Solvation dynamics in DMPC vesicle in the presence of a protein

ARTICLE *in* CHEMICAL PHYSICS LETTERS · DECEMBER 2003

Impact Factor: 1.9 · DOI: 10.1016/j.cplett.2003.10.091

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Chemical Physics Letters 382 (2003) 426–433
CHEMICAL PHYSICS LETTERS
www.elsevier.com/locate/cplett

Solvation dynamics in DMPC vesicle in the presence of a protein

Partha Dutta, Pratik Sen, Saptarshi Mukherjee, Kankan Bhattacharyya *

Physical Chemistry Department, Indian Association for the Cultivation of Science, Jadavpur, Kolkata 700 032, India

Received 9 July 2003

Abstract

Solvation dynamics of DCM is studied in DMPC vesicle in the presence of a protein, human serum albumin (HSA). In bulk water, solvation dynamics of DCM bound to HSA displays a 10 ns component. This component is absent when HSA is entrapped in the vesicle. This suggests that the protein does not undergo tumbling inside the vesicle. In the vesicle, solvation dynamics in the presence of HSA is slower compared to that in its absence. Solvation dynamics below the gel transition temperature ($T_c \approx 23$ °C) is about two times slower than that above it.

1. Introduction

Water molecules confined in a small region control many biological processes [1–3]. Perhaps, the most spectacular property of confined water molecules is the ultraslow component of solvation dynamics which is slower by 2–3 orders of magnitude compared to bulk water [1–3]. The dramatic retardation of solvation dynamics in a confined environment markedly slows down many polar reactions [2]. The slow component of solvation has been detected in many systems, e.g., protein [3–7], lipid vesicles [8,9], reverse micelles [10], DNA double helix [11] and in hydrophilic polymers [12,13]. Several theoretical models and computer simulations have been used to explain the slow solvation dynamics in confined environments. According to an analytical model, the biological systems involve a dynamic exchange between 'free' (i.e., fast) and 'bound' (essentially immobilized) water molecules [14]. More recently, several groups carried out large scale computer simulations on micelles [15,16], reverse micelles [17,18], liquid–liquid interfaces [19] and air–water surface [20]. These simulations also reveal a component of solvation substantially slower than that in bulk water.

A biological cell is highly crowded with many protein molecules packed in a small volume. In an attempt to understand behavior of a protein in a crowded environment several groups have investigated structural and biochemical activity of

* Corresponding author. Fax: (91)-33-2473-2805.
E-mail address: pckb@mahendra.iacs.res.in (K. Bhattacharyya).

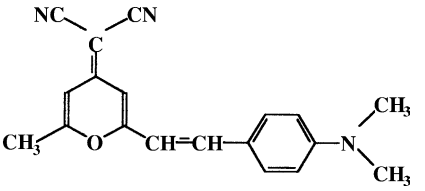
0009-2614/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.cplett.2003.10.091

many proteins inside the water pool of a reverse micelle using circular dichroism [21], NMR [22] and small angle X-ray and dynamic light scattering [23].

In a lipid vesicle, an aqueous volume ('water pool') is enclosed by a surfactant bilayer [24–28]. When many protein molecules are confined in one lipid vesicle, the system closely resembles a biological cell. In this Letter, we report on the solvation dynamics in a lipid, dimyristoylphosphatidylcholine (DMPC) containing many molecules of a protein, human serum albumin (HSA). HSA is an ellipsoidal molecule of dimensions 8 nm × 8 nm × 3 nm [25,29]. Considering the area of the head group of the lipid molecules is 0.48 nm^2 at a lipid:protein weight ratio of 1:1, the entire lipid surface is covered by the HSA molecules and there are about 80 HSA molecules per vesicle [24,25]. Incorporation of HSA within DMPC vesicles results in a marginal change in the radius of the vesicle (≈15 nm) [24].

An important feature of the lipid vesicles is the phase transition from gel to liquid crystalline phase below a phase transition temperature (T_c) [26–28]. The head group area of a vesicle increases from ≈40 $Å^2$ in the gel phase to ≈60 $Å^2$ in the liquid crystalline phase [28]. According to a MD simulation [27], in a vesicle each surfactant molecule is connected to 4–5 water molecules by hydrogen bonds and about 70% of the surfactants remain connected by hydrogen bond bridges mediated by water molecules. Maeda et al. [28] found that with rise in temperature the relative contribution of the collective O–H stretch band in the lipid (C_x) to that of pure water (C_w) decreases abruptly around the T_c of DMPC vesicles. The relative contribution of the gauche form of the alkyl chain to that of the trans form increases from the gel phase to the liquid crystalline phase [28].

We have earlier reported solvation dynamics of a dye, 4-(dicyanomethylene)-2-methyl-6(*p*-dimethylaminostyryl)-4H-pyran (DCM, Scheme 1) bound to HSA [7] in bulk water and in DMPC vesicle in the absence of protein [9]. In this Letter, we discuss how the solvation dynamics is affected when many HSA molecules are incorporated in the lipid vesicle.



Scheme 1. Structure of DCM.

2. Experimental

DCM (laser grade, Exciton), HSA (Fluka) and DMPC (Sigma) were used as received. The unilamellar vesicle was prepared by methanol injection method as described elsewhere [9]. The final concentration of DMPC was 1 mM. The protein was added to the liposome (DMPC) at a lipid:protein weight ratio of 1:1 and kept for 1 h at 34 °C (above $T_c \approx 23$ °C). Both the steady-state and time-resolved experiments were carried out at two different temperatures, 26 and 15 °C. All solutions were made using 50 mM Tris buffer solution (pH 7.4). The steady-state absorption and emission spectra were recorded in a Shimadzu UV-2401 spectrophotometer and a Perkin–Elmer 44B spectrofluorimeter, respectively. For lifetime measurements, the samples were excited at 405 nm using a picosecond diode (IBH Nanoled-07). The emission was collected at a magic angle polarization using a Hamamatsu MCP photomultiplier (2809U). The time correlated single photon counting (TCSPC) set up consists of an Ortec 935 QUAD CFD and a Tennelec TC 863 TAC. The data is collected with a PCA3 card (Oxford) as a multichannel analyzer. The typical FWHM of the system response is about 80 ps.

For anisotropy measurements, a polarizer was placed before the sample. The analyzer was rotated by 90° at regular intervals and the parallel ($I_\parallel$) and the perpendicular ($I_\perp$) components of the fluorescence decay were collected for equal times, alternatively. Then, $r(t)$ was calculated using the formula,

$$r(t) = \frac{I_\parallel(t) - GI_\perp(t)}{I_\parallel(t) - 2GI_\perp(t)}. \quad (1)$$

The G value of the set up was determined using a probe whose rotational relaxation time is very fast, e.g., nile red in methanol.

3. Results

3.1. Steady-state spectra

In the absence of HSA, with decrease in temperature, the emission maximum of DCM in a DMPC vesicle shows a very small blue shift from 613 nm at 26 °C (i.e., above T_c) to 611 nm at 15 °C (i.e., below T_c). On addition of HSA to DMPC the emission maximum of DCM exhibits a blue shift by 7 nm to 606 nm at 26 °C. At 15 °C, addition of HSA to DMPC causes a blue shift of the emission maximum of DCM by 16 nm to 595 nm. Thus in a vesicle containing protein the emission maximum of DCM at 15 °C is blue shifted by 11 nm from that at 26 °C.

Static emission spectrum is a good indicator of the solvation experienced by the fluorescent probe. The blue shift in the emission maximum indicates inadequate or incomplete solvation so that the system emits from a higher energy state. Thus the blue shift suggests that the solvation dynamics in DMPC vesicles is slowed down on addition of HSA at 26 °C. The large blue shift from 26 to 15 °C for a vesicle containing HSA indicates that the solvation dynamics is further slowed down when the temperature is lowered to 15 °C.

The absorption maximum of DCM is found to be at 450 nm for DMPC vesicle both at 15 and 26 °C. The absorption maximum of DCM in DMPC vesicle remains unchanged on addition of HSA. In bulk water, for DCM bound to HSA exhibits an absorption maximum at 480 nm. The significant red shift in the absorption maximum of DCM bound to HSA in bulk water gives rise to a smaller dynamic shift in the emission spectrum. This will be discussed in detail later.

3.2. Time-resolved studies: solvation dynamics

In this section, we discuss our results on solvation dynamics of DCM in DMPC vesicles containing 80 HSA molecules at two temperatures, one (26 °C) above and one (15 °C) below the gel transition temperature (23 °C) and compare the results with those in DMPC vesicles without the protein (HSA).

Solvation dynamics of DCM in DMPC vesicles is manifested in the wavelength dependence of emission decays of DCM. It is readily seen that at the blue end there is only a decay while at the red end a rise precedes the decay. At 15 °C (below T_c), in the presence of HSA, at 500 nm (blue end) the fluorescence decay is biexponential with two decay components – 300 ps (80%) and 2.10 ns (20%). However, at 700 nm (red end), there is a rise of 350 ps followed by a decay of 2.90 ns. In the case of DMPC vesicles without the protein the time constants are different from those in the presence of HSA. At 15 °C, in DMPC vesicle in the absence of HSA, at the blue end (500 nm), the, fluorescence decay of DCM is biexponential with two decay components of 300 ps (80%) and 1.60 ns (20%), while at the red end (700 nm), the decay of time constant 3.10 ns is preceded by a distinct rise with a time constant of 300 ps. Fig. 1 shows the fluorescence decays of DCM in DMPC vesicles containing protein at 15 °C.

The time-resolved emission spectra (TRES) were constructed following the method prescribed by Maroncelli and Fleming [31]. Finally, we calculated the solvation correlation function $C(t)$ defined as

$$C(t) = \frac{v(t) - v(\infty)}{v(0) - v(\infty)}, \tag{2}$$

where $v(0)$, $v(t)$ and $v(\infty)$ are the peak frequencies at time 0, t, and ∞, respectively. TRES of DCM in DMPC vesicles in the presence of protein at 15 °C

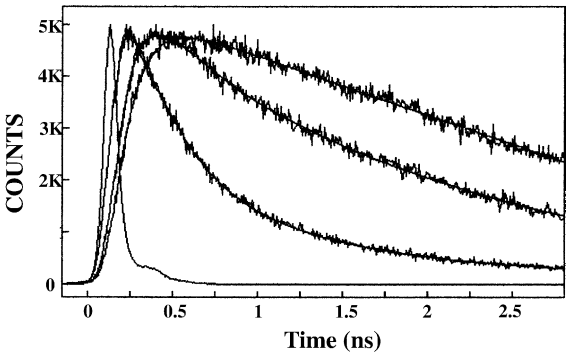


Fig. 1. Fluorescence decays of DCM in DMPC vesicles in the presence of HSA at 15 °C at (i) 500 nm, (ii) 580 nm and (iii) 700 nm.

are shown in Fig. 2. In the presence of protein, $C(t)$ of DCM in DMPC vesicles decays with an average solvation time ($\langle\tau_s\rangle$) of 1600 ± 100 ps at 26 °C and 3400 ± 200 ps at 15 °C. In the absence of protein, the average solvation time is found to be 950 ± 150 ps at 26 °C and 2000 ± 300 ps at 15 °C. Fig. 3 shows decay of $C(t)$ at 15 °C in DMPC vesicles in the presence and in the absence of the protein. Table 1 summarizes the decay characteristics of $C(t)$ under different conditions.

In a picosecond setup a significant portion of the total dynamic solvent shift is missed. The amount of solvation missed may be calculated using the procedure discussed by Fee and Maroncelli [34]. For this calculation, we used *n*-heptane as a non-polar solvent. In *n*-heptane the absorption and emission maxima of DCM are respectively, at 450 and 535 nm. We found that in the case of DCM bound to HSA in bulk water at 26 °C, about 10% of the total dynamic solvent shift

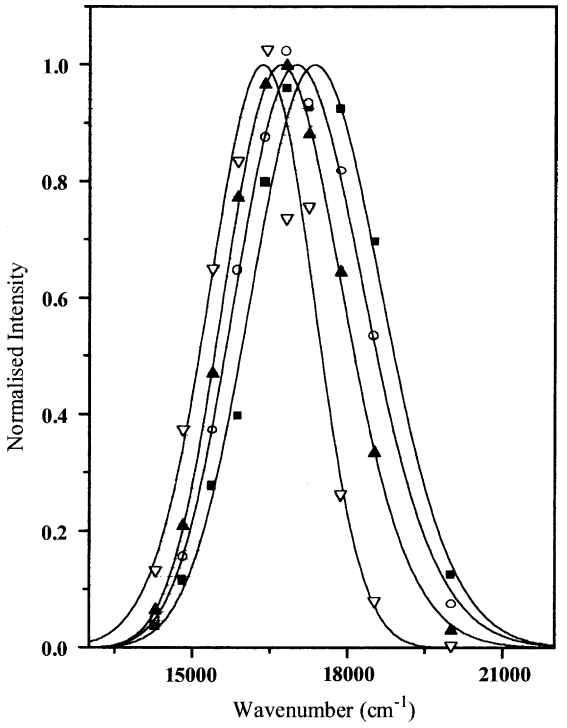


Fig. 2. Time-resolved emission spectra of DCM in DMPC vesicles in the presence of HSA at 15 °C at 0 ps (■), 300ps (○), 1800 ps (▲) and 22 000 ps (▽).

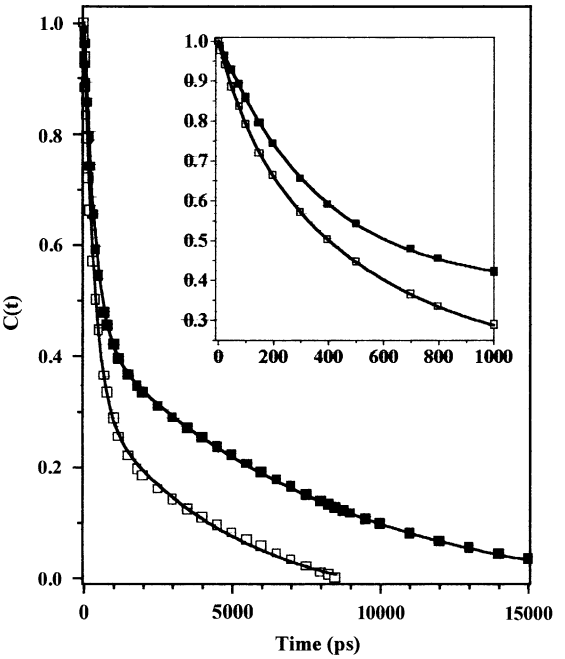


Fig. 3. Decay of response function, $C(t)$ of DCM in DMPC vesicles at 15 °C in the presence of HSA (■) and in the absence of HSA (□).The points denote the actual values of $C(t)$ and the solid line denotes the best fit to a biexponential decay. The initial parts of the decays of $C(t)$ are shown in the inset.

is missed in our picosecond set-up. When DCM is confined in a DMPC vesicle, about 50% of the total dynamic solvent shift is missed both in the presence and absence of HSA (Table 1).

Evidently, at both the temperatures, the average solvation time in the vesicle containing the protein is slower than that in a vesicle without protein. It is obvious that the presence of about 80 big protein molecules makes the motion of the water molecules more restricted compared to that in their absence. It may be recalled that in a recent work we have shown that the incorporation of polymer molecules inside the water pool of a microemulsion (reverse micelle) makes the solvation dynamics slower [10].

Solvation dynamics of DCM in DMPC vesicles in the presence of HSA is distinctly faster compared to that of DCM bound to HSA in bulk water. For HSA in bulk water, we [7] have earlier detected a component of 10 ns using DCM as a non-covalent probe while Buzády et al. [6] found

Table 1
Decay parameters of $C(t)$ of DCM in different systems

System	$\nu(0)$[a,b] (cm^{-1})	$\Delta\nu$[a,b] (cm^{-1})	$\Delta\nu$ (missed)[c] (cm^{-1})	a_1	τ_1 (ps)	a^2	τ_2 (ps)	$\langle\tau_s\rangle$[d] (ps)
DMPC, 26 °C	17 600	1300	50%	0.80	270	0.20	3600	950[e]
DMPC, 15 °C	17 500	1200	50%	0.70	350	0.30	6200	2000[e]
DMPC + HSA, 26 °C	17 350	1200	50%	0.50	340	0.50	2850	1600[e]
DMPC + HSA, 15 °C	17 350	1000	50%	0.60	340	0.40	7780	3400[e]
HSA, 26 °C	17 150	1000	10%	0.25	600	0.75	10 000	7650[e]

[a] Obtained from decay parameters.
[b] $\pm$150 cm^{-1}.
[c] As calculated by the Fee and Maroncelli procedure [34].
[d] $\langle\tau_s\rangle = a_1\tau_1 + a_2\tau_2$.
[e] $\pm$10%.

a component of 5 ns of using tryptophan as an intrinsic probe. Such a slow component (5–10 ns) of solvation dynamics is not observed when HSA is confined in the DMPC vesicle.

3.3. Fluorescence anisotropy decay

The fluorescence anisotropy decay of DCM in DMPC lipid and in HSA in bulk water exhibits a large residual anisotropy and a long decay. Fig. 4 shows the fluorescence anisotropy decay of DCM in DMPC lipids without protein at 26 °C. The anisotropy decays were fitted to a multi-exponential hindered rotor for which $r(t)$ is given by

$$r(t) = r(\infty) + [r(0) - r(\infty)]\left[a^{R}_{fast}\exp(-t/\tau^{R}_{fast}) + a^{R}_{slow}\exp(-t/\tau^{R}_{slow})\right]. \quad (3)$$

The results are tabulated in Table 2. The anisotropy decay may be analyzed using the 'wobbling-in-cone' model [35]. According to this model, the slow component (τ^{R}_{slow}) of anisotropy decay is related to the time constant (τ_D) for translational diffusion and that (τ_M) for overall tumbling time of the lipid as,

$$(\tau^{R}_{slow})^{-1} = (\tau_D)^{-1} + (\tau_M)^{-1}. \quad (4)$$

Since for the lipid, τ_M is very long (3500 ns), one may write $\tau_D \approx (\tau^{R}_{slow})$. Then the translational diffusion coefficient (D_T) is calculated using the relation $D_T = (r^2_M/6\tau_D)$ where for DMPC, $r_M \approx$ 15 nm [24].

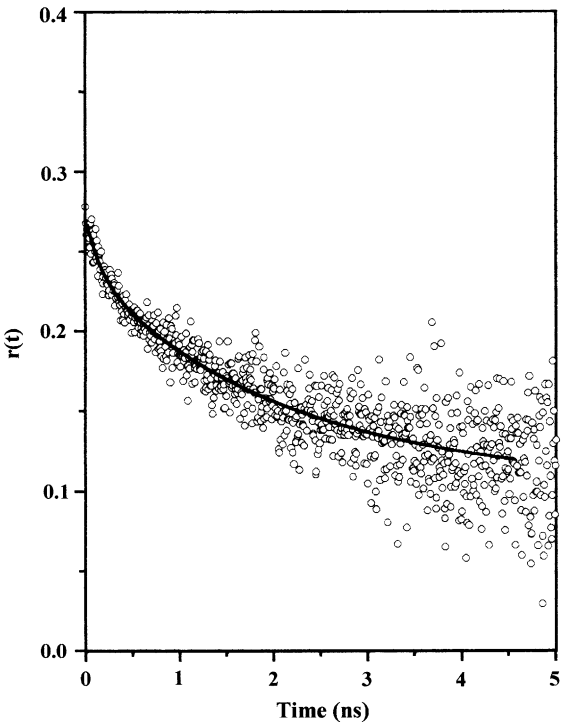


Fig. 4. Raw data along with fitted curve for fluorescence anisotropy decay of DCM in DMPC vesicles at 26 °C.

4. Discussion

It has been reported earlier that the dielectric relaxation in an aqueous solution of a protein exhibits components ranging from 10 to 100 ns [14,30,33]. The different components of dielectric

Table 2
Parameters of anisotropy decay of DCM (at 560 nm) in different systems using hindered rotor model

System	r_0	r_∞	a^R_{fast}	τ^R_{fast} (ns)	a^R_{slow}	τ^R_{slow} (ns)	D_T ($\times 10^9$ m^2 s^{-1})	d (nm)
DMPC, 26 °C	0.28	0.10	0.25	0.15	0.75	2.20	17.0	5.7
DMPC, 15 °C	0.28	0.11	0.25	0.14	0.75	6.80	5.5	4.7
DMPC + HSA, 26 °C	0.32	0.12	0.55	0.16	0.45	2.60	14.4	6.8
DMPC + HSA, 15 °C	0.30	0.14	0.50	0.16	0.50	8.14	4.6	5.6
HSA, 26 °C	0.27	0.10	0.10	0.44	0.90	15.70		

$d = (2D_T\langle\tau_s\rangle)^{1/2}$ where D_T is the coefficient for translational diffusion in the lipid vesicle and $\langle\tau_s\rangle$ is the average solvation time.

relaxation in aqueous solution of a protein has been classified as reorientation of bulk water (about 8 ps), relaxation of water molecules bound to the protein (20–200 ps) and overall tumbling of the protein (10–100 ns) [14,30,33]. According to the Stokes–Einstein relation, the overall tumbling time (τ_M) of a system of volume V is,

$$\tau_M = \eta V/k_B T, \tag{5}$$

where η is the viscosity of water. From this relation, τ_M is 25 ns for HSA in bulk water. The calculated τ_M for HSA in bulk water is in the range of the slow component of solvation dynamics [6,7,30]. Thus, the 10 ns component of solvation dynamics of DCM in HSA in bulk water earlier detected by us may be attributed to the overall rotation of the protein.

Inside the vesicle, the protein molecules can not tumble individually. Instead, the lipid vesicle may tumble as a whole. The radius r_M of the vesicle is about 15 nm [24]. Thus, the volume of the vesicle is about 140 times that of a single HSA molecule and τ_M is 3500 ns for the lipid vesicle. Evidently, tumbling of the lipid vesicle is too slow to occur within the lifetime (1–2 ns) of the fluorescent probe (DCM). It may be noted that Wand et al. [22] demonstrated earlier that when a protein is incorporated in the waterpool of a reverse micelle, tumbling of the protein is completely eliminated and this leads to line narrowing and a sharp NMR spectra. Thus, absence of the 10 ns component in the case of HSA in DMPC vesicles may be ascribed to the elimination of tumbling of individual protein molecules within the vesicle.

According to the Nandi–Bagchi model [14], the magnitude of the slow component of solvent relaxation depends on difference between the free energy of the bound (G^0_b) and the free water molecules (G^0_f), with $G^0_b < G^0_f$. In the limit of very high binding energy (i.e., $|\Delta G^0_{bf}|$), the slow component of solvation (τ_{slow}) is given by [1,14]

$$\tau_{slow} \approx k^{-1}_{bf}, \tag{6}$$

where, k_{bf} is the rate constant for bound-to-free interconversion,

$$k_{bf} = \left(\frac{k_B T}{h}\right)\exp\left(\frac{-(|\Delta G^0_{bf}| + \Delta G^*)}{RT}\right), \tag{7}$$

where ΔG^* is the activation energy for the conversion of free-to-bound water molecules. Using Eqs. (6) and (7) and the average solvation times ($\langle\tau_s\rangle$, Table 1), one may calculate the binding energy (ΔG^0_{bf}) in different systems. We used $\Delta G^* \approx 900$ cal mol^{-1}. In DMPC vesicles ΔG^0_{bf} is found to be −4.2 kcal mol^{-1} at 26 °C and −4.5 kcal mol^{-1} at 15 °C. In the presence of HSA in DMPC vesicles ΔG^0_{bf} is calculated to be −4.5 kcal mol^{-1} at 26 °C and −4.8 kcal mol^{-1} at 15 °C.

The overall dynamic emission spectral shift ($\Delta\nu$) of a solvation probe bound to a protein consists of many factors, e.g., motion of water molecules, tumbling of the protein, motion of the polar residues of the protein and self diffusion and may be expressed as

$$\Delta\nu = \sum_i \Delta\nu_i, \tag{8}$$

where $\Delta\nu_i$ denotes contribution of the ith component.

In HSA in bulk water fluorescence decay of DCM at the red end exhibits a rise of ≈50 ps [7]. However, in the case of HSA confined in DMPC vesicles the rise time ≈300 ps. This indicates that the initial part of solvation dynamics become

slower when HSA is confined in DMPC vesicles. This results in a blue shift of $\nu(0)$ for HSA in DMPC vesicles compared to HSA in bulk water. At 26 °C, $\nu(0)$ is blue shifted by 200 cm^{-1} from 17 150 cm^{-1} in HSA in bulk water to 17 350 cm^{-1} in HSA confined in DMPC vesicle. Thus though the very slow component (10 ns) of solvation dynamics of DCM bound to HSA in free water arising from tumbling is completely eliminated the total dynamic spectral shift is larger for HSA is entrapped inside the vesicles.

Another possible source of the nanosecond component of solvation dynamics is the self-diffusion of the probe. Since DCM is insoluble in bulk water, it is likely to reside at or near the membrane surface and not in the core of the water pool. On electronic excitation the DCM molecule become highly polar and more soluble in water. Thus after excitation all the DCM molecules move towards the core of the water pool.

The translational diffusion coefficient (D_T) of the probe in the lipid vesicle are given in Table 2. One may calculate the distance, $d = (2D_T\langle\tau_s\rangle)^{1/2}$ traversed by the probe within a period corresponding to the average solvation time, $\langle\tau_s\rangle$. For the different systems, d is found to be 4.7–6.8 nm (Table 2). This is less than the radius of the vesicle.

The full width at half maxima (Γ) of the TRES of DCM is found to vary with time. In DMPC vesicles, Γ exhibits a fast initial increase followed by a decay. The time constant of the decay in Γ is 1000 ps at 26 °C and 1200 ps at 15 °C (Fig. 5). In the presence of HSA in DMPC vesicles, Γ exhibits a decay with a time constant, 450 ps at 26 °C (Fig. 5) while at 15 °C, the overall decrease in Γ is only ~10%. Evidently, the time constants of decay in Γ are different from those of solvation dynamics.

The change in $\Gamma(t)$ reflects the change in local solvation environment. In the case bulk liquids, Maroncelli et al. [32] reported a fast growth in Γ (~15%) followed by a slight (~5%) decay. A large spectral width, Γ corresponds to a broad distribution of solvent environments for different probe molecules. A small Γ on the other hand, indicates a narrow distribution or a uniform environment.

The decay in Γ may arise from the self-diffusion of probe as follows. At $t = 0$, DCM molecules in different environments at the membrane surface are excited simultaneously. Due to the superposition of the emission spectra of DCM in different environments at short times, the spectral width (Γ) is very large. Because of self-diffusion at a sufficiently long time, all the DCM molecules reach the core of the water pool and all of them experience a more or less uniform environment. This results in a small Γ. However, as the time constants of decay in Γ are not same as those of solvation dynamics, self-diffusion is not the sole cause of slow solvation dynamics, in this case.

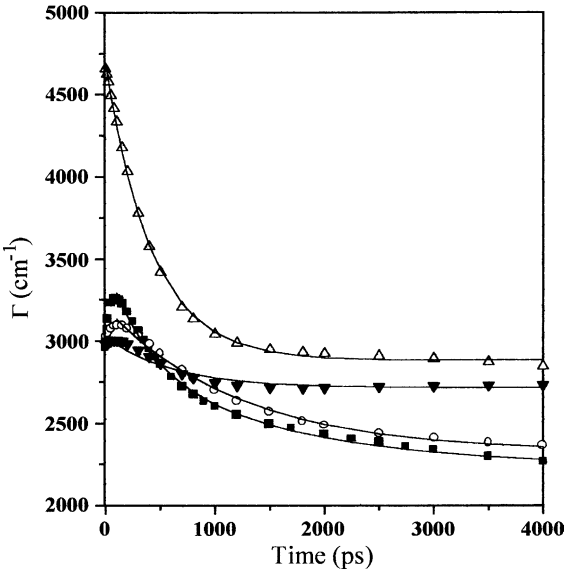


Fig. 5. The plot of full width at half maximum (Γ) of the TRES against time of DCM in DMPC vesicles both in the presence of HSA at 26 °C (△) and 15 °C (▼) and in the absence of HSA at 26 °C (■) and 15 °C (○). The points denote the actual values of Γ at different times and the solid line denotes the best fit to an exponential decay.

Acknowledgements

Thanks are due to Council of Scientific and Industrial Research (CSIR), and Department of Science and Technology (DST), Government of India for generous research grants. P.D. thanks CSIR for awarding fellowship and P.S. thanks DST for awarding fellowship. K.B. thanks Professor B. Bagchi and Dr. P.K. Das for many stimulating discussions.

References

[1] S.K. Pal, J. Peon, B. Bagchi, A.H. Zewail, J. Phys. Chem. B 106 (2002) 12376.
[2] K. Bhattacharyya, Ace. Chem. Res. 36 (2003) 95.
[3] N. Nandi, K. Bhattacharyya, B. Bagchi, Chem. Rev. 100 (2000) 2013.
[4] X.J. Jordanides, M.J. Lang, X. Song, G.R. Fleming, J. Phys. Chem. B 103 (1999) 7995.
[5] S.K. Pal, J. Peon, A.H. Zewail, Proc. Natl. Acad. Sci. (USA) 99 (2002) 1763.
[6] A. Buzády, J. Erostyák, B. Somogyi, Biophys. Chem. 88 (2000) 153.
[7] S.K. Pal, D. Mandal, D. Sukul, S. Sen, K. Bhattacharyya, J. Phys. Chem. B 105 (2001) 1438.
[8] J. Sykora, P. Kapusta, V. Fidler, M. Hof, Langmuir 18 (2002) 571.
[9] S.K. Pal, D. Sukul, D. Mandal, K. Bhattacharyya, J. Phys. Chem. B 104 (2000) 4529.
[10] S. Sen, P. Dutta, D. Sukul, K. Bhattacharyya, J. Phys. Chem. A 106 (2002) 6017.
[11] E.B. Brauns, M.L. Madaras, R.S. Coleman, C.J. Murphy, M.A. Berg, Phys. Rev. Lett. 88 (2002) 158101-1.
[12] L. Frauchiger, H. Shirota, K.E. Uhrich, E.W. Castner Jr., J. Phys. Chem. B 106 (2002) 7463.
[13] S. Sen, D. Sukul, P. Dutta, K. Bhattacharyya, J. Phys. Chem. B 106 (2002) 3763.
[14] N. Nandi, B. Bagchi, J. Phys. Chem. B 101 (1997) 10954.
[15] S. Balasubramanian, S. Pal, B. Bagchi, Phys. Rev. Lett. 89 (2002) 115505-1.
[16] S. Pal, S. Balasubramanian, B. Bagchi, J. Phys. Chem. B 107 (2003) 5194.
[17] S. Senapati, A. Chandra, J. Chem. Phys. 111 (1999) 1223.
[18] J. Faeder, M.V. Albert, B.M. Ladanyi, Langmuir 19 (2003) 2514.
[19] D. Michael, I. Benjamin, J. Chem. Phys. 114 (2001) 2817.
[20] D.A. Panatano, D. Laria, J. Phys. Chem. B 107 (2003) 2971.
[21] P.L. Luisi, Angew. Chem. 24 (1985) 439.
[22] A.J. Wand, M.R. Ehrhardt, P.F. Flynn, Proc. Natl. Acad. Sci. (USA) 95 (1998) 15299.
[23] G. Cassin, Y. Duda, M. Holovko, J.P. Badiali, M.P. Pileni, J. Chem. Phys. 107 (1997) 2683.
[24] I. Bardos-Nagy, R. Galántai, M. Laberge, J. Fidy, Langmuir 19 (2003) 146.
[25] R. Bartucci, M. Pantusa, D. Marsh, L. Sportelli, Biochim. Biophys. Acta 1564 (2002) 237.
[26] H. Heller, M. Schaefer, K. Schulten, J. Phys. Chem. 97 (1993) 8343.
[27] M. Pasenkiewicz-Gierula, V. Takaoka, H. Miyagawa, K. Kitamura, A. Kusumi, J. Phys. Chem. A 101 (1997) 3677.
[28] Y. Maeda, K. Kakinoki, H. Kitano, J. Raman Spectrosc. 27 (1996) 425.
[29] S. Sugio, A. Kashima, S. Mochizuki, M. Noda, K. Kobayashi, Prot. Engg. 12 (1999) 439.
[30] T. Hushcha, A. Peytcheva, U. Kaatze, J. Phys.: Condens. Matter 14 (2002) 9461.
[31] M. Maroncelli, G.R. Fleming, J. Chem. Phys. 86 (1987) 6221.
[32] M.L. Horng, J.A. Gardecki, A. Papazyan, M. Maroncelli, J. Phys. Chem. 99 (1995) 17311.
[33] E.H. Grant, R.J. Sheppard, G.P. South, Dielectric Behavior of Biological Molecules, Clarendon, Oxford, 1978.
[34] R.S. Fee, M. Maroncelli, Chem. Phys. 183 (1994) 235.
[35] S. Sen, D. Sukul, P. Dutta, K. Bhattacharyya, J. Phys. Chem. A 103 (2001) 7495.