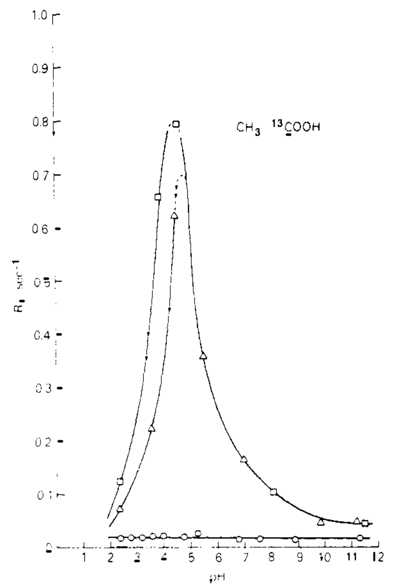


Figure 1. Spin-lattice relaxation rate of the carboxyl carbon atom of 90% enriched $CH_3{}^{13}COOH$ (1 *M* in 0.1 *M* NaCl solution) as a function of pH, alone (○), and in the presence of 3.8×10^{-4} *M* Cu^{2+} (△) and 7.6×10^{-4} *M* Cu^{2+} (□) ions.

Table I

pH	T_1	pH	T_1
3.95	42.2 ± 1.0	5.28	36.4 ± 2.4
4.14	33.1 ± 2.6	6.75	60.3 ± 1.4
4.35	35.8 ± 0.9	7.55	58.8 ± 2.0
4.74	44.6 ± 2.7		

rors of T_1 determinations were 10% or less. Results are reported as relaxation rates, R_1 ($\equiv 1/T_1$).[8]

The R_1 value measured for the carboxyl carbon of neat $CH_3{}^{13}COOH$ was 0.054 sec^{-1}, in good agreement with a previous value for an undegassed sample (0.05 sec^{-1}),[8] although this is naturally higher than the value recorded in degassed samples.[8,10] ^{13}C relaxation rates of 1 *M* aqueous solutions of acetic acid at different pH values are shown in Figure 1. A sample at pH 2.8 diluted to 0.15 *M* showed no significant change giving an R_1 value of 0.028 sec^{-1}.

No significant pH dependence is observed in the R_1 values. A good deal of scatter was observed in the T_1 values using several different samples (Table I). It is not considered warranted to make deductions on the basis of such differences (cf. ref 9) since long T_1 values are known to be very sensitive to the uptake of oxygen from the air and the presence of minute traces of paramagnetic metal ion impurities in solution.

On the addition of Cu^{2+} at 10^{-4} *M* concentration a distinct maximum is observed in the pH dependence of the ^{13}C R_1 value (Figure 1). At intermediate pH values the R_1 value is raised by complexation of Cu^{2+} with the carboxylate anions, whereas at low pH the Cu^{2+} ions are not bound to acetic acid since protons compete effectively, and at high pH hydroxyl ions compete with the acetate ions for the metal ions. The reason for the minimum being close to the p*K* value of the titrating group in this and the previous study[1] is under investigation. It is possible that the decrease in R_1 at high pH reflects a slow rate of exchange of Cu^{2+} between carboxylate anions.

These results do not eliminate spin rotation as a possible source of relaxation for carboxyl carbon atoms in the ostensible absence of paramagnetic metal ions. Nevertheless, it is concluded that low concentrations of paramagnetic metal ions can dominate relaxation in the intermediate pH range leading to a maximum in the spin-lattice relaxation rate.

Acknowledgment. We thank Roderick Wasylishen and Edwin Becker for helpful discussions.

References and Notes

(1) R. E. Wasylishen and J. S. Cohen, *Nature (London)*, **249**, 847 (1974).
(2) A. Allerhand, D. Doddrell, V. Glushko, D. W. Cochran, E. Wenkert, P. J. Lawson, and F. R. N. Gurd, *J. Am. Chem. Soc.* **93**, 544 (1971).
(3) V. Glushko, P. J. Lawson, and F. R. N. Gurd, *J. Biol. Chem.*, **247**, 3176 (1972).
(4) I. C. P. Smith, R. DesLauriers, H. Saito, R. Walters, C. Garrigou-Lagrande, H. McGregor, and D. Srantakis, *Ann. N.Y. Acad. Sci.*, **222**, 597 (1973).
(5) D. T. Browne, G. L. Kenyon, E. L. Packer, M. D. Wilson, and H. Sternlicht, *Biochem. Biophys. Res. Commun.*, **50**, 42 (1973).
(6) I. Chaiken, M. H. Freedman, J. R. Lyerla, Jr., and J. S. Cohen, *J. Biol. Chem.*, **248**, 844 (1973).
(7) D. A. Torchia and K. A. Piez, *J. Mol. Biol.*, **76**, 419 (1973).
(8) T. C. Farrar, S. J. Druck, R. R. Shoup, and E. D. Becker, *J. Am. Chem. Soc.*, **94**, 699 (1972).
(9) J. M. Armitage, H. Huber, H. Pearson, and J. D. Roberts, *Proc. Nat. Acad. Sci. U. S. A.*, **71**, 2096 (1974).
(10) T. D. Alger, D. M. Grant, and J. R. Lyerla, Jr., *J. Phys. Chem.*, **75**, 2539 (1971).

Jack S. Cohen*
Reproduction Research Branch
National Institute of Child Health
and Human Development
National Institutes of Health
Bethesda, Maryland 20014

Robert B. Bradley
Laboratory of Chemical Physics
National Institute of Arthritis,
Metabolism and Digestive Diseases
National Institutes of Health
Bethesda, Maryland 20014

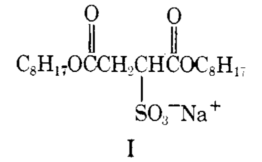
Thomas R. Clem
Division of Research Services
Biomedical Engineering and Instrumentation Branch
National Institutes of Health
Bethesda, Maryland 20014

Received September 7, 1974

Motional Freedom and Polarity within Water Pools of Different Sizes. Spin Label Studies

Sir:

Di-2-ethylhexyl sodium sulfosuccinate (I) has the ability to solubilize large amounts of water in heptane.[1] This water



$$\mathrm{C_8H_{17}O\overset{\overset{O}{\|}}{C}CH_2\underset{\underset{SO_3^-Na^+}{|}}{CH}\overset{\overset{O}{\|}}{C}OC_8H_{17}}$$

I

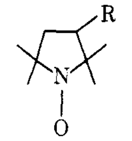
is confined in spherical "pools" encased by the surfactant. The size of the pools depends on the $[H_2O]/[I]$ ratio. For example, light scattering data[1] have revealed that ratios of 2.2, 8.9, and 36 lead to pools containing roughly 40, 400, and 6400 water molecules, respectively. We report here an ESR study of three spin labels (II) dissolved in the water pools. These nitroxide radicals display simple three-line spectra; the hyperfine coupling constants depend on the local polarity whereas the line widths reflect the rate of mo-

Table I. Dependence of the Hyperfine Splitting Constant, a_N, and Line Width Parameter, W, of Three Spin Labels on the Size of the Water Pools

Spin label	Solvent or $[H_2O]/[I]^a$	a_N, G (±0.7%)	W, G (±15%)
IIa	Water	16.27	0.03
IIa	1,0 M aq SBS[b]	16.26	0.02
IIa	4.7 M aq NaCl	16.30	0.04
IIa	Methanol[c]	15.30	0.04
IIa	1-Butanol[c]	15.31	0.17
IIa	1-Octanol[c]	15.23	0.42
IIa	Benzene	14.40	0.01
IIa	51.6	16.00	0.16
IIa	36,1	15.84	0.21
IIa	25.8	15.68	0.28
IIa	25.8[d]	15.63	0.24
IIa	15.5	15.51	0.39
IIa	12.9	15.42	0,43
IIa	10.3	15,26	0.46
IIa	10.3[e]	15.29	0.48
IIa	5.2	14.74	0.76
IIa	2.6	14.48	0.83
IIb	Water	17.31	0.03
IIb	36.1	16.60	0.31
IIb	10.3	16.06	0.32
IIb	2.6	15.68	0.60
IIb	Benzene	15.66	0.02
IIb	Heptane	15.30	0.01
IIc	Water	16.42	0.03
IIc	36.1	16.32	0.13
IIc	10.3	15.7[f]	h
IIc	10.3[g]	15.8[f]	h
IIc	2.6	14.41	0.68
IIc	Benzene	14.36	0.03

[a] [I] = 0.11 M in the heptane–water mixture. [b] SBS = sodium benzenesulfonate. [c] The viscosities in millipoises of methanol, 1-butanol, and 1-octanol at 20° are 5.93, 29.48, and 89.47, respectively. [d] This sample was degassed by the freeze–thaw method at 5×10^{-6} mm. None of the other samples was degassed. [e] The concentration of label was 2.1×10^{-5} M. In all other samples the concentration was 7.2×10^{-5} M. [f] These are approximate values. [g] The aqueous phase consisted of 0.001 M phosphate buffer, pH 7.00. [h] W values cannot be calculated because the high-field signal consisted of two lines.

lecular tumbling. Although spin labels have been widely used,[2,3] this seems to be the first investigation of a system in which it is possible to vary continuously the size of the concavity bearing the nitroxide. Table I lists nitrogen hyperfine



IIa, R = $CONH_2$
b, R = OH
c, R = COO^-

splitting constants (a_N)[4] and line width parameters (W)[5] as a function of $R = [H_2O]/[I]$. It is seen that as R decreases (i.e., as the pools become smaller) the coupling constants decrease and the line width parameters increase for all three labels. The similarity of the ESR parameters at high R values to those in pure water (Table I) proves that the labels are located within the pools rather than in the bulk heptane. This is reasonable because we found that less than 1% of IIa partitions into heptane when IIa is equilibrated with heptane and an equal volume of 4.7 M aqueous NaCl. At the low concentration of label used in our experiments (7.2×10^{-5} M), one can calculate[1] that there is only one label molecule for every nine pools when $R = 36$. As shown

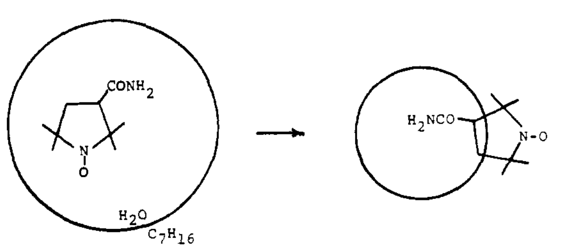


Figure 1. Schematic representation of the effect of pool size on the location of a spin label.

in Table I, reducing the label concentration 3.5-fold has an insignificant effect on the ESR parameters; thus, nitroxide-nitroxide interaction[2] is not a factor.

The trends evident in Table I can be explained readily in a qualitative manner. As the pool size diminishes from $R = 51.6$ to $R = 2.6$, the coupling constant a_N gravitates from that characteristic of water to that characteristic of heptane.[6] The coupling constant of IIa at $R = 10.3$ indicates a polarity equivalent to methanol.[7] Since the local environment of the nitroxide groups is heptane-like in the small pools ($R < 10$), the labels must reside either (1) entirely within the intermicellar heptane phase or (2) at the heptane-water interface with the nitroxide moiety penetrating into the hydrocarbon. The former possibility seems unlikely. First, as mentioned above, partitioning of the labels into heptane is unfavorable. Spin label IIc, bearing a carboxylate, would be particularly reluctant to dissolve in the heptane. The large increases in W with decreasing pool size (Table I) also point to interfacial adsorption. This broadening is caused by weak immobilization of the spin label and/or by time averaging of signals from polar and nonpolar media. Neither of these effects could explain the increases in W with decreasing R if spin labels relocated completely into the fluid heptane phase. Therefore, contraction of the pool volume must "squeeze" the spin label from the pool interior onto the interface (Figure 1). In this region the nitroxide groups can immerse themselves in the heptane while experiencing a small loss of motional freedom that frequently accompanies adsorption.[8,9] Comparing W values for IIa in small pools with those in alcohols of differing viscosities (Table I) shows that the apparent viscosity at the interface is greater than 90 mP.

At intermediate values of R, IIc shows spectral distortions not present with the two nonionic spin labels.[8] Most notably, the high-field peak of IIc at $R = 10.3$ is replaced by two lines. In this regard, the spectrum resembles that of 2,2,6,6-tetramethylpiperidinooxy radical in an aqueous phospholipid dispersion.[10] Buffering the water phase at pH 7.00 does not affect the ESR spectrum of IIc. The simplest explanation for the distortion is that signals from the probe molecules in the water pools and at the interface can be observed separately.[10,11] Although the high-field lines are poorly resolved, substantial amounts of each species are obviously present. These results imply that IIc molecules in the pool interior and at the water-heptane boundary interchange slowly relative to the ESR time scale ($k_{ex} < 10^7$ sec^{-1} when $\Delta a_N = 2$ G).[12] In contrast, the nonionic labels exchange environments rapidly to produce spectra which are weighted averages of those found in aqueous and hydrocarbon media.

Another explanation exists for the gradual decrease in a_N with decreasing pool size. The label may remain entirely within the water pool[13] whose dielectric constant varies over the whole polarity range. The large W values at $R < 10$ would then point to viscous micellar water (perhaps caused in part by the high concentration of Na^+, a "structure-forming" ion). This explanation seems unlikely because 4.7

M NaCl hardly perturbs either a_N or W (Table I). Moreover, we have shown previously[1] that the Z value for micellar water at $R = 3.1$ lies midway between that of bulk water and methanol. Fluorescent probe data likewise indicate a rather high water polarity at low R values.[1] Thus, presently available evidence is not consistent with water pools achieving a polarity approximating that of benzene.[14]

Acknowledgment. This work was supported in part by grants from the National Science Foundation and the National Institutes of Health.

References and Notes

(1) F. M. Menger, J. A. Donohue, and R. F. Williams, *J. Am. Chem. Soc.*, **95**, 286 (1973).
(2) O. H. Griffith and A. S. Waggoner, *Acc. Chem. Res.*, **2**, 17 (1969).
(3) H. M. McConnell and B. G. McFarland, *Q. Rev. Biophys.*, **3**, 91 (1970).
(4) The data were obtained with a Varian V-4500 ESR spectrometer (9.5 GHz) operated at ambient temperature. No saturation effects were observed. The a_N values were calculated from the separation of the first two peaks of the triplet on a scale calibrated with benzosemiquinone.
(5) $W = W_0[(W_1/W_0) - (W_{-1}/W_0)]$ where W_1, W_0, and W_{-1} are the peak-to-valley widths of the low, middle, and high field portions of the triplet, respectively. Values of W, which are proportional to rotational correlation times, were calculated by the "ratio of heights" method. See A. S. Waggoner, O. H. Griffith, and C. R. Christensen, *Proc. Nat. Acad. Sci., U. S. A.*, **57**, 1198 (1967).
(6) For solubility reasons we obtained ESR spectra for IIa and IIc in benzene but not heptane. However, the parameters for IIb in these solvents are similar (Table I).
(7) For a table of coupling constants of di-*tert*-butyl nitroxide in several solvents see T. Kawamura S. Matsunami, and T. Yonezawa, *Bull. Chem. Soc. Jpn.*, **40**, 1111 (1967). See also G. H. Dodd, M. D. Barratt, and L. Rayner, *FEBS Lett.*, *8*, 286 (1970).
(8) The spectra resemble closely that shown in Figure 1b in A. S. Waggoner, A. D. Keith, and O. H. Griffith, *J. Phys. Chem.*, **72**, 4129 (1968).
(9) M. J. Povich, J. A. Mann, and A. Kawamoto, *J. Colloid Interface Sci.*, **41**, 145 (1972).
(10) W. L. Hubbell and H. M. McConnell, *Proc. Nat. Acad. Sci. U. S. A.*, **61**, 12 (1968).
(11) O. H. Griffith, L. J. Libertini, and G. B. Birrell, *J. Phys. Chem.*, **75**, 3417 (1971).
(12) P. B. Ayscough, "Electron Spin Resonance in Chemistry," Methuen, London, 1967, p 309.
(13) Of course, the distinction between interfacial and aqueous adsorption of the spin labels becomes unclear at very low R values where micellar water is no doubt largely bound by the ionic portion of I.
(14) See D. C. Grahame, *J. Chem. Phys.*, *18*, 903 (1950) for a plot of dielectric constant of water as a function of distance from the center of a univalent ion.
(15) Recipient of a Camille and Henry Dreyfus Teacher–Scholar Grant and a National Institutes of Health Research Career Development Award.

F. M. Menger,*[15] G. Saito, G. V. Sanzero, J. R. Dodd
Department of Chemistry, Emory University
Atlanta, Georgia 30322

Received July 29, 1974

Ammonia Formation from Molybdenum-Bound Dinitrogen under Weakly Reducing Protic Conditions

Sir:

Conversion of transition metal bound dinitrogen (N_2) to ammonia (NH_3) by means of mild reducing agents is regarded as a fundamental and challenging problem in inorganic chemistry. Past reports in the biologically relevant molybdenum area concern $^{15}NH_3$ formation from the comparatively stable *trans*-$Mo(^{15}N_2)_2(dppe)_2$ (**1**)[1] when acted upon in THF by naphthalene radical anion (2.60 V)[2] or by the combination $[Fe_4S_4(SC_2H_5)_4]^{3-}$–fluoranthene radical anion (1.77 V).[3] While studying the effect of solvent on such reductions,[4] we observed that abundant amounts of $^{15}NH_3$ are generated when the complex **1** alone is merely treated with aqueous acid in *N*-methylpyrrolidone (NMP) or propylene carbonate as solvent, a reaction in which presumably the transition metal itself also acts as the reductant.

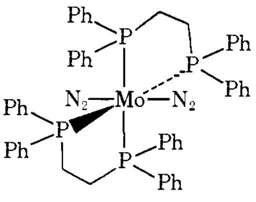

1

Table I. Effect of Solvent on Reduction of Complex 1[a]

Solvent	Acid	Concn (10^{-1} *M*)	Mol of NH_3/mol of Mo
NMP	HBr	4.5	0.286
Propylene carbonate	HBr	2.3	0.190
Triethyl phosphate	HBr	4.5	0.014
THF	HBr	4.5	0.000_9
THF	HCl	6.4	0.007_5

[a] $[1] = 2.3 \times 10^{-3}$ *M*, $T = 25°$, 29 hr.

Table II. Effect of Acid on Reduction of Complex **1**[a]

Acid	Concn (10^{-1} *M*)	Temp, °C	Mol of NH_3/mol of Mo
HCl	6.0	25	0.002
HCl	1.2	50	0.079
HBr	2.4	25	0.368
HBr	4.5	25	0.286
p-TSA	3.4	25	0.045
CF_3SO_3H	2.8	25	0.003

[a] $[1] = 2.4\text{–}5 \times 10^{-3}$ *M*, 29 hr.

In a typical experiment, 100 mg of complex **1**[5] was dissolved in 50 ml of NMP. After degassing with argon, 5 ml of redistilled aqueous HCl or HBr was added dropwise, and stirring was continued at 25° for ca. 30 hr. In the work-up, NH_4Cl and water were added, and the solution was rotoevaporated above room temperature nearly to dryness. The residue was taken up in water, and $^{14/15}NH_3$ was distilled under Kjeldahl conditions and trapped in aqueous HCl. The $^{15}NH_3$ content was determined by mass spectral analysis, in which procedure background $^{15}NH_3$ values were determined for NMP alone, and for complex **1** without use of mineral acid (0.003 mol of NH_3/mole of Mo).

The solvents $PO(OC_2H_5)_3$ and THF were much less efficacious for NH_3 production (Table I), while DMF and DMSO underwent serious side reactions. Hydrobromic acid was more effective than hydrochloric, trifluorsulfonic, or *p*-toluenesulfonic acid (Table II), preliminary data suggesting an optimal HBr:Mo ratio of about 100:1. Even after a few hours reaction time, the NH_3 yields were considerable. In no experiment could product hydrazine be detected (>5%) with *p*-dimethylaminobenzaldehyde. The complex $Fe^{15}N_2H_2[PC_2H_5(C_6H_5)_2]_3$[7] was found not to yield $^{15}NH_3$ under any conditions assayed. The course of the $^{15}NH_3$ producing reaction is adversely affected by impurities; for example, those present in NMP which is not freshly distilled from $NaOCH_3$, or in complex **1** prepared through use of Na sand rather than Na/Hg.

Since the N_2 complex **1** is converted to the species $MoBr_2(N_2H_2)(dppe)_2$ (**2**) under conditions[8] similar to those described herein for the generation of NH_3 from **1**, it seems reasonable that **2** mediates the latter process. In keeping with this interpretation, we found that subjection of **2** alone to reaction conditions used with **1** (as described above) resulted at least 0.15 mol of $^{15}NH_3$/mol of Mo, and that equimolar amounts of ^{15}N-labeled **2** and ^{14}N **1** gave rise to 0.48 mol of $^{15}NH_3$/mol of Mo (**1** + **2**)[9]. The high NH_3 yield in this experiment supports the intermediacy of **2** in the overall process **1** → NH_3; a second Mo may simply act as a reducing agent or may form a binuclear complex, e.g.,