Structural Information on Probe Solubilization in Micelles by FT–IR Spectroscopy

JOSÉ VILLALAÍN*, JUAN C. GÓMEZ-FERNANDEZ*, AND MANUEL J. E. PRIETO†,[1]

*Departamento de Química y Bioquímica, Facultad de Veterinaria, Universidad de Murcia, E-30071, Spain; and †Centro de Química Estrutural, Complexo I, Av. Rovisco Pais, 1096 Lisboa Codex, Portugal

Received October 13, 1986; accepted July 21, 1987

The carbonyl stretching vibration of *n*-(9-anthroyloxy)stearic acid probes was monitored in Triton X-100 and SDS micelles, and it was concluded that (i) the radial distribution function is more displaced to the micellar core when the alkyl chain between the terminal acid group and the chromophore increases and (ii) in SDS micelles a large fraction of probes is in all cases at the micellar interface.

INTRODUCTION

Molecular extrinsic probes have been intensively used to report the properties of heterogeneous media using a variety of techniques, namely fluorescence (1) and ESR (2).

When a carbonyl group is involved in hydrogen bonding, its stretching vibration is affected and a new band, assigned to the bound structure, appears at a lower frequency. The asymmetry observed in this new band is explained on the basis of a 1:2 complex, i.e., a carbonyl group involved in hydrogen bonding with two molecules of a protic solvent (3). Hence, the relative intensities of the free and bound carbonyl absorptions can in principle be used to assess the solvent concentration.

It is the purpose of the present work to monitor the water concentration in the vicinity of suitable probes, when solubilized in micelles, via FT–IR spectroscopy.

MATERIALS AND METHODS

Methyl benzoate and Triton X-100 (scintillation grade) were obtained from BDH (Poole, England), D_2O (99.8%) from Sigma (Poole, England), *n*-(9-anthroyloxy)stearic acids (*n*-AS) (n = 2, 6, 9, 12) from Molecular Probes (Eugene, OR) sodium dodecyl sulfate (SDS) from Merck (Darmstadt, FRG), and diglyme from Fluka (Buchs, Switzerland). They were used as received.

Surfactant solutions of Triton X-100 and SDS were prepared in order to obtain a concentration of micelles ca. 5×10^{-3} M (in phosphate buffer pH 7.4), assuming aggregation numbers of 143 and 85 and critical micellar concentrations of 2.4×10^{-4} and 1.9×10^{-3} M for Triton X-100 (4, 5) and SDS (6, 7), respectively. Mean occupancy numbers ([probe]/[micelle]) were kept below 7 in order to minimize micellar perturbation. The solubilization of the probes in the micelles was accomplished by bath sonication and gentle warming and the solutions were allowed to stand for 24 h before measurements.

D_2O was used, since with this solvent a window situated at 1650–1770 cm^{-1} is available, in the region where the stretching vibration of the carbonyl is observed. The conclusions obtained can be generalized to micelles in H_2O, since the alterations due to isotopic substitution of the solvent, such as effects of hydrophobicity (8), do not significantly modify the structure of the micelles (9).

[1] To whom all correspondence should be addressed. Present address: Centro de Química Física Molecular Complexo I, Av. Rovisco Pais, 1096 Lisboa Codex, Portugal.

0021-9797/88 $3.00

FT–IR spectra were obtained at 25°C in a Nicolet MX-1 spectrometer, assisted by a Nicolet 1200 S computer. The suspensions were injected into a Beckman FH-01 CFT cell, equipped with CaF_2 windows and using 25-μm Teflon spacers. The spectra were obtained after 27 accumulations and blank absorption (micellar solutions without probe) was substracted. In the experiments with the *n*-AS probes, the absorption of the acid terminal group was taken into account.

RESULTS AND DISCUSSION

The carbonyl stretching vibration of methyl benzoate in Triton X-100 is shown in Fig. 1a, the main absorption band at 1724.4 cm^{-1} evidencing a shoulder ca. 1710 cm^{-1}, due to the hydrogen-bound carbonyl.

In order to evaluate the concentration of water in the vicinity of the probe when solubilized in the micelle, a calibration was made using a solvent which is considered to have dielectric properties similar to those of the localization site of methyl benzoate in the micelle. Assuming that a polar molecule such as methyl benzoate is near the micellar surface (10), diglyme was chosen, as the outer part of the Triton X-100 micelle is essentially a polyether.

The calibration plot was obtained following the evolution of the absorption band corresponding to the carbonyl stretching vibration, in diglyme, at different water concentrations. This absorption band in neat diglyme is symmetric, showing the known Gaussian plus Lorentzian profile (11). When D_2O is added, the contribution of the new band is obtained through a curve decomposition, assuming the symmetry of the free oscillator curve. The systematic error introduced in this way is canceled out, once the same criterion is used for the calibration plot and for the experiments with micelles. As the asymmetry of the lower energy absorption band points to the existence of 1:2 complexes (3), the consideration of a simple kinetic scheme, ignoring water self-association, water–diglyme association, and activity coefficients, leads to the relationship

$$I_{bound}/I_{free} = K_1[D_2O] + K_1K_2[D_2O]^2, \quad [1]$$

where K_1 and K_2 are the association constants for the 1:1 and 1:2 complexes and I_{bound} and I_{free} refer to the integrated intensities of the bound and free carbonyl absorptions, the total integrated intensity being constant (12).

The application of this equation to the system methyl benzoate/D_2O in diglyme (Fig. 2) leads to $K_1 = 3.1 \times 10^{-2}$ $dm^3 \cdot mole^{-1}$ and $K_2 = 4.8 \times 10^{-2}$ $dm^3 \cdot mole^{-1}$ with K_2 higher than K_1, as was found by Kagarise and Whetsel (3) for the system acetone/*p*-cresol in cyclohexane. It must be stressed that the determination of the equilibrium constants is not necessary, as a plot such as the one shown in Fig. 2 can be used as a calibration curve.

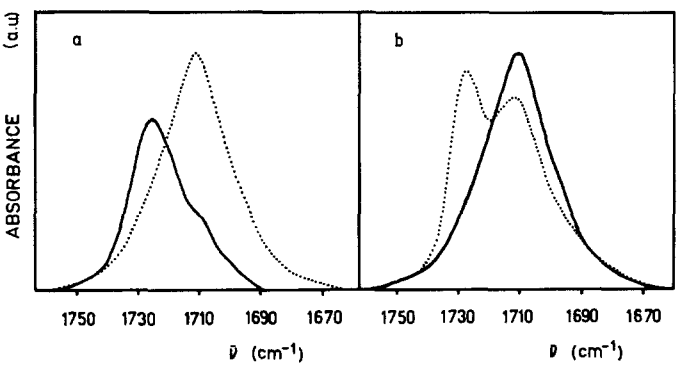

FIG. 1. FT–IR spectra of methyl benzoate in Triton X-100 and SDS micelles. (a) Methyl benzoate carbonyl stretching vibration in Triton X-100 (—) and SDS (· · ·) micelles. (b) Methyl benzoate carbonyl stretching vibration in SDS micelles with mean occupancy numbers of 4:1 (—) and 68:1 (· · ·).

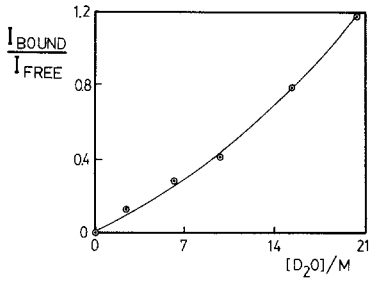

FIG. 2. Plot of I_{bound}/I_{free} versus $[D_2O]$ for methyl benzoate in diglyme with experimental points, ⊙, and fitting to Eq. [1], (—), where $K_1 = 3.1 \times 10^{-2}$ dm^3 mole^{-1} and $K_2 = 4.8 \times 10^{-2}$ dm^3 mole^{-1}.

From the previous treatment a value of 5 M of D_2O in the vicinity of methyl benzoate in Triton X-100 micelles was found.

The water concentration was also evaluated from a calibration curve, obtained by plotting $\tilde{\nu}_{max}$ for the free carbonyl band versus the D_2O concentration (Fig. 3), and from this procedure a similar value of 3.5 M D_2O was obtained, lower than previously reported (5).

Quantitative determination of the water in the case of the SDS micelles is not possible due to the difficulty of mimicking the water–hydrocarbon interface. In any case, the spectrum of methyl benzoate in the SDS micelles shows a much greater intensity of the bound structure (Fig. 1a) than that of Triton X-100. This striking difference could be ascribed to the more open structure of the SDS micelles (13), but recent evidence from small angle neutron scattering (14) conflicts with this opinion, since it was claimed that water does not enter any further than the γ-CH_2 of the surfactant. In this way, a localization of methyl benzoate near the interface, and eventually greater values of K_1 and K_2, would explain the observed difference.

When the mean occupancy number of the probe is significantly increased, as shown in Fig. 1b for methyl benzoate in SDS, a totally different pattern with a high fraction of nonbound carbonyl is observed. An alteration of the micellar system occurs in this case, the system being composed of a droplet of methyl benzoate stabilized by adsorbed surfactant. In this situation most of the probe is in a very hydrophobic environment and nonexposed to water.

The experiments hitherto reported should not be affected by significant methyl benzoate concentration in the water phase, as partitioning of this type of probe between water and micelles favors the latter (15, 16). A quantitative evaluation of this effect can be obtained by comparing methyl benzoate with benzene. For this molecule the distribution constant (SDS micelles/water) is $K = 1.6 \times 10^3$ M^{-1} (17), so under our experimental conditions 10% of the benzene molecules would be in the water phase. Considering that the solubility of methyl benzoate in water, 1.2×10^{-3} M (18), is one order of magnitude lower than that of benzene, 2.3×10^{-2} M (17), it can be concluded that the residual concentration of methyl benzoate in the water phase is negligible.

A very important point regarding the use of "extrinsic" probes is the eventual perturbations, i.e., structural alterations, that they induce on the system under study (19, 20). Moreover, in the present study, this perturbation could have a direct effect on the vibration of the carbonyl group, if it is assumed that the polar group would pull water toward its vicinity (19). While it cannot be excluded, this effect is probably nondominant, as shown from a ^{13}C NMR chemical-shift study (21).

An extension of this study was made using a set of *n*-(9-anthroyloxy)stearic acids. For these probes, which are structurely similar to the surfactant, a regular variation on the photophysical properties of the fluorophore was

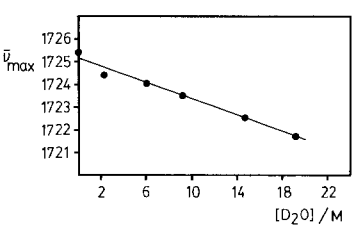

FIG. 3. Plot of $\tilde{\nu}_{max}$ of the free carbonyl stretching vibration of methyl benzoate in diglyme versus $[D_2O]$.

observed, and accordingly a gradual inner location was postulated, when the alkyl chain between the acid and the aromatic group increased (22–25). This graded series of positions was also reported from an energy-transfer study (20).

In Fig. 4 the results obtained for the $n = 2$, 6, 9, and 12 substituted compounds solubilized in Triton X-100 micelles are shown. For the 2-AS and 6-AS probes a contribution of the bound species is evident on the spectra, but the 9-AS and 12-AS report an essentially hydrophobic environment. In the Triton X-100 micelle the acid terminal group of the *n*-AS probe is eventually located near the phenoxy group of the surfactant, in the transition region between the outer part (polyether type) of the micelle and its hydrocarbon core. In this way, the 9- and 12-AS probes sense a hydrophobic core devoid of water. In addition they show that the effect of the carbonyl group, dragging water inside the micellar structure, is not significant for the case of the Triton X-100.

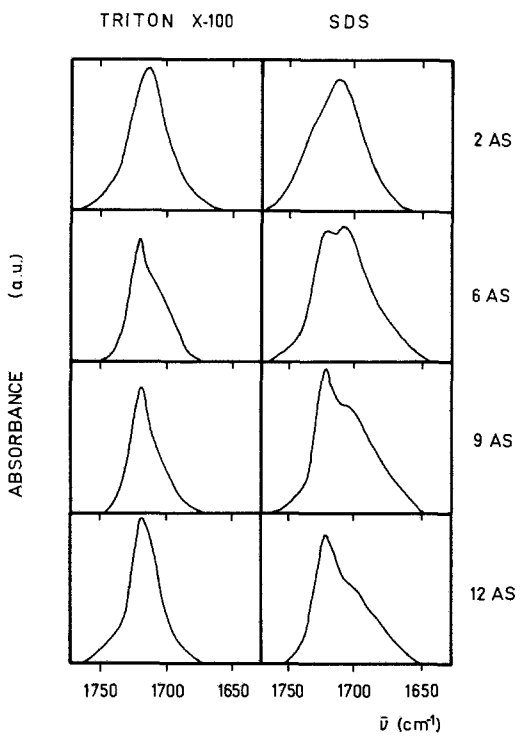

FIG. 4. Carbonyl stretching vibration of *n*-(9-anthroyloxy)stearic acids ($n = 2, 6, 9, 12$) in Triton X-100 and SDS micelles.

In contrast with the Triton X-100 micelle, the spectra in SDS, Fig. 4, exhibit strong absorption in all cases due to the bound carbonyl group, even for the 9- and 12-AS probes.

Recent work by neutron scattering (14, 26, 27) proved the absence of significant water penetration in micelles. In agreement with this, the low-frequency C–H stretching observed for the surfactants, when they are organized in micelles (28), indicates that there is little or no hydrocarbon chain–water contact.

In this way the trend of variation depicted in Fig. 4 is due to the fraction of probes localized at the micellar surface. This would imply that while the radial distribution function has its maximum displaced toward the micelle interior when the alkyl chain increases, the fraction of probes at the surface is in all cases very high. This effect is greater in SDS micelles than in Triton X-100 micelles, as the smaller the micelle, the greater the incidence of end groups at the surface (29).

The relative intensities of bound and free carbonyl absorptions for each probe give an underestimation of the fraction of probes at the micellar surface. For the SDS micelle this would point to very high values, but it should again be stressed that the micellar surface for this situation is a spherical shell (from the interface to the γ-CH_2 of the surfactant), of complex structure.

The relative values for the set of probes in each micelle are 1:4:6 for the 12-, 9-, and 6-AS in Triton X-100 and 1:1.2 for 12- and 9-AS in SDS.

Two main advantages of the infrared absorption approach used are (i) a correct report of the fraction of superficial probes, in contrast with fluorescence experiments where the molecule diffuses during its excited state lifetime sensing different environments, and (ii) the appearance of a new band ascribed only to a protonated species; this specificity for reporting protic solvents (e.g., water) is an advantage, when compared with the observation of

shifts in absorption or emission spectra, which have a complex dependence on solvent properties, e.g., polarizability (30).

SUMMARY

The carbonyl stretching vibration of aromatic probes solubilized in Triton X-100 and SDS micelles was monitored. The intensities of hydrogen-bound and free oscillator absorptions were ascribed to the fractions of probes at the micellar surface relative to those in the interior. The study of a set of functionalized probes, *n*-(9-anthroyloxy)stearic acids, *n*-AS, shows that (i) the radial distribution function is more displaced to the micellar core when the alkyl chain between the acid group and the chromophore increases, and (ii) in SDS micelles a large fraction of probes is in all cases at the micellar interface.

ACKNOWLEDGMENTS

This work was supported in part by grants from the CAICYT (Spain) to J.G.G.F. (No. 3401(01)-83) and from Fundação Calouste Gulbenkian to M.J.E.P. Computer software from J. H. Teles is gratefully acknowledged.

REFERENCES

1. Badley, R. A., *in* "Modern Fluorescence Spectroscopy" (E. L. Wehry, Ed.), Vol. 2, pp. 91–163. Plenum, New York, 1976.
2. Devaux, P. F., and Seigneuret, M., *Biochim. Biophys. Acta* **822,** 63 (1985).
3. Kagarise, R. E., and Whetsel, K. B., *Spectrochim. Acta* **18,** 315, 329, 341 (1962).
4. Kalyanasundaram, K., and Thomas, J. K., *J. Amer. Chem. Soc.* **99,** 2039 (1977).
5. Robson, R. J., and Dennis, E. A., *Acc. Chem. Res.* **16,** 251 (1983).
6. Lianos, P., and Zana, R., *J. Phys. Chem.* **84,** 3339 (1980).
7. Mazer, N. A., Benedek, G. B., and Carey, M. C., *J. Phys. Chem.* **80,** 1075 (1976).
8. Plonka, A., and Kevan, L., *J. Phys. Chem.* **88,** 6348 (1984).
9. Candau, S., Hirsch, E., and Zana, R., *J. Colloid Interface Sci.* **88,** 428 (1982).
10. Thomas, J. K., *Acc. Chem. Res.* **10,** 133 (1977).
11. Ramsay, D. A., *J. Amer. Chem. Soc.* **74,** 72 (1952).
12. Wexler, A. S., *Appl. Spectrosc. Rev.* **1,** 29 (1967).
13. Menger, F. M., *Acc. Chem. Res.* **12,** 111 (1979).
14. Dill, K. A., Koppel, D. E., Cantor, R. S., Dill, J. D., Bendedouch, D., and Chen, S.-H., *Nature (London)* **309,** 42 (1984).
15. Simon, S. A., McDaniel, R. V., and McIntosh, T. J., *J. Phys. Chem.* **86,** 1449 (1982).
16. Alauddin, M., and Verrall, R. E., *J. Phys. Chem.* **88,** 5725 (1984).
17. Almgren, M., Grieser, F., and Thomas, J. K., *J. Amer. Chem. Soc.* **101,** 279 (1979).
18. Hodgman, Charles D. (Ed.), "Handbook of Chemistry and Physics," 32nd ed. Chem. Rubber Publ. Co., Cleveland, 1950.
19. Wennerstrom, H., Lindman, B., *J. Phys. Chem.* **83,** 2931 (1979).
20. Berberan-Santos, M. N., and Prieto, M. J. E., *J. Chem. Soc. Faraday Trans. 2* **83,** 1391 (1987).
21. Menger, F. M., Jerkunica, J. M., and Johnston, J. C., *J. Amer. Chem. Soc.* **100,** 4676 (1978).
22. Blatt, E., Ghiggino, K. P., and Sawyer, W. H., *J. Chem. Soc. Faraday Trans. 1* **77,** 2551 (1981).
23. Chalpin, D. B., and Kleinfeld, A. M., *Biochim. Biophys. Acta* **731,** 465 (1983).
24. Blatt, E., and Sawyer, W. H., *Biochim. Biophys. Acta* **822,** 43 (1985).
25. Blatt, E., Ghiggino, K. P., and Sawyer, W. H., *Chem. Phys. Lett.* **144,** 47 (1985).
26. Cabane, B., and Zemp, T., *Nature (London)* **314,** 385 (1985).
27. Zemb, T., and Charpin, P., *J. Phys. (Les Ulis Fr.)* **46,** 249 (1985).
28. Unemura, J., Mantsch, H. H., and Cameron, D. G., *J. Colloid Interface Sci.* **83,** 558 (1981).
29. Dill, K. A., *Nature (London)* **313,** 603 (1985).
30. Liptay, W., *in* "Excited States" (E. C. Lim, Ed.), Vol. I, pp. 129–229. Academic Press, New York, 1974.