the dielectric constant along the membrane normal

Earlier NMR studies showed [1] that the dielectric constant linearly decreases from the membrane–water interface to about two-thirds of the membrane half layer (Fig. 1) where there is a transition into the flat area of low and equal dielectric constant. TMA-DPH is located with its hydrophobic part of the fluorophore within the membrane while the polar trimethylammonium group protrudes into the polar region. In this way, the probe reflects the region roughly of the length of about one-half of the acyl chain, viz. C-1 to C-10 [13]. Thus, one can assume

Table 1
Two-component distribution analysis of DPH and TMA-DPH fluorescence emission decay in egg PC/cholesterol mixtures in H_2O and in D_2O

Probe	Molar % of cholesterol	τ_1	w_1	f_1	τ_2	w_2	f_2
DPH	0	7.85	1.11	0.97	1.55	0.05	0.01
(H_2O)	20	8.75	0.66	0.95	1.50	0.05	0.04
	35	9.59	0.05	0.93	1.15	0.05	0.04
DPH	0	8.11	0.12	0.92	2.90	1.12	0.07
(D_2O)	20	8.99	0.05	0.93	2.78	0.05	0.05
	35	9.89	0.05	0.94	2.67	0.08	0.06
TMA-DPH	0	3.25	0.36	0.93	0.51	0.40	0.04
(H_2O)	20	4.61	0.47	0.85	2.47	0.06	0.12
	35	5.85	0.40	0.89	2.76	0.05	0.09
TMA-DPH	0	3.55	0.12	0.89	1.14	0.05	0.09
(D_2O)	20	5.00	0.05	0.85	2.23	0.27	0.14
	35	6.15	0.05	0.90	2.89	0.05	0.10

τ, center of lifetime distribution; w, FWHM of Lorentzian lifetime distribution; f, fractional intensity of lifetime components.

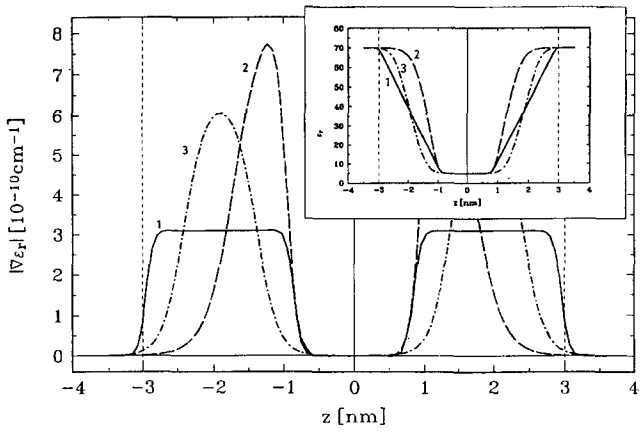

Fig. 1. Gradient of relative permittivity ($\nabla\varepsilon_r$) along the normal of the membrane. Curve 1, original model of Griffith [1]; curve 2, model based on present experiments; curve 3, effect of cholesterol on gradient.

that TMA-DPH 'feels' the dielectric constant gradient more than DPH which is distributed homogeneously across the bilayer. Surprisingly, the FWHM of DPH decreased much more than the FWHM of TMA-DPH (from 1.11 and 0.36 NS, respectively, to 0.12 NS; Table 1). This clearly reveals the higher homogeneity of the dielectric constant at the membrane–water interface. Thus, the shape of distribution is more complicated and is definitely not a simple slope.

We summarize our results combined with earlier NMR studies [1] in Fig. 1. The change of the dielectric constant is not a constant as one would expect for a linear decrease but consists of three parts: the first flat zone of at least 1.3 nm (roughly the region scanned by TMA-DPH; see also below) is due to the penetration of water molecules into the membrane bilayer. This zone is followed by a steep decrease of the dielectric constant which reaches the third area at a depth of ~3.0 nm where the dielectric constant is equal to the value in the lipid phase (in accordance with [1] and [5]). We wish to emphasize that the profiles of the gradient of the dielectric constant have an illustrative character, their exact profiles are the subject of future studies. For simplification, we chose the Gaussian profiles which we feel describe best the real situation; the assumption of a constant integral of functions in normal or heavy water was employed.

3.3. Effect of cholesterol on the dielectric constant

The presence of cholesterol shifts up the center of the distribution and narrows the FWHM both for DPH and TMA-DPH (Table 1) in accordance with earlier observations [11] that the presence of cholesterol decreases the amount and depth of penetration of water molecules into the membrane. At 20% cholesterol decreased the FWHM of the longer component of DPH to about one-half its original value (Table 1). However, the same concentration of cholesterol (20%) did not decrease but rather increased the FWHM for TMA-DPH (Table 1). This result is in accordance with an earlier observation of redistribution of DPH in the presence of cholesterol. Namely, DPH is more or less homogeneously distributed along the membrane normal in the absence of cholesterol while in the presence of cholesterol it is localized in the inner part of the bilayer [14]. The higher concentration of cholesterol (35%) resulted in a

virtually exponential decay (the minimal distributional width was 0.05 NS) for DPH but not for TMA-DPH. While DPH located in the inner part of the membrane did not feel any gradient of the dielectric constant, TMA-DPH located on the membrane–water interface was able to detect a heterogeneous dielectric constant environment.

Heavy water decreased the FWHM both for DPH and TMA-DPH. The lower concentration of cholesterol (20%) already led to FWHM indistinguishable from the exponential decays. This indicates that the drop of the dielectric constant along the membrane normal was right at the membrane–water interface. Deep in the membrane where the reporting fluorophore group of TMA-DPH is located (1.3 nm), lies a region with a constant relative permittivity. In other words, the peak of the first derivative of the dielectric constant is shifted to a more external part of the membrane, while at a depth of 1.3 nm it is almost flat (Fig. 1).

3.4. Conclusions

(a) A decrease of the gradient of the dielectric constant brings about an increase of the central value of the lifetime distribution of the main component of both DPH and TMA-DPH and reduces the width of the distribution of its main component.

(b) The profile of the dielectric constant along the membrane normal is not a monotonic and linear gradient, but consists of three parts.

(c) Since the conformation of integral proteins should be highly affected by the change of the dielectric constant (because of the change of electric multipole–multipole interactions), the present results show that penetration of water molecules (as influenced by cholesterol), by affecting the relative permittivity, can mediate the effects of lipids on membrane protein conformation.

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