

CONCENTRATION OF OXYGEN IN LIPID BILAYERS USING A SPIN-LABEL METHOD

WITOLD K. SUBCZYNSKI AND JAMES S. HYDE

National Biomedical ESR Center, Department of Radiology, Medical College of Wisconsin, Milwaukee, Wisconsin 53226

ABSTRACT The concentration of oxygen in the hydrocarbon region of lipid bilayer has been determined using a novel electron spin resonance (ESR) nitroxide-radical spin-probe method. For dimyristoylphosphatidylcholine (DMPC), the partition coefficient above the main transition temperature is ~ 3 . Rapid decrease to 0.2 occurs below the pretransition temperature indicating exclusion of oxygen in the crystalline phase. The differences of molar free energy, enthalpy, and entropy of mixing between water and lipid have been determined for each phase.

It is customary to assume that data on oxygen partition coefficients between water and olive oil obtained by Batino et al. (1) are relevant to lipid bilayers. Direct support for this assumption, however, is lacking. Moreover, in recent years a number of reports based on radioactive isotope labeling have indicated that partition coefficients of noble gases and CO_2 in anisotropic organized media such as lipid bilayers are much lower than in isotropic media such as olive oil (2–4). There are no convenient radioactive oxygen isotopes, and therefore we have developed a new method using nitroxide radical spin probes for the measurement of the average oxygen partition coefficient between the hydrocarbon region of lipid bilayers and water. The method has been applied to dimyristoylphosphatidylcholine (DMPC) (Sigma Chemical Co., St. Louis, MO) liposomes as a function of temperature. DMPC exhibits a main phase transition at 23.6° and a pretransition at 12° . For each of the three phases, the data permit the calculation of the difference of molar free energy, enthalpy, and entropy of mixing between water and lipid.

Oxygen transport in membranes is poorly understood, even though it is obviously of critical importance in cellular respiration. Diamond and Katz (5) have derived an expression for the permeability, P , of oxygen across a bilayer:

$$1/P = -\frac{C_w'' - C_w'}{J} = r' + \int_{x=0}^{x=x_0} \frac{dx}{K(x)D(x)} + r'' \quad (1)$$

Here J is the oxygen flux across the bilayer that occurs because of a difference in concentration in water on either side, $(C_w'' - C_w')$. The partition coefficient $K(x) = ([\text{O}_2(\text{membrane})]/[\text{O}_2(\text{water})])$ and the diffusion constant $D(x)$ are allowed to vary across the bilayer from $x = 0$ to $x = x_0$. Into the resistances r' and r'' are lumped, conceptually,

the problem of transport across the polar head-group regions of the membrane.

Apparently no measurements of P have been made. Nothing is known of r' or r'' . Several studies do exist where the product $K(x) \cdot D(x)$ has been measured. These reports (6–9) are based on measurements of the bimolecular collision rate ω between a probe and oxygen, using the Smoluchowski equation:

$$\begin{aligned} \omega &= 4\pi R(D_0 + D_p)[\text{O}_2(\text{membrane})] \\ &\approx 4\pi R D_0 K[\text{O}_2(\text{water})]. \end{aligned} \quad (2)$$

Here D_0 and D_p are the diffusion constants of oxygen and the probe, and R is an interaction distance, usually $\sim 4 \text{ \AA}$. Normally D_p is negligible. We believe the present work is the first in which the product of D and K has been systematically separated over a wide temperature range. Peters and Kimmich and Peters et al. (10, 11) have reported two data points, one on each side of the main phase transition of dipalmitoyl lecithin using an NMR method.

Our method is illustrated in Fig. 1, which shows a two-compartment vessel, the outer walls of which are stainless steel. The two compartments are separated by a machineable methylpentene plastic known as TPX (12). This plastic has an unusually high ratio of oxygen permeability to water permeability. It thus can be used for oxygen dialysis with either a liquid or a gas on the far side of the barrier.

Air-saturated water containing a spin probe was put in the stainless steel container, Fig. 1 *A*. Oxygen was removed from the sample of interest, Fig. 1 *B*, by initially exposing it to water-saturated nitrogen gas. The sample was then inserted into the container and sealed, Fig. 1 *C*. After a prolonged time, oxygen concentrations in the two compartments reached equilibrium. The remaining oxygen in the outer chamber was then determined using the spin-probe

Dr. Subczynski is on leave from the Department of Biophysics, Institute of Molecular Biology, Jagiellonian University, Krakow 31-001, Poland

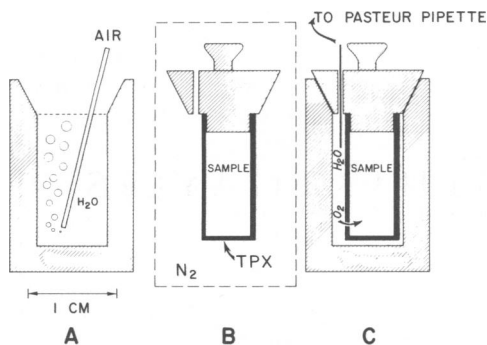


FIGURE 1 The double chamber method. *A*, air-saturated water containing spin probe in stainless steel vessel. *B*, sample in TPX degassed by equilibration with water saturated nitrogen gas. *C*, sealed assembly. After O_2 equilibrium is reached, the water is sampled and examined by ESR.

method described below. The partition coefficient, K_L is determined from the readily derivable expression:

$$K_L = \frac{V_1 C_1}{V_2 f C_2} - \frac{V_1 + V_2(1-f) + K_T V_T}{V_2 f} \quad (3)$$

where V_1 = volume of air saturated water (0.48 ml); V_2 = volume of TPX container (0.53 ml); V_T = volume of TPX walls (0.14 ml); C_1 = oxygen concentration in air saturated water from published tables (13); C_2 = measured oxygen concentration in water after equilibrium is reached; K_T = oxygen partition coefficient between TPX and water; and f = weight (or volume) fraction of hydrocarbons in the sample (~ 0.3).

To measure oxygen concentration in water, the so-called CTPO probe (Aldrich Chemical Co., Inc., Milwaukee, WI), Fig. 2, was used at a concentration of 1.1×10^{-4} M in doubly deionized water. Observation was made with a Varian ESR spectrometer (Varian Associates, Palo Alto, CA) in normal configuration. The resolution of the super-hyperfine coupling from the protons of the probe is dependent on the bimolecular collision rate with oxygen. Molecular oxygen is paramagnetic, interacting with the spin probes through Heisenberg exchange. This method was

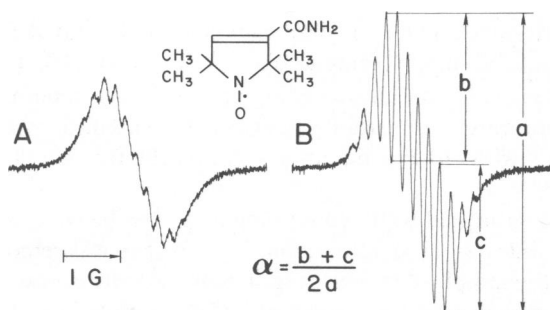


FIGURE 2 ESR spectrum of the spin probe CTPO (1.1×10^{-4} M) in air-saturated water (*A*) and deoxygenated water (*B*) at 10° . The definition of α , the parameter used in calibration charts to determine O_2 , is indicated.

originally introduced by Backer et al. (14) and has been carefully calibrated by Lai et al. (15). After equilibration, a sample was taken with a Pasteur pipette, Fig. 1 *C*, the parameter α (see Fig. 2) was measured, and the concentration of oxygen determined from Lai's charts.

Various problems were encountered. Initially a brass chamber was made, but it consumed oxygen by an undefined mechanism all by itself. In early experiments 0.1 M HEPES and 0.2 M phosphate buffers were tried for the liposomal suspension, but these buffers slowly consumed oxygen. Therefore liposomes were prepared in doubly deionized water. To avoid consumption of oxygen by bacteria, both inner and outer compartments contained 3 mM NaN_3 . Great care was taken that no air bubbles were trapped.

Deoxygenation, Fig. 1 *B*, at 37° , was essentially complete in 2 h and prolonged deoxygenation to 14 h had no further effect. Incubation, Fig. 1 *C*, at $37^\circ C$ was essentially complete in 2 1/2 h and prolonged incubation to 10 h had no further effect. Incubation was carried out at the lowest temperatures reported here for up to 25 h, until we verified that equilibrium had been reached. With water in both chambers, the partition coefficient of TPX, K_T , was found to be 1.4; it was temperature independent between 15° and $35^\circ C$. To further test the method, the partition coefficient of commercial grade olive oil was determined. We obtained 3.95 at $25^\circ C$ and 4.45 at $40^\circ C$, to be compared with Battino et al.'s values of 4.1 at $25^\circ C$, 4.8 at $35^\circ C$, and 5.5 at $45^\circ C$. Considering the uncertain nature of olive oil, this is felt to be excellent agreement.

Data on DMPC liposomes are shown in Fig. 3. The percent by weight of lipids in the sample was determined after each experiment by weighing a portion of the sample before and after drying under vacuum for 60 h. We assumed that the densities of water and lecithin are about

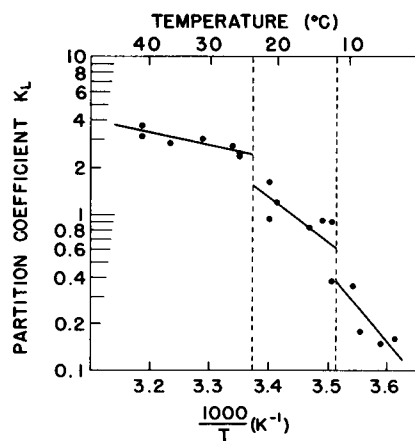


FIGURE 3 The partition coefficient of oxygen in the hydrocarbon region of DMPC liposomes as a function of T^{-1} . Vertical broken lines indicate the main- and pre-transition temperatures. The straight lines were calculated by a linear least-squares fit to the experimental data in a logarithmic reciprocal-temperature display.

TABLE I

PARTITION MOLAR FREE ENERGIES (ΔG), ENTHALPIES (ΔH), AND ENTROPIES (ΔS) OF OXYGEN PARTITION FOR DMPC/H₂O

	ΔG	ΔH	ΔS
	<i>cal · mol⁻¹</i>	<i>Kcal · mol⁻¹</i>	<i>cal · mol⁻¹ · T⁻¹</i>
23.6° > T_m^*	-519		
23.6° < T_m^*	-254		
11.6° > T_p^\ddagger	270		
11.6° < T_p^\ddagger	540		
> T_m^*		3.6	14
$T_m > T > T_p^\ddagger$		12	43
< T_p^\ddagger		20	70

* T_m is the main-transition temperature.‡ T_p is the pre-transition temperature.

equal (16). We assumed that the partition coefficient between water and the polar head group region of the bilayer is unity. The hydrocarbon chains of the DMPC bilayer constitute 55% of the total weight. Deoxygenation and saturation of water by air were done at 37°C. ESR measurements were made at 10°C, which is an optimum temperature for the method. The incubation temperature was systematically varied.

Least-squares fits were used (Fig. 3) for each temperature region, and no attempt was made to connect the fits across the phase-transition temperatures.

The partition coefficient of oxygen in the hydrocarbon region of DMPC bilayers above the phase transition is ~30% less than in olive oil, and drops sharply at each phase-transition temperature to a value as low as ~0.2. If regions of biological membranes exist that are in the solid phase, our data suggest that they are at greatly reduced oxygen concentration relative to fluid regions.

The partition coefficient is related to the partial molar standard free energy change on transferring oxygen from water to the lipid phase, ΔG , by

$$K = \exp(-\Delta G/RT). \quad (4)$$

The partial enthalpy, ΔH , and entropy, ΔS , on transferring oxygen from water to the lipid phase are related to ΔG by

$$\Delta G = \Delta H - T\Delta S. \quad (5)$$

Making the reasonable assumption that ΔH and ΔS are independent of temperature, all three thermodynamic quantities can be determined for each of the three temperature intervals. The results of the calculation are given in Table I.

Both ΔH and ΔS are large and positive and change abruptly at the main- and pre-transition temperatures. The change in ΔG is smaller, but nevertheless significant. The increase in positive enthalpy observed below the main transition temperature can be attributed to the energy that is required to disrupt hydrophobic interactions. The

increase in positive entropy can be attributed to the disorder from the introduction of oxygen into the crystal line region of the hydrocarbon chains. Similar dependence at the main transition temperatures were reported for nonelectrolyte solutes in DMPC liposomes by Katz and Diamond (17) and for small spin probes in DMPC and dipalmitoylphosphatidylcholine (DPPC) by Dix et al. (18, 19).

Many helpful discussions were held with Dr. A. Kusumi. This work was supported by grants GM-22923 and RR-01008 from the National Institutes of Health.

Received for publication 29 May 1982 and in revised form 18 October 1982.

REFERENCES

- Battino, R., F. D. Evans, and W. F. Danforth. 1968. Solubilities of seven gases in olive oil with reference to theories of transport through the cell membrane. *J. Am. Oil Chem. Soc.* 45:830-833.
- Power, G. G., and H. Stegall. 1970. Solubility of gases in human red blood cell ghosts. *J. Appl. Physiol.* 29:145-149.
- Katz, Y., and S. A. Simon. 1977. Physical parameters of the anesthetic site. *Biochim. Biophys. Acta.* 471:1-15.
- Simon, S. A., and J. Gutknecht. 1980. Solubility of carbon dioxide in lipid bilayer membranes and organic solvents. *Biochim. Biophys. Acta.* 596:352-358.
- Diamond, J. M., and Y. Katz. 1974. Interpretation of nonelectrolyte partition coefficients between dimyristoyl lecithin and water. *J. Membr. Biol.* 17:121-154.
- Fischkoff, S., and J. M. Vanderkooi. 1975. Oxygen diffusion in biological and artificial membranes determined by the fluorochrome pyrene. *J. Gen. Physiol.* 65:663-676.
- Windrem, D. A., and W. Z. Plachy. 1980. The diffusion-solubility of oxygen in lipid bilayers. *Biochim. Biophys. Acta.* 600:655-665.
- Subczynski, W. K., and J. S. Hyde. 1981. The diffusion-concentration product of oxygen in lipid bilayers using the spin-label T_1 method. *Biochim. Biophys. Acta.* 643:283-291.
- Kusumi, A., W. K. Subczynski, and J. S. Hyde. 1981. An oxygen transport parameter in membranes as deduced by saturation recovery measurements of spin-lattice relaxation times of spin label. *Proc. Natl. Acad. Sci. U. S. A.* 79:1854-1858.
- Peters, A., and R. Kimmich. 1978. The heterogeneous solubility of oxygen in aqueous lecithin dispersions and its relation to chain mobility. A NMR relaxation and wide-line study. *Biophys. Struct. Mech.* 4:67-85.
- Peters, A., R. Kimmich, and K.-H. Spohn. 1981. Investigation of the solubility profile of small nonelectrolyte molecules in lecithin bilayers. *Bull. Magn. Reson.* 2:398.
- Popp, C. A., and J. S. Hyde. 1981. Effects of oxygen on EPR spectra of nitroxide spin-label probes of model membranes. *J. Magn. Reson.* 43:249-258.
- Hitchman, M. L. 1978. Measurement of Dissolved Oxygen. John Wiley & Sons, Inc., New York.
- Backer, J. M., V. G. Budker, S. I. Eremenko, and Yu. N. Molin. 1977. Detection of the kinetics of biochemical reactions with oxygen using exchange broadening in the ESR spectra of nitroxide radicals. *Biochim. Biophys. Acta.* 460:152-156.
- Lai, C.-S., L. E. Hopwood, J. S. Hyde, and S. Lukiewicz. 1980. ESR studies of O₂ uptake by Chinese hamster ovary cells during the cell cycle. *Proc. Natl. Acad. Sci. U. S. A.* 79:1166-1170.
- Lecuyer, H., and D. G. Derivichian. 1969. Structure of aqueous mixtures of lecithin and cholesterol. *J. Mol. Biol.* 45:39-58.

17. Katz, Y., and J. M. Diamond. 1974. Thermodynamic constants for nonelectrolyte partition between dimyristoyl lecithin and water. *J. Membr. Biol.* 17:101-120.
18. Dix, J. A., J. M. Diamond, and D. Kivelson. 1974. Translational diffusion coefficient and partition coefficient of a spin-labeled solute in lecithin bilayer membranes. *Proc. Natl. Acad. Sci. U. S. A.* 71:474-478.
19. Dix, J. A., D. Kivelson, and J. M. Diamond. 1978. Molecular motion of small nonelectrolyte molecules in lecithin bilayers. 1978. *J. Membr. Biol.* 40:315-342.