Association of Spin-Labeled Substrate Molecules with Poly(sodium 10-undecenoate) and the Sodium 10-Undecenoate Micelle[1]

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Abstract: Poly(sodium 10-undecenoate) is intuitively expected to have some of the characteristics of micelles in aqueous solution. Its ability to associate with substrate molecules has been tested with spin-labeled probes. Electron spin resonance (ESR) measurements show unambiguously that the polymer readily associates with both amphiphilic and hydrophobic substrates, as does the sodium 10-undecenoate micelle. Analysis of the ESR spectra allows a comparison of the associated species formed in polymer solution with those formed in micelle solution. Both similarities and differences are observed.

During the early 1930's, Hartley developed a simple model of surfactant micelles: in aqueous solution, surfactant monomers aggregate to form spheroidal colloids pictured as liquid hydrophobic droplets surrounded by hydrophilic shells.[2] This model has evolved in its details over the past 50 years, but has remained the basis for understanding the physical properties of aqueous micelles.[3-5] Recently, Dill and Flory proposed an important refinement of this model.[6] On the basis of a statistical lattice theory,[7] they concluded that the core of a spherical micelle is relatively ordered (crystal-like) near the center and relatively disordered (liquid-like) away from the center. Direct confirmation of their calculations has not yet been made.

The experiments described in this paper take advantage of a micelle/polymer pair formed from the same surfactant monomer. Sodium 10-undecenoate has been shown to undergo radiation-induced polymerization in aqueous solutions of the monomer, provided the concentration exceeds the critical micelle concentration (cmc).[8] The polymer ($\sim$10-mer) has the primary structure

$$-CH_2-CH-$$
$$|$$
$$(CH_2)_8$$
$$|$$
$$COO^- \quad Na^+$$
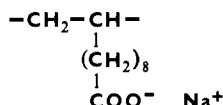
with a short hydrocarbon backbone and relatively long side chains terminated by ionic headgroups. The intrinsic viscosities of the polymer and micelle in aqueous solution at 20.00 °C are indistinguishable, implying a close similarity in the shape and degree of hydration of the two species in solution.[9] Local ordering in the core of the polymer is guaranteed by the covalent bonding of the undecenoate tails.

The primary object of this study was to determine whether the ordering of the polymer core would prevent the binding of substrate molecules. Electron spin resonance (ESR) spectra of both amphiphilic and hydrophobic spin-labeled probes in aqueous solution and in solution with the micelle and polymer were obtained. Binding was observed for both substrates to both the micelle and the polymer. Additional information about the microenvironment of the bound spin labels was obtained from a more detailed analysis

of the spectra.[10,11] Evidence consistent with ordering in the micelle was found; the extent of the ordering appeared less in the micelle than in the polymer.

Experimental Section

Water was prepared by batch distillation of dilute sulfuric acid (five drops of concentrated sulfuric acid per liter of tap water). The middle third of each distillation was retained. Practical grade 10-undecenoic acid was purified by vacuum distillation (3.5 torr, fraction collected at 144–145 °C). Aqueous solutions of sodium 10-undecenoate were prepared by adding the appropriate amount of a sodium hydroxide solution to the weighed acid and diluting to the final concentration. Stock solutions of sodium hydroxide were prepared and standardized by the usual methods.[12] Poly(sodium 10-undecenoate) was prepared as described previously.[8] Surfactant concentration during irradiation was 0.1 mol/dm³, and total dose from the cobalt-60 source was 4 Mrad at a dose rate of 0.14 Mrad/h. The polymer was separated from unreacted monomer by precipitation in ethanol. Aqueous solutions of the polymer were prepared by combining weighed quantities of the polymer and water. Methanol (Fisher Spectranalyzed) and heptane (MCB Spectroquality Reagent) were used as received. Sodium 5-doxylstearate was prepared by neutralization of 5-doxylstearic acid, 2-(3-carboxypropyl)-4,4-dimethyl-2-tridecyl-3-oxazolidinyloxy, which was used as received from Syva. Its structural formula is
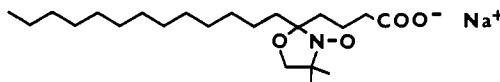
A spin-labeled testosterone was obtained from Professor H. B. Halsall of this department. It has the structural formula
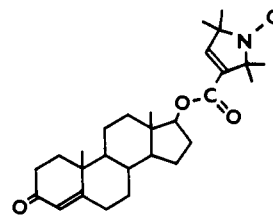
Samples for ESR measurements were prepared from stock solutions of the labeled substrates in methanol. Aliquots were placed in vials, the methanol was evaporated, a measured amount of solvent, micelle solution, or polymer solution was added, and the resulting solution was degassed by bubbling nitrogen through for several minutes.

ESR measurements were performed on a Varian E-4 spectrometer under the control of a microcomputer system.[13] Samples were contained in a flat, aqueous sample cell at room temperature, 23 °C. Care was taken to adjust all instrumental parameters so as to avoid distortion of the ESR signals.[14] A nominal microwave power level of 2 mW was

(1) Based in part on the Ph.D. thesis of C.E.L. Portions of this work were presented at the American Society of Biological Chemists/Biophysical Society National Meeting in New Orleans, June, 1980.

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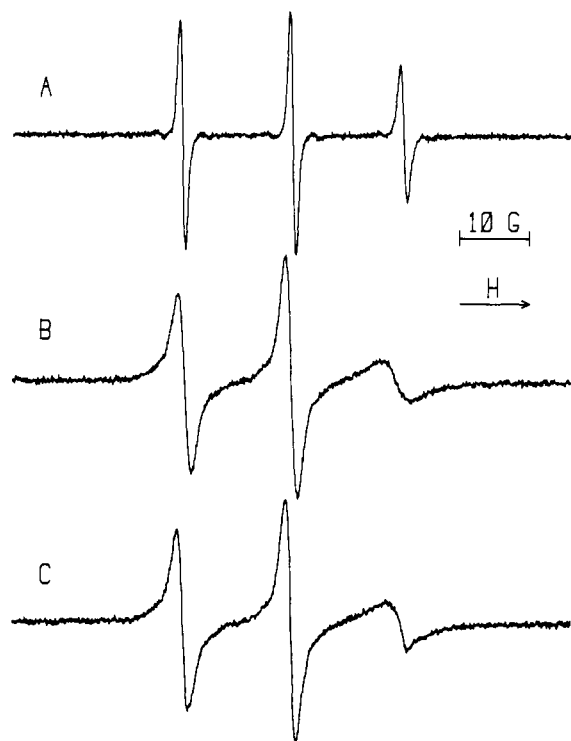(14) See: Jost, P.; Griffith, O. H. in ref 10, Chapter 7.

Figure 1. ESR spectra of sodium 5-doxylstearate in (A) water, (B) sodium 10-undecenoate solution, and (C) poly(sodium 10-undecenoate) solution. See the Experimental Section for details.

employed, and the modulation amplitude did not exceed 25% of the narrowest experimental peak-to-peak line width. In order to obtain a satisfactory spectrum from the nearly insoluble labeled testosterone in water, however, it was necessary to increase these values somewhat (to 10 mW and 50% of the line width). A saturated solution of the labeled testosterone in water was used. In all other samples the substrate concentration was either 8×10^{-5} mol/dm^3 for the labeled testosterone or 1.1×10^{-4} mol/dm^3 for sodium 5-doxylstearate. The total sodium 10-undecenoate concentration in the micelle solutions was 155 g/dm^3. The poly(sodium 10-undecenoate) concentration was 12.0 g/dm^3. The magnetic field sweep was calibrated with Fremy's salt (potassium nitrosodisulfonate) in 0.05 mol/dm^3 potassium carbonate solution, where the two outermost lines in the spectrum are separated by 26.182 G.[15,16] For each quantity measured on the experimental spectra (line widths, line heights, etc.) 95% confidence limits were estimated. Straightforward propagation of errors[17] led to the uncertainties which are presented with the results.

Results

ESR spectra for sodium 5-doxylstearate in water, in sodium 10-undecenoate solution, and in poly(sodium 10-undecenoate) solution are presented in Figure 1. A corresponding set for the labeled testosterone is shown in Figure 2. It is immediately apparent from the qualitative appearance of the ESR spectra that both of the spin-labeled substrates readily associate with both the micelle and the polymer. The loss of motional freedom which occurs upon association with the larger species results in differential broadening of the three lines in the spectrum, and this is
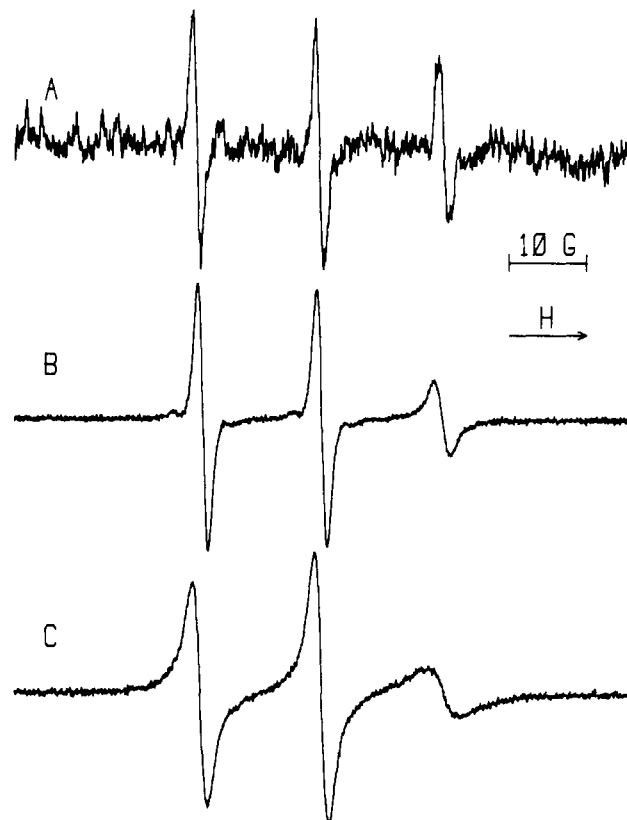
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(16) In saturated potassium carbonate solution substantial differential line broadening was observed, and the spacing between the outermost lines was about 1.5% greater. Differential line broadening was very slight in the 0.05 mol/dm^3 potassium carbonate solution. With the sweep calibrated as indicated, the hyperfine splitting measured for the *p*-benzosemiquinone anion in alkaline ethanol agreed, within experimental uncertainty, with the well-established value of 2.368 G (see: Venkataraman, B.; Segal, B. G.; Fraenkel, G. K. *J. Chem. Phys.* **1959**, *30*, 1006–16). This may explain the discrepancy in hyperfine splitting constants reported in Table I of an earlier study (see: Lim, Y. Y.; Fendler, J. H. *J. Am. Chem. Soc.* **1978**, *100*, 7490–4).

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Figure 2. ESR spectra of spin-labeled testosterone in (A) water, (B) sodium 10-undecenoate solution, and (C) poly(sodium 10-undecenoate) solution. See the Experimental Section for details.

Table I. Hyperfine Splitting Constants and Rotational Correlation Times in Various Media[a]

medium	a_N, G	$\tau_c \times 10^{10}$, s	$R \times 10^{10}$,[b] m
Sodium 5-Doxylstearate			
water	15.87 ± 0.04	2.06 ± 0.13	6.00 ± 0.14
methanol	15.04 ± 0.06		
heptane	14.08 ± 0.06		
micelle solution	15.11 ± 0.07	17.0 ± 1.1	12.1 ± 0.3
polymer solution[c]	15.39 ± 0.16	15.6 ± 2.6	11.8 ± 0.7
Labeled Testosterone			
water	16.07 ± 0.07	1.2 ± 0.5	5.0 ± 0.7
methanol	15.10 ± 0.06		
heptane	14.09 ± 0.06		
micelle solution	15.51 ± 0.04	6.7 ± 0.3	8.89 ± 0.15
polymer solution	15.74 ± 0.11	16.9 ± 1.0	12.1 ± 0.3

[a] For experimental conditions and explanation of the uncertainties listed, see the Experimental Section. [b] See the Results section for the definition of R. [c] Values calculated after applying an approximate correction for the weak signal due to substrate free in solution.

strongly reflected in their relative heights. Since the solubility of the labeled testosterone in water is so low, spectra B and C in Figure 2 are due entirely to labeled testosterone associated with micelle or polymer. Sodium 5-doxylstearate is relatively soluble, however, so its ESR spectrum consists, in general, of overlapping spectra due to bound substrate and substrate free in solution. Concentrations were chosen which yielded essentially only the bound species, although a weak signal from free substrate is still evident in spectrum C of Figure 1.

Having ascertained that the substrates do indeed bind to the micelle and the polymer, the ESR spectra were analyzed in the usual way to gain information on the microenvironment of the labels. The nitrogen hyperfine splitting constant, a_N, was taken to be one-half the separation, measured in gauss (10^4 G = 1 T), between the outermost lines in the three-line spectrum. Values

from these spectra and others from methanol and heptane solutions are reported in Table I. The rotational correlation time, τ_c, was calculated from

$$\tau_c = 5.89 \times 10^{-10} \Delta H_0 [(h_0/h_1)^{1/2} + (h_0/h_{-1})^{1/2} - 2]\ \text{s} \quad (1)$$

where ΔH_0 is the peak-to-peak width of the central line in gauss, and h_1, h_0, and h_{-1} are the heights of the low-field, central, and high-field lines, respectively.[10,11,18] The constant term results from the use of the hyperfine tensor elements for 5-doxylstearic acid.[19] Since there is little variation in these from one nitroxide radical to another, the same constant was used in the analysis of the spectra of the labeled testosterone. The values found for τ_c are listed in Table I.

Equation 1 is based on the assumption of fast, isotropic, rotational motion for the nitroxide label. All of the spectra in this study consist of relatively sharp, symmetrical lines, and this is often taken as sufficient evidence that the motion is indeed rapid and isotropic. On the other hand, the magnetic properties of nitroxide radicals are such that in X-band measurements (employed here) the low-field line is unlikely to be narrower, and therefore taller, than the central line, if the motion is isotropic.[20] The appearance of the spectrum of the testosterone bound to the micelle therefore suggests the possibility of anisotropic motion.

Equation 1 is derived from the quadratic term in the theoretical expression for the line width as a function of nuclear spin state,[18,20,21] and an alternative expression can be derived from the linear term. A comparison of correlation times calculated both ways is sometimes used to check for anisotropic motion.[20] For all three of the 5-doxylstearate spectra and that of the testosterone bound to the polymer, the correlation time from the linear term equation is a little lower than that from eq 1, an average of 13%. (Values ranged from 10 to 16%). For the testosterone bound to the micelle, it is 11% higher. The signal-to-noise ratio for the testosterone in water is insufficient to permit a reliable comparison. Since the two correlation times are in reasonably good agreement for each spectrum, it is concluded that the rotational motion is essentially isotropic for all cases examined here, although the difference between the testosterone bound to the micelle and all of the others may be real.

One further quantity is included in Table I. It is the effective radius of the rotating species, calculated by means of the Stokes equation

$$\tau_c = 4\pi\eta R^3 / (3kT) \quad (2)$$

where R is the radius of the particle, assumed spherical, k is the Boltzmann constant, T is the absolute temperature, and η is the viscosity of the solvent.[21] For each of these aqueous solutions at 23 °C, a value of 9.3×10^{-4} Pa·s was used for η.[22]

Discussion

The primary question posed in this study has been unambiguously answered. A qualitative examination of the ESR spectra has shown that both the amphiphilic 5-doxylstearate and the bulky, hydrophobic, labeled testosterone associate readily with both poly(sodium 10-undecenoate) and the sodium 10-undecenoate micelle in aqueous solution. The rather severe constraints imposed by covalently linking the tails of the surfactant monomers are clearly insufficient to prevent the binding of substrate molecules. Thus, a liquid-like core is not a requirement for substrate binding. Having established this essential fact, the remainder of the discussion is concerned with the additional information which can be obtained from a closer examination of the ESR results.

Spin-labeled molecules have found widespread use in chemistry and biology,[10,11] including experiments on micelles, especially in studies concerned with the microenvironment of the solubilized species.[23] Care is required, however, in the interpretation of such

experiments. In a recent paper, Lindman et al. state "Firstly, to be able to deduce information about the molecular environment in the micelle one must have independent information on the distribution of the probe in the micelle. Secondly, the relation between the spectroscopic parameter in question and the nature of the molecular environment of the probe should either have a sound theoretical basis or it should be experimentally tested in detail."[24] We add a reminder that the possibility must also be kept in mind that the probe may significantly perturb the system being examined. The ability to compare results from the micelle and polymer experiments in this study was very useful in minimizing these problems.

The shape of the ESR spectrum, expressed in terms of the rotational correlation time, is a reflection of the total motional freedom of the nitroxide label. This includes the overall motion of the micelle–substrate or polymer–substrate species and any local motion of the label, such as movement of the labeled substrate within the micelle or polymer, or movement of the spin-label group with respect to the remainder of the substrate molecule itself. Examination of the structural formulas for the labeled substrates shows the possibility of the latter type of motion to be greater for the labeled testosterone, in which the spin-label group is attached to the steroid through three single bonds, than for 5-doxylstearate, in which the doxyl group is bonded directly into the stearate chain. The relative time scales for each of the possible types of motion determine which has the most effect on the ESR spectrum in a particular case.

In spite of the substantial differences in the structures of the two labeled substrates, their ESR spectra when bound to the polymer are nearly identical. The rotational correlation times are the same, within the experimental uncertainty. This indicates that the dominant motion is the overall rotation of the polymer–substrate species, with little contribution from local motion of the label. In view of the internal order forced on the polymer by the covalent bonding, this is not an unexpected result. Further support for this interpretation is given by the radii calculated for these species by means of eq 2. The values are found to be about 12 $\times 10^{-10}$ m, which is only a little less than the length of the fully extended undecenoate anion, between 13 and 14 $\times 10^{-10}$ m.

The spectrum of the 5-doxylstearate bound to the micelle is very close to that for either substrate bound to the polymer. Rotational correlation times and calculated radii are indistinguishable. This is most readily explained by assuming that the motion observed is again that of the overall species, which is apparently very similar to the analogous polymer–stearate species. This is not what would be expected if the interior of the micelle were purely liquid-like. Compared with the covalently linked core of the polymer, a liquid-like interior should permit significantly greater internal freedom of motion, even with the ionic headgroup fixed at the micelle surface. The observed similarity between polymer and micelle is understandable if the stearate chain spends much of its time in an ordered, crystal-like region in the interior of the micelle. With both ends of the chain thus held in place in the micelle, the observed motion would be primarily that of the overall species.

The spectrum of the labeled testosterone bound to the micelle is substantially different from the others. The correlation time and calculated radius are much lower, too low to correspond simply to overall motion of the micelle–substrate species. It is concluded that there is significant motion of the spin-label group and/or substrate itself within the micelle. Perhaps the "crystallinity" of the micelle core is insufficient to immobilize the bulky, labeled, testosterone molecule, or perhaps the testosterone disrupts the order previously existing in the micelle core, similar to the effect of cholesterol on the fluidity of lipid bilayers at temperatures below the lipid phase transition.[25]

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The nitrogen hyperfine splitting constant is sensitive to the polarity of the microenvironment of the nitroxide spin label. Both labeled substrates exhibit the typical variation in this parameter as the medium is changed from highly polar aqueous to nonpolar hydrocarbon solution.[26] When either substrate is bound to the micelle or the polymer, the polarity experienced by the nitroxide is intermediate, but relatively polar, as observed in earlier studies on micelles.[23] Two patterns are observed. First, the polarity experienced by either label is greater in the polymer than in the micelle. This suggests that the substrates are less able to penetrate the nonpolar core of the polymer than that of the micelle. Second, the nitroxide group in the 5-doxylstearate experiences a less polar environment than that in the labeled testosterone in either polymer or micelle. It is not possible to specify unambiguously the cause of this difference. It is, however, consistent with a radial disposition of the 5-doxylstearate with the ionic headgroup located at the surface, forcing the polar nitroxide group a short distance into the interior.

In summary, the 5-doxylstearate appears to be "fixed" to the micelle, just as it is to the polymer, so the stearate tail seems to be located in an ordered, crystal-like region in the micelle interior. The labeled testosterone, on the other hand, experiences a more

liquid-like micelle interior, although it too appears "fixed" to the polymer. It is possible, however, that the testosterone simply disrupts the order previously existing in the micelle. Both substrates are excluded somewhat more from the polymer core than from the micelle core. The covalent bonds apparently impose a higher degree of order than does the packing of the chains in the micelle. While this study does not provide a direct confirmation of the calculations of Dill and Flory,[6] all of the results obtained here are consistent with their general conclusions.

Conclusion

Poly(sodium 10-undecenoate), which is intuitively expected to have some of the characteristics of micelles in solution, has been shown to readily associate with amphiphilic and hydrophobic substrates. This establishes that a liquid-like core is not necessary for substrate binding. All of the results could be interpreted in a manner consistent with the recent conclusions of Dill and Flory[6] on the ordered interiors of micelles.

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Charge-Transfer Spectra of Metallophthalocyanines: Correlation with Electrode Potentials

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Abstract: Electrode potentials are reported for first-row transition-metal phthalocyanines for redox processes occurring both at the phthalocyanine ring and at the central metal ion. Procedures are developed for calculating the potentials of couples which cannot be directly observed. Electronic spectra are reported for many of these species and charge-transfer spectra identified. In many cases such absorption occurs at low energies in the near-infrared region. A model is developed to relate the electrode potentials with the observed charge-transfer spectra. This model provides an assignment for two allowed ligand-to-metal charge-transfer (LMCT) bands and affords calculated energies generally within 2000 cm^{-1}, of those observed. Both 0–0 and 1–0 vibrationally excited LMCT bands are observed. These data permit mapping of the electronic energies of metallophthalocyanines, allow determination of the redox energies of excited states, and hence lead to the purposeful design of metallophthalocyanines as solar energy conversion catalysts.

The phthalocyanine (Pc) ring is subject to successive one-electron oxidation or one-electron reduction to yield cation and anion radicals, respectively. The potentials at which these processes occur have been well documented.[1–6] With redox-active metals, metal oxidation or reduction may also occur. If one or more metal redox processes occur at potentials lying between the phthalo-

cyanine oxidation and reduction, then we may infer that one or more metal d levels lie between the phthalocyanine HOMO (π) and LUMO (π*) orbitals. In such circumstances metal-to-ligand (MLCT) and/or ligand-to-metal (LMCT) charge-transfer transitions may be observed.

Since the separation of the phthalocyanine ring HOMO and LUMO orbitals is only about 1.5–1.7 eV,[4] such charge-transfer transitions may lie at very low energies, probably in the near-infrared region. Fielding and MacKay have previously investigated this region in a number of metallophthalocyanines and report[7,8] weak transitions below 10000 cm^{-1} (1 eV = 8065 cm^{-1}) and ascribed to d–d bands or vibrational overtones. Fortuitously, they

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