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Biradical Spin-Labeled Micelles

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A biradical spin-label, *N,N'*-di-[4-(1-oxyl-2,2,6,6-tetramethylpiperidyl)] urea, has been used for studying the dynamic structure of a micelle, sodium dodecyl sulfate (SDS). Analysis of the ESR spectra of the label has shown that the biradical was incorporated in the micelle. The tumbling motion of the label in the micelle was not strongly inhibited, suggesting a dynamic character of the micelle structure. The exchange rate of the biradical between the micelle and the water phases was slower than 10^7 sec^{-1}. The partition coefficient expressed as the ratio of the concentration of the biradical in the micelle to that of the biradical in the water phase was approximately 6×10^5 in the SDS concentration lower than 2×10^{-2}M. The biradicals form aggregate in the micelle, when they are present in excess in the solution.

In the present paper we describe the results of a preliminary study of the paramagnetic resonance of a biradical acting as a solubilizate (spin-label) in a micelle system. Micelles may be a simple model of biological membranes, and are chosen in this study to illustrate a possible application of a biradical, such as I, to biological systems.

(I)

The spin-label technique has yielded a great deal of information on the dynamic properties of biological systems such as hemoglobin, enzymes, and nucleic acids.[1] More complex systems, for example membranes, are also examined.[2-4] Labels used for the investigations have been mostly nitroxide *monoradicals*. The rate of tumbling motion of the labels is sensitively reflected in the ESR spectra and indicates some of the dynamic and structural characteristics of the environment of the labels. *Biradicals* can also be used as spin-labels and their ESR spectra can yield more information on the environment, since the spectra depend on the intramolecular spin-spin exchange interaction, in addition to the *g*-factor and hyperfine interaction. The exchange interaction is determined by the molecular configuration which, in turn, may be affected by the environment. Hsia and Piette[5] were the first to use biradical spin-labels in the study of hapten-antibody interactions.

The results of the present investigation complement and extend those of Waggoner *et al.*[6] who used nitroxide monoradical spin-labels to study the same micelle system.

Materials and Methods

The spin-label used for the present study is the nitroxide biradical (I), *N,N'*-di-[4-(1-oxyl-2,2,6,6-tetramethylpiperidyl)] urea. This label was prepared by mixing stoichiometric amounts of 2,2,6,6-tetramethyl-4-aminopiperidine-1-oxyl and phosgene in benzene. After several days the reaction mixture was washed with water and dried. The product was purified first by recrystallization from benzene and then by column chromatography on alumina using benzene, ether, and ethanol, needle crystals of mp 145°C (uncorrected) being obtained. Sodium dodecyl sulfate (SDS) was used as the micelle forming compound in water. Its critical micelle concentration (CMC) and aggregation number in water are reported to be 8.1×10^{-3}M

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1) For example, see C. L. Hamilton and H. M. McConnell, "Structural Chemistry and Molecular Biology," ed. by A. Rich and N. Davidson, W. H. Freeman & Co., San Francisco, Calif. (1968), pp. 115—149.

2) V. K. Koltover, M. G. Goldfield, L. Ya. Hendel and E. G. Rozantzev, *Biochem. Biophys. Res. Commun.*, **32**, 421 (1968).

3) W. L. Hubbel and H. M. McConnell, *Proc. Nat. Acad. Sci. U. S.*, **61**, 12 (1968).

4) A. D. Keith, A. S. Waggoner and O. H. Griffith, *ibid.*, **61**, 819 (1968).

5) J. C. Hsia and L. H. Piette, "Recent Developements of Magnetic Resonance in Biological System," ed. by S. Fujiwara and L. H. Piette, Hirokawa Publishing Co., Inc., Tokyo (1968), pp. 74—82.

6) A. S. Waggoner, O. H. Griffith and C. R. Christensen, *Proc. Nat. Acad. Sci. U. S.*, **57**, 1198 (1967).

and 62, respectively.[7] Commercial SDS from Wako Chemical Co. was purified by recrystallization from hot ethanol.

ESR spectra of the biradical aqueous solutions containing various amounts of SDS were measured at room temperature with a commercial X-band spectrometer (JEOLCO Model JES-P-10S).

Results and Discussion

ESR Spectrum of the Biradical Aqueous Solution. The ESR spectrum of the biradical in water shown in Fig. 1a, can be accounted for in terms of the spin-Hamiltonian,[8]

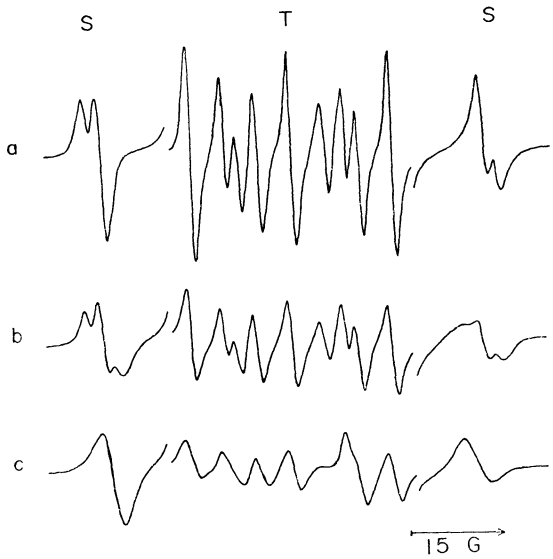

Fig. 1. ESR spectrum of biradical I in a) water, b) aqueous SDS solution of 1.4×10^{-2}M, and c) aqueous SDS solution of 6.9×10^{-2}M. Concentration of the biradical, R_0, is 4.4×10^{-5}M. The "S" resonance spectra are recorded at 6 times higher gain than that of the "T" resonances, since the S resonances are due to the "forbidden" transitions.

$$\mathcal{H}_s = g\beta\mathbf{H}\cdot(\mathbf{s}_1+\mathbf{s}_2) + a_N(\mathbf{I}_1\cdot\mathbf{s}_1+\mathbf{I}_2\cdot\mathbf{s}_2) + J\mathbf{s}_1\cdot\mathbf{s}_2$$

where a_N is the isotropic hyperfine coupling constant of the nitroxide N^{14} nucleus and J is the exchange interaction between the two unpaired electrons in a biradical. The spectra in Fig. 1 consist of the "triplet"-"triplet" or T resonances and the "singlet"-"triplet" or S resonances. The J/a_N value can be easily estimated from the relative S resonance positions. The value obtained for the biradical in water was $J/a_N=1.35$ resulting $J=23.1$ G, since $a_N=17.1$ G. The J values of the biradical in organic solvents (for example 18 G in hexane) were always smaller than that in water, suggesting that the biradical assumes a folded configuration in water and more or less extended forms in organic media.[9] This is consistent with the hydrophobic nature of the biradical as judged from solubility data.

Effect of SDS on the Biradical Spectrum. A series of ESR spectra were recorded with aqueous solutions containing a fixed concentration (R_0) of the biradical and various concentrations of SDS. The ESR spectra remained unchanged until the concentration of SDS became larger than the critical value, 7×10^{-3}M, which is very close to the reported CMC of SDS, 8.1×10^{-3}M. At higher concentrations, the spectrum changed in both T and S resonance regions and new peaks became observable at inner field positions (Fig. 1b) in the latter. As the SDS concentration increased, these new peaks grew in intensity at the expense of the initial resonances. The final spectrum is broad (Fig. 1c), slightly asymmetric with respect to the field and corresponds to a slower rate of tumbling motion of the biradical. Moreover, since the new peaks locate at inner field positions, the J value of the biradical is smaller in the presence of micelles ($J/a_N=1.18$, $a_N=16.9$ G, $J=19.9$ G). The biradical has smaller J values in organic solvents. The results suggest that the biradical is incorporated in the micelle, has organic surroundings and experiences a slower rate of tumbling motion.

State of the Biradical in Micelle. The biradical is not strongly immobilized in the micelle, since the spectrum in Fig. 1c is similar to that of the biradical in ethyleneglycol at *ca.* 30°C. A rough estimation of the correlation time for the tumbling motion in the ethyleneglycol medium gives a value of the order of 10^{-9} sec. If the biradical were to tumble with the micelle as a whole, the correlation time would be of the order of 10^{-8} sec and the breadth of the spectral components would be much larger. This reflects the dynamic character of the micelle structure, in agreement with conclusions drawn from a monoradical spin-label study.[6] Exchange of the biradical between micelle and water, however, is not so fast as to average out the two spectra in Fig. 1a and 1c. The exchange rate is, therefore, slower than the difference in J and a_N, *i. e.* 10^7 sec^{-1}.

The S resonance portions of the spectra were recorded on an expanded scale with solutions containing various concentrations of SDS and $R_0=4.4\times10^{-5}$M. It can be seen from Fig. 2 that isosbestic points are present in the superposition

7) For example, see K. Shinoda, T. Nakagawa, B. Tamamushi and T. Isemura, "Colloidal Surfactants," Academic Press, New York and London (1963), Chap. 1.

8) S. H. Glarum and J. H. Marshall, *J. Chem. Phys.*, **47**, 1374 (1967).

9) T. Iwaoka, T. J. R. Cyr, and S. Ohnishi, unpublished results.

of the S resonance spectra. This demonstrates that the biradicals are present in only two environments, *i. e.* in the micelle and in the water phase. The fraction of the biradical in each phase is easily obtained from Fig. 2 and some values are given in Table 1.

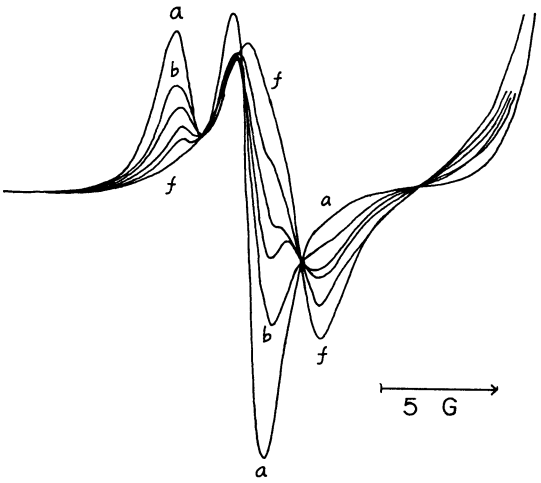

Fig. 2. Superposition of the S resonances of the biradical-SDS solutions. $R_0=4.4\times10^{-5}$M. Concentration of SDS, c_{SDS}, is a) 0, b) 1.0, c) 1.4, d) 1.7, e) 2.1, and f) 14×10^{-2}M.

Table 1

c_{SDS} 10^{-2}M	c_m 10^{-4}M	f_m/f_w a	f_m/f_w b	R_m/c_m a	R_m/c_m b	$P\times10^{-5}$ a	$P\times10^{-5}$ b
1.0	0.6	0.54	0.37	0.27	2.0	5.0	3.5
1.4	1.2	1.2	1.5	0.21	2.3	5.6	7.2
1.7	1.7	1.9	2.6	0.17	1.9	6.0	8.3
2.1	2.3	4.0	3.6	0.16	1.5	11	8.8
2.4	2.8	7.3	5.0		1.3	14	9.8
2.8	3.4	12	6.7	0.12	1.1	19	11
3.1	4.0	14	6.1	0.1	0.96	19	8.5
3.5	4.5	19	5.3	0.095	0.82	23	6.5
6.9	10		19		0.41		10

c_m: Assumed concentration of micelle calculated by $c_m=(c_{SDS}-CMC)/62$, where CMC is 6.7×10^{-3}M.

R_m: Mole of biradical in micelle per liter of aqueous micellar solution.

f_m and f_w: Estimated by comparison of the observed spectra shown in Fig. 2, with calculated spectra assuming various ratios of the fractions.

a and *b*: In these columns are given the data for $R_0=4.4\times10^{-5}$M and $R_0=4.4\times10^{-4}$M, respectively. R_m/c_m and P for $R_0=2.8\times10^{-3}$M solutions are 10 and 7.2×10^5 for $c_m=2.1\times10^{-4}$M, respectively.

P: The partition coefficient defined as $(f_m/f_w)(c_w/c_m)$. The relative error in the evaluation of f_m/f_w is equal to δ/f_mf_w, where δ is the error in f_m and f_w.

Aggregation of the Biradicals in a Micelle. When more concentrated biradical solutions were employed in the above measurements, the isosbestic

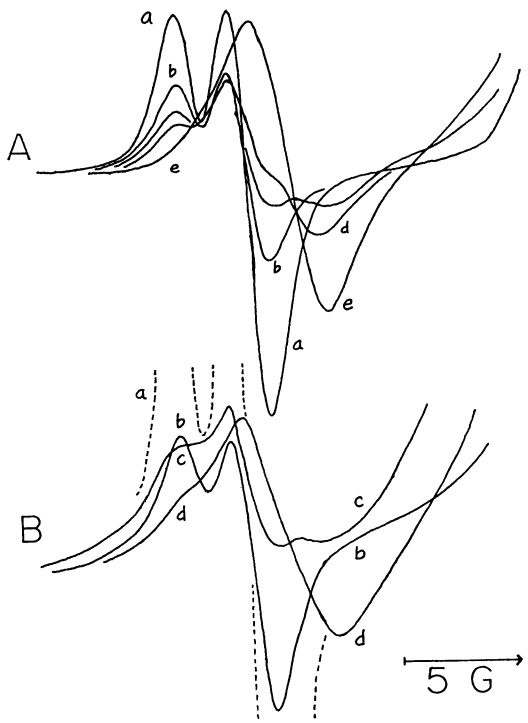

Fig. 3. Superposition of the S resonances of the biradical-SDS solutions.
A: $R_0=4.4\times10^{-4}$M. c_{SDS}; a) 0, b) 1.4, c) 1.7, d) 2.4, e) 3.5×10^{-2}M.
B: $R_0=2.8\times10^{-3}$M. c_{SDS}; a) 0, b) 1.9, c) 5.6, d) 6.9×10^{-2}M. Curve a (dotted) is calculated spectrum.

character was removed. This is evident in Fig. 3 where the S resonance spectra for $R_0=4.4\times10^{-4}$M and $R_0=2.8\times10^{-3}$M are shown. We should note that the solubility limit of the biradical in water is approximately 1×10^{-3}M, and that, in the presence of micelles, the excess biradical beyond this limit is quickly solubilized. Other features seen only at higher biradical concentrations were the decrease in the apparent spectral intensity (*cf.* Fig. 4) and the presence of a background signal increasing monotonously in the field range shown in Fig. 3. The background signal is most pronounced in the spectrum c in Fig. 3B, and from the full spectrum we see that the background is the tail part of a broad single line spectrum. The broadening is interpreted to be due to the interbiradical spin-spin exchange interaction, and can be expected when more than one biradicals dissolve in a micelle particle and the distances among them become small. In Table 1 we listed the number of the biradicals per micelle particle, which was around 2 at low SDS concentrations for $R_0=4.4\times10^{-4}$M. In such cases, the apparent S resonance intensities decrease by an amount equal to the number of biradicals in aggregation. The isosbestic character, observed at lower value of R_0, diappears since there are at

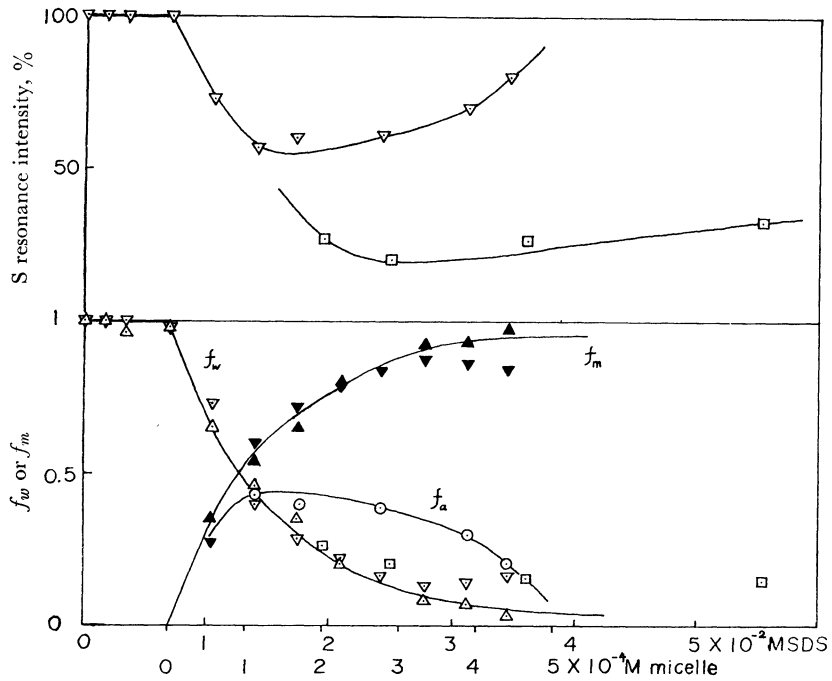

Fig. 4. Upper: The relative S resonance intensity *vs.* SDS concentration. ▽ for $R_0=4.4\times10^{-4}$M and ⊡ for $R_0=2.8\times10^{-3}$M.
Lower: The fraction of biradical in water, f_w, and micelle, f_m, *vs.* SDS concentration. △ and ▲; f_w and f_m for $R_0=4.4\times10^{-5}$M. ▽, ▼, and ⊙; f_w, f_m, and f_a for $R_0=4.4\times10^{-4}$M. ⊡; f_w for $R_0=2.8\times10^{-3}$M. f_a is the fraction of biradical aggregates in micelle.

least three different spectra of various intensities depending on the micelle concentration.

The results suggest that the biradicals in the micellar solutions can be divided into three fractions: those remaining dissolved in water (f_w), those incorporated individually in micelles (f_s), and those forming aggregates in micelles so that the biradicals give exchange-broadened ESR spectra (f_a). The fraction of biradical in a micelle, f_m, is equal to f_a+f_s. The ratio, f_s/f_w, can be estimated from the S resonance spectral shape, and f_a from the decrease in the total S resonance intensity. The values obtained for biradical solutions of various concentrations are given in Table 1 and are plotted in Fig. 4 against the SDS concentration. The micelle concentration, also given on the ordinate, is calculated by assuming that all the SDS molecules form micelles of molecular weight 62×288 above CMC. In the most dilute biradical solution, there is no apparent decrease in the S resonance intensity, suggesting that the biradicals do not aggregate within micelles. At the first measurement above CMC, the calculated micelle concentration, 6×10^{-5}M, is already larger than the biradical concentration, 4.4×10^{-5}M. On the other hand, in the more concentrated biradical solutions, there are fewer micelles than biradicals and the radicals aggregate. The f_a *versus* micelle-concentration curve, after reaching a maximum value, decreases towards zero at higher micelle concentration. If the f_a curve is extrapolated, f_a reaches zero at about 5×10^{-4}M micelles, which is close to the biradical concentration 4.4×10^{-4}M. The radicals, therefore, appear to distribute themselves fairly evenly among the micelles and aggregate only when there are not enough micelles present.

The Partition Coefficient. The f_w and f_m *versus* SDS-concentration curves for the various biradical concentrations fall on the same curve, indicating that the partition of the biradicals in the water and micelle phases is independent of the biradical concentration. The presence of biradical aggregates does not appear to affect the partition. We can define the partition coefficient P as $P=(f_m/f_w)(c_w/c_m)$, where c_w and c_m represent the concentrations of water and micelle in the solution, respectively. The calculated coefficients are fairly constant, 6×10^5 within experimental error, in the smaller SDS concentration region ($<\sim2\times10^{-2}$M) (see Table 1). P seems to tend to increase, however, in the larger SDS concentration region, although the relative error in the evaluation of P becomes greater for larger f_m or f_w values. This might suggest some change in the mode of micelle formation in more concentrated SDS solutions, since, in the calculation of P, we assumed a monotonous linear increase in the micelle concentration.