Interactions of Benzoquinone with a Model Membrane Bilayer As Reported by a Positronium Probe

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Abstract: Techniques of positronium chemistry have been used to examine the properties of model bilayer membranes in the presence and absence of *p*-benzoquinone at temperatures below and above the transition temperature of the phospholipid aggregate. The chemical reactivity between positronium atoms and *p*-benzoquinone molecules in the bilayer systems (water–DL-α-dipalmitoylphosphatidylcholine) was found to vary as a function of DPPC concentration. By comparison of the rate constants of the reaction with the rate constants for *p*-benzoquinone in various pure solvents, we are able to suggest that the location of the *p*-benzoquinone molecules in DPPC bilayers is in a region of high polarity. Positronium lifetimes and formation probabilities were measured at 25 and 45 °C for a variety of DPPC concentrations. The variation of *o*-Ps lifetime as a function of DPPC concentration was found to be greater for aggregates in the liquid crystalline phase than for those in the gel phase. The *o*-Ps formation probabilities were found to increase as a function of DPPC concentration in the liquid crystalline phase but to decrease in the gel phase. The implications of these aspects of positronium annihilation in this bilayer membrane system are discussed in relation to the charge transfer properties of *p*-benzoquinone.

Introduction

In recent years, considerable research effort has been made to further the understanding of structure and function relationships in biological membranes. The "fluid-mosaic" membrane model of Singer and Nicolson[2] pictures a variety of proteins which are immersed to some degree in a laterally mobile matrix of polar lipid. The biological activity of membrane-bound enzyme systems is usually dependent on the degree to which the lipid bilayer matrix is fluidized. This may in part be due to specific mechanical requirements of the enzyme, for example, for a requisite conformational change in the protein during its operation. In addition, the activity of membrane enzyme systems may be dependent on the permeability properties of the membrane toward inorganic ions or organic substances. These permeability characteristics in themselves may be related to the local fluidity of the bilayer as recently demonstrated for DPPC–water aggregates.[3] These physical properties are closely related to many important biological functions, including the charge-transfer reactions involving redox components in specialized membranes. An important aspect in the evaluation of the related physical and structural properties of biological membranes is the study of the location, disposition, and orientation of such charge-transfer molecules or functional groups in the membrane.

Recently, the structure of biological membranes has been investigated in terms of the lipid bilayer model.[4] The generally accepted phospholipid bilayer model is one in which two polar exterior surfaces containing the various charged head groups of phospholipid molecules sandwich a hydrocarbon-like bilayer. The bilayer region has characteristically progressively higher viscosity in a direction along the hydrocarbon chain from the methyl terminal end toward the backbone. While much of the understanding of structure and properties of membranes is reasonably satisfied in terms of this bilayer model, more detailed information is needed about the dynamics of the charge-transfer molecules and the locations of solubilization in the membrane.

Various physical methods such as nuclear magnetic resonance, electron spin resonance, differential scanning calorimetry, X-ray diffraction, and fluorescence polarization have been employed[5] to study the structure, the local properties, and the dynamics of bilayer membranes. However, positronium (Ps) annihilation, which we have employed in this investigation, is a relatively new technique. In the past few years, Ps atoms have been employed as nuclear probes to study the structure of chemical and biological systems.[6] Notable experimental successes include the determination of macro- and micro-phase transformations, such as liquid crystals and their polymorphisms,[7] micellar formations,[8] and membrane phase transitions.[9-12] Although the underlying basis for these successes is not yet fully understood, the advantages of positronium annihilation applications to biological systems are well recognized in several areas. First, the destructive effect of the radiation on the membrane system is negligible, and the degree of perturbation to the system by the positron probe is found to be minimal. The typical concentration of positrons required for experimentation is about 10^{-16} M, which is some orders of magnitude less than the most sensitive conventional probes. Secondly, the detection limit of a microphase transformation in terms of concentration is extremely high. Typically, detection at a concentration better than 10^{-4} M is possible, for example, in the determination of critical micelle concentration,[8] and in phase transitions of dilute solutions of aqueous vesicles.[13]

In the first part of this study, we show for the first time that this probe is able to identify the probable location of a charge-transfer molecule in a model bilayer membrane system. The special property of Ps upon which this determination depends is that of the differing chemical reactivity of the Ps atom as a function of its local environment. Certain function groups react strongly with Ps atoms. For example, Ps reacts with benzoquinone molecules with a chemical rate constant whose value depends on the para, meta, or ortho isomerism of the quinone.[14] Again, the unique chemical reactivity of Ps has been demonstrated in a study of molecular association constants and binding properties of protein molecules with members of the vitamin K_1 series.[15] This aspect of positronium technique is very important to biological applications since one can accurately study properties at specific

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Table I. Positron Lifetime Results for the DPPC-Water-Benzoquinone Bilayer Membrane System

temp, °C	system	τ_1, ns	τ_2, ns	τ_3, ns	I_2 %	I_3 %
25	DPPC (0 to 64%) in water (gel)	0.145 ± 0.025	0.513 ± 0.021	vary from[a] 1.76 ± 0.02 to 2.08 ± 0.021	49.5 ± 4.2	vary from[b] 23.6 ± 0.6 to 15.3 ± 1.2
45	DPPC (0 to 64%) in water (liquid crystal)	0.143 ± 0.020	0.597 ± 0.032	vary from[a] 1.75 ± 0.02 to 2.62 ± 0.02	50.9 ± 4.0	vary from[b] 24.0 ± 0.7 to 11.3 ± 1.5
	DPPC (0 to 64%) + *p*-benzoquinone (20 mM) in water at 25 and 45 °C	0.140 ± 0.026	0.537 ± 0.030	from 1.26 ± 0.02[c] to 2.10 ± 0.03	55.0 ± 5.0	vary from[d] 19.0 ± 1.3 to 11.0 ± 2.0

[a] As shown in Figure 2. [b] As shown in Figure 3. [c] The lifetime of *o*-Ps decreases due to chemical reactions between Ps and quinone molecules. [d] The I_3 % values decrease slightly due to the presence of quinones as a result of inhibition in Ps formation (ref 6).

functional sites in complex biological molecules by measuring the Ps reactivity with the selected functional groups. On this basis, therefore, we have measured the chemical reactivities between Ps and *p*-benzoquinone molecules as a function of the lipid concentration in a DPPC–water system. We interpret the data to indicate that the benzoquinone is not randomly distributed in the bilayer, but rather it is concentrated at specific polar regions of the bilayer.

The second part of the study involves measurement of the change of *o*-Ps annihilation characteristics both at 25 °C and at 45 °C as a function of the lipid concentration in the DPPC–water system. We consider that data from these experiments are consistent with formation of multilayer aggregates from suspended vesicular aggregates as the lipid concentration is raised from 0% to 6%. In addition, the data give indication of change of characteristics in the water bound to the phospholipid aggregate above and below the transition temperature of the lipid.

Experimental Materials and Methods

Materials. Anhydrous DPPC (DL-α-dipalmitoylphosphatidylcholine, >99%) was purchased from Sigma Chemical Co. (St. Louis, MO) and was used without further purification. The purity of the lipid was assessed by thin-layer chromatography in two solvent systems. In each case, the lipid appeared as a single spot. After alkaline deacylation of the lipid, gas-liquid chromatographic analysis of the fatty acid released showed that fatty acid composition was >99.6% palmitic acid. *p*-Benzoquinone was supplied from the Aldrich Chemical Co. (Milwaukee, WI) (>99%) and was used without further purification.

The positron source was 5 μCi ^{22}Na (in ^{22}NaCl solution from New England Nuclear, MA). The lipid aggregates were prepared by mixing the appropriate amounts of DPPC (approximately 30 mg), the desired proportion of water, and the ^{22}Na radioisotope (approximately 5 μCi in 1 μL). The mixed samples (50–100 mg) were degassed by freezing and thawing six times in order to remove all oxygen without changing the composition of the aggregates and were subsequently sealed in the glass vial. The thickness of sample was about 1.5 mm, which was sufficient to stop >99% of positrons emitted from the ^{22}Na decay. The prepared samples were heated to 50 °C and maintained at that temperature 24 h in order to form clear liquid-crystalline aggregates. The liquid-crystalline DPPC bilayer membranes were allowed to cool to room temperature, thus reverting them to the gel phase. No decomposition of the lipid could be detected by TLC analysis. The positronium lifetime measurements were made at 25 °C for the gel phase study and at 45 °C for the liquid-crystalline phase study. The sample temperatures were determined to better than ±0.1 °C by a thermometer in a thermostated Dewar oil bath. The samples of DPPC–water aggregates containing *p*-benzoquinone were premixed and prepared in an analogous way. These experiments were performed by cycling temperature at least twice to detect any hysteresis effect. The results were reproducible from cycle to cycle.

Positron Lifetime Measurement. The positronium lifetime and its formation probability were measured by a standard fast-fast coincidence method. The decay of ^{22}Na emits a positron and a simultaneous 1.28-MeV photon, the latter serving as starting signal. The annihilated photon (0.51 MeV) then registers as stopping signal within a resolving time of 100 ns. The resolution of the lifetime spectrometer was determined to be 380 ps by measuring the coincident photons from a ^{60}Co source. The actual resolution of lifetime spectra was found to be slightly larger (i.e., 430–480 ps) than the results obtained by the ^{60}Co by fitting the lifetime spectra into a multiexponential function computer program, POSITRONFIT.[16] The positron source corrections were made in the data analysis process by setting 5% of positron annihilation on the wall of the containing vial (Pyrex). This correction was estimated from a lifetime result from a sample which contained an excess of *p*-benzoquinone (>200 mM). This concentration gave the Ps lifetime for a completely quenched sample and permitted a determination of the proportion of positrons annihilated on the container wall as 5%. A detailed discussion of the data obtained is given in the next section.

Data Analysis of Positron and Positronium Lifetime

The positron lifetime spectra were fitted to a multinegative exponential function with two, three, and four terms in the POSITRONFIT[16] computer program. Two-component results give a short lifetime of about 0.4 ns and also a long lifetime of Ps atoms. The χ^2 results of the two-component fit are consistently larger than the results of three-component fit. The results reported in this paper are from three-component fittings, where the χ^2 values are between 1.1 and 0.9. The results of four-component fit give as good χ^2 value as results of three-component fit, but some of the standard deviations are much larger than for the three-component fit and are therefore considered less reliable than those of the three-component fit. The resolved three different lifetimes, $\tau_1 \sim 0.12$ ns, $\tau_2 \sim 0.5$ ns, and $\tau_3 \sim 1.5$ ns, correspond to different positron and positronium states with the probabilities I_1 %, I_2 %, and I_3 %, respectively. The results of positron lifetime analysis in DPPC bilayers and in the presence of p-benzoquinone are summarized in Table I.

The short-lived component (lifetime $\tau_1 \sim 0.12$ ns) is due to the contribution of the annihilation of *p*-Ps (singlet Ps, theoretical lifetime = 0.125 ns), epithermal positrons, and positron- or positronium molecules. The intermediate-lived component (lifetime $\tau_2 \sim 0.5$ ns) represents mainly the annihilation due to thermalized free positrons (theoretical lifetime in condensed matter = 0.5 ns), and some of the positron bound molecules. The long-lived component (lifetime $\tau_3 > 1.0$ ns) is due to the annihilation of the *o*-Ps atoms (triplet Ps). It is this *long-lived o-Ps component* which contains the most useful information for the study of structure and chemical reactions in bilayer membrane systems. Detailed descriptions of the processes of positronium formation and annihilation can be found in a recent text.[6]

Results and Discussion

Quinone Chemical Reactivity in the Aqueous DPPC Bilayer System. The rationale for the incorporation of *p*-benzoquinone into the DPPC bilayer was that the quinone[17] would serve as a simple model for the coenzyme of the ubiquinone type. This is known to be involved in hydrogen atom transfer in redox reactions in some membrane electron transport chains. The chemical reaction rate constants between quinone molecules and Ps atoms can be calculated from the equation derived from a Ps-complex formation[14] scheme as

$$\lambda = \lambda_0 + k_{Ps}[M] \quad (1)$$

where λ and λ_0 are the measured Ps annihilation rates ($\lambda = 1/\tau_3$, $\lambda_0 = 1/\tau_3^0$, and τ_3 is the *o*-Ps lifetime as defined in the above section) in the presence and the absence of quinone molecules in the membrane, and [M] is the quinone molar concentration. The values of k_{Ps} (quinones) vs. wt % DPPC concentrations are shown in Figure 1 for temperatures above and below the membrane main

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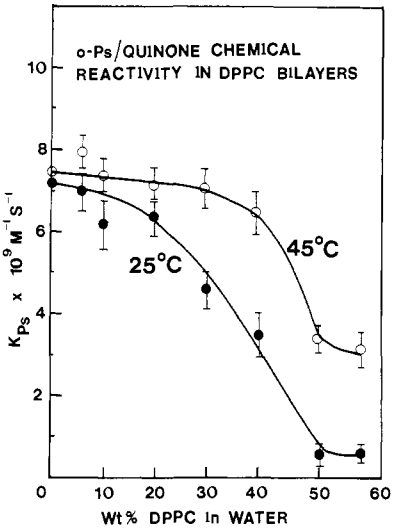

Figure 1. The observed chemical reaction rate constants between Ps atoms and quinone molecules in various DPPC concentrations (eq 1 of the text). The lines are drawn for eye-guide only.

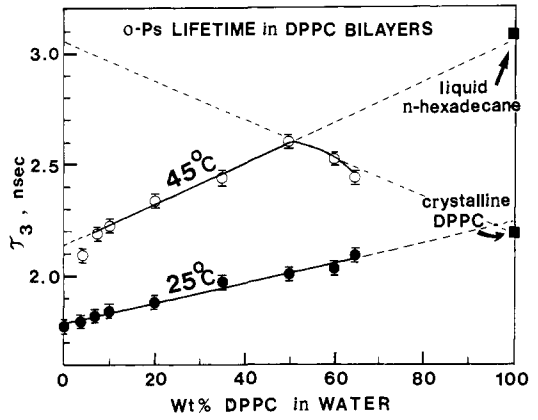

Figure 2. Variations of *o*-Ps lifetimes vs. wt % DPPC in water. The lines are fitted according to eq 2 and 3.

transition temperature (45 and 25 °C, respectively). The chemical reactivity of *p*-benzoquinone with Ps atoms, as expressed by k_{Ps}, decreases as a function of the lipid concentration. Since the reaction between the bilayer membrane itself and Ps atoms is only a weak-interaction process as shown in Figure 2, where the chemical reactivities between DPPC molecules and Ps were calculated to be less than 10^8 M^{-1} s^{-1}, the k_{Ps} values of Figure 1 represent the chemical rate constant of *p*-benzoquinone with Ps atoms in such a DPPC bilayer membrane system. In order to interpret the chemical reactivities of quinones with Ps atoms in this system, we need to know the k_{Ps} values between quinones and Ps in various pure solvents. The k_{Ps} values measured for *p*-benzoquinone in pure solvents are listed in Table I. They vary significantly according to the physical properties of the solvents, the solvents with high polarizability and with low viscosity usually giving high k_{Ps} values. By comparing the observed k_{Ps} in bilayers (Figure 1) with the k_{Ps} values in Table II, we are able to define the probable locations and the strength of the charge-transfer process of quinones in such a system.

Since the solubility of quinones in hydrocarbon solvents is higher than in aqueous solvents, one would first predict that quinone molecules are more likely to be located inside the membranes than in the aqueous phase. The actual location of *p*-benzoquinone within the membrane can in fact be assessed in a similar way to the comparison method which has been employed to locate the site of the solubilization in micelles.[18] First, the k_{Ps} values are obtained at sufficiently high lipid concentration (>50 wt % DPPC) to establish that most of the p-benzoquinone molecules are inside

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Table II. Chemical Reactivity (k_{Ps}) of Benzoquinone in Various Environments

solvents	k_{Ps} (Ps reactivity with quinones) × 10^9 M^{-1} s^{-1}
water (25 °C)[a]	7.3 ± 0.2
n-hexadecane[a] liquid (25 °C)	11.7 ± 0.2
n-hexadecane solid (5 °C)	<0.1
liquid glycerol (25 °C)	0.31 ± 0.05
liquid glycerol (45 °C)	0.45 ± 0.05
liquid benzene (25 °C)	52.8 ± 0.5
membranes[b] (45 °C)	3.1 ± 0.8
membrane (25 °C)[b]	0.5 ± 0.3

[a] k_{Ps} values at 45 °C are essentially the same as the values at 25 °C for quinones in water or in *n*-hexadecane. [b] When wt % DPPC ≥ 40%.

the membranes. These limiting k_{Ps} values (from Figure 1) are found to be 3.1 × 10^9 M^{-1} s^{-1} and 0.5 × 10^9 M^{-1} s^{-1} for the temperatures of 45 and 25 °C, respectively. As is evident from Table II, the k_{Ps} values (45 °C) agree with neither the k_{Ps} value in pure liquid hexadecane (k_{Ps} = 11 × 10^9 M^{-1} s^{-1}) nor with that in water (7.3 × 10^9 M^{-1} s^{-1}). A similar behavior was observed for the comparison of the k_{Ps} (25 °C) and the k_{Ps} value in solid hexadecane. Therefore, we can eliminate the likelihood of a location either in the center of the bilayer or in the aqueous region outside the bilayer. A location in the hydrocarbon chain milieu is also considered to be unlikely in light of the large difference of k_{Ps} in liquid *n*-hexadecane. The Ps reactivity is probably not reduced to one-third of its k_{Ps} value from the local viscosity difference alone, since k_{Ps} values for *p*-benzoquinone have reached a diffusion-controlled value in liquid hydrocarbons. The most likely location of *p*-benzoquinone, at least in the absence of protein, is suggested to be near the hydrated polar region of the lipid bilayer. In this polar region, it is possible that a partial charge-transfer reaction may have occurred between the acceptor, the *p*-benzoquinone, and the donor, the polar head, thus significantly reducing the effective reactivity of *p*-benzoquinones with *o*-Ps atoms, as we have observed.

Variation of *o*-Ps Lifetime as a Function of DPPC Concentration. We have performed experiments at two temperatures (25 and 45 °C), one being below and the other being above two known membrane phase transition[5] temperatures of aqueous DPPC systems. (Main transition at approximately 41 °C, and pretransition at 35 °C). The DPPC bilayers are in different physical states at these two temperatures, a liquid-crystalline state at 45 °C and a gel state at 25 °C.

For fairly low concentration of DPPC molecules (<1%) in aqueous solution, DPPC molecules aggregate into micellar forms [critical micelle concentration (cmc) ~ 10^{-8} M], and then may form multilamellar bilayers suspended in the aqueous phase. Unless externally agitated, the bilayers aggregate together to give particles of diameters ranging from a few hundreds to thousands of Å. Further addition of DPPC molecules increases the number of bilayer aggregates but does not necessarily influence the size of the individual aggregates in the system. The state of the DPPC system reported here (wt % of DPPC from 3% to 64%) is thus mainly a multi-lamellar bilayer state. The plots of τ_3 and I_3 % values vs. DPPC concentration are shown in Figure 2 and 3. As shown in the Figure 2, we observe a substantially different variation of τ_3 vs. wt % of DPPC for those aggregates in the liquid-crystalline phase (45 °C) than for those gel phase (25 °C), where the variation monotomically increases as a function of the wt % DPPC. This relationship represents the rigid structure of DPPC bilayers in water matrices in the gel phase. If we consider DPPC–water as a binary-mixture system, the measured Ps annihilation rate, λ_3 (= $1/\tau_3$), can be represented as a combination of the annihilation due to water and that due to crystalline DPPC at a weight percentage x as

$$\lambda_3 = \lambda_3^{water}(1 - x) + \lambda_3^{DPPC}x \qquad (2)$$

where λ_3^{water} and λ_3^{DPPC} are the Ps annihilation rates for pure water

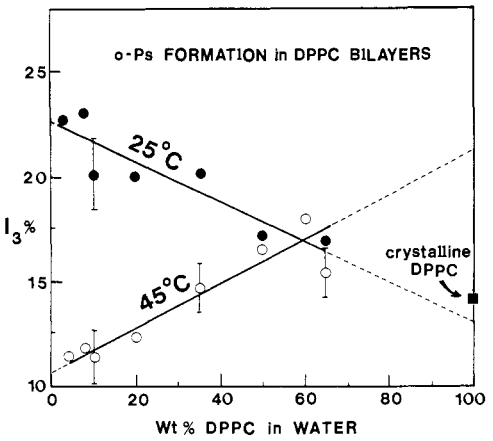

Figure 3. Variations of *o*-Ps formation probability vs. wt % DPPC in water. The lines are fitted according to eq 2 and 3 by replacing τ_3 as I_3 % (see the text).

and pure DPPC solids at 25 °C, respectively. They are simply obtained by taking the reciprocal values of *o*-Ps lifetime τ_3 in pure water and in DPPC (crystalline), respectively (τ_3^{water} = 1.78 ns and τ_3^{DPPC} = 2.18 ns). The fitted τ_3 from eq 1 is plotted as a line in Figure 2. The close agreement between the fitted line and the experimental data further suggests that Ps atoms are diffusing randomly in the gel matrices in the annihilation process.

Bilayers in the liquid-crystalline state (45 °C) show a variation of τ_3 vs. wt % DPPC that is more complicated than that in the gel phase. As shown in Figure 2, τ_3 increases sharply at low DPPC concentration and more slowly up to 50% DPPC, but then decreases above 50 wt % DPPC. The rapid increase of τ_3 values in the range 0% to 6% DPPC is probably due to a structural change of the system from suspended vesicular aggregates to multilayer aggregates, and so we have concentrated on the variations occurring above 6 wt % DPPC which involve multilamellate structures in the liquid-crystalline state.

We further interpret the data expressed in Figure 2 both in terms of water structure perturbation and of the degree of fluidity of the bilayer as follows. The data points of τ_3 between 6% and 50% for temperature at 45 °C of Figure 2 lie on a fairly straight line. If we apply the model equation (2) from the gel phase treatment to the case of liquid crystalline phase, then we write the measured λ_3 as

$$\lambda_3 = \lambda_3^{polar}(1 - x) + \lambda_3^{apolar}x \qquad (3)$$

where λ_3^{polar} and λ_3^{apolar} are the Ps annihilation rates in the polar and in the nonpolar region of membranes, respectively, and x is the weight fraction of apolar component in the system. If we assume x = wt % DPPC as a good approximation due to the abundance of hydrophobic character in DPPC, and fit the data points of λ_3 between 6% and 50% in eq 3, we obtain a straight line as shown in Figure 2. The extrapolated values of τ_3^{polar} (= $1/\lambda_3^{polar}$) and τ_3^{apolar} (= $1/\lambda_3^{apolar}$) are found to be 2.12 and 3.05 ns, respectively. It is interesting that one observes a different value of τ_3^{polar} (2.12 ns) from τ_3^{water} (1.78 ns) in the liquid-crystalline phase and in the gel phase. The value of τ_3^{polar} (2.12 ns) lies between τ_3 values of water (1.78 ns) and that of polar organic liquids[19] (varies from 2.5 to 4.0 ns). This could be interpreted to indicate that the behavior of water molecules bound to lipid in the liquid-crystalline phase differes from their behavior when bound to lipid in the gel phase. Judging from extrapolated τ_3^{polar} and τ_3^{water} values, it appears that the characteristics of the water bound to the liquid-crystalline lipid have changed from those of pure water toward those of polar organic liquids. However, the characteristics of the water molecules bound to the gel phase lipid appear to retain bulk water characteristics. Since it is known[19] that the lifetime of *o*-Ps in different liquid hydrocarbons is inversely correlated with the polarizability of the hydrocarbon, a likely interpretation of the difference in these extrapolated τ_3 values is that the water molecules become less polarizable when they are bound to liquid-crystalline lipid. The value of τ^{apolar} (3.05 ns) is also interesting because it is close to the τ_3 value in hexadecane (measured $\tau_3^{hexadecane}$ = 3.12 ns). This value is distinctly different from the τ_3^{DPPC} value obtained in the gel phase (τ_3^{DPPC} = 2.18 ns). We interpret this difference as indicating that the nonpolar region of the membrane is a liquid hydrocarbon-like environment in the liquid-crystalline phase (45 °C) while the gel phase is still a solid-like environment, an interpretation which is consistent with the commonly accepted view of the main transition phenomenon. At temperatures higher than that of the main transition, hydrocarbon chains across the membrane obtain considerable rotational freedom; therefore the physical state within the bilayer resembles liquid hydrocarbon rather than crystalline lipid. It has been established[5] that there exists a local differential increase of viscosity along the hydrocarbon chain toward the membrane surfaces. The hydrocarbon chains in the center of the membrane move as freely as liquid hydrocarbon while the volume near the polar backbone (membrane surface) still retains relatively high molecular rigidity.

The decrease of τ_3 above 50 wt % DPPC (Figure 2) is difficult to rationalize in view of the known behavior in DPPC–water systems as studied by conventional techniques. Two tentative explanations are suggested. First, Ps atoms may diffuse differently when the bulk viscosity becomes very high, such as above 60% of DPPC, and secondly, it is possible that there is a phase transformation above the 50% DPPC–water system which has escaped detection by the conventional methods.

Variations of *o*-Ps Formation Probability vs. wt % DPPC. The relationship between *o*-Ps formation probability, I_3 % vs. wt % DPPC is shown in Figure 3 for both liquid crystalline (45 °C) and gel (25 °C) phases. The variations of I_3 % vs. wt % DPPC are very different from the variation of τ_3 vs. wt % DPPC as one compares Figure 2 with Figure 3. This is due to the distinct difference in the time scale of observations employed. Ps formation takes place in 10^{-12} s while the Ps annihilation occurs in 10^{-9} s. It is known from experimental observations that I_3 % and τ_3 respond differently for different phase transformations in different systems. The underlying reason is still a current research topic in Ps chemistry. In the micellar system I_3 % values show[8] an abrupt decrease at the critical micelle concentration (cmc) but only a slow variation of τ_3. In the case of liquid-crystal systems,[7] τ_3 changes at the phase transition temperature rather than I_3 %.

For the current DPPC–water system, we have studied a system involving both micellar and liquid-crystalline phases within a range of DPPC concentrations. At very low concentrations of DPPC above the phase transition temperature (45 °C), we observe a I_3 % drop from that observed for pure water (24%) to 11% due to micelle formation (Figure 3). In the liquid-crystalline regions we observe a monotonical increase of I_3 % vs. wt % of DPPC. These data are fitted in a simple linear equation similar to eq 3 where I_3 is used instead of τ_3. Since there are no other lyotropic phase transitions in this region, such a simple linear relationship is to be expected, as shown in the line of Figure 3. For the gel phase, the decrease of I_3 % vs. wt % DPPC can be interpreted as a linear combination of pure $I_3^{H_2O}$ % and pure I_3^{DPPC} % at different values of wt % as in eq 2. This result again confirms the rigidity of the gel phase in which the overall Ps formation probability could be considered as arising from the additive contributions of pure water and of crystalline DPPC. In the case of the liquid crystalline phase, the extrapolated values I_3^{polar} % (11%) and I_3^{apolar} % (22%) are again distinctly different from the corresponding values in the gel phase. The variations of I_3 % can be used as a means of detecting phase transitions in membranes, since marked changes in I_3 % are observed between 25 and 45 °C at each concentration of DPPC below 35 wt %. In our previous study, in which we reported the use of the positronium probe in measuring temperatures of pre- and main-transitions for a DPPC–water system, we found no change in I_3 % values between the gel and liquid-crystalline states. It is now clear that this result was simply fortuitous: we happened to choose for study a DPPC concentration of 60 wt % which corresponds to the single instance where I_3 % has the same value

(19) P. R. Gray, C. F. Cook, and G. P. Sturm, Jr., *J. Chem. Phys.*, **48**, 1145 (1968).

both for the gel state and for the liquid-crystalline state (Figure 3). We are currently investigating DPPC mixture of low weight percent in our laboratory.

Conclusions

High Ps formation probabilities in lipid bilayers enable us to apply the positronium annihilation technique to study the chemical and physical properties of biomembranes. The correlations between membrane fluidity and the Ps annihilation characteristics, particularly those of I_3 % and τ_3, show that Ps atoms are able to distinguish between various microstructural properties at least within a DPPC bilayer, and they reveal the hydrocarbon-like interior. If we assume the comparison can be made between the observed K_{Ps} values in the DPPC bilayer and in the values of various solvents, then we can suggest that the location of the *p*-benzoquinone charge transfer molecule is near the backbone polar region in the interior of the bilayers. These observations thus suggest that Ps atoms are potentially sensitive nuclear probes to study the dynamic behavior of charge transport and microstructures within biomembranes.

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Mechanisms of the Dismutation of Superoxide Catalyzed by the Copper(II) Phenanthroline Complex and of the Oxidation of the Copper(I) Phenanthroline Complex by Oxygen in Aqueous Solution

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Abstract: By using the technique of pulse radiolysis to generate O_2^-, it is demonstrated that copper 1,10-phenanthroline ($(op)_2Cu^{2+}$) is capable of catalytically dismutating O_2^- with a "turnover" rate constant $k_{cat.} = (5.1 \pm 0.9) \times 10^8$ M^{-1} s^{-1}. The rate constants of the reduction of $(op)_2Cu^{2+}$ and of the oxidation of $(op)_2Cu^+$ by O_2^- have been determined to be $(1.93 \pm 0.07) \times 10^9$ and $(2.95 \pm 0.3) \times 10^8$ M^{-1} s^{-1}, respectively. The kinetic results of the oxidation of $(op)_2Cu^+$ by molecular oxygen in aqueous solution are interpreted by a mechanism that proceeds via a superoxide intermediate, and the rate constant $k_{-10} = (5.0 \pm 0.3) \times 10^4$ M^{-1} s^{-1} has been determined. The rate constant of the oxidation of $(op)_2Cu^+$ by H_2O_2 was measured to be $k_{22} = (937 \pm 20)$ M^{-1} s^{-1}.

Enzymatic catalysis of superoxide dismutation has been a matter for investigation since the work of McCord and Fridovitch.[1] It has been suggested that the mechanism of the catalytic dismutation of O_2^- by bovine superoxide dismutase (SOD) involves alternate reduction and oxidation of copper ions:[2,3]

$$\text{enzyme-Cu}^{2+} + O_2^- \xrightarrow{k_1} \text{enzyme-Cu}^{+} + O_2 \qquad (1)$$

$$\text{enzyme-Cu}^{+} + O_2^- + 2H^+ \xrightarrow{k_2} \text{enzyme-Cu}^{2+} + H_2O_2 \qquad (2)$$

The "turnover" rate constant, $k_{cat.} = (2.4 \pm 0.3) \times 10^9$ M^{-1} s^{-1}, is pH independent over the range 4.8–9.5.[4-6]

A large number of copper compounds have been tested for the rate at which they react in aqueous solution with O_2^- to catalyze its dismutation. The aquo complex and the copper chelates of some amino acids[7-9] and salicylates[10] exert almost the same catalytic activity as SOD.

Recently, it has been demonstrated that degradation of deoxyribonucleic acid (DNA) by 1,10-phenanthroline requires Cu(II), a reducing agent, and O_2. Other metal ions do not substitute for Cu(II), and the degradation of DNA is inhibited by metal ions that can form stable complexes with 1,10-phenanthroline as well as by chelators that can bind copper. The degradation of DNA is also inhibited by either SOD or catalase, suggesting that O_2^- and H_2O_2, respectively, are required for the breakdown of the DNA.[11-14]

In view of the role of $(op)_2Cu^{2+}$ in the degradation of DNA, and the possible role of O_2^- in this process, we have decided to study the catalytic effect of $(op)_2Cu^{2+}$ upon the disproportionation of O_2^- and the kinetics and the reaction mechanism of the oxidation of $(op)_2Cu^+$ by molecular oxygen in the presence and in the absence of H_2O_2.

Experimental

Pulse radiolysis experiments were carried out on a Varian 7715 linear accelerator. The pulse duration ranged from 0.1 to 1.5 μs with a 200-mA current of 5 MeV electrons. Either a 200W Xe–Hg or a 150W Xenon arc were used as the analytical light source. We used 2.0 or 4.0 cm long irradiation optical cells with one or three light passes. Appropriate light filters were used to eliminate any scattered light. The detection system included a Bausch & Lomb grating monochromator Model D330/D331

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