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Solvation Dynamics of DCM in Dipalmitoyl Phosphatidylcholine Lipid

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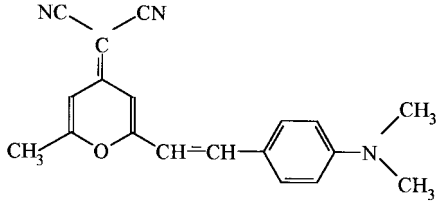
Abstract—Solvation dynamics of 4-(dicyanomethylene)-2-methyl-6-(*p*-dimethylaminostyryl) 4*H*-pyran (DCM) in dipalmitoylphosphatidylcholine (DPPC) vesicles in water is studied using picosecond emission spectroscopy. The solvation dynamics of DCM in DPPC vesicles is found to be bi-exponential with two components of 120±20 ps (20%) and 5.5±0.5 ns (80%). This indicates slow relaxation of the water molecules inside the water pool of the lipid vesicles.

Introduction

Chemistry in organized environments differs significantly from that in many simple liquids. As a result, photochemistry and photophysics in constrained and confined media has been a subject of vigorous activity in recent years.[1,2] Water molecules confined in various self-organized molecular assemblies play a key role in the structure, function and dynamics of many biological systems. Solvation dynamics of water molecules in organized assemblies, i.e. reorganization of water molecules around an instantaneously created dipole has received special attention in recent years.[3–13] In bulk water, solvation dynamics occurs in sub-picosecond time scale.[3a,c] However, in almost all organized assemblies the solvation dynamics of water is observed to be slower by several orders of magnitude. Several groups have studied solvation dynamics and dielectric relaxation of water molecules in many organized assemblies such as cyclodextrin,[3] proteins,[4,5] DNA,[6] microemulsions,[7–9] micelles,[10] water surface,[11] sol–gel matrix[12] and lipid vesicles.[13] Among these organized media, the lipid vesicles resemble most closely a biological cell and hence, study of solvation dynamics in lipid is of fundamental importance to understand the behavior of biological water. In a vesicle, an aqueous volume is surrounded by a bilayer membrane and is dispersed in bulk water.[14–16] So far there are few studies on solvation dynamics in lipids.[13a–b] In order to develop a complete picture of solvent relaxation in lipids it is necessary to study solvation dynamics of more than one probe in the same lipid and also to vary the lipid. To find out the probe dependence of solvation dynamics in lipids, we have studied earlier solvation dynamics of coumarin 480 (C-480)[13a] and 4-(dicyanomethylene)-2-methyl-6-(*p*-dimethylaminostyryl) 4*H*-pyran (DCM, Scheme 1)[13b] in the lipid, DMPC. In order to ascertain how the solvation dynamics of the same probe varies in different lipids, in the present work we have studied solvation dynamics of DCM in dipalmitoyl-phosphatidylcholine (DPPC) vesicles.

According to the structure of DCM molecule, it may undergo several ultrafast processes, such as, photoisomerization about the olefinic double bond, twisted intramolecular charge transfer (TICT), and solvation dynamics. Because of the intramolecular charge transfer process, the excited state dipole moment of DCM (23.6 Debye) is very much higher than that in the ground state.[13,14] Recent femtosecond studies indicate that in methanol DCM exhibits ultrafast solvation dynamics with an inertial component on the 100 fs timescale and a slower component of a few ps.[17,18] It is obviously of interest to find out how these ultrafast processes are affected in an organized assembly. The TICT process is usually slowed down in various organized assemblies,[19] and thus it is important to know whether the TICT process of DCM is retarded sufficiently to exhibit dual emission in a lipid.



Scheme 1. Structure of DCM.

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Experimental

DCM (laser grade, Exciton) and DPPC (Sigma) were used as received. For recording the absorption and emission spectra we used respectively, a JASCO 7850 and a Perkin–Elmer 44B instruments, respectively. For time resolved study, the sample was excited at 300 nm with the second harmonic of a cavity dumped rhodamine 6G dye laser (Coherent 702-1) pumped by a cw mode locked Nd:YAG laser (Coherent Antares 76s). The emission was detected at magic angle polarization, using a Hamamatsu MCP photomultiplier (2809U). The full width at half maximum of the instrument response at 300 nm is ≈80 ps. Fluorescence decays were deconvoluted using a global lifetime analysis software (PTI).

For the preparation of the lipid, the ethanol injection method[14c,d] was followed. Briefly, 2 mg DPPC was at first dissolved in 50 μl of a ethanolic solution of DCM. Then the whole solution was rapidly injected using a microlitre syringe into 2 ml of tris buffer of pH 7.4 at 45°C. This method produces a high proportion of small unilamellar vesicles of diameter ca. 50 nm.[14c,d] After injection the mixture is kept for 1 h at 45°C (i.e. above the gel transition temperature of DPPC). The concentration of the final solution of DPPC was 1.3 mM and DCM concentration was 1×10^{-5} M so that the probe to lipid ratio was ≈1:80. All steady state and time resolved measurements were done at 20°C, i.e. below the gel transition temperature of DPPC (41°C).[14]

Results

Steady state spectra

In the DPPC lipid, DCM does not exhibit dual emission and displays a single, strong emission band at 615 nm (Fig. 1). As reported in the literature, the emission maximum of DCM displays a red shift with increase in solvent polarity, from 530 nm in *n*-heptane to 620 nm in methanol. DCM is insoluble in water and hence, the steady state spectral properties of DCM in lipid could not be compared with those in water. However, it is evident that the observed emission maximum of DCM in lipid is similar to that in methanol.

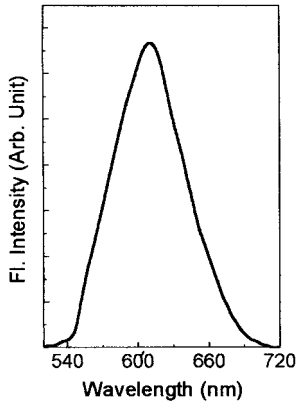

Figure 1. Emission spectrum of DCM in 1.3 mM DPPC lipid.

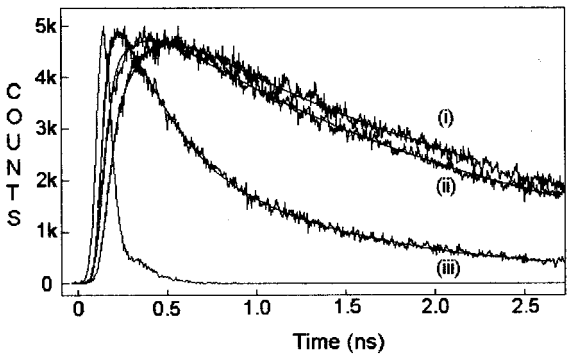

Figure 2. Fluorescence decays of DCM in 1.3 mM DPPC lipid at (i) 700 nm, (ii) 615 nm, (iii) 540 nm.

Time resolved studies

In DPPC vesicles, the fluorescence decays of DCM are found to be markedly dependent on the emission wavelength. At short wavelengths, a fast decay is observed while at long wavelengths (red end) the decay is preceded by a growth (Fig. 2). Such a wavelength dependence clearly indicates that the DCM molecules undergo solvation dynamics in the DPPC lipid.[20] Using the parameters of best fit to the fluorescence decays and the steady state emission spectrum, the time resolved emission spectra (Fig. 3) of DCM in DPPC were constructed following the procedure described by Fleming and Maroncelli.[20c] According to this method if $\nu(0)$, $\nu(t)$ and $\nu(\infty)$ respectively denote the observed emission energies (frequencies) at time zero, t and infinity, one first calculates the solvent response function $C(t)$ which is defined as

$$C(t) = \frac{\nu(t) - \nu(\infty)}{\nu(0) - \nu(\infty)}$$

The solvation dynamics is then described by the decay of $C(t)$ as shown in Fig. 4. The decay parameters of $C(t)$ are summarized in Table 1. It is readily seen that the total Stokes shift $\Delta\nu=\nu(0)-\nu(\infty)$, of DCM in DPPC is 1000 ± 100 cm^{-1} and the decay of $C(t)$ is bi-exponential

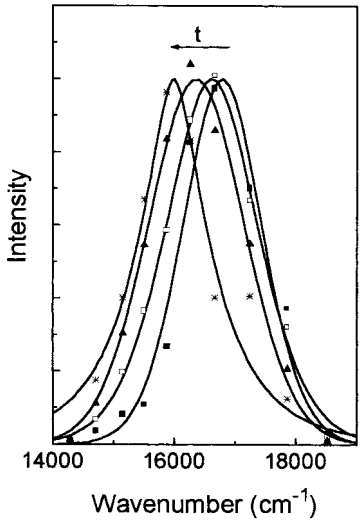

Figure 3. Time resolved emission spectra of DCM in 1.3 mM DPPC lipid at 0 ps (■), 200 ps (□), 2000 ps (▲), 7000 ps (*).

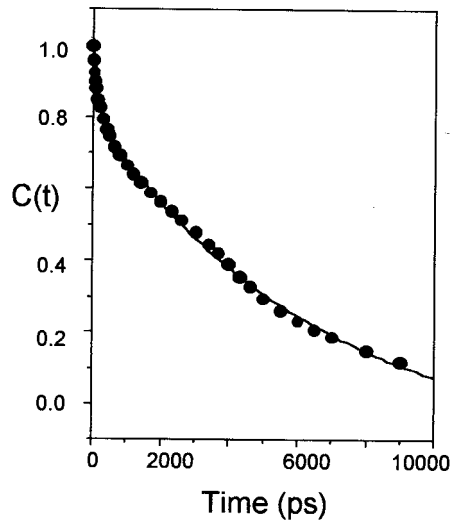

Figure 4. Decay of response function, *C(t)* of DCM in 1.3 mM DPPC lipid. The points denote the actual values of *C(t)* and the solid line denotes the best fit to a bi-exponential decay.

with two components 120±20 ps (20%) and 5.5±0.5 ns (80%).

Discussion

It is evident that DCM does not exhibit dual emission in the DPPC lipid. The observed emission of DCM in DPPC at 615 nm is very close to that of the TICT band of DCM in polar solvents like methanol.[17,18] This indicates that the TICT process of DCM remains ultrafast in the lipid.

The time dependent Stokes shift (TDSS) indicates that DCM exhibits a $\Delta\nu$=1000±100 cm^{-1} in the DPPC lipid. It may be noted that recent femtosecond studies indicate DCM exhibits a $\Delta\nu$=3800 cm^{-1} in methanol and 2400 cm^{-1} in ethylene glycol.[17b,c] In our apparatus of time resolution ~80 ps, we have most likely missed a large part of the solvation which occurs in the femtosecond time-scale.[17,18] Nevertheless, it is evident that the solvation dynamics of DCM in DPPC exhibits a component which is much slower than the sub-picosecond component of DCM or other probes in methanol or water.[3,17,18,22]

In a lipid vesicle, there are three possible locations for the spectroscopic probe DCM, e.g. bulk water, inner water pool, and the bilayer. Since DCM is insoluble in water, the possibility of DCM staying in bulk water is ruled out. In a hydrocarbon, the emission quantum yield of DCM is extremely weak and the lifetime is very short (<50 ps).[7b] Further, in *n*-heptane the emission maximum of DCM is very much blue shifted to 530 nm and the decay does not exhibit a wavelength dependence.[7b] The observed emission maximum of DCM in DPPC at 615 nm and the wavelength dependent emission decays suggest that the probe (DCM) does not stay in the 'dry' hydrocarbon like bilayer of the DPPC lipid. Thus the observed solvation dynamics appears to be due exclusively to the DCM molecules in the inner water pool of the DPPC lipid. Kaatze and coworkers[21] reported that the lipid vesicles exhibit two prominent dielectric relaxation time (τ_D) in 10 and 0.1 ns time scale. They assigned the 10 ns relaxation component to the solvent relaxation. Since in the continuum model[20b] solvation time, $\tau_S=(\epsilon_\infty/\epsilon_0)\ \tau_D$, if one assumes ϵ_∞ of the inner water pool same as that of water i.e. 5[20b] and ϵ_0 is of the inner water pool same as that of methanol (~30), one immediately calculates a solvation time of (5/30)×10 ns, i.e. 1.67 ns. In the present case, the average solvation time of DCM in DPPC vesicles is 4.6 ns which is of the same order of magnitude.

Table 1. Decay parameters of *C(t)* of DCM in DPPC lipid

$\Delta\nu$ (cm^{-1})	a_1	τ_1 (ps)	a_2	τ_2 (ps)
1000±100	0.20	120±20	0.80	5500±500

In DMPC, we observed earlier that the decay of *C(t)* is bi-exponential for both C-480 and DCM.[13] For C-480, there is a short component of 600 ps (40%) and another very long component of 11 ns (60%).[13a] In the case of DCM the components are 230 ps (40%) and 1600 ps (60%).[13b] In the present case for DCM in DPPC, the decay is bi-exponential with components of 120±20 ps (20%) and 5.5±0.5 ns (80%). It should be noted that the $\Delta\nu$ observed for DCM in DPPC (~1000 cm^{-1}) in the present work is very similar to that of DCM in DMPC (1250 cm^{-1}).[13b] As pointed out earlier, we are obviously missing a large part of solvation which occurs in a time scale faster than the response time of our setup (~80 ps). It is however, evident that irrespective of the probe (DCM or C-480) or of the lipid (DMPC or DPPC) the solvation dynamics in the lipids, is bimodal with one component in 100–600 ps time scale and the other in 1–10 ns time scale. Both of these components are substantially shorter than the sub-picosecond relaxation observed in ordinary water.[3a,c]

The components of solvation dynamics detected in the present work are too slow to be explained in terms of any vibrational modes. To explain the slow component of dielectric relaxation in aqueous solution of proteins, Nandi and Bagchi[3b] proposed a dynamic exchange between the 'bound' and the free water molecules. The bound water molecules are those which are attached to a bio-molecule through strong hydrogen bonds. Their motion is coupled with that of the bio-molecules and hence, they are quite slow. The dynamic exchange thus corresponds to the rupture of hydrogen bonds and this may be responsible for the nanosecond component of solvation dynamics in an organized assembly like lipid.

Conclusion

The present work shows that DCM does not exhibit dual emission in the DPPC lipid. This suggests that the TICT process of DCM remains ultrafast in lipids. The time dependent Stokes shift of DCM indicates that in the inner water pool of the DPPC lipid, the water molecules relax with two components of 120±20 ps (20%) and 5.5±0.5 ns (80%). Evidently the dynamics of the water molecules present in lipids is substantially slower than that of ordinary water molecules. Since the water molecules inside the water

pool of vesicles resemble those in biological cells, the present study demonstrates that the mobility of biological water molecules is severely constrained compared to ordinary water molecules.

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