Motional properties of copper(II) tetraphenylporphyrin and copper(II) hematoporphyrin IX in lipid bilayers: An ESR study

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TABLE V: Enthalpies of Transport of Aqueous NaOH (1) + NaCl (2) at 25 °C[a]

$\bar{m}_1$	$\bar{m}_2$	$\partial \ln \gamma_{\pm 1}/\partial \ln m_1$	$\partial \ln \gamma_{\pm 1}/\partial \ln m_2$	$\partial \ln \gamma_{\pm 2}/\partial \ln m_2$	H_1^*	H_2^*
0.000	0.020			−0.058		2.6[b]
0.005	0.015	−0.01	−0.045	−0.043	15.2	0.7
0.010	0.010	−0.031	−0.030	−0.029	16.2	0.7
0.015	0.005	−0.046	−0.015	−0.014	17.1	2.9
0.020	0.000	−0.061			16.2	

[a] Units: $\bar{m}_i$ in mol kg^{-1}; H_i^* in kJ mol^{-1}. [b] Reference 8.

In order to derive enthalpies of transport from the measured ternary Soret coefficients, eq 25 can be extended to provide[13]

$$H_1^* = RT^2\left[1 + \frac{m_1}{m_1 + m_2} + 2\frac{\partial \ln \gamma_{\pm 1}}{\partial \ln m_1}\right]\sigma_1 + RT^2\left[\frac{m_2}{m_1 + m_2} + 2\frac{\partial \ln \gamma_{\pm 1}}{\partial \ln m_2}\right]\sigma_2 \quad (27)$$

$$H_2^* = RT^2\left[\frac{m_1}{m_1 + m_2} + 2\frac{\partial \ln \gamma_{\pm 2}}{\partial \ln m_1}\right]\sigma_1 + RT^2\left[1 + \frac{m_2}{m_1 + m_2} + 2\frac{\partial \ln \gamma_{\pm 2}}{\partial \ln m_2}\right]\sigma_2 \quad (28)$$

The values of H_i^* calculated by using averages of the ternary Soret coefficients that were measured at each composition are listed in Table V. The ±0.0004 K^{-1} uncertainty in σ_1 and σ_2 leads to an uncertainty of about ±1 kJ in the derived values of H_1^* and H_2^*.

The experiments reported here suggest remarkable differences between the thermal diffusion behavior of ternary NaOH + NaCl solutions and the behavior of the binary solutions of the electrolytes. The results would gain credibility if it could be shown that the measured ternary Soret coefficients are consistent with accepted theory. Conveniently, the enthalpies of transport of dilute 1-1 electrolytes are approximately additive.[8,12] Ternary Soret coefficients can therefore be estimated by solving eq 27 and 28, using the known enthalpies of transport of the binary electrolytes. The estimates of σ_i obtained by this procedure (solid lines) are compared with the measured ternary Soret coefficients in Figure 3. The agreement lends qualitative support to the correctness of the present experimental results.

Finally, what is the physical basis for the strong concentration dependence of the ternary Soret coefficients of NaOH + NaCl solutions? It is well-known that diffusion of electrolytes produces an electric field. When aqueous NaOH diffuses, for example, a relatively strong electric field is required to slow down the highly mobile OH^- ions and to speed up the Na^+ ions so that they travel at the same speed, thereby maintaining electroneutrality. When NaOH and NaCl diffuse in the same solution, the electric field generated by the diffusion of NaOH produces a countercurrent coupled flow of Cl^- ions.

When a temperature gradient is applied to an aqueous solution containing both NaOH and NaCl, the OH^- ions, with their large intrinsic enthalpies of transport,[12] show the strongest tendency to diffuse to the solution's cooler regions. The electric field which tends to slow down the OH^- ions is sufficiently strong to drive a counterflow of Cl^- ions to the warmer regions of the solution. Evidently the inherent tendency of Cl^- ions to diffuse to the cooler regions is outweighed by the flux of Cl^- to the warmer regions due to the electric field.[13] Electrostatic interactions between diffusing ions should provide mixed electrolytes with an especially wide range of interesting thermal diffusion properties.

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Motional Properties of Copper(II) Tetraphenylporphyrin and Copper(II) Hematoporphyrin IX in Lipid Bilayers: An ESR Study

Abbas Pezeshk,*,†

Department of Chemistry, Moorhead State University, Moorhead, Minnesota 56560

Marta Pasenkiewicz-Gierula,‡,§ Witold K. Subczynski,‡,§ and William E. Antholine*,§

Department of Biophysics, Institute of Molecular Biology, Jagiellonian University, Krakow, Poland, and National Biomedical ESR Center, Medical College of Wisconsin, Milwaukee, Wisconsin 53226

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Copper(II) tetraphenylporphyrin, CuTPP, and di-spin-labeled copper(II) hematoporphyrin IX, Cu-heme-SL_2, are readily taken up into phospholipid bilayers of dimyristoylphosphatidylcholine, DMPC, and egg-yolk phosphatidylcholine, EYPC. Motional effects from the ESR data from both copper complexes are evident. These effects are parametrized in terms of a Cu-motion parameter, which is a measure of the degree of resolution of the nitrogen superhyperfine structure. Abrupt changes of the Cu-motion parameter at the main transition temperature of the DMPC bilayer demonstrate that hydrophobic copper complexes are sensitive probes of membrane fluidity. The nitrogen superhyperfine structure in the ESR spectra for CuTPP and Cu-heme-SL_2 in light paraffin oil are particularly well resolved in the rigid-limit and fast-tumbling regions. The features of the ESR spectrum of CuTPP in DMPC liposomes are unusual and not attributed entirely to a rigid-limit or fast motion but to slow motion that needs further modeling.

Introduction

Electron spin resonance of spin-labels and/or of paramagnetic transition-metal ions offers a powerful method for investigating a number of features of the structure, motion, and chemistry of model and biological membranes. In spite of a large volume of work in the area of spin-labels in membranes, very few papers have appeared on the interaction of paramagnetic metal complexes with model membranes.[1-6] Recently, it was shown that derivatives

† Moorhead State University.
‡ Jagiellonian University.
§ Medical College of Wisconsin.

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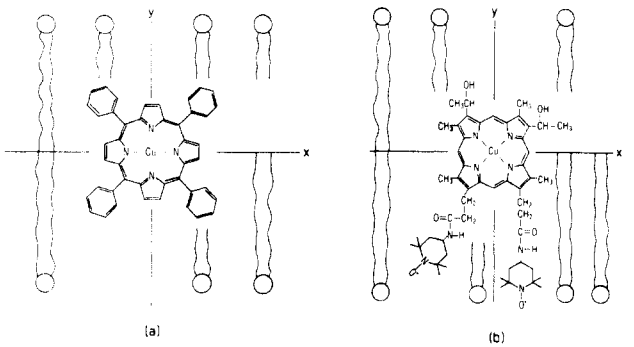

Figure 1. Schematic for CuTPP (a) and Cu-heme-SL_2 (b) in an artificial bilayer. The g value is assumed to be invariant in the plane of this figure, i.e., $g_\perp$. The axis parallel to the axial direction is $g_\parallel$.

of bis(thiosemicarbazonato)copper(II) complexes partition favorably into dimyristoylphosphatidylcholine (DMPC) vesicles and, by analogy, to the membrane of cells.[3,4,6] From analysis of ESR data, it was concluded that the copper complex intercalated into the bilayer.[3-6]

Copper metalloporphyrins and phthalocyanines have been extensively studied by ESR spectroscopy for two decades.[7-9] However, the ESR measurements were carried out on these copper complexes in organic solvents or in the solid state. Localization and restriction of motion of metalloporphyrins in model membranes is interesting in itself for information concerning transport in membranes. This information is useful for understanding the phototherapy effects of porphyrins.[10] Insertion of copper in the porphyrin ring is intended to serve as a probe to measure the physical interaction between porphyrin and membrane. Copper porphyrins are more favorable than iron porphyrins because the ESR studies can be extended to temperatures where slow and fast motion occurs. The system chosen is characterized by a fast relaxing copper that influences the T_1 of the spin-label primarily through a dipole–dipole mechanism rather than through Heisenberg exchange.[11] The spin-labels in the absence of copper are about 16 nm apart (unpublished data). This paper describes the partitional and motional properties of copper(II) tetraphenylporphyrin, CuTPP, and di-spin-labeled copper(II) hematoporphyrin IX, Cu-heme-SL_2 (Figure 1), in two lipid bilayers, dimyristoylphosphatidylcholine, DMPC, containing saturated hydrocarbon chains, and egg-yolk phosphatidylcholine, EYPC, containing unsaturated hydrocarbon chains in the cis conformation.

Experimental Section

Materials. Dimyristoylphosphatidylcholine, DMPC, and egg-yolk phosphatidylcholine, EYPC, were purchased from Sigma (St. Louis, Missouri). Paraffin oil (light) was purchased from MCB, Manufacturing Chemists, Inc., Germany. The complex

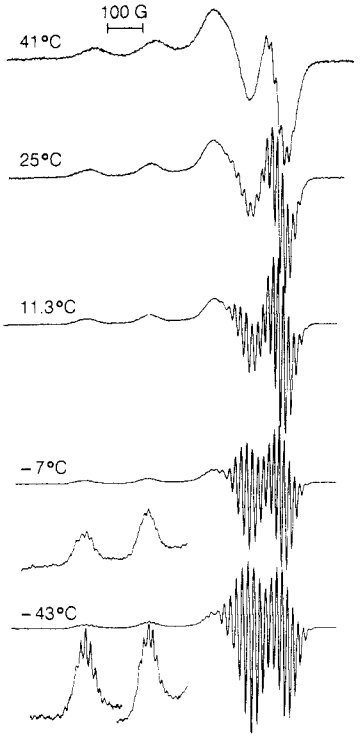

Figure 2. ESR spectra of CuTPP (2 mM) in light paraffin oil as a function of temperature. Spectrometer conditions: modulation amplitude, 5 G; modulation frequency, 100 kHz; microwave power, 50 mW; microwave frequency, 9.131 GHz. Note the gains vary from bottom to top as follows: 1.25×10^2, 1.25×10^2, 2.5×10^2, 4×10^2, and 6.3×10^2, respectively. Gains for the expanded spectrum at −7 and −43 °C for $M_I = -3/2$ and $-1/2$ lines in the $g_\parallel$ region are 2.5×10^3 and 2×10^3, respectively.

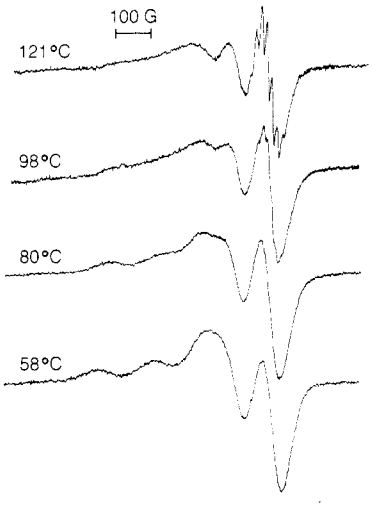

Figure 3. ESR spectra of CuTPP (2 mM) in paraffin oil as a function of temperature. Spectrometer conditions are given in the legend of Figure 2. Gains from bottom to top spectra: 8×10^2, 8×10^2, 10×10^2, and 10×10^2.

CuTPP was prepared according to a published method.[8] The compound di-spin-labeled hematoporphyrin IX was prepared according to the method of Asakura et al.[12] The complex Cu-heme-SL_2 was prepared as described for the preparation of CuTPP.

Sample Preparations. A chloroform mixture of lipid (1×10^{-5} mol) and copper complex (1×10^{-7} mol) in chloroform was first dried with a stream of nitrogen and the residual solvent removed by placing the sample under a reduced pressure (0.1 mmHg) for at least 12 h. Buffer (0.1 mL of reagent-grade phosphoric acid

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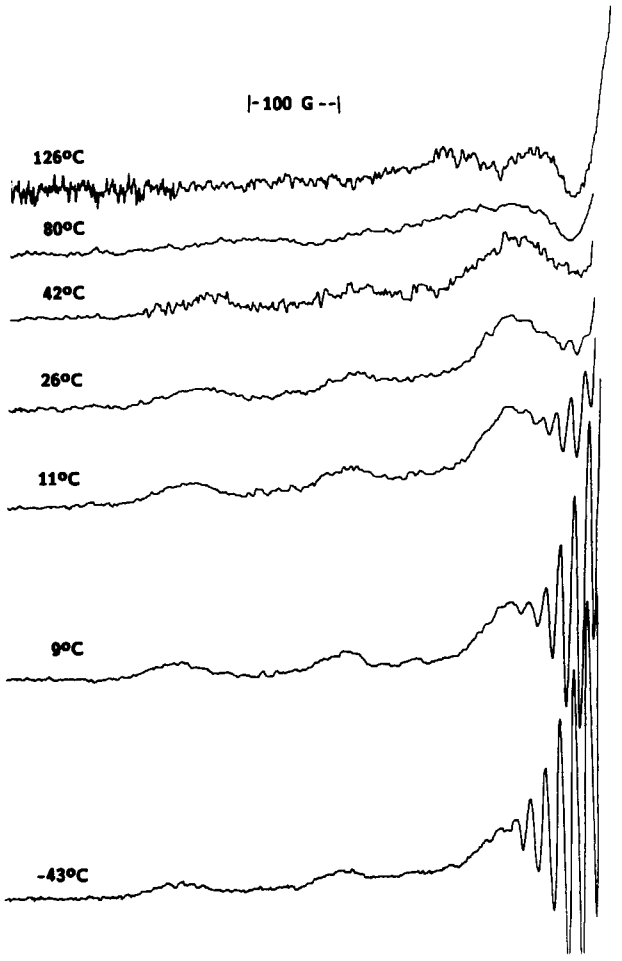

Figure 4. ESR spectra of Cu-heme-SL_2 in paraffin oil as a function of temperature. Spectrometer conditions given in the legend of Figure 2 except the microwave frequency is 9.120 GHz. Gains from top to bottom: 1.25×10^4, 3.2×10^4, 1.6×10^4, 1.6×10^4, 1.6×10^4, 1.6×10^4, 2×10^4. The three lines assigned to spin-label are off scale.

at pH 7) was added to dried lipid at about 43°C and vortexed vigorously to obtain a multilamellar dispersion of lipids. The lipid dispersion was centrifuged, and the loose pellet was transferred to a Pasteur pipette and placed inside the ESR dewar insert of the temperature-controlling system.

ESR Measurements. ESR spectra were obtained with a Varian Model E109 X-band spectrometer with variable temperature control accessories and a Model E-231 Varian multipurpose cavity. The ESR spectra were simulated with a modified program from Dr. John Pilbrow.[13] This program uses resonance frequencies and not resonance fields. The Gaussian half-width in this program is

$$\sigma_\nu = \{\sigma_R^2 + [(\Delta g/g)\nu_0(H) + \Delta A_{m_I}]^2\}^{1/2}$$

where σ_R^2 is the residual line width and $\Delta g/g$ and ΔA are terms for g and A "strain".

Results

Figures 2 and 3 show variable-temperature ESR spectra of CuTPP in paraffin oil, and Figure 4 shows spectra for Cu-heme-SL_2 in parafin oil. The data demonstrate that the spectrum of CuTPP gradually changes from a spectrum for which CuTPP is immobilized at −43 °C to a spectrum for which CuTPP is in the slow-tumbling domain at 58 °C to a spectrum for which CuTPP tumbles rapidly at 121 °C. Paraffin oil is particularly suitable in dynamic studies of hydrophobic molecules because,

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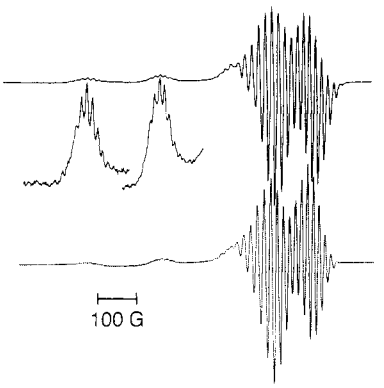

Figure 5. ESR spectrum of CuTPP (2 mM) in paraffin oil at −43 °C (top) and simulated ESR spectrum (bottom), best-fit spectrum following minimization technique. Spectrometer conditions are given in the legend of Figure 2. Parameters for computer simulation: $g_\perp = 2.055$; $g_\parallel = 2.180$; $A_\perp^{Cu} = 26$ G; $A_\parallel^{Cu} = 176$ G; $A_x^N = 15.5$ G; $A_y^N = A_z^N = 13.5$ G; line width, $W_x = W_y = 3.75$ G, $W_z = 3.25$ G; strain parameter, $C_1(x) = C_1(y) = -0.002$, $C_1(z) = +0.008$, $C_2(x) = C_2(y) = +3.0$, $C_2(z) = +0.008$.

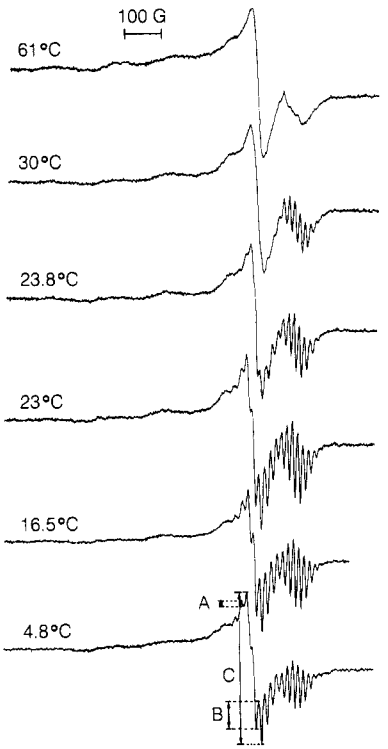

Figure 6. ESR spectra of CuTPP in DMPC lipsomes as a function of temperature (mole ratio of CuTPP/DMPC = 1/100). Spectrometer conditions: modulated amplitude, 5 G; modulated frequency, 100 kHz; microwave power, 50 mW; microwave frequency, 9.141 GHz; gain, 2.5×10^3. The Cu-motion parameter is $(A + B)/C$.

on the one hand, it consists of carbon chains that surround the complex much like acyl chains in the lipid bilayer and, on the other, it lacks the ordered structure of the membrane. The carbon chains therefore can undergo an isotropic reorientation. The range of viscosities obtained with paraffin oil as the temperature changes from −20 to 120 °C makes possible the determination of a wide range of rotational correlation times for a single probe in a single solvent. At −43 °C, the spectrum for CuTPP is well resolved. The parameter set taken from the nitrogen hyperfine structure in both the $g_\parallel$ and $g_\perp$ regions can be fitted reasonably well (Figure 5) with the parameter set typical for copper complexes given in the legend of Figure 5. The value of 176 G for the copper hyperfine coupling constant along the z-axis ($A_\parallel$) for CuTPP in paraffin oil is less than the value for $A_\parallel$ in doped crystals.[8] This fact most likely reflects the difference in environment in doped crystals and paraffin oil. The resolution of the nitrogen hyperfine

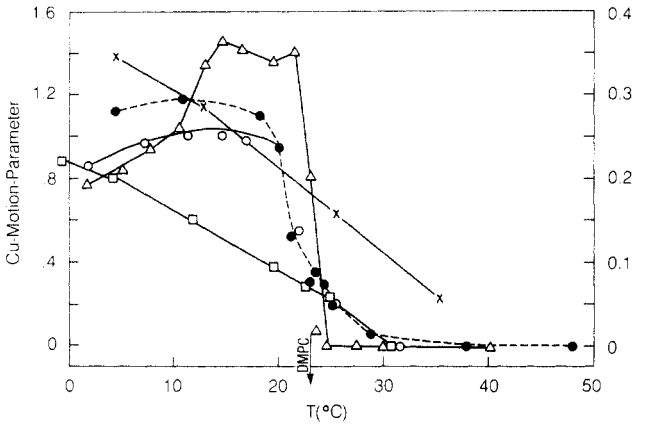

Figure 7. Plot of Cu-motion parameter versus temperature for CuTPP in DMPC liposomes (△), for CuTPP in EYPC liposomes (□), for Cu-heme-SL_2 in DMPC liposomes (●), and for Cu-heme-SL_2 in EYPC liposomes (×). The data for $CuKTSM_2$ in DMPC liposomes (○) is given, but the copper motion parameter for Cu-heme-SL_2 has been redefined.[2] Therefore, the spectral parameters of each copper complex may be compared only qualitatively. The Cu-motion parameter is equal to $(A + B)/C$ where A–C are defined in Figure 6 and 8. The axis on the right side is for the Cu-motion parameter for Cu-heme-SL_2 where A–C were taken from hyperfine lines from the spectrum of Cu-heme-SL_2, which were not obscured by the nitroxide signal (Figure 8).

structure diminishes as the temperature increases from −43 to 41 °C more quickly in the $g_\parallel$ region than in the $g_\perp$ region (Figure 2). The $g_\parallel$ lines and the $g_\perp$ lines move toward positions expected for isotropic ESR values as the temperature reaches 121 °C. These motional changes observed in the ESR spectra are in accord with changes that have been described previously for metal complexes.[15]

Figure 6 shows the ESR spectra of CuTPP in DMPC liposomes. The resolution of the nitrogen hyperfine structure in the $g_\perp$ region of the spectra is diminished as the temperature increases from 4.8 to 61 °C. Decreased resolution can be attributed to slow, reorientation of the copper complex in the membrane. This rotational motion, reflected by the broadening of the nitrogen hyperfine structure of the ESR spectrum as the temperature increases, has been described as the "Cu-motion parameter".[3] This parameter is equal to $(A + B)/C$, where A, B, and C are defined in Figure 6, and is used as a measure of the resolution of the hyperfine structure. The Cu-motion parameter is related to the rotational correlation time. The Cu-motion parameter for CuTPP in DMPC liposomes changes abruptly at the main phase-transition temperature (Figure 7). As expected, a gradual change of the Cu-motion parameter is observed for CuTPP in EYPC liposomes over the same temperature range because of the lack of a phase transition for EYPC in this range of temperatures (Figure 7). Similar results are obtained for the three-spin system, Cu-heme-SL_2 (Figures 7 and 8). However, in this latter case, the Cu-motion parameter is modified to contain only the heights of the nitrogen hyperfine lines that are not superimposed over the nitroxide lines. The presence of features assigned to $g_\parallel$, $g_\perp$, and overshoot lines in the ESR spectrum for CuTPP and Cu-heme-SL_2 in EYPC liposomes (spectra not shown) and paraffin oil (Figures 2–4) is a result of the near rigid limit and slow motion of the complexes in these media. In spite of the reduced intensity of the lines in the $g_\parallel$ region with respect to the lines in the $g_\perp$ region, the motion of the CuTPP in DMPC bilayers is apparently slow. This is reflected in the line shapes of the overshoot lines and the lines in the $g_\perp$ region, where little change was observed when the temperature increases from 4.8 to 23 °C (Figure 6). In contrast, the change in the resolution of the superhyperfine structure is more gradual for Cu-heme-SL_2 (Figure 8). The bulkiness of the hydrocarbon chains of the spin-labels of Cu-heme-SL_2 and of the

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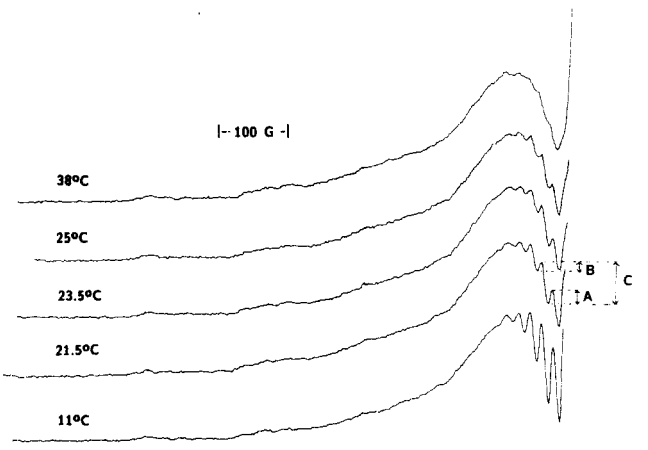

Figure 8. Partial ESR spectra of Cu-heme-SL_2 in DMPC as a function of temperature. Spectrometer conditions are as given in Figure 6. The Cu-motion parameter, $(A + B)/C$, is not the same as the motion parameter in Figure 6 because the signal from the spin-label is off scale in this region.

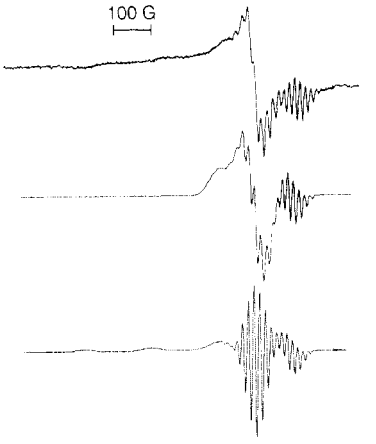

Figure 9. Experimental ESR spectrum for CuTPP in DMPC bilayers at 4.8 °C (top). Spectrometer conditions are as shown in Figure 6. Simulated spectrum (middle) with only $M_I = +1/2$ and $+3/2$ lines. Full simulated spectrum (bottom) with strain parameters included: $g_\perp$ = 2.053; $g_\parallel$ = 2.176; $A_\perp^{Cu}$ = 33 G; $A_\parallel^{Cu}$ = 177 G; A_x^N = 15.5 G; A_y^N = A_z^N = 13 G; line widths, $W_x = W_y$ = 3.5 G, W_z = 4.5 G; strain parameters, $C_1(x) = C_1(y)$ = −0.0008, $C_1(z)$ = +0.0008, $C_2(x) = C_2(y)$ = +14, $C_2(z)$ = −5.0.

tetraphenyl groups of CuTPP is consistent with a slow rotational motion of these complexes in the membrane.

The ESR spectrum of CuTPP in DMPC liposomes cannot be simulated with either the powder or fast anisotropic motion programs. Figure 9 shows the experimental ESR spectrum of CuTPP in DMPC liposomes at 4.8 °C and a simulation using a modified version of a program written by John Pilbrow,[13] Monash University, Australia, which includes g- and A-strain contributions to the line width. The parallel and perpendicular components for the $M_I = +3/2, +1/2$, and $M_I = -1/2, -3/2$ lines were generated separately and summed to produce the bottom spectrum in Figure 9. The sum resembles the spectrum for CuTPP in paraffin oil, but there is little resemblance to the experimental data for CuTPP in DMPC liposomes. The overall shape of only the $M_I = +1/2$ lines superimposed on only the $+3/2$ lines (middle spectrum, Figure 9) is similar to the experimental spectrum at 4.8 °C but with the $M_I = +1/2$ lines in the $g_\perp$ region of the simulated spectrum somewhat better resolved (Figure 9). It is anticipated that inclusion of slow motion into the simulation will improve the fit.

Discussion

Both hydrocarbon chains of the spin-label of Cu-heme-SL_2 and the tetraphenyl groups of CuTPP cause the slow rate of rotational motion of these complexes in the membrane. The Cu-motion parameter appears to be sensitive to restricted rotational diffusion

of these molecules in membranes. One comparison that can be made from the literature is to the motion of a bis(thiosemicarbazonato)copper(II) complex.[3] As the temperature increases, the changes in line shape for CuTPP in paraffin oil and in DMPC liposomes resemble the changes in line shape for [3-ethoxy-2-oxobutyraldehyde bis(N^4,N^4-dimethylthiosemicarbazonato)]copper(II), $CuKTSM_2$, but the temperature for the CuTPP sample for these changes is higher than for $CuKTSM_2$ for equivalent spectra (compare Figures 2, 3, and 6 with Figures 5–7 in ref 16 and Figure 4 in ref 3). This indicates that the rotational motion for CuTPP is slower than for $CuKTSM_2$. Assuming that better resolution of the lines in the $g_\perp$ region reflects slower motion[3], the better resolution of the lines in the $g_\perp$ region indicates that the larger Cu-heme-SL_2 complex rotates slower than CuTPP in both lipid bilayers at equivalent temperatures. The rotational motion of Cu-heme-SL_2 and CuTPP is faster in fluid-phase DMPC membranes than in fluid-phase EYPC membranes (Figure 7 for temperatures above 23.6 °C). It is likely that the unsaturated bonds in the cis conformation introduce rigid bent hydrocarbon chains of about 30° and cause additional restriction of rotational motion. Similar effects of unsaturation have been observed for translational diffusion of a fluorescent-labeled phosphatidylethanolamine.[17] Also, nitroxide moieties of Cu-heme-SL_2 are polar (3D)[19] and possibly more "anchored" in the polar head group region and may account for more restricted motion (Figure 1). An abrupt change in the Cu-motion parameter at the main phase-transition temperature in DMPC liposomes is consistent with the idea that low molecular weight copper complexes may serve as probes of the physical properties of the membrane.

Oriented multilayers of these lipids have not yet been studied, primarily because of the absence of well-defined features in the $g_\parallel$ region. It seems most likely that both CuTPP and Cu-heme-SL_2 are oriented in the membrane with the square plane of the complex parallel to the acyl chains and perpendicular to the surface of the bilayer as shown in Figure 1.[2] Given the orientation in Figure 1, the CuTPP can possibly rotate about its y-axis (parallel to the bilayer normal) and its z-axis. However, the rotation about its x-axis is hindered. If anchoring occurs, the Cu-heme-SL_2 most likely rotates only about its y-axis parallel to the bilayer normal.

The frozen solution spectra of copper phthalocyanines and porphyrins have been extensively studied,[7-9] but, to our knowledge, the loss of intensity of the low-field $g_\parallel$ region with respect to the intensity of the $g_\perp$ region has not been discussed. At least three factors, g strain, T_1, and motion, can be involved in this phenomena. In paraffin oil, the height of the $M_I = -1/2$ lines is about 1.3 times the height of the $M_I = -3/2$ lines at −43 °C. This indicates little strain (spectral simulation supported this observation) and suggests a homogeneous environment for CuTPP in paraffin oil. The simulated ESR spectrum for CuTPP in paraffin oil has a line width of 3.25 G for the parallel region and 3.75 G for the perpendicular region, which is consistent with a T_1 of about 10^{-7} s. The nitrogen lines in the $g_\perp$ region dominate the ESR spectrum, leaving $g_\parallel$ lines less intense. The ESR spectrum of CuTPP in DMPC is not simulated because it is difficult to fit the reduced intensity of the $M_I = -3/2$ and $-1/2$ lines in the $g_\parallel$ region. It appears that a simulation program needs to incorporate the effect of both g strain and motion on the line width. These simulations need to account for the orientation of CuTPP in the bilayer. However, the bilayer itself is randomly oriented. The Moro and Freed slow-motion approach[18] is warranted but, without oriented bilayers, requires supercomputing capability.

An alternate explanation for an "unusual" ESR spectrum of CuTPP in DMPC might involve the formation of ordered aggregates of CuTPP along the z-axis. Dipolar interaction between adjacent copper atoms would broaden lines in one direction (presumably the axial direction), leaving lines in other directions ($g_\perp$ region) unchanged. But, this explanation can be ruled out as the ESR spectrum of the concentrated CuTPP crystals has well-resolved nitrogen hyperfine structure with no sign of dipolar broadening.[8] Also, a 5-fold decrease of the concentration of CuTPP in DMPC did not alter the spectrum (data not shown) although the low S/N in the $g_\parallel$ region prevented quantitative analysis.

Averaging of the **g** tensor is evident from the M_I-dependent averaging for CuTPP in paraffin oil. As the temperature increases from 58 to 121 °C, first the $M_I = +3/2$ lines (the high-field lines) move from the rigid limit through slow motion to fast motion. Then, the $M_I = +1/2$, $-1/2$, and $-3/2$ lines change sequentially in the above M_I-dependent order from slow to faster motion (Figure 3). The nitrogen superhyperfine structure is also M_I dependent. The high-field line ($M_I = +3/2$) is better resolved at 121 °C than the $M_I = +1/2$, $-1/2$, and $-3/2$ lines (Figure 3). This is consistent with more complete averaging of the **g** and **A** tensors for the $M_I = +3/2$ lines. At higher temperatures or lower microwave frequencies, the nitrogen superhyperfine structure for the $M_I = +1/2$ lines is often resolved. As the temperature decreases from +58 to −43 °C, first the resolution of the $M_I = +3/2$ lines and the overshoot lines at high field are resolved at 41 °C and next the $M_I = +1/2$ lines at 25 °C in the $g_\perp$ region and then the $M_I = -3/2$, $-1/2$, and $+1/2$ lines in the $g_\parallel$ region at −7 °C have resolved superhyperfine structure. In retrospect, a similar trend has been observed for $CuKTSM_2$ in paraffin oil.[16]

The basis for the change in the resolution of the nitrogen superhyperfine structure in the $g_\perp$ region from +58 to −43 °C is not as obvious to us as the changes in the $g_\parallel$ region. It is of concern because the resolution of primarily the $M_I = +1/2$ lines superimposed on overlapped lines with $M_I = -1/2$ and $3/2$ lines, overshoot lines, and forbidden transitions has become the criterion for the Cu-motion parameter. It is estimated that a motion corresponding to about 30 G is incompletely averaged at 25 °C. In accordance, the low-field lines in the $g_\parallel$ region begin to move upfield (Figure 2). It is possible to explain the better resolution of the high-field lines by assuming additional anisotropy for the in-plane values of the **g** and **A** tensors. If the configuration becomes more rhombic as the temperature increases, i.e., $g_x = 2.03$, $g_y = 2.06$, $A_x = 35$ G, and $A_y = 20$ G, the ESR parameters would be consistent with the M_I-dependent line broadening as observed in Figure 2. That is, the $M_I = +3/2$ lines would broaden last as the temperature increases. In many instances, it is difficult to determine a change in the in-plane parameters when the isotropic g and A values are similar for both the square-planar and more rhombic configuration. Nevertheless, as evident from the plots in Figure 7, the Cu-motion parameter is sensitive to the physical properties of the bilayer, including the phase-transition temperature.

Additional studies are needed to understand the origin of constraints and the mode and rate of reorientation of copper probes in the membrane. Restricted rotational diffusion, but in oriented membranes, can be used to determine the degree of orientation of CuTPP in the bilayer. It is presumed that CuTPP intercalates along the acyl chains of the bilayer as shown in Figure 1. If additional studies confirm this orientation for CuTPP, then the kinetics for more complicated interactions between oriented copper complexes and, for example, thiols from cysteine residues of protein oriented at the interface of the protein–lipid barrier could be interpreted in terms of restricted motion and one-dimensional orientation of the copper complex. Translational diffusion, either laterally along the membrane or across the membrane, can be studied by saturation-recovery ESR. The bimolecular collisions between a copper complex and stearic acid spin-labels at either the polar head group, several carbon atoms down the acyl chain, or at the center of the bilayer are used to determine translational diffusion constants.[3] In order to have a clear understanding of this data, it is helpful to know if the rotational motion of the copper complex is restricted.

In summary, it is now evident that the motion of CuTPP and Cu-heme-SL_2 in artificial bilayers is near the rigid limit at ambient temperatures, and the motion is slow as the temperature is raised to 50 °C. ESR spectra of these copper complexes are sensitive

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probes of membrane fluidity, but the features of the spectra are unusual and need further modeling. The restricted rotational motion is slower for Cu-heme-SL_2 than for CuTPP, and additional studies need to be completed to determine if functional groups like the nitroxide moiety anchor the complex to the polar head group.

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Micelle Size Distribution and Free Monomer Concentration in Aqueous Solutions of Octyl Glucoside

Robert W. Roxby* and Brian P. Mills

Biochemistry Department, University of Maine, Orono, Maine 04469 *(Received: March 28, 1989)*

Sedimentation equilibrium measurements show that the concentration dependence of molecular weight of the detergent octyl β-D-glucoside in aqueous solution conforms closely to that expected of a reversible, high-order association reaction. Below the point at which micelle formation becomes detectable by this technique, molecular weights characteristic of the homogeneous monomer are obtained. The concentration of monomer can be determined at any total concentration in this system and therefore the contribution of the monomer to the average molecular weights can be subtracted, making it possible to determine average micelle sizes with high precision and to obtain information about micelle size distributions. Micelles are homogeneous in size at the critical micelle concentration and have an aggregation number of 70. As total concentration increases, micelles become larger and somewhat heterodisperse.

Introduction

Nonionic detergents are important tools in the fractionation, manipulation in aqueous solution, and reconstitution of components of biological membranes.[1] The detergent octyl glucoside, introduced for membrane studies by Baron and Thompson,[2] has become widely used because of its ability to solubilize membranes and to maintain the activity of membrane enzymes dispersed in aqueous solutions. It also has the advantages of a high critical micelle concentration, which facilitates removal and replacement by dialysis, transparency in the ultraviolet, and a well-defined chemical structure. We report here studies of micelle formation by octyl glucoside using the technique of sedimentation equilibrium, with associated densimetric measurements of partial specific volumes of detergent monomer and micelles. This detergent offers a reversibly associating system which lends itself to analysis by the rigorous mathematical theory underlying the technique of sedimentation equilibrium, from which precise estimates of monomer concentration, micelle size, and micelle size distribution may be obtained. These data may be helpful in the interpretation of hydrodynamic studies of detergent–protein complexes. It should be noted, however, that many such studies reported in the current literature have employed experimental procedures and methods of data analysis sufficiently faulty that conclusions based on those works are invalid. Alternative procedures having a sound theoretical basis are suggested here.

Materials and Methods

Octyl β-D-glucopyranoside, lot no. 80012, was used as supplied by Calbiochem, La Jolla, CA. All measurements reported in this paper were made at 20 °C on solutions prepared by weight in water, with volume concentrations calculated from partial specific volume and water density. A few measurements carried out in 0.05 M NaCl, for which the data are not shown, gave similar results.

Partial specific volumes of detergent were measured in two ways. Density measurements were made in a Mettler-Paar densimeter as a function of concentration, and partial specific volumes were determined from the limiting relationship

$$d\rho/dc = (1 - \bar{v}\rho) \quad (1)$$

At concentrations below the cmc, the partial specific volume is that of the detergent monomer. (The term "cmc", or critical micelle concentration, is used in the conventional sense, to mean the concentration at which micelles become detectable, although the term has no exact meaning in treatment of the data applied here.) At concentrations above the cmc, virtually all detergent added to the solution forms micelles and the slope therefore gives the micelle partial specific volume.

Because the cmc of octyl glucoside is quite high, about 6 mg/mL, it is possible to choose conditions for sedimentation equilibrium experiments in which only monomer is present. Since the molecular weight of the detergent monomer is known to be 292.4 from the chemical composition, the partial specific volume can be obtained from the measured concentration distribution at sedimentation equilibrium from the relationship

$$d \ln C/(\omega^2 dr^2) = M(1 - \bar{v}\rho)/2RT \quad (2)$$

Sedimentation equilibrium measurements were carried out by the low-speed method in Beckman Model E ultracentrifuges.[3]

TABLE I: Experimental Parameters for Sedimentation Equilibrium Measurements on Octyl Glucoside

expt no.	optical system[a]	rotor speed, rpm	initial concn, mg/mL
308	I	52000	5.01
101	I	52000	6.12
313	I	52000	6.16
302	I	36000	7.21
304	S	20000	19.66
310	S	14000	29.54
315	S	10000	55.99
316	S	8000	85.07

[a] I = interference optics. S = schlieren optics.

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