Study of Organized Media Using Time-Resolved Fluorescence Spectroscopy

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Many photophysical processes which occur on an ultrafast time scale in ordinary liquids become significantly retarded in organized assemblies, by two to three orders of magnitude. Recently many groups have applied ultrafast laser spectroscopy and theoretical methods to elucidate this dramatic phenomenon. Although the implications of this phenomenon in biology and chemistry are not yet fully understood, it has been demonstrated that ultrafast time-resolved fluorescence spectroscopy is a very powerful tool to study the microscopic properties of the organized assemblies and that water or other liquids confined inside these assemblies are fundamentally different from the corresponding liquid in bulk.

KEY WORDS: Organized media; time-resolved fluorescence spectroscopy.

INTRODUCTION

One of the long-standing goals of chemistry is to unravel the structure, dynamics, and reactivity in complex biological systems. Most natural and biological systems consist of self-organized molecular assemblies [1–6]. The organized assemblies are molecular aggregates which are formed spontaneously in nature and are held together by weak intermolecular attractions. Examples of such assemblies are abundant in nature, e.g., the DNA double helix, enzyme–substrate complex, and micellar aggregates. Study of such assemblies constitutes an emerging frontier of contemporary research because of their implications in biology, catalysis, and advanced materials. The chemistry of a species confined inside an organized assembly is often unusual and is drastically different from that in any bulk fluid. Inside a small region, of dimension a few nanometers, around the confined probe, the local properties such as polarity, viscosity, and pH are significantly different from those in a bulk liquid. Further, the local interactions (e.g., hydrogen bonding with a macromolecule) seriously hinder the molecular motions.

Among the various techniques for studying dynamics in organized assemblies, time-resolved fluorescence spectroscopy stands out for its superior time resolution down to femtosecond time scale. In the present article, we discuss some recent applications of time-resolved fluorescence spectroscopy to study chemical dynamics in the nanoenvironments inside various organized assemblies.

Among the various organized assemblies, the most popular are the micelles, reverse micelles and microemulsions, cyclodextrin cavities, lipid vesicles, proteins, and DNA. Structures of these systems are described in many recent reviews [4,5]. In recent years, many groups have carried out computer simulations to understand the structure and function of different organized assemblies [6]. In Section 2, we give a very brief outline of the structure of various organized assemblies. In Section 3, we discuss the dynamics of various ultrafast processes in organized assemblies.

By far the most interesting finding of the recent ultrafast time-resolved studies in organized assemblies is the detection of a component of solvation dynamics which is two to three orders of magnitude slower than that in a bulk polar liquid (water, acetonitrile or formamide).

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Since solvation plays a fundamental role in many biological charge or proton transfer processes, the slow solvation dynamics has profound chemical and biological implications. Though the effect of the slow component of solvation dynamics on various biological processes has not yet been understood fully, dynamics of many processes is observed to be dramatically retarded in many organized assemblies. We discuss some of these results.

STRUCTURE OF VARIOUS ORGANIZED ASSEMBLIES

Micelles

Micelles are nearly spherical aggregates of surfactants formed in water or other highly polar solvents. Recent small-angle neutron scattering (SANS) studies indicate that the central region or core of a micelle is essentially "dry" and contains only the hydrocarbon chains [7]. The core is surrounded by the Stern layer, which consists of the ionic head groups, bound counter ions and water molecules [7].

Reverse Micelles and Microemulsions

The reverse micelles (or microemulsions) are elegant examples of confined water molecules [8]. The reverse micelles refer to the aggregates of surfactants formed in a nonpolar solvent, in which the polar head groups of the surfactants point inward and the hydrocarbon chains project outward into a nonpolar solvent. The reverse micelles possess a remarkable ability to encapsulate fairly large amount of water to form what is known as a microemulsion. For a reverse micelle containing the surfactant, AOT (aerosol-OT, dioctyl sulfosuccinate, sodium salt) up to 50 water molecules per molecule of the surfactant can be trapped in this manner. Such a surfactant coated nanometer-sized water droplet dispersed in a nonpolar liquid is known as a "water pool" [8]. The radius (r_w) of the water pool varies linearly with the water-to-surfactant mole ratio, w_0. For AOT, r_w is approximately equal to $2w_0$ (Å) in n-heptane [8c].

Polymer–Surfactant Aggregates

In aqueous solutions, many polymers form relatively well-defined aggregates with selected surfactants [9]. According to the "necklace" model, the polymer–surfactant aggregates consist of a series of spherical micelles surrounded by polymer segments and connected by polymer strands [9]. In such a polymer–surfactant aggregate, the structure and composition of the surface region of the micelles are seriously perturbed because of the surrounding polymer chain.

Lipid Vesicles. A lipid vesicle resembles most closely a biological cell. A vesicle is an aqueous volume ("water pool") entirely enclosed by a membrane and dispersed in bulk water [10]. The membrane is basically a bilayer of lipid molecules. In the case of unilamellar vesicles (radius, $\approx$50 nm), there is only one such bilayer while a multilamellar vesicle (radius, >250 nm) consists of several concentric bilayers. Unilamellar vesicles can be produced by breaking the multilamellar vesicles through sonication or by rapid injection of a concentrated ethanolic solution of the lipid to a buffered aqueous medium.

TIME-RESOLVED STUDIES OF ULTRAFAST PROCESSES IN ORGANIZED MEDIA

In this section we discuss how different ultrafast processes are affected in various organized media. For a non-covalently attached fluorescent probe, there is always a slight uncertainity about its exact location as it undergoes excursion over a region of space within its excited-state lifetime. For ordinary organic molecules in water, the diffusion coefficient (D) is 0.05 Å^2 ps^{-1} [8e]. Since the mean square displacement $\langle z^2 \rangle = 2D \langle t \rangle$, the probe travels a distance of $\approx$10 Å in 1 ns [8e]. Thus a probe with lifetime $\approx$1 ns reports the property of a region of radius of the order of 10 Å. Evidently, if the probe is covalently attached to a selected site within an organized assembly the slight uncertainty about its position is eliminated.

Solvation Dynamics

Solvation refers to the interaction between a solute molecule and the surrounding solvent molecules. The dynamics of this process, i.e., how quickly the solvent dipoles rearrange around an instantaneously created charge (electron) or dipole, is known as solvation dynamics. The creation of a dipole may be done most conveniently as follows. There are many molecules whose dipole moment is zero or very low in the ground electronic state, while it is very large in the electronically excited state [11]. When such a solute molecule in a solution is excited by an ultrashort light pulse, the excited molecule serves as an instantaneously created dipole. Initially (at $t = 0$), the solvent dipoles remain randomly oriented around the solute dipole and the energy of the system remains high. With an increase in time, the solvent dipoles gradually reorient and the energy of the system decreases.

Thus, if one records the emission energy of the excited dipole as a function of time, it is observed that with increase in time the emission maximum shifts to a lower energy, i.e., toward a longer wavelength. This phenomenon is known as time-dependent Stokes shift. The solvation dynamics is monitored by the decay of the solvation time correlation function $C(t)$, which is defined as

$$C(t) = \frac{v(t) - v(\infty)}{v(0) - v(\infty)} \tag{1}$$

where $v(0)$, $v(t)$, and $v(\infty)$ denote the observed emission energies (frequencies) at time 0, t, and infinity, respectively. If the decay of $C(t)$ is single exponential, e.g., $C(t) = \exp(-t/\tau_s)$, the time constant (τ_s) of the decay is defined as the solvation time. If the decay of $C(t)$ is multiexponential, i.e., $C(t) = \Sigma\, a_i \exp(-t/\tau_i)$, one uses the average solvation time $\langle \tau_s \rangle = \Sigma\, a_i \tau_i$.

According to the continuum theory, the solvation time, τ_s, is $(\varepsilon_\infty/\varepsilon_0)\tau_D$, where ε_∞ and ε_0 are the high-frequency and static dielectric constant of the solvent, respectively, and τ_D is its dielectric relaxation time [4]. For water, τ_D is 8.3 ps [12], while ε_∞ and ε_0 are about 5 and 80, respectively. Thus according to the continuum theory, the solvation time of pure water is about 0.6 ps. Actual experimental results are very close to this. In the first study of solvation dynamics in water, Barbara *et al.* reported that the solvation dynamics is biexponential, with two components, of 0.16 ps (33%) and 1.2 ps (67%), for coumarin 343 (C343) [13a]. Later, using better time resolution, Fleming *et al.* detected a Gaussian component of frequency 38.5 ps^{-1} and a biexponential decay with time constants of 126 and 880 fs, respectively [13b]. Several other studies on solvation dynamics of large dye molecules as well as electrons in water demonstrate that the dynamics of solvation in water is, indeed, ultrafast and becomes complete within 1 ps [13–15]. Bagchi *et al.* attributed the ultrafast components of solvation dynamics in water to the intermolecular vibration and libration modes of water [14a].

Solvation Dynamics in Organized Media

In aqueous solutions, proteins and many other organized assemblies exhibit a component of dielectric relaxation on the 10-ns time scale, in addition to the 10-ps component [16,17]. The 10-ns component of dielectric relaxation gives rise to a component of solvation dynamics, three orders of magnitude slower compared to bulk water. The slow component usually constitutes 10–30% of the total decay of $C(t)$. Recently several groups have detected such a dramatically slow component of solvation

dynamics in many organized assemblies. We discuss these results in the following section.

Cyclodextrin. Cyclodextrins (CD) are cyclic polymers of the sugar α-amylose. They are highly soluble in water and can encapsulate suitably sized organic molecules along with solvent molecules [20]. The structure of guest–host complexes involving cyclodextrins have wide implications and have been studied using many experimental techniques and computer simulations [20]. Vajda *et al.* first studied solvation dynamics of C480 and C460 in a γ-CD cavity, using time-dependent Stokes shift [21]. They found that the initial component of solvation in γ-CD is similar to that in bulk water. However, at longer times, the solvent response in γ-CD shows a component which is nearly three orders of magnitude slower. In γ-CD the slow relaxation is described by three components, of 13, 109, and 1200 ps, for C480 [21]. Nandi and Bagchi [22] carried out a theoretical analysis of the solvation dynamics in γ-CD using a multishell continuum model (MSCM) and molecular hydrodynamic theory (MHT). They ascribed the slow component to the freezing of the translational motion of the water molecules in the γ-CD cavity.

Micelles. Bhattacharyya *et al.* studied solvation dynamics in neutral [Triton X-100 (TX)], cationic [cetyl trimethyl ammonium bromide (CTAB)], and anionic [sodium dodecyl sulfate (SDS)] micelles using several probes [23]. It was observed that in the micelles the average solvation times is three orders of magnitude slower than that in bulk water and is only slightly faster than the longest component of solvation dynamics in γ-CD [21].

For all the probes, it is observed that the solvation times for the neutral micelle TX is slower than that in the cationic (CTAB) or anionic (SDS) micelles. The difference in the solvation times in the three micelles may be explained in terms of the differences in their structures [7]. The hydrated shell for TX (25 Å) is thicker than that for SDS and CTAB (6–9 Å). Thus for SDS and CTAB, the probe remains exposed to bulk water, while for TX, it remains well shielded from bulk water. This may be the cause for the slower solvation dynamics in the case of TX.

Confined Water Pool of Microemulsions. Many groups have studied solvation dynamics in a confined water pool of microemulsions [24–30]. Bhattacharyya *et al.* studied solvation dynamics of three probes C480 [24a], 4-AP [24b], and DCM [24c], in AOT/*n*-heptane/water microemulsions. A distinct rise time was observed at the red end of the emission spectrum for all of them. This clearly indicates that in the microemulsions, solvation dynamics occurs on the nanosecond time scale. In a small

water pool ($w_0 = 4$), the solvation time is 8 ns, while in a very large water pool ($w_0 = 32$) the response is bimodal, with a fast component of 1.7 ns and a slower component of 12 ns [24a]. Bright *et al.* studied the solvation dynamics of acrylodan-labeled human serum albumin in AOT microemulsions using phase fluorimetry [27]. They found that the solvation time is about 8 ns in a small water pool ($w_0 = 2$) and 2 ns in a large water pool ($w_0 = 8$). The appearance of the 2-ns component in the large water pools indicates that even in large water pools of the microemulsions, the water molecules are about 6000 times slower compared to bulk water.

A semiquantitative explanation of the nanosecond component may be as follows. The static polarity or the dielectric constant of the water pool of AOT microemulsions may be estimated from the position of the emission maximum of the probes [24]. For all the probes, the water pool resembles an alcohol-like environment, with an effective dielectric constant of 30–40. One may make the reasonable assumption that the infinite-frequency dielectric constant of water in the water pool of the microemulsions is the same as that of ordinary water, i.e., 5. Then using the experimentally determined dielectric relaxation time of the microemulsion of about 10 ns [31], the solvent relaxation time is calculated to be $(5/30) \times 10 \approx 1.67$ ns. This is close to the observed 2-ns component of solvation dynamics in AOT microemulsions.

One might argue that the nanosecond dynamics observed in the water pool is not due to the slower water molecules but is because of the solvation of the probe by the Na^+-counterions present in the water pool for the AOT microemulsions. To eliminate the role of ions, several groups have studied solvation dynamics in microemulsions consisting of neutral surfactants [29]. Mandal *et al.* studied solvation dynamics of 4-AP in a microemulsion containing a neutral surfactant, TX, where no ions are present in the water pool [29a]. The TX microemulsion exhibits nanosecond solvation dynamics. This shows that the ionic solvation dynamics has little or no role in the solvation dynamics observed in the water pool.

Levinger *et al.* studied the solvation dynamics of a charged dye, coumarin 343 (C343), in lecithin and AOT microemulsions using femtosecond upconversion [25]. For lecithin microemulsions, they observed that the solvent relaxation is very slow and does not become complete within 477 ps [25a]. This is consistent with the nanosecond dynamics detected by Bright *et al.* [27] and by Bhattacharyya *et al.* [24]. For Na-AOT, the solvation dynamics reported by Levinger *et al.* [25b] for the charged probe C343 is much faster than that reported by previous workers [24,27].

More recently, several groups studied solvation dynamics of nonaqueous solvents such as formamide [28a], acetonitrile [28b], and methanol [28b] in AOT microemulsions. Shirota and Horie observed that in AOT microemulsions, the solvation dynamics of acetonitrile and methanol is nonexponential and each is about 1000 times slower than that in the pure solvents [28b]. They attributed the nonexponential decay to the inherent inhomogeneous nature of the solvent pools.

Lipids. In a lipid, 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, ≈ 50 nm) than those of the water pool of the reverse micelles (radius, < 10 nm). The state of solvation of a fluorescent probe in the ground state in unilamellar and multilamellar vesicles is often studied by red-edge excitation spectroscopy (REES) [31]. REES is based on the fact that in such an inhomogeneous medium, the probe molecules in different regions remain in different states of solvation and, as a result, exhibit different absorption and emission characteristics. This gives rise to the gradual shift in the emission maximum as the wavelength of excitation is changed. Evidently, REES arises as a result of the different extent of solvation of the probe molecules in the ground state and gives no information on the excited-state relaxation properties inside the vesicles. Several groups have addressed the important issue of the dynamics of water molecules inside the water pool of unilamellar vesicles [32,33]. Hof *et al.* used time-resolved fluorescence and NMR to study solvent relaxation in many lipids [32]. Bhattacharyya *et al.* observed that irrespective of the probe or the lipid, the solvation dynamics in lipid vesicles is biexponential with one component in the range 100–600 ps and another of 1–11 ns [33a–33c]. This result is consistent with the dielectric relaxation studies of lipids [33d,33e]. This is also very similar to the solvation dynamics of the same probes in the large water pools of AOT microemulsions. Since the solvation dynamics in bulk water is much faster, the slow solvation dynamics clearly demonstrates the restricted motion of the water molecules in the inner water pool of the vesicles.

Proteins. Among all the organized media, study of the water molecules in the immediate vicinity of a protein molecule is most relevant in understanding the behavior of biological water. Very recently many groups have studied ultrafast processes in various protein environments [34–38]. Pierce and Boxer [33a] and Bashkin *et al.* [33b] studied solvation dynamics in protein environments using dynamic Stokes shift and reported solvation times of the order of 10 ns. Most recently, Fleming *et al.* [35a] and Beck *et al.* [35b] employed three-photon echo peak shift

(3PEPS) and transient grating spectroscopy, respectively, to study the dynamic properties of the protein environment. Before discussing their results, it should be emphasized here that in the case of a protein, because of the presence of many charged side groups, even the definition of the dielectric constant is not straightforward. Recent simulations indicate that the static dielectric constant of a protein varies with position and depends quite strongly on what is taken into account explicitly in the model [38,39]. Because of these difficulties, Fleming *et al.* considered several dielectric continuum models to explain the solvation dynamics of eosin bound to lysozyme in aqueous solutions [35a]. They detected a slow component of 530 ps, which is absent in the case of free eosin in bulk water. This demonstrates that the motion of the water molecules in the immediate vicinity of the protein is highly constrained. Bhattacharyya *et al.* have recently studied solvation dynamics of DCM bound to human serum albumin and observed that the solvation dynamics is biexponential, with one component of 600 ps and another component of 10 ns [37]. Hochstrasser *et al.* used the femtosecond pump-dump method to study the solvation dynamics in the ground and excited state of a coumarin dye in aqueous protein solution [36]. In their ultrafast setup, they detected only a bulk water-like component and did not observe any long component as detected by Fleming *et al.* [35a] and Bhattacharyya *et al.* [37].

DNA. Most probes which intercalate in DNA do not exhibit solvation dynamics. Very recently, Brauns *et al.* introduced solvation dynamics as a technique to study the microenvironment within DNA [40]. For this purpose, they attached a probe C480 unit covalently to DNA. Such a covalently attached C480 probe can examine the local dynamics within a specified region of DNA. It is observed that in DNA the covalently attached C480 exhibits a slow biexponential solvation dynamics, with two components of decay, of 300 ps (47%) and 13.4 ns (53%). These components are very similar to those reported by Bhattacharyya *et al.* in aqueous protein solutions [37].

Polymer Hydrogels, Sol-Gel Matrixes, and Nanoparticles. Several macroscopically solid materials trap a large amount of water. The most common examples are the microporous polymer hydrogel and sol-gel matrix. The microporous synthetic polymer hydrogels refer to certain polymers which are inherently insoluble in water but can entrap a considerable amount of water within their polymer networks [41]. Among the various types of hydrogels, polyacrylamide (PAA) hydrogel is most suitable for photophysical studies, as it is optically transparent over a wide range of concentrations of the monomer and the cross-linker.

The bulk viscosity of most polymers and, particularly, semirigid hydrogels is very high. Thus at first sight, one expects a very slow relaxation of the water molecules in the polymer matrices and the polymer hydrogels. Contrary to this expectation, Bhattacharyya *et al.* observed that in the polyacrylamide hydrogel, both solvation dynamics and fluorescence depolarization are extremely fast and occur in <50-ps time scale [42]. The surprisingly fast solvation and rotational dynamics (which causes fluorescence depolarization) of small probe molecules in hydrogels have been attributed to the extensively porous structure of the hydrogels. In a hydrogel, these pores are so big that even large biomolecules pass through them quite easily. The high mobility inside hydrogels is also reported by other workers. Claudia-Marchi *et al.* found that for titania gels at the sol-gel transition point (when the bulk viscosity increases sharply), the emission anisotropy does not change perceptibly [43]. Thus the microviscosity of the gel is low in spite of the high bulk viscosity. NMR [44a] and simulation [44b] studies indicate that the diffusion coefficient of water molecules in polymer hydrogels is not appreciably slow compared to ordinary water and is lower at most by a factor of 2 than that in ordinary water. A similar high mobility in polyacrylamide hydrogel has been observed in a fluorescence microscopy study by Moerner *et al.* [45]. They found that almost all (98%) of the probe molecules (Nile red) remain highly mobile in polyacrylamide hydrogels.

The inorganic sol-gel composite obtained from the hydrolysis of tetra-alkyl orthosilicate acts as a good host for many biological materials [46]. Many enzymes can be encapsulated in a biologically active form for a very long period in a sol-gel glass. Sol-gel glasses doped with biomolecules have potential applications as chemical sensors. It is obviously interesting to find out the dynamics occurring in such an interesting material. Bright *et al.* studied relaxation of acrylodan labeled BSA in a sol-gel matrix using phase fluorimetry and reported that the protein molecule remains highly mobile in this matrix [47]. Pant and Levinger employed femtosecond upconversion to study the solvation dynamics of C343 adsorbed to zirconia particles in water and observed that the solvation time in zirconia particles is 0.24 ps, which is similar to that in bulk water [48]. Fourkas and co-workers carried out a optical Kerr effect (OKE) study of methyl iodide and acetonitrile in sol-gel glasses of different pore sizes [49]. They observed that for both the liquids the decay of the OKE signal in a sol-gel glass is multiexponential with a major component similar to that in bulk liquid and an additional component which is about four times slower. Most recently, Bhattacharyya *et al.* have studied solvation dynamics of water molecules trapped in tetra-ethyl ortho-

silicate (TEOS) sol-gel matrix using C480 as a probe [49]. They found that even in the rigid sol-gel matrix, the solvation dynamics is very fast. The average solvation time is 220 ± 30 ps. This is about 200 times slower than the slow component of solvation of the same probe in bulk water. The rotational relaxation study suggests that the probe C480 remains highly mobile within the sol-gel matrix.

Origin of the Slow Decay in Organized Assemblies

The origin of the almost universally observed slow component of solvation dynamics in organized assemblies is yet to be understood fully. It is obvious that the slow dynamics in the nanosecond time scale is too slow to be described in terms of any vibrational mode. The nanosecond time scale corresponds to a chemical process. Nandi and Bagchi showed that the dielectric relaxation properties of aqueous protein solutions may be explained if one assumes a dynamic exchange between the so-called "free" and "bound" water molecules [16]. The bound water molecules are those which are bound to the protein molecules quite strongly by one or more hydrogen bonds. Their motion is coupled with that of the large biomolecule, and hence, they are very slow. The dynamic exchange mechanism is consistent with ^{17}O NMR relaxation dispersion [18] and nuclear Overhauser effect (NOE) studies [19]. Fischer *et al.* carried out a reaction path calculation for the rotation of a water molecule bound to a protein [51]. Their calculations indicate that the motion of the bound water molecules involves the rupture of two water hydrogen bonds.

Recently several groups have carried out computer simulations to understand the static and dynamic properties of water molecules confined in various organized assemblies. Balasubramanian and Bagchi carried out a molecular dynamics simulation to elucidate the solvation dynamics in micelles [53c]. Their simulations clearly reveal a fast component similar to bulk water and a two to three orders of magnitude slower component of relaxation. They studied relaxation dynamics at different regions of the micelles.

In an interesting MD simulation, Senapathy and Chandra modeled the water pool of a microemulsion as a Stockmeyer liquid confined in a smooth spherical cavity [53a]. This model is rather oversimplified, as it ignores the presence of any ions (both the ionic surfactants and the counterions). They found that the dielectric constant increases as the cavity size (size of water pool) increases and solvation dynamics slows down nearly five times on confinement [53a]. Thus, this simulation reproduces at least qualitatively some of the features of solvation

dynamics in microemulsion. More recently, Faeder and Ladanyi used a model with a more realistic representation of the AOT and taking into account many experimental quantities explicitly [53b]. In this model, the surfactants were considered to be a pair of atomic ions and the interaction potential as a sum of point charge and Lennard–Jonnes terms. They used the SPC/E model of water for both charge distribution and Lennard–Jonnes interaction. Faeder and Ladanyi reported that at a low w_0 ($=1$), nearly all the counterions remain bound to the surfactant. With a rise in w_0, solvent-separated ion pairs are formed, and an increasing amount of the counterions become detached from the surfactant. By $w_0 = 10$, the surface lattice (formed by surfactants, counter ions, and trapped water molecules) was found to be completely destroyed and density of water at the interface exceeds that in bulk. Surprisingly, the preliminary results reported by Faeder and Ladanyi do not indicate slowing-down of the solvation dynamics in the water pool. This is apparently in conflict with the experimental results. It is possible that Faeder and Ladanyi missed the slow relaxation component on a nanosecond time scale, as the simulation was not carried out long enough to detect slow dynamics [53b].

Excited-State Proton Transfer (ESPT) Processes

Acid–base properties of many organic molecules in the excited state differ markedly from those in the ground state. Many of them become very strongly acidic in the excited state and readily undergo deprotonation as

$$ROH^* \rightarrow RO^{-*} + H^+$$

Solvation of the ejected proton and the anion is a precondition for this process. When a probe is transferred from bulk water to the interior of an organized medium, the solvation of proton becomes markedly inhibited because of two factors. First, an adequate number of water molecules may not be available inside an organized assembly. Second, the solvation process becomes too slow compared to bulk water as explained in the previous section.

In ordinary water, excited-state proton transfer (ESPT) of 1-naphthol occurs in 35 ps as detected by the decay of the emission of neutral 1-naphthol (at 360 nm) and the rise time of the emission of naptholate anion (at 460 nm) [54]. However, Fleming and co-workers reported that when 1-naphthol is encapsulated inside the β-cyclodextrin cavity, the ESPT of the "photoacid," 1-naphthol, is significantly retarded, by about 30 times, and occurs on a 930-ps time scale [55]. Bhattacharyya *et al.* observed that in micelles the ESPT of 1-naphthol is suppressed to a much greater extent [56]. The rise times of the anion emission is, respectively, 600, 600, and 1900 ps for

CTAB, SDS, and TX reduced micelles [56]. Bhattacharyya et al. also studied the ESPT of 1-naphthol in a polymer surfactant aggregate formed by polyvinyl pyrrolidone (PVP) and SDS [57]. In the PVP–SDS aggregates, two distinct sites were detected, in one of which the ESPT process is totally suppressed so that the neutral form decays with a very long lifetime of 5300 ps. In the other site, the ESPT occurs on a 1600-ps time scale, which is nearly 45 times slower than that in bulk water [57]. Petrich et al. studied light-activated antiviral and antitumor agents, such as hypericin and its analogues [58]. By studying these molecules embedded in micelles or in cells, his group has demonstrated that protons are ejected subsequent to light absorption and this acidifies the immediate neighborhood of the probes [59]. Zewail et al. studied excited—state intramolecular proton transfer processes in cyclodextrin [60a], micelles [60b], and protein [60b] and found marked differences in the dynamics of proton transfer dynamics in organized assemblies, compared to bulk water.

The local pH inside AOT microemulsion varies quite drastically over a short distance due to the negative charge of the AOT surfactant. Fendler et al. studied the ESPT process of a tri-negatively charged probe, hydroxypyrene trisulfonate in AOT microemulsion [61]. They observed that in large water pools the negatively charged probe remains in the central region of the water pool, far away from the AOT anions and exhibit an almost bulk water-like ESPT process. In small water pools, the very different local pH around AOT anions renders the ESPT process quite different from that in bulk water [61]. Bhattacharyya et al. [62] studied the ESPT process of the well-known DNA probe, ethidium bromide [63]. They observed that while in bulk water hydroxide ions quench the emission of ethidium bromide very efficiently by the ESPT process, no such quenching is observed inside the water pool of AOT microemulsion [62]. This shows that the hydroxide anion cannot access the ethidium cation, which stays near the AOT anion.

Micropolarity: Twisted Intramolecular Charge Transfer (TICT)

Charge transfer (CT) processes play a crucial role in many biological processes. The dynamics of intramolecular CT processes in molecules where the electron donor and the acceptor are joined by a flexible covalent bonds is a very active area of current research [64–69]. When such a molecule is excited to a "nonpolar" excited state, it undergoes intramolecular CT as well as twisting about the single bond connecting the donor and the acceptor to give rise to the twisted intramolecular CT

(TICT) state [64]. In many cases, dual emission is observed from both the nonpolar [locally excited (LE)] state and the TICT state.

The rate of TICT may be obtained from the decay of the emission from the LE state or from the rise of the emission from the TICT state. In many systems, the TICT state is nonfluorescent. For them, TICT is the predominant nonradiative pathway and the rate of TICT (k_{TICT}) may be obtained from the observed quantum yield of emission (ϕ_f) and fluorescence lifetime (τ_f) as $k_{TICT} \approx k_{NR} = (1 - \phi_f)/\tau_f$ [67]. The rate of the TICT process increases markedly with an increase in the polarity of the medium. Eisenthal et al. showed that the activation barrier (E_B) for the TICT process decreases linearly with an increase in the empirical polarity parameter, $E_T(30)$ [66]. Many groups have applied this relation to estimate the microscopic polarity of organized assemblies using the observed rate of the TICT process [67–69]. The observed system includes cyclodextrin [67,69], micelles [67], protein [67], zeolite [68a], linear dextrin and dextran [68b], and microemulsions [68c]. Interaction of surfactant with cyclodextrin has also been studied using a TICT probe [70].

Microviscosity: Isomerization vs Fluorescence Depolarization

The microviscosity of organized assemblies has been the subject of many studies [11]. In a homogeneous solution, the rotational diffusion coefficient is inversely related to the viscosity. This principle has been used in many early works on fluorescence depolarization to estimate the microviscosity of confined environments [11,72]. However, recently it has been pointed out that the rotational dynamics of a probe in an organized assembly is quite complex [72]. A major complicating feature of the fluorescence depolarization studies in organized assemblies is that the overall motion of the macromolecules is superimposed on the rotational dynamics of the probe solute [72].

The microviscosity of an organized assemblies can also be estimated from the rate of cis–trans isomerization of an olefin in the excited electronic state. The isomerization process involves the motion of one-half of the probe molecule against another and, hence, is free from the complications arising from the motion of the macromolecules. The cis–trans isomerization plays an important role in many natural processes including the vision process [71]. The isomerization process is controlled by the viscosity (friction) of the medium according to Kramer's relation [71]. At very high viscosities (Smoluchowski limit), Kramer's relation reduces to a simple formula

where the rate of isomerzation is inversely proportional to the viscosity. This has been utilized to estimate the microviscosity of micelles [73a], protein [73b], and DNA [73b]. Recently, it has been pointed out that in some cases (e.g., for merocyanine 540) the isomerization dynamics is controlled by the polarity of the medium [74]. Since the polarity of the organized assemblies is very different from that of the bulk liquid, the isomerization dynamics of merocyanine 540 is a good probe for the microscopic polarity of the medium.

CONCLUSIONS

The ultimate goal of ultrafast laser spectroscopy is to unravel the dynamics in biological and natural assemblies. The results outlined in the present article summarize the recent progress in this direction. Because of the remarkable sensitivity of the dynamics of ultrafast photophysical processes to the environment, time-resolved emission spectroscopy is a very sensitive tool to study the microscopic properties of the organized assemblies. The dramatic differences observed in organized assemblies demonstrate that water or other liquids, when confined in a small volume, are fundamentally different from the corresponding liquid in bulk. The complete understanding of such confined liquids constitutes one of the major challenges of contemporary research. The more important aspect will be to understand the implications of the unusual properties of the organized assemblies in various chemical processes. The vast majority of the works carried out so far and discussed in this review involve noncovalently attached fluorescent probes. This has two disadvantages. First, as pointed out earlier, because of the excursion of the probes there is an inherent uncertainty about the exact location of the probe. Second, while a covalently attached probe remains attached to a macromolecule all the time, a noncovalent probe may become detached during many processes, e.g., unfolding of a protein. Future studies with covalently attached probes are expected to yield more detailed information on selected sites within an organized assembly and during many important biological processes, e.g., folding or unfolding of protein and binding of enzymes on substrates.

ACKNOWLEDGMENTS

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