Solvation Dynamics of Coumarin 480 in Vesicles

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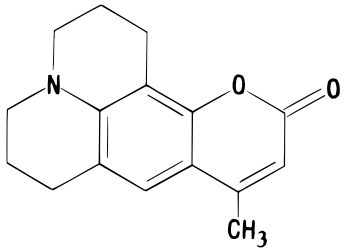
Solvation dynamics of Coumarin 480 (C-480) in dimyristoylphosphatidylcholine (DMPC) vesicles in water is studied using picosecond time-resolved Stokes shift. In sonicated unilamellar DMPC vesicles C-480 exhibits wavelength dependent fluorescence decays. At short wavelengths a fast decay is observed while at the long wavelengths a growth in the nanosecond time scale precedes the decay. The solvation dynamics of C-480 in DMPC vesicles is found to be bimodal with two components of 0.6 and 11 ns, which is similar to the solvation dynamics of C-480 in the large water pools of AOT/*n*-heptane/water (AOT = aerosol OT) microemulsions.

1. Introduction

The water molecules present in confined environments play a key role in many natural and biological processes. The structure, reactivity, and dynamics of the biomolecules are essentially governed by the water molecules present in organized assemblies. As a result, the relaxation behavior of water molecules in organized environments has been the subject of several recent studies.[1−5] Ordinary water molecules exhibit solvation dynamics in the subpicosecond time scale.[1a,b] However, in many organized media such as cyclodextrins,[1a,2a] reverse micelles[3a−c] or micelles[3d] the solvation dynamics of water molecules occur in a time scale slower by several orders of magnitude. Surprisingly, in the case of semirigid gels (orthosilicate[4a] or polyacrylamide[4b]), with very high bulk viscosity, it is observed that the solvation dynamics is very fast and occurs in the time scale of less than 50 ps. The nanosecond solvation dynamics in the organized media is consistent with the dielectric relaxation and pulsed NMR studies of the biological systems.[5] These studies reveal that while the dielectric relaxation of ordinary water occurs in the time scale of 10 ps, in many biological systems the dielectric relaxation of water is bimodal with one component of ≈10 ps and another about 10 ns. The solvation time is governed by the longitudinal relaxation time, τ_L, which is less than the dielectric relaxation time by a factor of $\epsilon_\infty/\epsilon_s$, where ϵ_∞ and ϵ_s are, respectively the dielectric constants at high and zero (static) frequencies.[6,7] The polarity or the static dielectric constant of the biological systems is often less than that of ordinary water by a factor of 2−3. The 10 ns component of dielectric relaxation of biological systems, which is 1000 times slower compared to water, causes a several thousand-fold reduction in the solvation dynamics of water in many organized assemblies.[1a,2a,3] Earlier workers ascribed the bimodal dielectric relaxation behavior of water in the organized assemblies to the presence of two kinds of water molecules, "bound" and "free", relaxing, respectively, in the nano- and picosecond time scales.[5] However, very recently Nandi and Bagchi proposed a two state model, according to which the slow component of the dielectric relaxation arises not due to the inherent slowness of the bound water molecules but due to a dynamic exchange between the bound and the free water.[2b]

CHART 1: Structure of Coumarin 480



Inspired by these results, we have decided to probe the microenvironment of the vesicles using time-resolved fluorescence spectroscopy of a well-known solvation probe, coumarin 480 (C-480, Chart 1). In a vesicle an aqueous volume ("water pool") is entirely enclosed by a membrane which is basically a bilayer of the lipid molecules.[8−13] In the case of the unilamellar dimyristoylphosphatidylcholine (DMPC) vesicles (radius ≈ 250 nm) there is only one such bilayer while a multilamellar vesicle (radius ∼ 1000 nm) consists of several concentric bilayers.[10] A unilamellar vesicle is usually produced by breaking the multilamellar vesicles using ultrasonic irradiation. Since the vesicles are formed by dispersing the lipid molecules in aqueous medium, in such a system there are two kinds of water molecules present—those in the bulk and those entrapped within the water pool of the vesicles. The entrapped water pool of a small unilamellar DMPC vesicle is much bigger (radius ∼ 250 nm) than those of the water pool of the reverse micelles[3a−c] (radius less than 10 nm). In recent years several groups studied chain dynamics of lipids using ESR of spin-labeled lipids[8a,b] and fluorescence of pyrene-labeled lipids.[8c] Recent molecular dynamics (MD) simulations,[9a,b] NMR,[9c] crystal structure,[9c,d] and other studies[9f] revealed detailed information on the structure of DMPC vesicles and the water molecules in its neighborhood. The most recent MD simulation indicates that above the transition temperature (≈23 °C) each DMPC molecule is hydrogen bonded to about 4.5 water molecules which form an inner hydration shell of the polar headgroup of the lipids and about 70% of the DMPC molecules remain connected by the water bridges.[9a] Time-resolved emission spectroscopy has been

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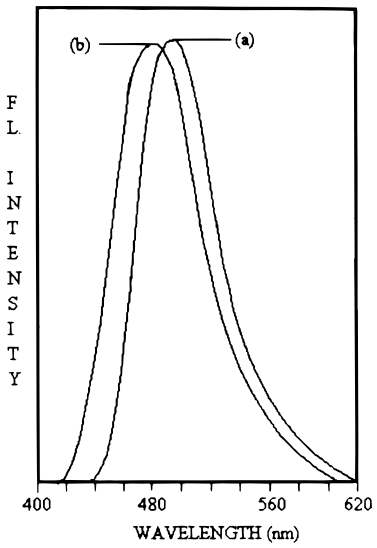

Figure 1. Emission spectra of 1.2×10^{-5} M C-480 in tris buffer of pH 7.4 (a) in the absence and (b) in the presence of 1 mM DMPC: $\lambda_{ex} = 300$ nm.

employed by many workers to study optical anisotropy,[11a,b] isomerization,[11b,c] and intramolecular charge transfer[12] processes in vesicles. Several groups also studied red edge excitation spectroscopy in multilamellar and unilamellar vesicles which gives information on the solvation of the fluorescent probes in the ground state.[13] However, to the best of our knowledge, there has been no attempt to study the solvation dynamics of the water molecules trapped inside the vesicles using picosecond spectroscopy. In the present work we report on the solvation dynamics of coumarin 480 in sonicated unilamellar DMPC vesicles.

2. Experimental Section

DMPC (Sigma) and Coumarin 480 (laser grade, Exciton) were used as received. All solvents were of spectroscopy grade. The sonicated vesicles were prepared following literature procedures.[10] Briefly a solution of DMPC in a 1:1 (v/v) mixture of chloroform and methanol was slowly evaporated at room temperature to produce a thin film of the lipid molecules on the inner wall of the container. To the thin film of the lipid a solution of 1.2×10^{-5} M C-480 in tris buffer of pH 7.4 containing 0.1 M NaCl was added. The volume was adjusted to make the concentration of the final solution of DMPC 1 mM so that the probe-to-lipid ratio was 1:86. The sonicated unilamellar vesicles were produced by repeated cycles of sonications for 2 min at 1 min intervals for 45 min. Then the solution was allowed to stand for 1 h at 26 °C, i.e., above the transition temperature (≈23 °C)[8−10] of DMPC. All measurements were performed at 26 °C. The laser system and the single photon counting apparatus are described in earlier publications.[3b−d] The response time of the setup is about 50 ps. The wavelength of excitation for the time-resolved studies is 300 nm.

3. Results

A. Steady-State Emission Results. C-480 exhibits intense emission with an emission maximum at 490 nm in aqueous solution and at 410 nm in hydrocarbon medium (e.g., *n*-heptane).[3b,d,14] In DMPC vesicles the emission maximum of C-480 is observed to be at 480 nm, which is blue shifted by about 10 nm from that in water and red shifted by 70 nm from that in the hydrocarbon medium (Figure 1). This indicates that

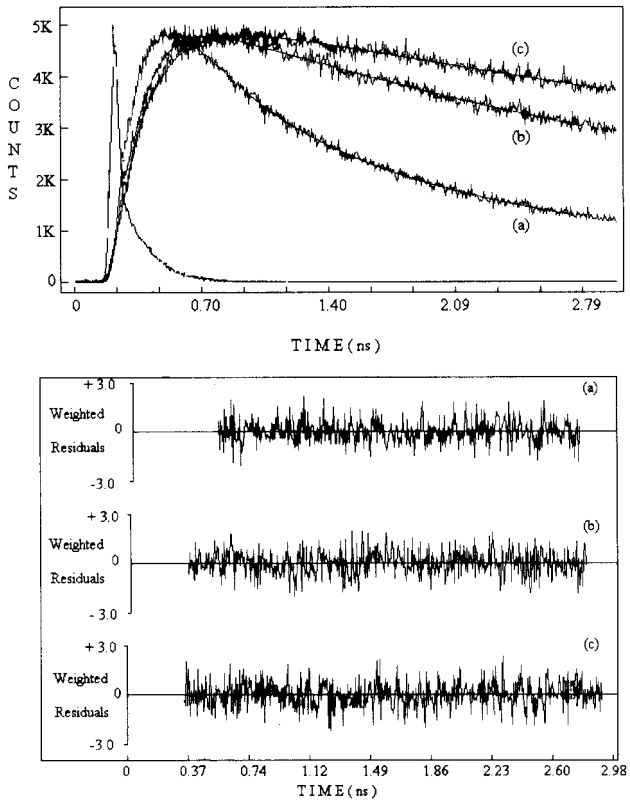

Figure 2. Fluorescence decays of 1.2×10^{-5} M C-480 in DMPC vesicles at (a) 430, (b) 460, and (c) 550 nm: $\lambda_{ex} = 300$ nm.

in the presence of the vesicles the C-480 molecules stay neither in the bulk water nor in the hydrocarbon-like region between the alkyl chains of the bilayers. The position of the emission maximum is close to that of C-480 in alcohol (473 nm)[14] or in the water pool of reverse micelles[3b] or in the micelles.[3d] This suggests that C-480 stays in a region which is very much more polar than ordinary hydrocarbons but is less polar than ordinary water. Thus, the steady-state studies indicate that in the DMPC vesicles the C-480 molecules reside in the water pool of the vesicles.

B. Time-Resolved Emission. In the DMPC vesicles, the fluorescence decays of C-480 are found to be wavelength dependent. At the blue end (430−470 nm), C-480 exhibits only fast decay while at the red end (500−560 nm) the decays are much longer and are preceded by a growth in the nanosecond time scale (Figure 2). Such a wavelength dependence of the fluorescence decay indicates that C-480 exhibits time-dependent Stokes shift in the vesicles. From the parameters corresponding to the best fits to the fluorescence decays, time-resolved emission spectra (Figure 3) are constructed and the emission maxima at different times are extracted by fitting to a log-normal function following the procedure of Fleming and Maroncelli.[6a] Using the emission frequencies at time zero, t and ∞ (respectively $\nu(0)$, $\nu(t)$, and $\nu(\infty)$), the solvent response function $C(t)$ defined by

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

is constructed. Finally, the solvation times are obtained from the decay of $C(t)$ with time (Figure 4). The decay charactersitics of $C(t)$ are summarized in Table 1. It is readily seen that the solvation time in the vesicles is biexponential with one component of 0.6 ns and another of 11 ns.

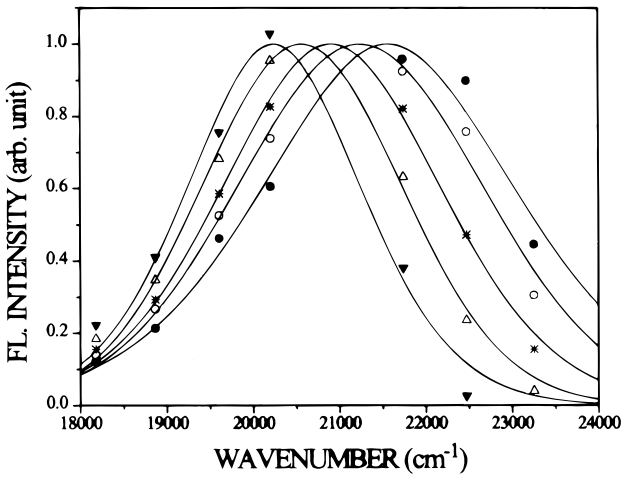

Figure 3. Time-resolved emission spectra of 1.2×10^{-5} M C-480 in DMPC vesicles at (a) 0 (●), (b) 400 (○), (c) 2000 (*), (d) 9000 (△), and (c) 34000 ps (▼).

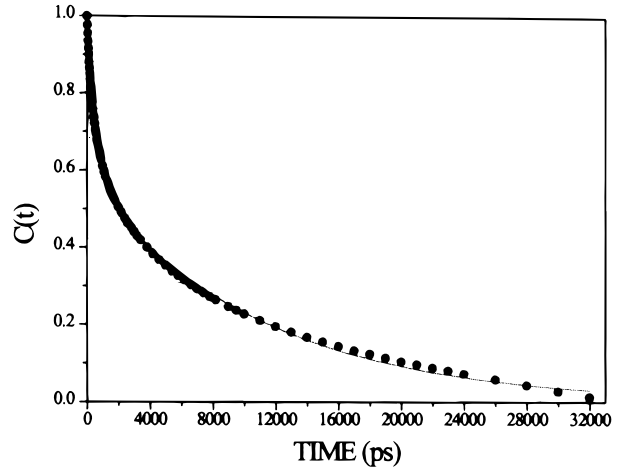

Figure 4. Decay of the response function, $C(t)$ of 1.2×10^{-5} M C-480 in DMPC vesicles. The points denote the actual values of $C(t)$, and the solid line denotes the best fit to a biexponential decay.

TABLE 1: Decay Characteristics of $C(t)$ of C-480 in DMPC Vesicles

$\Delta\nu$ (cm^{-1})	a_1	τ_1 (ps)	a_2	τ_2 (ps)
1327	0.4	600	0.6	11 000

4. Discussion

Both the steady-state and the time-resolved studies indicate that C-480 experiences a microenvironment different from the bulk water and the hydrocarbon region of the bilayers. This suggests that the probe C-480 molecules are located in the inner water pool of the DMPC vesicles. It is obvious that if the C-480 molecules were located in the bulk water, the solvation dynamics would have been so fast that we would not have seen the growth in the nanosecond time scale, at the long wavelengths. Since C-480 does not exhibit wavelength-dependent decay in *n*-heptane, the observation of the wavelength-dependent decay also rules out the presence of the dye in the hydrocarbon region of the bilayer. The observed solvation dynamics indicates that the water molecules, present in the confined water pool of the vesicle, relax in a time scale much slower than that in bulk water. It may be recalled that the observed bimodal solvation dynamics is very similar to that observed earlier, in the case of the large water pool of the reverse micelles.[3b] For the reverse micelles, containing a large water pool of radius ~ 6 nm, the solvation dynamics of C-480 exhibited two components one of ~2 ns and another of ~12 ns.[3b] In the case of lipids, the two components are respectively of 0.6 and 11 ns. It should, however, be pointed out that, in the microemulsions, the water pool is dispersed in a bulk hydrocarbon medium while, in the case of vesicles, the water pools are dispersed in a bulk aqueous medium. It is important to note that the solvation dynamics in lipids is at least 10 times slower than that in micelles.[3d] This indicates that the water molecules in the vesicles are much more constrained compared to the micelles. The observed slow solvation dynamics in the vesicles cannot be attributed to the motion of DMPC headgroups as the ESR study of spin-labeled DMPC indicates chain dynamics in greater than a 100 ns time scale.[8a]

At this stage, it is difficult to quantify the nonexponentiality of the nanosecond solvation dynamics in the vesicles, reported in this work. In bulk water, the solvent response is strongly bimodal, with one ultrafast component (<100 fs) arising from the intermolecular vibration and librations, followed by a biexponential decay of subpicosecond components due to diffusive motions.[1a,b,2c] In our setup with a time resolution ≈ 50 ps, we are obviously missing these ultrafast components. Nandi and Bagchi attributed the nanosecond component of the dielectric relaxation of aqueous protein solutions to the dynamic exchange between the free and the bound water molecules.[2b] However, their model cannot explain the observation of two nanosecond components of the solvation dynamics in the vesicles. The nonexponentiality may arise from the inherent inhomogeneity in the immediate neighborhood of the probe, C-480 molecules in the vesicles. The recent MD simulations[9a,b] indicate that about 12−13 water molecules per DMPC molecule remain "ordered" of which 4.5 form direct hydrogen bonds with the DMPC oxygen atoms.[9f] Evidently, the local dielectric constant and the relaxation properties of the water molecules, close and hydrogen bonded to the polar headgroup of the bilayer, are expected to be quite different from those in the region, far away from the polar headgroups. Castner et al. earlier proposed an "inhomogeneous continuum" model, which involves a dielectric constant $\epsilon_0(r)$, dependent on the distance (r) from the probe.[6b] Although, such a model, appears quite attractive for the present case, it is diificult to apply, as the exact functional form of $\epsilon_0(r)$, cannot be determined. Obviously, much more experiments are needed to get more information on the vibrational frequencies and the microscopic dielectric properties of such complex systems, and such information, when available, will lead us to a better understanding of the relaxation behavior of such complex systems.

5. Conclusions

The present work shows that C-480 is a sensitive fluorescent probe for the microenvironment of the DMPC vesicles. From the steady-state and the time-resolved studies it is inferred that the probe C-480 molecule is located in the water pool inside the vesicles. The time-resolved Stokes shift indicates that the solvation dynamics of water in the water pool of DMPC vesicles is bimodal with two components of 0.6 and 11 ns. Since DMPC vesicles are known to undergo a phase transition at ≈23 °C, it will be interesting to find out how the solvation dynamics is affected in the more compact phase below 23 °C. Temperature variation and other aspects of solvation dynamics in vesicles will be the subject of our future study.

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