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THE DIFFUSION-SOLUBILITY OF OXYGEN IN LIPID BILAYERS

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Summary

The product, $D_o\alpha$, of the oxygen diffusion coefficient, D_o, and the oxygen solubility, α, is determined in phosphatidylcholine bilayers at temperatures above the lipid phase transitions from ESR spin-exchange measurements. The resulting values of $D_o\alpha$ are in good agreement with those obtained from fluorescence-quenching experiments. The use of fatty acid spin labels makes it possible to measure $D_o\alpha$ as a function of the coordinate perpendicular to the bilayer surface. The results indicate that $D_o\alpha$ is a strong function of this coordinate; it is greatest in the bilayer center and least near the bilayer head groups.

Introduction

Several studies of molecular oxygen in lipid bilayers or membranes have appeared in the literature. Knopp and Longmuir [1] investigated the feasibility of using fluorescence quenching to measure oxygen concentrations in biological systems, and Fischkoff and Vanderkooi [2] used this technique to obtain the product of the diffusion coefficient and the solubility (the 'diffusion-solubility') of oxygen in sonicated phosphatidylcholine vesicles and in erythrocyte plasma membranes. Kimmich and Peters [3,4] estimated the oxygen solubility in sonicated phosphatidylcholine vesicles from proton NMR relaxation rates. The use of ESR spin exchange to determine the diffusion-solubility of oxygen was suggested by Povich [5,6], and Backer et al. [7] used spin exchange to follow oxygen consumption in respiring mitochondria. In the work described

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Abbreviations: DMPC, dimyristoyl-L-α-phosphatidylcholine; DPPC, dipalmitoyl-L-α-phosphatidylcholine; DSPC, distearoyl-L-α-phosphatidylcholine.

here, spin exchange between oxygen and nitroxide spin labels was used to determine the diffusion-solubility of oxygen in unsonicated phosphatidylcholine bilayers at temperatures above the main lipid phase transitions.

The diffusion-solubility of oxygen in membranes is significant because it is proportional to the membrane permeability to oxygen in the simplest model [8] and to the rate at which diffusion-controlled reactions with oxygen occur in membranes. The precise definition of the diffusion-solubility of oxygen in bilayers is complicated by the anisotropy of the bilayer medium. Both the solubility and the diffusion coefficient (average of the diagonal values of the diffusion tensor) are expected to vary along the coordinate perpendicular to the bilayer surface [9]. The use of spin labels which are located at different positions within the bilayer makes it possible to measure the diffusion-solubility as a function of this coordinate.

Methods and Materials

Line broadening and diffusion-solubility

The oxygen-nitroxide spin-exchange frequency is related to the oxygen broadening of the nitroxide absorption line [10]. If the line is Lorentzian, then the relationship is [11,12]:

$$\omega_E = \frac{\sqrt{3}}{2} |\gamma_e| W_o \tag{1}$$

in which ω_E is the spin-exchange frequency, γ_e is the electron gyromagnetic ratio, and W_o is the broadening (in G) of the peak-to-peak width of the first-derivative spectrum due to spin exchange with oxygen. The spin-exchange frequency is related to ω_c, the frequency at which a nitroxide molecule collides with oxygen, by the expression [10]:

$$\omega_E = p\omega_c \tag{2}$$

in which p is the probability for spin exchange during a collision. The collision frequency is related by the Smoluchowski equation [11,12]:

$$\omega_c = 4\pi R(D_o + D_n)N_o \tag{3}$$

to the distance R between oxygen and nitroxide centers upon collision, the sum of the oxygen and nitroxide diffusion coefficients, and the number (N_o) of oxygen molecules per unit volume of solution. Eliminating ω_E and ω_c from Eqns. 1—3 gives:

$$W_o = \frac{8\pi}{\sqrt{3}|\gamma_e|} pR(D_o + D_n) N_o \tag{4}$$

N_o is given by:

$$N_o = \frac{N_A \alpha P_o}{760 V_o}$$

where N_A is Avogrado's number, α is the Bunsen coefficient of solubility [13], P_o is the oxygen partial pressure in Torr, and V_o is the molar volume of oxygen gas at 0°C and 1 atm [14]. Substituting this expression into Eqn. 4, letting

$U = pR(1 + D_n/D_o)$, and combining constants gives:

$$W_o = QUD_o\alpha P_o$$

with

$$Q = \frac{8\pi N_A}{\sqrt{3}|\gamma_e| 760 V_o} = 2.9159 \cdot 10^{10}\ \mathrm{G \cdot s \cdot Torr^{-1} \cdot cm^{-3}}$$

Neglecting the linewidth contribution due to the oxygen-nitroxide dipolar interaction (see Results and Discussion) and letting W_a equal the linewidth in the absence of oxygen, the total linewidth, W, is given by:

$$W = W_a + QUD_o\alpha P_o \tag{5}$$

For measurements in lipids, it was found that W_a and $D_o\alpha$ could be adequately fitted by polynominal functions of the temperature:

$$W_a = A_0 + A_1T + A_2T^2$$

$$D_o\alpha = B_0 + B_1T + B_2T^2$$

With these substitutions, Eqn. 5 was fitted to the experimental data (W, P_o, T) to obtain the coefficients A_i and B_i; the B_i coefficients allow the evaluation of $D_o\alpha$ as a function of T.

Linewidth measurements

Linewidth measurements as a function of temperature and oxygen partial pressure were made in unsonicated, 10%-by-weight, aqueous dispersions of three phospholipids: dimyristoyl-L-α-phosphatidylcholine (DMPC), dipalmitoyl-L-α-phosphatidylcholine (DPPC) and distearoyl-L-α-phosphatidylcholine (DSPC). Four spin labels were used: perdeuterated di(*t*-butyl)nitroxide and 5-, 12- and 16-doxylstearic acids (doxyl: 4′,4′-dimethyloxazolidine-*N*-oxyl). Linewidths were measured at temperatures ranging from just above the main lipid phase transitions, which occur at 24°C (DMPC), 42°C (DPPC) and 55°C (DSPC) [15], to about 60—70°C. Linewidths of perdeuterated di(*t*-butyl)-nitroxide were measured at oxygen partial pressures of 0, 0.21, 0.5, 0.75 and 1 atm, and linewidths of the doxylstearic acid spin labels were measured at oxygen partial pressures of 0 and 1 atm.

Spectra of dilute perdeuterated di(*t*-butyl)nitroxide in deoxygenated and air-saturated aqueous dispersion of DMPC have already been published [16]. The perdeuterated di(*t*-butyl)nitroxide partitions between the lipid and water environments to give two spectra, each split into three ^{14}N hyperfine lines, one spectrum corresponding to each phase. The spin-exchange information on the diffusion-solubility of oxygen in the bilayer lies in the widths of the lipid lines. Linewidths were determined from the high-field nitrogen hyperfine lines, since these are the best-resolved lines from the two phases. In order to measure the widths of these lines, perdeuterated di(*t*-butyl)nitroxide spectra were digitized and fitted, using an iterative, least-squares program [17], to a sum of Lorentzian functions which included the four largest ^{13}C satellites of each main line. The measured ^{13}C hyperfine constants for perdeuterated di(*t*-butyl)-nitroxide in water are 5.34 G (α-^{13}C) and 4.70 G (β-^{13}C), and the hyperfine constants for perdeuterated di(t-butyl)nitroxide in lipids are 4.94 G (α-^{13}C) and 4.35 G (β-^{13}C).

In order to determine accurately the spin-exchange broadening, it is necessary to correct the observed width of the nitrogen hyperfine line for the inhomonogenous broadening due to the unresolved hyperfine splitting of the 18 equivalent deuterons [12,18,19]. This correction was made in the following way. The spectra of perdeuterated di(t-butyl)nitroxide in water and in lipids which were taken in the absence of oxygen, when the lines were the narrowest and the resolution and non-Lorentzian character were therefore the greatest, were fitted by least-squares to a function composed of one main multiplet and four ^{13}C-satellite multiplets for each phase. Each multiplet consisted of 37 Lorentzian deuteron hyperfine lines. The deuteron hyperfine constants for perdeuterated di(t-butyl)nitroxide in the two phases were treated as variable parameters in the fit, and the resulting values are a_D(water) = 13 ± 2 mG and a_D(lipid) = 20 ± 4 mG. These values are reasonable in view of the known proton hyperfine constants for proton di(t-butyl)nitroxide in solvents of similar polarities [12,19] and the ratio of the deuteron and proton magnetic moments [20]. Using these hyperfine constants, 37-line multiplets were simulated as functions of the widths of the individual deuteron hyperfine lines (assumed to be equal). Each simulation was fitted by a single Lorentzian to obtain the relationship between the width of the deuteron hyperfine lines and the width of the fitted Lorentzian. The deuteron-hyperfine widths and the fitted-Lorentzian widths were then related by least-squares to the empirical equation:

$$W_L - W_D = C_1\exp(-C_2W_L) + C_3\exp(-C_4W_L)$$

in which W_L is the width of the fitted Lorentzian and W_D is the width of the deuteron hyperfine components. This correction is shown in Fig. 1 and amounts to about 20% of the linewidth in water and 10% of the linewidth in lipid in the case of the narrowest W_L values actually recorded (approx. 190 and 390 mG, respectively) and decreases rapidly as W_L increases. The C_i values for perdeuterated di(t-butyl)nitroxide in water are 0.204, 11.89, 0.0243 and 1.24,

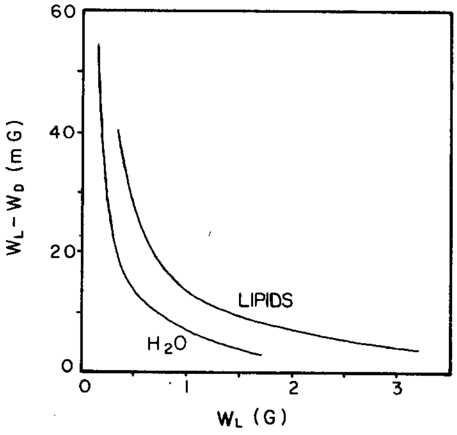


Fig. 1. Correction of perdeuterated di(t-butyl)nitroxide linewidth for deuteron hyperfine splitting.

respectively, and the C_i values for perdeuterated di(*t*-butyl)nitroxide in lipid are 0.0964, 4.15, 0.0205 and 0.541.

When the doxylstearic acid labels are present in a lipid suspension, they dissolve almost entirely in the lipid phase. The spectrum consequently consists of a single, 3-line pattern. Only the width of the most intense (center-field) nitrogen hyperfine line was measured, and no correction was made for the unresolved proton hyperfine structure.

Estimating the U parameter

The parameter, $U = pR(1 + D_n/D_o)$, can be estimated as follows. There is evidence that $p \approx 1$ in solvents of viscosity greater than or equal to that of water [19,21,22]. R can be estimated from the Stokes-Einstein radius of di(*t*-butyl)nitroxide in pentane ($3.2 \cdot 10^{-8}$ cm [19]) and the van der Waal's radius of oxygen ($1.40 \cdot 10^{-8}$ cm [23]) or the Stokes-Einstein radius of oxygen in water ($1.06 \cdot 10^{-8}$ cm [24]) to be about $4.5 \cdot 10^{-8}$ cm. $D_n/D_o \approx 0.3$ in water at 25° C [24,25], so that $6 \cdot 10^{-8}$ cm is a reasonable estimate of U.

U can be determined experimentally from linewidth measurements in solvents in which D_o and α are known. Unfortunately, D_o has been measured in only a few solvents, and most of the values that have been determined are probably subject to large uncertainties [26]. The published diffusion coefficients [24,27—31] and solubilities [32—34] of oxygen in several solvents are listed in Table I along with the values of U for perdeuterated di(*t*-butyl)nitroxide which were determined from linewidth measurements. The values vary from $4 \cdot 10^{-8}$ to $12 \cdot 10^{-8}$ cm. It is not known if this variation indicates a real solvent-dependence of U or if it is the result of imprecise D_o values. In any case, water is the only solvent in which D_o is known reasonably accurately and over a significant temperature range [24,26]. Therefore, it was decided to set U for perdeuterated di(*t*-butyl)nitroxide in the lipids equal to its measured value in water and to set U for all three doxylstearic acid labels equal to its measured value for 16-doxystearic acid in water. U for perdeuterated di(*t*-butyl)nitroxide in water was found to be a linear function of the temperature to within ±1%, ranging from $4.76 \cdot 10^{-8}$ cm at 20° C to $3.77 \cdot 10^{-8}$ cm at 70° C. U for 16-doxylstearic acid in water was found to be a constant over the same

TABLE I

THE *U* PARAMETER FOR PERDEUTERATED DI(*t*-BUTYL)NITROXIDE

Solvent	T (°C)	$10^5 \times D_0$ (cm^2/s)	α	$10^8 \times U$ (cm)
Methanol	20	1.88 [27]	0.231 [32]	12.2
Ethanol	20	1.64 [27]	0.226 [32]	12.2
Ethanol	29.6	2.64 [28]	0.222 [32]	8.6
2-Propanol	20	1.41 [27]	0.229 [33]	11.7
CCl_4	25	3.82 [30]	0.279 [32]	5.2
CCl_4	25.4	2.71 [31]	0.279 [32]	7.3
Dimethylsulfoxide	25	3.23 [29]	0.0496 [32]	3.9
Benzene	29.6	2.89 [28]	0.208 [32]	10.0
Cyclohexane	29.6	5.31 [28]	0.259 [32]	4.9
Water	25	2.31 [24]	0.0285 [34]	4.66

temperature range; $U = 2.09 \cdot 10^{-8}$ cm ±1%: (the diffusion and Bunsen coefficients necessary for these determinations were taken from Refs. 24 and 34, respectively).

Materials

The perdeuterated di(*t*-butyl)nitroxide was synthesized by using the method of Chiarelli and Rassat [35]. Gas-liquid chromatography indicated that the product was about 90% pure, and ESR spectra of the product in a variety of solvents gave no evidence of paramagnetic impurities. Comparison of the integrated NMR intensities of the methyl and t-butyl protons of the *t*-butylacetamide precursor showed that the t-butyl groups were more than 98% deuterated.

A standard solution of perdeuterated di(*t*-butyl)nitroxide in glass-distilled water was used for experiments involving perdeuterated di(*t*-butyl)nitroxide in water and for making up lipid suspensions containing perdeuterated di(*t*-butyl)nitroxide. The concentration of this solution was determined by ultraviolet spectroscopy to be $4.4 \cdot 10^{-4}$ M [36]. After 6 months, the concentration had decreased by 10%. The doxylstearic acid labels were purchased from Syva Corp., Palo Alto, CA.

The lipids were obtained from three sources: Calbiochem, San Diego, CA; Sigma Chemical Co., St. Louis, MO; and Applied Science Laboratories, State College; PA. They were the best grades of lipids produced by these companies and were used without further purification.

Mixtures of gases containing 50 and 75% oxygen were made up by mixing dry air and oxygen. The flow rates of the two gases into a common tube were controlled by regulators, and the mixture was homogenized by passing it through a U-tube containing Raschig rings. A small amount (100 ml/min) of the homogenized gas flowing to the temperature controller was diverted through a Beckman E2 paramagnetic oxygen analyzer to determine the oxygen content. This instrument is accurate to within 0.5% of full scale.

Sample preparation

The standard solution of perdeuterated di(*t*-butyl)nitroxide in water described above was used directly for the water measurements. Lipid suspensions containing perdeuterated di(*t*-butyl)nitroxide were made up in 100-μl round-bottomed flasks. Typically, 7 mg of lipid were weighed into the flask and 70 μl of standard aqueous perdeuterated di(*t*-butyl)nitroxide solution were added. The flask was capped, and the bilayer suspension was formed by agitating the flask on a vortex mixer for 10 min while using a hot-air gun to keep the flask 10° C above the main lipid phase transition.

The organic solvents used for the *U* determinations were either spectral or reagent grade and were dried over Linde 3A molecular sieves. Solutions of perdeuterated di(*t*-butyl)nitroxide in these solvents were made up to be about $1 \cdot 10^{-3}$ M.

The aqueous solution of 16-doxylstearic acid was prepared by placing a chloroform solution of the label in a flask, removing the chloroform by rotary evaporation, and redissolving the label in 0.05 M aqueous Tris-HCl buffer (pH 7.4) to give a solution which was $5 \cdot 10^{-4}$ M in spin label.

Lipid suspensions containing doxylstearic acid spin labels were prepared by weighing out 7 mg of lipid into a 100 μl flask and adding the spin label dissolved in chloroform to form a solution with a label : lipid mole ratio of about 0.01. The chloroform was removed by rotary evaporation to leave a label-lipid film on the walls of the flask, and 70-μl of 0.05 M Tris-HCl buffer (pH 7.4) were added. Bilayers were formed by using the vortex mixer and hot-air gun as described above.

Instrumentation

A description of the ESR sample tubes used in this study has already been published [16]. They were constructed from thin-walled polytetrafluoroethylene tubing. This tubing is permeable to oxygen and nitrogen gases, but it is impermeable to water and only slightly permeable to organic liquids, allowing the oxygen concentration in the sample to equilibrate rapidly with its partial pressure outside the sample tube. The oxygen concentration in the sample was controlled by adjusting the mole fraction of oxygen in the gas stream used to control the temperature.

The temperature was measured with a copper-constantan thermocouple placed in the microwave cavity adjacent to the sample. The thermocouple was calibrated with an ASTM thermometer, and the two devices agreed to within 0.1°C. The temperature was stable to within 0.1°C, and the temperature gradient over the sample varied from 0.1°C near room temperature to 4°C at 70°C.

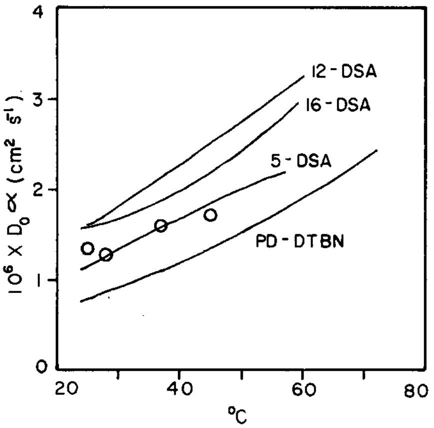
All ESR spectra were recorded on a Varian E-12 X-band spectrometer equipped with a 30.5 cm electromagnet, a rectangular TE_{102} microwave cavity, and a Varian E-257 temperature controller. The spectrometer was interfaced to a Hewlett-Packard 9830A programmable calculator. Digitized spectra were recorded by the calculator, stored on magnetic tape, and fitted to obtain the widths of the lines. These digitized spectra contained 200 points with at least 10 points between the maximum and minimum of the narrowest derivative line. Spectra of perdeuterated di(*t*-butyl)nitroxide in water and in lipids which were taken in the absence of oxygen were transferred to a CDC Cyber 174 time-share computer system in order to determine the deuteron hyperfine constants.

Most spectra were recorded at 3 mW incident microwave power with the modulation amplitude equal to 5% of the width of the narrowest line in the spectrum and with the time constant equal to 5% of the time required to sweep the field through the peak-to-peak width of the narrowest line. In some cases, because of low signal-to-noise, 10 mW microwave power, a modulation amplitude equal to 10% of the width of the narrowest line and a time constant equal to 10% of the time required to sweep through the narrowest line were used. The field sweep was calibrated using an Alpha Magnetometer, model 576, and a Systron-Donner frequency counter, model 1037, and found to be within 1% of the nominal value. The modulation frequency was 100 kHz in all cases.

Results and Discussion

Since it is known that the polarity [37] and fluidity [38—44] of a phospholipid bilayer vary along the coordinate perpendicular to the bilayer surface, with polarity increasing and fluidity decreasing from the center toward the glycerol group, it is expected that the oxygen diffusion-solubility also varies along this coordinate. We have attempted to measure this variation with the doxylstearic acid spin labels. These labels are known to orient themselves in bilayers with their average chain orientation parallel to the fatty acid chains of the lipids and with their carboxyl groups in the polar head-group region of the bilayer [38—40]. Since the doxyl group of each different doxylstearic acid label is attached to a different carbon of the fatty acid chain, the frequency at which these labels exchange spin with oxygen should be a measure of the diffusion-solubility of oxygen at different depths of the bilayer. On the other hand, $D_0\alpha$ measured by perdeuterated di(*t*-butyl)nitroxide should be an average weighted by the concentration function of perdeuterated di(*t*-butyl)nitroxide across the bilayer.

The diffusion-solubilities measured by the four spin labels in the three lipids are plotted in Figs. 2—4 along with the fluorescence results of Fischkoff and Vanderkooi [2]. Because oxygen is about 10 times as soluble in nonpolar solvents as it is in water [32,34] and the bilayer is least polar in its center [37], the oxygen solubility is expected to be highest there. Since the fluidity is greatest in the center [38—44], D_0 might also be expected to be greatest there. Therefore, it is expected that $D_0\alpha$(16-doxylstearic acid) $\geqslant$ $D_0\alpha$(12-doxylstearic acid) $\geqslant$ $D_0\alpha$(5-doxylstearic acid), which is observed in the case of DSPC (Fig. 4). The results in DMPC (Fig. 2), in which $D_0\alpha$(12-doxylstearic acid) $\geqslant$ $D_0\alpha$(16-doxylstearic acid), and DPPC (Fig. 3), in which $D_0\alpha$(12-doxylstearic acid) $\approx$ $D_0\alpha$(16-doxylstearic acid), may be because the 18-carbon spin labels do not fit well in bilayers composed exclusively of 14- or 16-carbon chains, so that the positions of the doxyl groups relative to the lipid fatty acid chains are not the same in the three different lipids. This explanation is suggested by the evidence that lipids with different chain lengths form nonideal solutions [15,45—47].



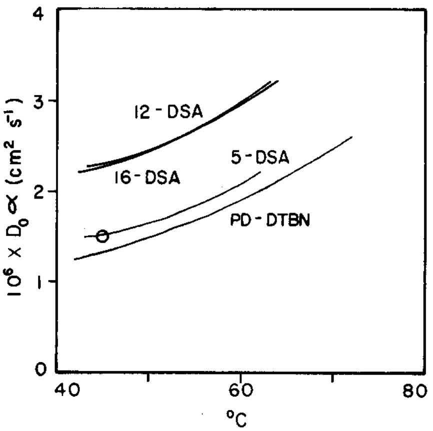
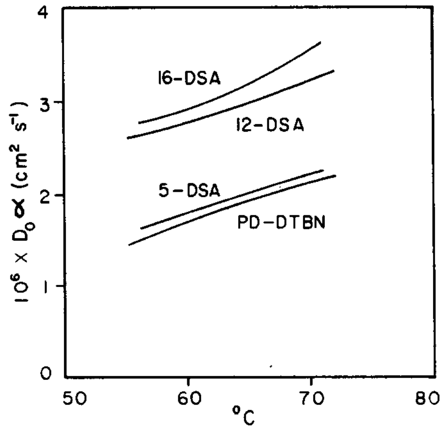


Fig. 2. The diffusion-solubility of oxygen in DMPC. 5-DSA, 12-DSA and 16-DSA, 5-, 12- and 16-doxylstearic acid, respectively; PD-DTBN, perdeuterated di(*t*-butyl)nitroxide. The curves are reproducible to within 4%. (○) Fluorescence results from Ref. 2.

Fig. 3. The diffusion-solubility of oxygen in DPPC. All other details as in Fig. 2.



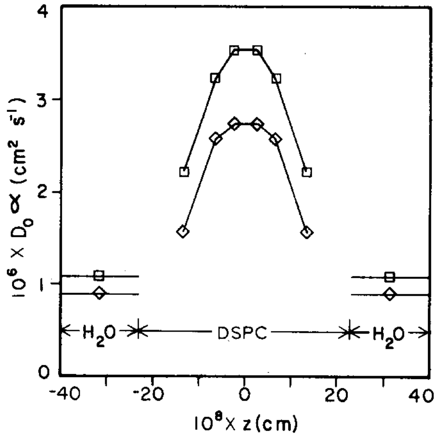


Fig. 4. The diffusion-solubility of oxygen in DSPC. All other details as in Fig. 2.

Fig. 5. The diffusion-solubility profile of oxygen in DSPC at 55°C (◇) and 70°C (□).

It is reasonable to suppose that the $D_0\alpha$ value measured by the doxylstearic acid spin labels in DSPC can be correlated with the coordinate perpendicular to the bilayer, that is, that $D_0\alpha$(5-doxylstearic acid) is equal to $D_0\alpha$ at C-5 of DSPC and so forth. The thickness of the DSPC bilayer above its melting point can be inferred from X-ray and neutron-diffraction studies of DPPC to be about $46 \cdot 10^{-8}$ cm [48,49]. Thus, the bilayer bounds are $z = \pm 23 \cdot 10^{-8}$ cm, where z is the coordinate perpendicular to the bilayer with its origin at the bilayer center. Each fatty acid methylene unit contributes close to $1 \cdot 10^{-8}$ cm to this thickness [48,49], so that C-18 lies at $z \cong \pm 0.5$, and the 5- , 12- and 16-doxyl substituents are located at $z \cong \pm 13.5$, ± 6.5 and $\pm 2.5 \cdot 10^{-8}$ cm, respectively. Plots of $D_0\alpha$ at these points at two different temperatures are given in Fig. 5. These plots show that $D_0\alpha$ is a strong function of the z coordinate. A maximum value of $D_0\alpha$ occurs in the bilayer center, and minima occur near the head groups.

$D_0\alpha$(perdeuterated di(*t*-butyl)nitroxide) is an average of $D_0\alpha$ over the bilayer weighted by the concentration function of perdeuterated di(*t*-butyl)nitroxide over the bilayer. Therefore, $D_0\alpha$(perdeuterated di(*t*-butyl)nitroxide) must lie between the minimum and maximum values of $D_0\alpha$ in the bilayer. The fact that $D_0\alpha$(perdeuterated di(*t*-butyl)nitroxide) is less than the $D_0\alpha$ values recorded by any of the doxylstearic acid spin labels seems to indicate that the perdeuterated di(*t*-butyl)nitroxide concentration is a strong function of the z coordinate with maxima near the head groups, where $D_0\alpha$ is low. This is consistent with the observation of Dix et al. [9] that the relaxation enhancement of ^{13}C nuclei of DPPC due to added di(*t*-butyl)nitroxide is greatest at the carbonyl carbon (C-1).

Berner and Kivelson [50] have recently questioned the validity of using linewidths to determine diffusion coefficients in systems as viscous as lipid bilayers may be (approx. 1—10 P) [51]. They assume that diffusion is described by the Stokes-Einstein equation and argue that when $T/\eta \leqslant 1000$ K/P, the relative translational motions of the interacting radicals are sufficiently slow so that dipolar broadening, which is inversely proportional to the sum of the diffusion coefficients of the interacting radicals, competes with spin exchange as a broadening mechanism. They present experimental evidence which tends to support their argument, although there are serious discrepancies between their theory and their data. Their evidence is based on the homospin exchange of radicals larger than oxygen and may not apply to such a small diffusant. That the dipolar interaction is not the dominant relaxation mechanism is indicated by the following evidence. (1) The diffusion-solubilities of oxygen in bilayers obtained from ESR, assuming that spin exchange is the only hetero-radical broadening mechanism, agree with those obtained by fluorescence quenching (Figs. 2 and 3) [2]. (2) Povich [6] measured the oxygen broadening of nitroxide ESR spectra as a function of temperature in solvents of viscosities ranging from 0.01 to 10 P and T/η ranging from 40 to 40 000 K/P. These experiments include the T/η range in which, according to Berner and Kivelson [50], the linewidth should be relatively insensitive to T or even decrease as T increases. However, Povich found that the oxygen broadening invariably and sharply increases with T. He also found that the oxygen broadening is essentially independent of the viscosity. (3) Oxygen broadening in bilayers (Figs. 2—4) also increases with T, although this does not necessarily contradict the theory of Berner and Kivelson, since the temperature dependence of the oxygen solubility in bilayers is unknown. If the oxygen solubility in bilayers increases with T, as it does in most nonpolar solvents [32], then it would tend to mask any decrease or plateau in broadening as predicted by Berner and Kivelson.

In conclusion, ESR spin exchange appears to provide a means for determining oxygen diffusion-solubility in lipid bilayers and in living systems [7]. It is capable of measuring oxygen diffusion-solubility as a function of location within bilayers, and it should be capable of measuring the diffusion-solubility as a function of lipid composition, phase equilibria, the concentration of anesthetics, and other variables of interest. If further work indicates that the parameter, U, is relatively independent of the solvent, then spin exchange will offer an accurate way to measure D_o in those solvents in which the oxygen solubility is known.

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