Proc. Indian Acad. Sci. (Chem. Sci.), Vol. 98, Nos 5 & 6, June 1987, pp. 469–478.
© Printed in India.

Microenvironmental differences amongst micelles of various shapes and structures

J SHOBHA and D BALASUBRAMANIAN*
Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad 500 007, India

Abstract. Micelles of different amphiphiles adopt different shapes and internal packing arrangements in water, depending on their chemical structures and the conditions of the medium. Two microenvironmental features, namely the polarity and the microviscosity that the aggregate offers to a solubilized molecule, have been monitored using extrinsic fluorescence probes. While the differences between micelles of spherical and rod-like shapes are not always distinct, stacked micelles and peptide micelles offer distinctly lower polarity and higher microviscosity to solubilizates than the others.

Keywords. Micellar polarity; micellar microviscosity; shapes of micelles; fluorescence probes; vibronic emission intensity; emission anisotropy.

1. Introduction

A variety of amphiphiles aggregate in water to produce hydrophobic assemblies or micelles. The structure of spherical micelles has recently been reviewed (Gruen 1985). It is however known that micelles can adopt different shapes and internal packing depending on the chemical structures of the constituent monomers. Linear alkyl chain surfactants with an ionic or polar headgroup such as sodium dodecyl sulphate (SDS), cetyltrimethylammonium bromide (CTAB) or *n*-octylglucoside (OG) micellize as spherical particles at low or intermediate concentrations. Ionic micelles such as SDS or CTAB also change their shapes from spherical to rod-like aggregates when the headgroup charge interactions are attenuated by salt addition to the medium (Imae *et al* 1985; Ikeda *et al* 1981). The polyoxyethylene based non-ionic detergent Triton X-100 appears to micellize into oblate ellipsoidal or egg-shaped aggregates (Robson and Dennis 1977). On the other hand the molecular structures of bile salts do not allow them to exhibit the distinct end-to-end polarity which characterizes many aqueous surfactants – but allows them to aggregate at moderate concentrations in water to form micelles. They seem to aggregate as molecular stacks into primary micelles, which further aggregate as secondary micelles at high salt concentrations (Small 1971). Some protein molecules also associate to form hydrophobic assemblies. The surface-active amphiphilic peptide melittin aggregates at high concentrations and in high salt containing aqueous medium to produce tetramers, the crystal structure and solution conformation of which have been determined (Terwilliger and Eisenberg 1982; Brown *et al* 1980). It has been shown that the milk protein κ-casein also forms micellar aggregates (Payens and Vreeman 1982). The cyclic oligosaccharides α-cyclodextrin, β-cyclodextrin and γ-cyclodextrin (cyclohexa-, cyclohepta- and cyclooctaamylose) contain hydrophobic cavities in them which accommodate lipophilic compounds in aqueous solution. Cyclodextrins are not surfactants but are

*To whom all correspondence should be addressed

considered to be simple models of hydrophobic solubilizers and while both cyclodextrins and surfactant micelles catalyze reactions, the cyclodextrins are simpler and smaller than surfactant micelles. The structure of cyclodextrins is known from x-ray crystallographic analysis (James *et al* 1959).

While all these systems are grossly thought of and used as hydrophobic hosts to incorporate solubilizates, it is of interest to investigate whether they would display features characteristic of their fine structural differences in their sizes or shapes. Some of these features would be the solubilization capacity, the site of localization of the solubilizate and the microenvironment offered by them to the solubilizate in its immediate or cybotactic region (Kosower 1968). Incorporation of polar, polarizable and ionic solubilizates occurs largely around the headgroup region of the host micelles and the microenvironment these solubilizates experience is that of this region (Ganesh *et al* 1982, 1984). Since most micelle-catalyzed reactions involve such solubilizates, it is of interest to monitor the microenvironmental differences, if any, in the solubilization regions of micelles of various sizes and shapes. We present here our attempts to monitor some fine structural differences between micelles of various shapes such as spheres, rods, eggs, stacks, protein aggregates and also the hydrophobic basket-like cavities of α, β- and γ-cyclodextrins. To our knowledge such an attempt has not been made earlier, though the solubilization capacities of spherical and rod-like micelles have recently been compared (Ozeki and Ikeda 1985). The method chosen is the use of different types of fluorescent probes solubilized in host micelles. The spectroscopic probe technique necessarily assumes that the probe molecule does not perturb the local environment or structure. Usually this is hopefully ascertained by choosing a very low probe:surfactant mole ratio such that most micelles are 'free', several micelles are singly housed with the probe and only a negligible number of micelles are doubly occupied, following the Poisson distribution. Such a situation obtains when fluorescent probes are used wherein micromolar concentrations of probes are used with decimolar surfactants, and the sensitivity of the fluorescence method allows this low probe: surfactant ratio. The principle behind this technique is that it utilizes the changes in the fluorescence of the electronically excited probe molecule to elucidate specific properties of the host system such as its polarity and fluidity. Fluorescence probes used in micellar systems can also be used in biological systems such as membranes, liposomes and proteins.

The fluorescence spectrum of the neutral arene molecule pyrene has been used to monitor the medium polarity. The relative intensity ratio of the third and the first vibronic components I_3/I_1 (the so-called Ham effect) of the emission spectrum of pyrene is a measure of the polarity experienced by the probe molecule around its environment (Nakajima 1976). 8-Anilino-1-naphthalene sulphonic acid (magnesium salt) (ANSA) is another probe whose fluorescence emission wavelength (λ_{max}) and intensity (I_f) are indicative of the polarity of the environment surrounding it (Stryer 1968). Diphenyl hexatriene (DPH) is a fluorescence probe whose emission quantum yield gets enhanced upon incorporation from water into a micelle. In addition the fluorescence polarization (P) and anisotrophy (r) of DPH allows us to estimate the microviscosity ($\bar{\eta}$) of the medium in which the fluorophore is placed (Shinitzky and Barenholz 1978). DPH has been used in our studies to monitor, through its anisotropy, the microviscosity of the host micellar systems. Zachariasse *et al* (1982) have introduced a very useful probe, (dipyrenyl)-methyl

ether (DPME). This molecule displays the Ham effect in its emission around 370–400 nm (the monomer emission) and also forms an intramolecular excimer whose emission is a broad band around 450 nm. The efficiency of excimer formation is directly governed by the fluidity of the medium. Thus the relative intensity ratio of the excimer band to that of the monomer band, Ie/Im, is an index of the microviscosity of the medium; the higher the microviscosity the lower the ratio. In addition, the vibronic band ratio I_3/I_1 of the monomer emission is sensitive to the polarity of the medium. We have used this two-in-one probe to monitor several micellar systems.

2. Experimental section

Sodium dodecylsulphate (SDS, from Biorad) was recrystallized twice from ethanol. Cetyltrimethylammonium bromide (CTAB), sodium deoxycholate, sodium cholate, pyrene, DPH and ANSA were obtained from Sigma and used without further purification. Cetyltrimethylammonium chloride (CTAC) was made in the laboratory from CTAB by extensive dialysis using sodium chloride. Melittin (Serva), α-, β-, γ-cyclodextrin (Aldrich) and CHAPSO (a zwitterionic detergent 3-[3-(cholamidopropyl)-dimethyl-ammonio-]-2-hydroxy-1-propanesulphonate) (Calbiochem) were used as such. κ-casein was isolated from milk and purified by HPLC. (Dipyrenyl)-methyl ether was a gift from Dr K A Zachariasse. Stock solutions of pyrene, DPME and ANSA were made in methanol, while that of DPH was made in tetrahydrofuran (distilled and passed over an alumina column). The concentration of the probes pyrene, DPME and DPH was 1 μM in each micelle solution, whereas in the case of ANSA we have used 10 μM. We have taken care to see that the probe to micelle ratio is as low as 1:100 so that the probe perturbs the micellar structure only negligibly. Direct addition of an organic solution of the probe to water leads to the formation of microcrystals that are responsible for low fluorescence intensities. This can be avoided by extensive sonication and incubation. The sample solutions were made and kept for incubation for 8–10 hrs before recording their fluorescence spectra. The fluorescence signal of systems containing DPH decreases with time on exposure to the excitation light. Upon shutting off the excitation light for periods of 15–30 seconds the system regains its original fluorescence signal. The phenomenon, which originates from reversible photoisomerization of DPH, was eliminated by reducing the period of exposure to excitation light to less than 10 seconds so as to ensure that it is in the all *trans* form.

Fluorescence spectra were measured using a Hitachi 650-10S instrument (at 297 K). The excitation wavelengths for pyrene, DPME, ANSA and DPH were 334, 335, 365 and 357 nm, respectively. Fluorescence polarization (P) and anisotropy (r) values were calculated from the intensities obtained at 0–0°, 0–90°, 90–90° and 90–0° angle settings of the excitation and emission polarizers respectively and using a correction factor for I (Azzi 1974). Microviscosities were calculated using standard equations (Shinitzky and Barenholz 1978). The polarities were estimated using the pyrene band ratio values measured in methanol:water and methanol:n-octanol mixtures of varying volume ratios, using the same instrument and experimental conditions. The polarities of the host systems are thus compared to those of aqueous methanol or methanol:octanol mixtures.

3. Results and discussion

The fluorescence intensities of the several vibronic fine structures in the pyrene monomer fluorescence show strong solvent dependence. The strong perturbation in the vibronic band intensities of pyrene is more dependent on the solvent dipole moment than on the bulk solvent dielectric constant (Dong and Winnick 1982). The strong perturbation of the vibronic band intensities has been used as a probe to determine the polarity of the medium surrounding pyrene. Out of the five vibronic bands exhibited by pyrene emission in the 365–400 nm region, the vibronic component peak 3 shows the maximum variation in intensity relative to that of the 0–0 band (peak 1). Hence the relative intensity of peak 3 to that of peak 1, referred to as the I_3/I_1 ratio, is used here to monitor the environmental effects of micelles on pyrene monomer fluorescence. The peak ratios are quite reproducible ($\pm$ 0·02). The sharpness of the five vibronic frequencies as well as the I_3/I_1 ratio increase as one goes through a series of simple polar solvents, (the ratio ranges from 0·5 to 0·8) aromatic solvents (0·8 to 1·0), hydrocarbon solvents (around 1·6 to 1·8) and saturated perfluorocarbon solvents where it is around 2.

Dong and Winnick (1982) have analyzed the vibronic intensity ratio of pyrene emission in a variety of homogenous media and attempted to correlate those with the empirical solvent polarity parameters E_T^{30} (Reichardt and Dimroth 1968) and their own *Py* values. Kalyanasundaram and Thomas (1977) have measured the pyrene emission ratio I_3/I_1 in micellar systems. We have used 1 μM pyrene as a solubilizate in a variety of host micelles, proteins and cyclodextrins and monitored its I_3/I_1 emission ratios in these. As with other aromatic solubilizates reported earlier (Ganesh *et al* 1982, 1984), pyrene is expected to be solubilized and located largely in the headgroup region of the host micelles and would report on the polarity of this region. Table 1 lists the results obtained in such a study in several systems using pyrene, and estimates the polarities in terms of comparable homogeneous solvents and solvent mixtures. The polarity experienced by pyrene decreases from water in the order α-cyclodextrin, casein micelles, the egg-shaped micelles of Triton X-100, CTAB micelles, SDS micelles, melittin tetramers – the least polar being the stacked micelles of the bile salts and their derivatives.

We wish to comment in passing about the suggestion (Dong and Winnick 1982) that in anisotropic systems we might see differences in the Ham effects of the absorption and emission spectra of pyrene. The rationale behind this argument is that such a difference would imply a net change in the environment of the excited pyrene subsequent to absorbing a photon and prior to emitting a photon. Accordingly we measured the extinction coefficient ratio $\varepsilon_{362}/\varepsilon_{377}$ values of pyrene in spherical, rod-like, egg-shaped and stacked micelles and compared them with the I_3/I_1 emission ratio in the corresponding system. In all these cases the former was uniformly found to be about twice the value of the latter and no distinction was discernible based on the shape of the host aggregate.

The emission quantum yield and the wavelength maximum of the fluorescence of ANSA have also been used to monitor the polarity of the medium surrounding this fluorophore molecule (Stryer 1968). The wavelength of maximum emission reflects the overall dipolar character of the solvent, whereas Q is sensitive to more localized deactivating processes. Of the two parameters we have chosen to limit our attention to λ_{max} since the intensities of emission of this anionic probe could vary

Table 1. Polarity of host assemblies monitored by pyrene and by ANSA.

System	Pyrene I_3/I_1*	λ_{max} ANSA**, nm	Remarks on polarity
Water	0·58	515	
Apolar cavities			
α-Cyclodextrin	0·59	495	water
β-Cyclodextrin	1·07	495	30% MeOH in octanol
Spherical micelles			
SDS, 25 mM	0·92	490	95% MeOH 5% octanol
CTAB, 25 mM	0·79	485	35% MeOH 65% water
Rod-like micelles			
SDS + NaCl	1·01	490	55% MeOH 45% octanol
CTAB + NaBr	0·80	480	30% MeOH 70% water
Egg-shaped micelles			
Triton X-100, 10 mM	0·74	485	50% aq. methanol
Stacked micelles			
Na DOC, 10 mM	1·37	490	*n*-butyl ether (?)
Na DOC + NaCl	1·44		
Na cholate, 10 mM			
Na cholate + NaCl	1·22	48	*n*-butyl ether (?)
CHAPSO, 10 mM	1·13	475	neat octanol
Peptide micelles			
Melittin tetramers	1·06	465	35% MeOH 65% octanol
Casein micelles	0·6	470	water

* 1 μM pyrene, excited at 334 nm, slit width 5 nm, 1 cm cell, room temperature (297 K).
** 10 μM ANSA, excited at 365 nm, slit width 5 nm, 1 cm cell, room temperature (297 K).

depending upon the coulombic interactions with the headgroup charges of the host micelles and the extent of partitioning and solubilization in them. Being aromatic and charged, ANSA would be expected to be solubilized not in the core of the host micelle but near the headgroup region and thus report on the polarity of this region (Ganesh *et al* 1982, 1984). Table 1 compares the λ_{max} of ANSA in a variety of micelles with trends that are roughly the same as those obtained with the other probe pyrene.

ANSA reports the polarities of the cyclodextrin hydrophobic cavities to be higher than any of the micelles we have studied. The polarity of both the cavities is seen to be roughly that of 10% dioxane in water. It is possible that ANSA does not accommodate itself well into either of the cavities. The rod-like micelles appear to display slightly reduced polarities than spherical ones. The stacked micelles offer the least polar environment that is accentuated further in secondary micelles of the bile salts in high salt media.

The results on polarities obtained using ANSA are not identical with those using pyrene, which is not surprising in the light of the different mechanisms that operate in the two cases. But the general trend that emerges out of such polarity probing is that the polarity of the microenvironment decreases in order: cyclodextrin cavities are the most polar, then are the spherical, egg-shaped and rod-like micelles, and

the least polar of the surfactant micelles being the stacked assemblies. The peptide micelles of melittin tetramers and of the protein casein offer the least polar environment of all for ANSA, though pyrene sees them to be more polar than bile salt micelles. This difference in the behaviour of ANSA and pyrene in the proteins might perhaps be due to differences in their binding sites, a point that needs to be established.

It is important to reiterate that the polarity being studied is not that of the interior or the core of the micelle, but that of the region near the headgroup or at best a few methylenes beneath. The polarity of the core would be roughly that of a liquid alkane in spherical micelles, as the standard picture implies (Gruen 1985). Any probe that is used, being polar or polarizable as ANSA and pyrene are, would tend to locate itself largely near the headgroup region of the host micelle and would report on its immediate or cybotactic region. It is thus of interest to note that even in this region, cyclodextrin cavities, spherical and rod-like micelles differ in their polarity features from stacked micelles, and from protein micelles. While the polarity difference between spherical and rod-like micelles is not substantial, it is so in bile salt micelles.

The other parameter of interst is the microenvironmental viscosity or the microviscosity of the micellar aggregate. This can be estimated (Shinitzky and Barenholz 1978) by using the fluorescence anisotropy or polarization of the probe diphenylhexatriene (DPH). Table 2 summarises the anisotropy values and the environmental microviscosity estimates in a variety of systems. The emission intensity increases perceptibly upon incorporating the DPH into host systems, though no definite shape-dependent trends emerge. This at least in part is due to the variation seen in the intensity upon changing the detergent concentration, since we cannot use the same number of molecules of the detergent in the micelle in each case. The microviscosities we have measured agree for CTAB spheres and for Triton X-100 micelles with those reported using other probes, around 50 cP for CTAB (Blatt *et al* 1982) and by 145 cP for Triton X-100 (Watkins and Selinger 1979).

The estimated microviscosities increase in the general order: spherical micelles, α-cyclodextrin, rod-like micelles, egg-shaped micelles, β-cyclodextrin, melittin tetramer, the most viscous being the stacked micelles of bile salts. One would also expect DPH to be intercalated within the stacks of the bile salt micelles leading to the greater degree of immobilization that is seen here. In comparison, its binding to the hydrophobic patch of melittin tetramers or the rod-like micelles would offer it higher mobility. It is interesting however that Triton X-100 micelles seem to immobilize DPH better than rod-like micelles. One must however add here that the only example of an egg-shaped micelle we have is that of polyoxyethylene-based Triton X-100. Whether the increased microviscosity seen by DPH here is due to the inherent packing mode of the micelle or to the influence of polyoxyethylene, a known medium of higher viscosity, is not clear. The other polyoxyethylene-based nonionic detergent, Brij 35, whose shape is not known yet, appears to be four times more viscous than Triton X-100. In any event, it is seen that microviscosity differences between the various assemblies seem to be more marked and better distinguishable than their polarity differences.

The composite probe (dipyrenyl)-methyl ether (DPME) displays not only the emission bands of the constituent pyrene moieties with their vibronic fine structure

Table 2. Fluorescence features of diphenylhexatriene (DPH)* solubilized in various media.

System	Intensity (a.u.)	Anisotropy (r)	Microviscosity (poise)
Water	2·5		
Spherical micelles			
SDS, 25 mM	50·4	0·080	0·66
CTAB, 25 mM	443	0·072	0·58
Rod-like micelles			
SDS + NaCl	208	0·094	0·82
CTAB + NaBr	376	0·078	0·77
Egg-shaped micelles			
Triton X-100, 10 mM	470	0·116	1·11
Peptide micelles			
Melittin tetramers	462	0·191	2·64
Stacked micelles			
Na DOC	220	0·228	4·03
Na cholate	203	0·263	6·26
CHAPSO	162	0·102	1·05
Apolar cavities			
α-Cyclodextrin	35	0·081	0·68
β-Cyclodextrin	56	0·172	2·15

*DPH = 1 μM in THF excited at 357 nm, emission at 430 nm, ambient temperature (297 K); 1 cm cell, slit width 4 nm. Solution kept in dark for 30 s prior to measurement so as to ensure that the probe is exclusively in the *trans* isomeric form. The estimated values of $\bar{\eta}$ are accurate to $\pm$ 15%.

(monomer fluorescence), but also an intramolecular excimer emission band beyond 450 nm. The vibronic intensity ratio of the monomer fluorescence of DPME monitors the polarity of the medium just as the I_3/I_1 ratio of pyrene itself does. In addition, the intensity ratio of the excimer band to that of the monomer band (Ie/Im) depends on the viscosity of the environment of DPME; the higher the environmental viscosity (or microviscosity of the host aggregate in which DPME is incorporated), the lower the excimer: monomer intensity ratio Ie/Im (Zachariasse *et al* 1978; Zachariasse 1986). Use of DPME might be advantageous in comparison to the use of separate probes to measure the polarity (pyrene, ANSA) and the microviscosity (DPH) of host assemblies. It is more frequently used to compare the relative microviscosities or fluidities of the same assembly under different conditions.

Table 3 presents the results on the polarity and microviscosity profiles of several of the test assemblies, using DPME as the composite probe in them. It is to be pointed out that the vibronic ratios obtained using DPME need not be the same as those with pyrene itself, due to the fact that the two molecules differ in structure (the I_3/I_1 ratios for DPME and for pyrene in water are 0·71 and 0·58 respectively) and also the possibility that the two probes may not be located in identical regions of the host assembly (DPME gives a value of 0·59 in β-cyclodextrin while with

Table 3. Fluorescence features of DPME* in various assemblies

System	I_3/I_1 monomer band	Ie/Im
Apolar cavities		
α-Cyclodextrin	–	–
β-Cyclodextrin	0·59	–
γ-Cyclodextrin	0·84	0·13
Spherical micelles		
SDS, 25 mM	0·57	0·32
CTAC, 25 mM	0·67	0·33
Rod-like micelles		
SDS + NaCl	0·66	0·38
CTAC + NaCl	0·67	0·28
Egg-shaped micelles		
Triton X-100, 10 mM	0·73	0·21
Stacked micelles		
Na cholate, 10 mM	0·84	0·13
Na DOC, 10 mM	0·94	0·17
Na cholate + NaCl	0·92	0·25
Na DOC + NaCl	1·16	0·07
Peptide micelles		
Melittin tetramers	1·07	0·66

*1 μM DPME; excited at 335 nm, emission of monomer band in the 370–400 nm region and of the intramolecular excimer band above 450 nm; 1 cm cell; slit width, 4 nm; ambient temperature (297 K).

pyrene it is 1·07). Yet the trend seen in the polarity profile of these systems is largely the same as with pyrene. DPME was not found to be solubilized in α-cyclodextrin solutions, perhaps because of its size. Even in β- and γ-cyclodextrins, it seems to be accommodated only partially. The excimer band of DPME could not be measured in the former, and it also has a smaller intensity compared to that of the monomer band in the latter.

The excimer : monomer emission ratios, Ie/Im, of DPME in these systems is only partly in line with those expected based on their microviscosities measured by DPH, and there are exceptions. For example, the microviscosity of the DPH binding site in melittin tetramers is more than that of Triton X-100. Yet the Ie/Im ratio of DPME in melittin is the highest amongst all the systems studied. Similarly there is a substantial increase in the excimer emission of DPME in SDS rods compared to that in SDS spheres, and in the cholate secondary micelles as compared to its primary micelles. In DPME, the intensity of the monomer emission is sensitive to the medium polarity while that of the excimer band is not. Thus there would be variations in the Ie/Im ratios that depend not only on the microviscosity but on the polarity of the medium as well. This would be responsible for the differential responses of DPME and of DPH to medium microviscosities.

4. Conclusions

In conclusion, it appears possible by fluorescence probe methods to monitor the microenvironmental differences amongst micelles of different shapes. The caveat is that the environment being monitored is not the micellar core but the region a little beneath the headgroup (since that is largely where most polar and polarizable probes are solubilized in micelles).. Even in this region of different micelles, the polarity and the microviscosity differ in different systems. The polarity appears to decrease in the order – cyclodextrin cavities, spherical and rod-like ionic micelles, bile salt micelles and protein self-aggregates. The fluidity of the medium surrounding solubilized DPH appears to decrease in the order – spherical mielles, rod-like micelles, egg-shaped micelles, melittin micelles, with the bile salt stacks offering the most microviscous environment for DPH.

Clear differences arise between stacked micelles on the one hand and other micelles of surfactants on the other. It would be interesting to try the fluorescence approach to predict the possible shape of aggregates whose shapes are not known. Aggregates of surface-active or membrane-active peptides and proteins could also be analyzed using this approach.

Acknowledgements

Financial assistance from DST and from CSIR (in the form of a fellowship to JS) is gratefully acknowledged. We thank V Srinivas for help with experiments and Drs Ch Mohan Rao and K A Zachariasse for their advice.

References

Azzi A 1974 *Methods Enzymol.* **32** 234
Blatt E, Ghiggino K P and Sawyer W H 1982 *J. Phys. Chem.* **86** 4461
Brown L R, Lauterwein J and Wuthrich K 1980 *Biochim. Biophys. Acta* **231** 622
Dong D C and Winnick M A 1982 *Photochem. Photobiol.* **35** 17
Ganesh K N, Mitra P and Balasubramanian D 1982 *J. Phys. Chem.* **86** 4291
Ganesh K N, Mitra P and Balasubramanian D 1984 in *Surfactants in solution* (eds) K L Mittal and B Lindman (Plenum Press: New York) vol.1, 599
Gruen D W R 1985 *Prog. Colloid Poly Sci.* **70** 6
Imae T, Kamiya R and Ikeda S 1985 *J. Colloid Interface Sci.* **105** 215
Ikeda S, Hayashi S and Imae T 1981 *J. Phys. Chem.* **85** 106
James W J, French D and Rundle D E 1959 *Acta Cryst.* **12** 385
Kalyanasundaram K and Thomas J K 1977 *J. Am. Chem. Soc.* **99** 2039
Kosower E M 1968 *Introduction to physical organic chemistry* (New York: John Wiley)
Nakajima 1976a *J. Lumin.* **11** 429
Nakajima 1976b *J. Mol. Spectrosc.* **61** 467
Ozeki S and Ikeda S 1985 *J. Phys. Chem.* **89** 5088
Payens T A J and Vreeman H J 1982 in *Solution behavior of surfactants* (eds) K L Mittal and E J Fendler (New York: Plenum Press) vol. 1, p. 543
Reichardt C and Dimroth K 1968 *Fortschr. Chem. Forsch.* **11** 1
Robson R J and Dennis E A 1977 *J. Phys. Chem.* **81** 1075
Small D M 1971 in *The bile acids: Chemistry, physiology and metabolism* (eds) R P Nair and D Kritchovsky (New York: Plenum Press) vol. 1, 249

Shinitzky M and Barenholz Y 1978 *Biochim. Biophys. Acta* **515** 367
Stryer L 1968 *Science* **162** 526
Terwilliger T C and Eisenberg D 1982a *J. Biol. Chem.* **257** 6016
Terwilliger T C and Eisenberg D 1982b *Biophys. J.* **37** 353
Watkins A R and Selinger B K 1979 *Chem. Phys. Lett.* **64** 250
Zachariasse K A 1986 in *Excited state probes in biochemistry and biology* (ed.) A Szabo (New York: Plenum Press) (in press)
Zachariasse K A, Vaz W L C, Sotomayor C and Kuhnle W 1982 *Biochim. Biophys. Acta* **688** 323