

Electron Spin–Lattice Relaxation Times of Spin Probes in Aqueous Dispersions of a Unique Amphiphilic Compound Obtained by a Saturation Recovery Method

Kouichi Nakagawa

RI Research Center, Fukushima Medical University, 1 Hikarigaoka, Fukushima 960-1295

Received January 25, 2008; E-mail: nakagawa@fmu.ac.jp

Electron spin–lattice relaxation times (T_{1e}) of a unique nonionic amphiphilic compound, (poly(oxyethylene) hydrogenated castor oil, HCO) in aqueous dispersions, were investigated by electron paramagnetic resonance (EPR) and saturation recovery (SR) spectroscopies. The spin probes, 5-doxylstearic acid (5-DSA) and 3β-doxyl-5α-cholestane (CHL), were used to obtain T_{1e} values for the head group region in the HCO membrane. Analysis of SR signals from both probes showed that the T_{1e} value ($\approx$5 μs at 20 °C) indicates relatively faster relaxation behavior in the region than that of 12-DSA. No abrupt change (such as phase transition) of the relaxation time was observed in the temperature region studied. Thus, the present T_{1e} results indicate relative flexibility for both probe moieties in the membrane throughout the temperatures studied.

Quantitative knowledge of the internal dynamics of amphiphilic membranes provides important clues for a detailed understanding of various properties. Especially, the behavior of the head group region may play an important role in controlling water molecules in-and-out of the membrane. Dynamic information of various positions in the membrane can be obtained by electron paramagnetic resonance (EPR) as well as fluorescence spectroscopy.[1,2] EPR spectral shapes are useful monitors of motion and environment of the probe. The interpretation of EPR line shapes can be done by the rotational diffusion coefficients, order parameters, and relaxation times.[3–6] The investigation of the head group dynamics in the membranes have also been performed using fluorescence spectroscopy in conjunction with aromatic probes. The probes having a large aromatic ring and very different chemical structure relative to the membrane components are normally used.

EPR is a powerful technique for the study of dynamics over the range from picoseconds to milliseconds in tumbling times. Conventional continuous-wave (CW) EPR gives motional information with the observed spectral analyses. Advanced time-domain EPR yields more insight into the spin system because less averaging of relaxation times is done.[7] It is interesting to note that various motional processes contribute to electron spin–lattice relaxation such as tumbling motion and intramolecular motions.[8] The direct measurement of spin probe moieties in a membrane can be performed using a saturation recovery (SR) apparatus. SR is the technique for electron spin–lattice relaxation measurements in EPR.[7–9] With long and intense microwave pulses, the spin system approaches a steady state, at which the population of spins at each energy level tend to be equalized. After the saturating pulse, the recovery signal of the system to Boltzmann equilibrium is observed with non-perturbing microwave observing power. Thus, the SR signal represents a direct relation to motion and environment of the probe radicals.

For current SR investigations, one can use a long-pulse to saturate almost the entire EPR signal and the so-called true T_{1e} is able to be extracted because spectral diffusion processes including nitrogen nuclear spin flips have less impact on SR observation.[8] The recovery signal for each delay time between the intense saturating pulse and observing pulse was recorded with magnetic field in a way similar to the determination of T_1 for nuclear spin in NMR experiments. From this viewpoint, SR is an ideal instrument to measure T_{1e} in the membranes.

In this investigation, a newly modified SR apparatus was applied to reveal detailed probe behavior in the head group region of the HCO membrane. The spin lattice–relaxation times of spin probes (5-doxylstearic acid and 3β-doxyl-5α-cholestane) in aqueous dispersions of HCO membrane were analyzed as a function of temperature. The detailed probe environment in the membrane is also discussed in terms of the electron spin–lattice relaxation time.

Experimental

Materials and Sample Preparations. Poly(oxyethylene) hydrogenated castor oil (HCO) of the highest quality was donated by Nikko Chemicals Co., Ltd. (Tokyo, Japan) and used as received. A detailed description of the HCO is reported elsewhere.[10] The spin probes 5-doxylstearic acid (=2-[(3-carboxypropyl)-4,4-dimethyl-2-tridecyl]-3-oxazolidinyloxy, 5-DSA), and 3β-doxyl-5α-cholestane (=4′,4′-dimethylspiro[5a-cholestane-3,2′-oxazolidin]-3′-yloxy, CHL) were obtained from Sigma-Aldrich Co. (Tokyo, Japan). The chemical structures of HCO and the spin probes used in this study are depicted in Figure 1. Lecithin (phosphatidylcholine, PC) from egg was purchased from Wako Pure Chemical Ind. Ltd., Japan. All chemicals were of the highest grade obtainable and used as received.

Sample solutions were prepared as follows: A weighed amount of HCO was dissolved in a few milliliters of chloroform. The spin probe was dissolved in $\approx$0.3 milliliters of chloroform and mixed

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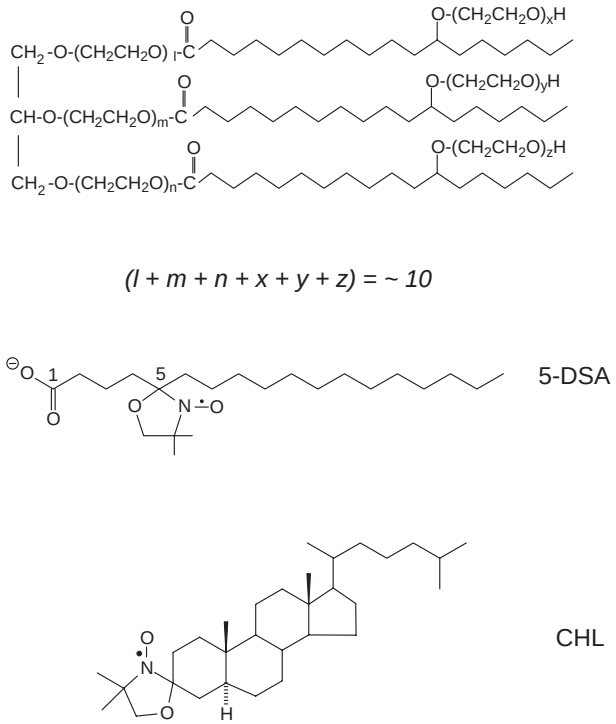

Figure 1. Chemical structures of poly(oxyethylene) (10) HCO and the spin probes (5-DSA and CHL) used are depicted.

with the HCO solution. After evaporation of the chloroform in a rotary evaporator, a 10 wt % dispersion of HCO/spin probe in distilled water (Wako Pure Chemical Ind. Ltd., Japan) was prepared. The test tube containing the dispersion solution was agitated on a vortex mixer until completely dispersed. An aliquot of the dispersion was used for EPR measurements. The final concentration of the spin probe was approximately 80 μmol dm^{-3} for the measurements. A detailed description of the sample preparation including lecithin (PC) from egg is also presented elsewhere.[1,9]

Deoxygenation. For CW EPR, the sample solutions (≈0.15 mL) were deoxygenated for about 15 min in an AtmosBag (Aldrich) and the solutions were put into capillaries (i.d., 0.9 mm; o.d., 1.4 mm; Nippon Rikagaku Kikai Co., Ltd., Japan). The sample capillary was inserted in a 3-mm EPR tube (JEOL Datum Co., Ltd., Japan) in the AtmosBag and taped around the tube cap.

EPR Measurements. EPR signals were measured by a JEOL FE1X X-band EPR spectrometer. This spectrometer is equipped with a cylindrical TE_{011} mode cavity. The sample temperature was controlled by nitrogen gas flow through the Dewar using a JEOL ES-DVT system. The microwave frequency was measured using an EMC-14 X-band microwave frequency counter (Echo Electronics Co., Ltd., Japan). Typical EPR conditions were the following: microwave frequency, 9.18 GHz; microwave power, 5 mW; modulation amplitude, 0.032 mT; time constant, 1 s; scan rate, 0.3125 mT per min.

Saturation Recovery (SR) Measurements. Electron spin–lattice relaxation time (T_{1e}) was measured by a home-built saturation recovery (SR) apparatus based on a Varian EPR spectrometer equipped with a 5-loop-4-gap resonator (LGR).[7,8] For this investigation, Teflon tubes to achieve degassing were used in the following manner: Two Teflon tubes were inserted side-by-side in a 4-mm EPR tube. One Teflon tube (i.d., 0.96 mm; o.d., 1.56 mm) contained the sample solution. Nitrogen was passed

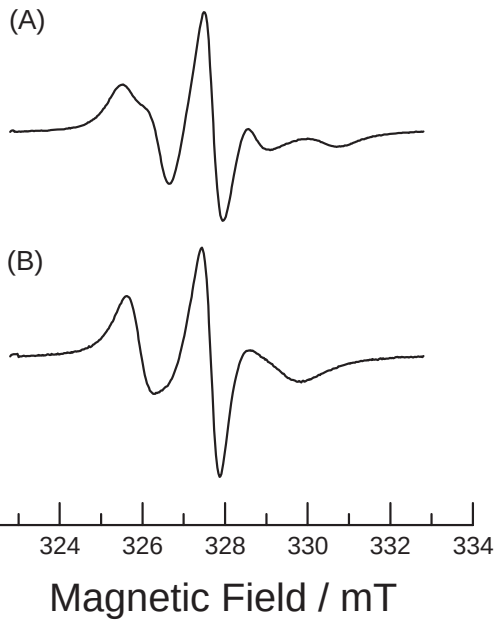

Figure 2. Representative experimental EPR spectra of (A) 5-DSA and (B) CHL in aqueous dispersions of HCO at 20 °C are presented.

through the second Teflon tube (i.d., 1.5 mm; o.d., 2 mm) to purge oxygen from the solution. Sample temperature was controlled by a Varian V-6040 and nitrogen gas passed over the LGR. Temperature was monitored with a thermocouple positioned immediately above the resonator. No magnetic field modulation was used. The applied magnetic field was set on the magnetic field that corresponds to the maximum intensity in the first-integral spectrum. The SR signal was observed as an exponential curve. SR curves can be fit with a single-exponential to obtain T_{1e} as follow:

$$SR = A[1 - \exp(-t/T_{1e})] \quad (1)$$

where t is the time, and A is adjustable constant.

Results and Discussion

In order to reveal the motional behavior in the head group region, 5-DSA and CHL were used as probes. Figure 2A shows the typical EPR spectrum of HCO/5-DSA in an aqueous dispersion at 20 °C. The EPR spectrum shows that the nitroxide probe is relatively immobilized in the membrane and clearly recognizes the parallel ($2A_{\parallel}$) and perpendicular ($2A_{\perp}$) hyperfine components as reported previously.[11] Figure 2B shows the EPR spectrum of HCO/CHL in an aqueous dispersion at the same temperature. The EPR spectral pattern is different from the one for 5-DSA because the axes of CHL are not the same along the membrane Z axis.[11]

Next, direct observations of interaction between spin probe and lattice (membrane) were made by SR spectroscopy.[9,12,13] Typical SR signals of 5-DSA and CHL obtained at 20 °C are presented in Figures 3 and 4. The spin–lattice relaxation times (T_{1e}) are sensitive to overall molecular tumbling of the probe at the specific position and to internal motion within the membrane. The recovery signal was obtained at the magnetic field that corresponds to the centerline. The T_{1e} values were calculated using a single-exponential fit for the SR signal using eq 1. The T_{1e} values obtained are listed in Table 1 together

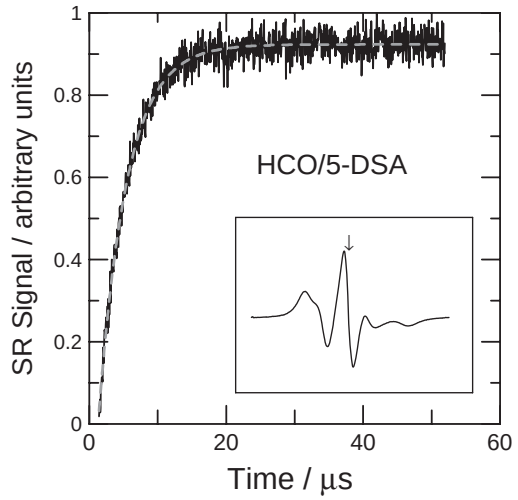

Figure 3. Typical saturation recovery signal from 5-DSA in H_2O dispersions of HCO at 20 °C. The arrow in the inset indicates the applied magnetic field to obtain the SR signal. The dotted line indicates a single-exponential fit to obtain T_{1e}.

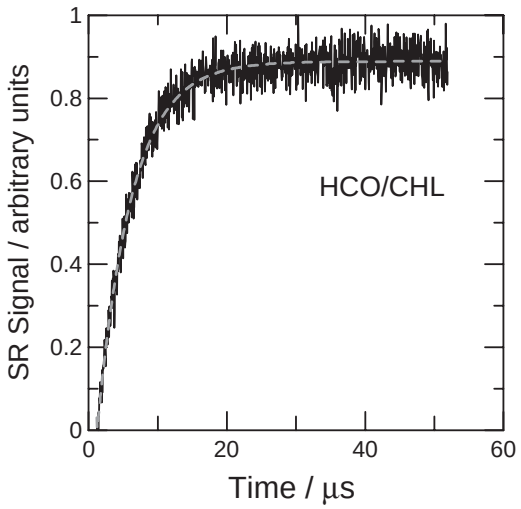

Figure 4. Typical saturation recovery signal from CHL in H_2O dispersions of HCO at 20 °C. The dotted line indicates a single-exponential fit to obtain T_{1e}.

with the various values obtained by EPR slow-tumbling simulation.[1,11,12] The T_{1e} for CHL is slightly longer than one for 5-DSA.

Figure 5 shows the T_{1e} values of 5-DSA and CHL as a function of the temperature examined. The values of 5-DSA and CHL at 20 °C were 4.6 and 5.2 μs, respectively. Both probes had relatively short relaxation times and showed no abrupt change in the temperature range. This suggests that spin probes are mobile in the membrane. The probe behavior increases as the temperature increases. The temperature dependence of T_{1e} is moderate, suggesting the probe motions change moderately in the temperature range.

In general, spin–lattice relaxation time is proportional to correlation time until $\approx 10^{-11}$ s for 9 GHz EPR.[12] When T_{1e} becomes shorter, the correlation time becomes shorter. The T_{1e} values (≈5 μs) of 5-DSA is in-between the values of 12- and 16-DSA at the same temperature as listed in Table 1.[1,11,12] The rotational diffusion coefficient ($R_\perp$) for 5-DSA is faster than the one for 12-DSA. The $R_\perp$ value of 5-DSA gives rotational correlation time (τ_R) $\approx 4.9 \times 10^{-9}$ s. The correlation time is shorter than $\approx 6.4 \times 10^{-9}$ s of 12-DSA. Interestingly, the present SR results are consistent with the previously obtained dynamic parameters from the slow-tumbling simulation analysis of the HCO dispersions.[11] Taking account of the values for the same series of DSA probes, 5-DSA can be located in or nearer the head group region than that of 7-DSA. Based on the results, the possible 5-DSA location in the HCO membrane is illustrated in Figure 6. Because of the oxyethylene group in the head region, H_2O molecules can always move in-and-out of the membrane. The 5-DSA probe might easily collide with H_2O molecules due to the location in the HCO membrane. The rest of the membrane components will change to a hydrophobic environment as the temperature rises. Thus, the T_{1e} and rotational diffusion values suggest that the organization around the probe in the region is relatively flexible. The flexibility can be associated with the physicochemical properties of the amphiphilic membrane such as oxyethylene groups in the head group region.

On the other hand, the T_{1e} value of CHL is slightly longer than that of 5-DSA. The relaxation time implies that the corre-

Table 1. Spin–Lattice Relaxation Time (T_{1e}), Rotational Diffusion Coefficient ($R_\perp$), and Order Parameter (S_0) of Various Nitroxide Spin Probes Incorporated in the HCO Membrane[a)]

Spin probe	T_{1e}/μs	$R_\perp$/s^{-1}	S_0	Reference
CHL	5.2	7.1×10^7	0.20	11
5-DSA	4.6	3.4×10^7	0.29	11
7-DSA	9.1	1.9×10^7	0.68	1
12-DSA	5.5	2.6×10^7	0.25	1
16-DSA	3.6	8.6×10^7	0.04	1

a) $R_\perp$ and S_0 values were previously obtained by the EPR simulation. Rotational correlation time (τ_R) $\approx 1/(6R_\perp)$.[3]

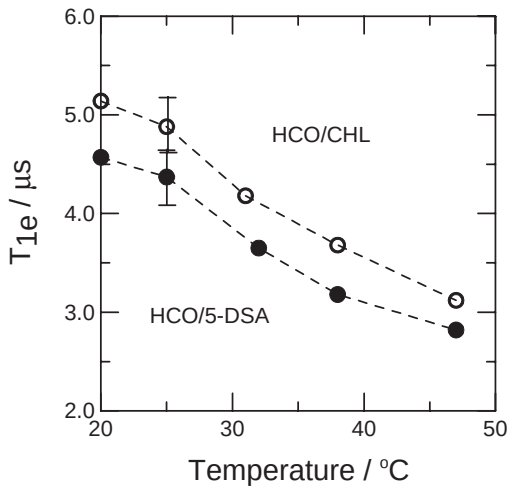

Figure 5. Plot of T_{1e} for 5-DSA (filled circles) and CHL (open circles) in H_2O dispersions of HCO as a function of the temperature. The experimental errors are also indicated.

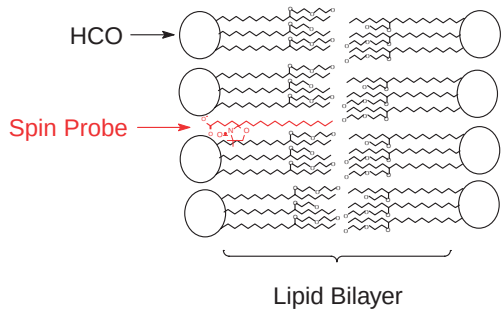

Figure 6. Schematic illustration of 5-DSA incorporated in the HCO membrane.

lation time must be longer than that of 5-DSA. However, the diffusion coefficient obtained by the simulation was not slow. The results can be due to different molecular motions of CHL having aromatic rings and its location in the membrane. It was also suggested that EPR and EPR simulation for the cholesterol derived probe remain preferentially in the liquid ordered region of the detergent-resistant membrane.[14] The experimental errors are presented in Figure 5. It is noted that the low sample concentration ($\approx$80 μmol dm^{-3}) of the probe provided a weak signal and was related to the errors.

In the previous research of HCO, the temperature dependence of rotational diffusion coefficient for 5-DSA and CHL showed a moderate decrease as a function of temperature.[11] In order to verify the results concerning the probes in the membrane, the probe behavior in egg PC, as a well-defined membrane, were examined. Both probes which incorporated PC from egg in aqueous dispersion were carried out in the same manner as the HCO membrane. The T_{1e} values obtained for PC/5-DSA and PC/CHL were approximately 2.3 μs at 20 °C. The T_{1e} of egg PC had a shorter relaxation time than those for HCO dispersion. It is also notable that the short relaxation time of $\approx$2.3 μs is close to the SR instrumental dead time.[15] The reliable relaxation time cannot be observed due to the short relaxation time. Thus, it is not possible to compare the relaxation behavior as a function of temperature with the egg PC which has phase transition at $\approx$37 °C.

Conclusion

The current results from the saturation recovery method provide quantitative insight into the dynamics of the head group region of the amphiphilic membrane. The quantitative relaxation behavior of both probes showed similar temperature behavior throughout the temperatures examined. The relaxation results are also consistent with the simulation analysis.[11] Therefore, the obtained T_{1e} values indicate the following: (1) the head group region is relatively mobile; (2) no abrupt probe motion such as phase transition is detected in the temperature range studied.

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15 A brief period after the pump pulse to allow the dispersion–solution system to recover from the transients is the "dead time" of the instrument.