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ELECTRON SPIN RESONANCE STUDY OF SPIN PROBES IN FROZEN AQUEOUS SOLUTIONS

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The ESR spectra of 12-nitroxide stearic acid (12-NS) in frozen aqueous solutions of sodium hydroxide(1N~0.005N) were examined in the temperature range from -22.0 to -0.1°C. The ESR line shapes of the spectra of 12-NS in ice exhibit two types spectra overlaped, a broad singlet and a sharp triplet. Some amounts of 12-NS molecules move rapidly in frozen aqueous solutions below the melting point of ice.

Nuclear magnetic resonance(NMR) spectroscopy was used to examine the behavior of mobile water molecules within frozen aqueous solutions.[1,2] Electron spin resonance (ESR) study of mobile molecules in frozen aqueous solutions has never been reported in the temperature range from -22.0 to -0.1°C. The spin labeling has been applied to wide variety of problems. This technique enables us to study the motion of a free radical in its microscopic environment by ESR.[3,4,5] In this experiment 12-NS molecule was used as a probe to examine the surroundings of the molecule. The purpose of this paper is to report temperature dependencies on the ratios of two states of 12-NS probes in frozen aqueous solutions.

The ESR spectra were recorded with a JES-3BS-X(X-band) spectrometer. A specially designed thermo control unit was made for measurement. The temperatures were regulated within ± 0.01°C and monitored with a small thermistor during a run. Once the spectrum was recorded, the temperature was changed to a new fixed point about 0.5°C higher(lower) with a heating(cooling) rate of 0.1°C/min. A sequence of the spectra was recorded at increasing and decreasing temperatures.

2-(10-Carboxydecyl)-2-hexyl-4,4-dimetyl-3-oxazolidinyloxyl (12-NS) was synthesized by the method of Waggoner et al.[6]

The solubility of 12-NS is very low. Therefore NaOH was added for dissolution. The concentrations of 12-NS were 1.9×10^{-4}M and those of NaOH were from 1 to 0.005N. Capillary tubings of the order of 1.0mm inner diameter were filled with the solution by capillary action and sealed at one end. No effort was made to exclude air since exposure to air did not affect the line shapes of 12-NS. The water used in the experiment was obtained by running distilled water through an ion exchange column and distilled again.

Figure 1 shows the ESR spectra of 12-NS at -7.35°C and -20.15°C. These spectra are attributed to the probe moving quite rapidly and isotropically in supercooled water,

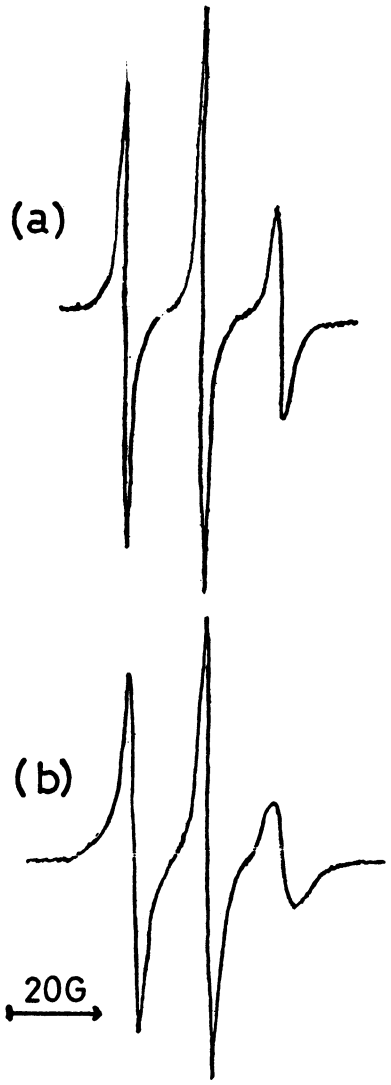

Fig.1. ESR spectra of 1.9×10^{-4}M 12-NS in supercooled solution of NaOH(0.5N). (a),T=-7.35°C; (b),T=-20.15°C.

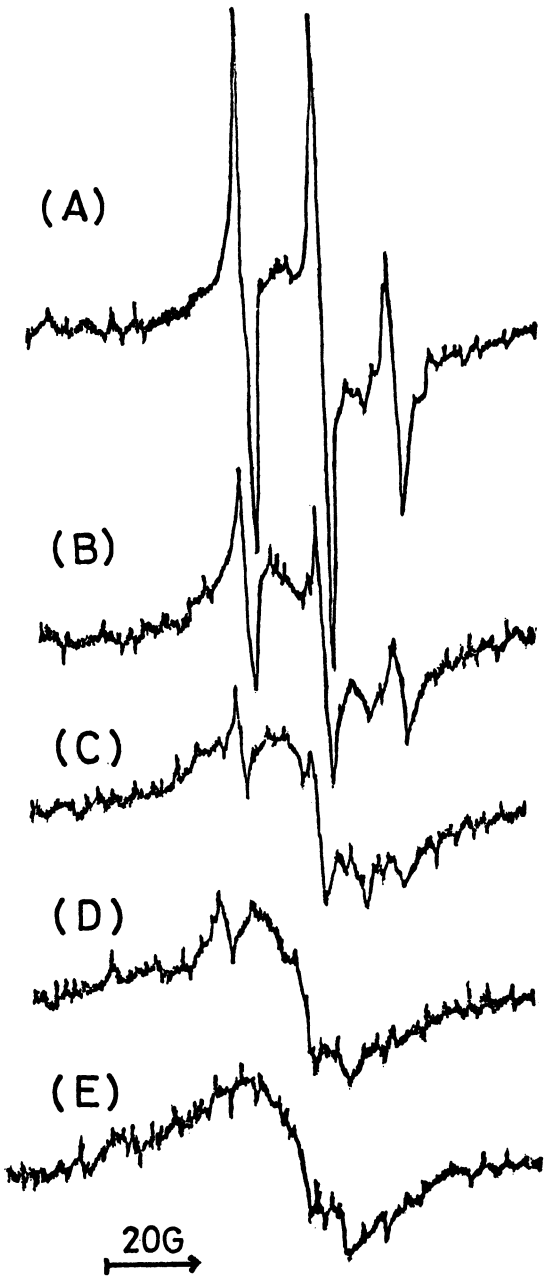

Fig.2. Variation of the spectra of 1.9×10^{-4}M 12-NS with temperature in frozen aqueous solution of NaOH(0.5N). (A),T=-4.85°C; (B),T=-6.05°C; (C),T=-7.45°C; (D),T=-8.35°C; (E),T=-9.25°C.

because the spectrum at about the same temperature starting from the frozen sample is very different from the one (Fig.2 C). When gradually cooled in a variable temperature accessory, freezing occurs suddenly from the bottom of the capillary tubing at about -23°C. The freezing of supercooled water depends on the existence of dust particles or capillary diameter of sample tubings. Ahn used very fine capillaries and his samples became frozen at -34°C.[7] When the sample is frozen, a drastic change in the spectrum occurs. A broad singlet appears instead of the sharp triplet. The ESR spectra of 12-NS in frozen aqueous solutions do not yield slow motional anisotropic line shapes conventionally found in the spectra of dispersed probes at low temperature. It suggests that solvent-solute segregation occurs in ice, resulting in associations of 12-NS probes.[8] As the result, spectra

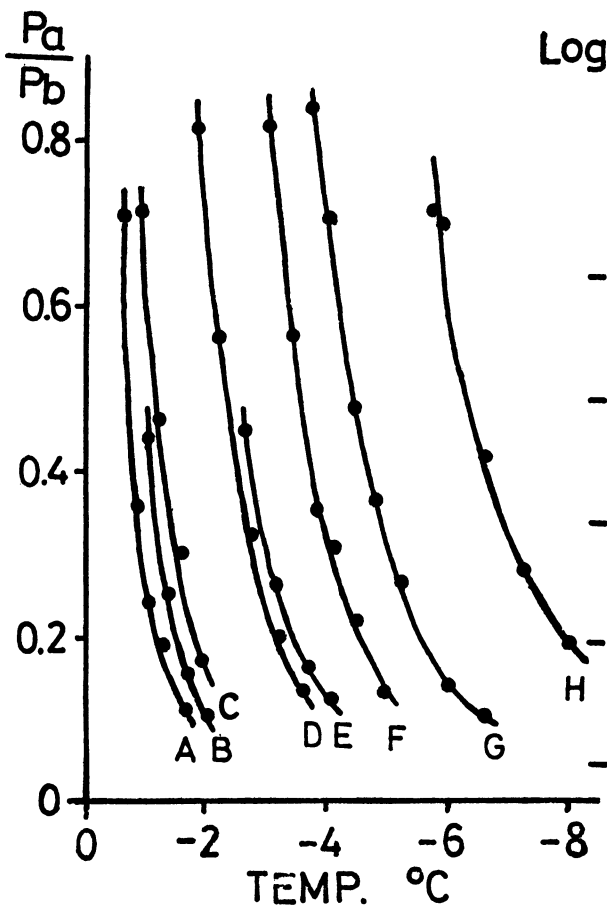

Fig.3. The ratios(Pa/Pb) vs. temperature. Concentrations of NaOH are 0.005N, 0.01N, 0.02N, 0.05N, 0.1N, 0.2N, 0.5N, and 1.0N for A,B,C,D,E,F,G, and H, respectively.

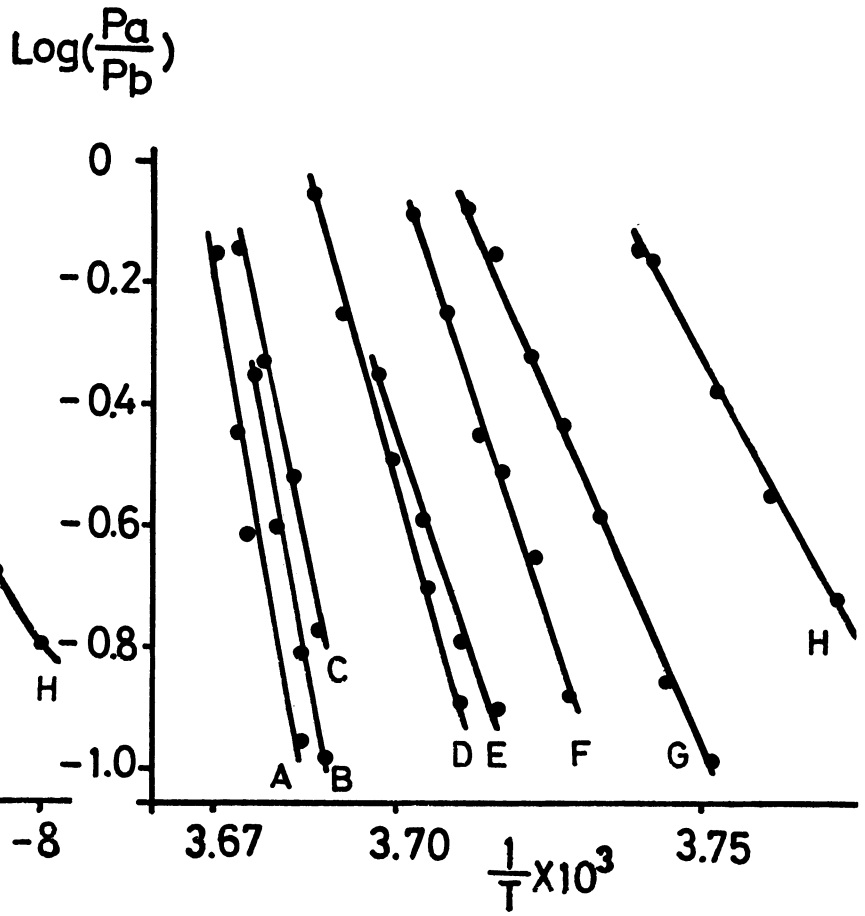

Fig.4. Common logarithm of the ratios(Pa/Pb) vs.1/T. A,B,C,D,E,F,G, and H as in Fig.3.

were broadened by dipolar and exchange interactions.

Sharp triplet emerges at about -8°C (Fig.2 D) overlapping on the broad singlet with increasing temperature. The triplet is due to rapid isotropic tumbling of the probes. In the course of the frozen sample, this phenomenon is reversible until the sample is melted, A⇄B⇄C⇄D⇄E (Fig.2). The reproducibility was examined by the different process of freezing. When the sample was frozen by dipping into liquid nitrogen and the temperature was raised nearly to the melting point, the spectra showed the same phenomenon. The intensity ratios of the triplet to the singlet increase with increasing temperature.

As the peak area is assumed to be propotional to the peak to peak intensity, the curves (Fig.3) represent the variations of the amounts of free rotating and associated probes with temperature measured at equilibrium. An equilibrium state between free rotating and associated probes remains unchanged at a fixed temperature for several hours. Pa and Pb are the corresponding peak area for the sharp triplet and the broad singlet, respectively. The ratios Pa/Pb increase exponentially toward the melting point of ice. The ratios in the case of the low concentrations of NaOH increase more rapidly than that of the high concentrations of NaOH. The increasing ratios which show

amounts of the mobile 12-NS molecules in ice increase with increasing temperature below the melting point. Therfore, the ice around the probes is expected to melt and the surrounding water molecules move rapidly with temperature.

Figure 4 shows the common logarithm of the ratios of the peak area (Pa/Pb) versus 1/T. How well these linear relatioships are met such a narrow temperature interval! Even though a detailed knowledge of this phenomenon is lacking, the result suggests that rapidly moving probes are in equilibrium with associated probes nearly below the melting point. Values of enthalpy(Δ H) of this phenomenon were calculated. For example, the calculated values are 95, 130, and 220 kcal/mol for 1, 0.2, and 0.01N NaOH solution, respectively. The values increase with decreasing concentrations of NaOH. It is interpreted that the ice lattice around the probes is destroyed by further addition of NaOH. However, even here the solvent-solute interaction is not well understood in ice.

Further studies are now in progress to make clear this phenomenon and detailes will be published elsewhere.

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