latex particle surface structure must also be involved. Such factors could include the interface structure, the water distribution at the interface, the chemical composition, and the surface potential. Of these factors the surface potential can be directly investigated experimentally.

The surface potential is a function of the latex particle charge and can be directly measured by a Z meter. Figure 4 shows that the Z potential becomes more negative for both latices as the SDS concentration increases. This is consistent with the adsorption of SDS leading to an increase in the negative surface charge. Quantitative values of the surface charge or charge variation are not directly determinable since the Z potential is the overall charge of the colloidal particle along with its electrical double layer.[18] From a qualitative point of view an increase in the negative surface charge between the TMB location and the bulk water should lead to a decrease in the photoionization yield.[8,16] This is opposite to what is observed in Figure 2, so the trend in photoionization yield is not correlated to the Z potential trend. This supports, at least for negatively charged particles, that the photoionization yield does not correlate with interfacial particle charge.

Therefore, the most important contribution to the TMB photoionization yield, in negatively charged particle systems, is the TMB^+–water interaction. This result confirms deductions from the mixed micellar system SDS/DTAC.[8] The difference of about 20% in the TMB^+ yield between latices A and B seems to be related to different interfacial structure perhaps associated with the different chemical compositions of the two latices since the TMB^+ yield in latex A versus latex B is not correlated to the deuterium modulation depth or to the Z potential.

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Conclusions

The photoionization yield of TMB^+ in frozen latices depends on the photocation–water interaction and is a function of the amount of SDS adsorbed at the latex interface. An increase in the negative charge of the latex particle does not affect the photocation yield, showing that the surface charge plays a secondary role for the photocation yield in negatively charged dispersed systems. The TMB^+ yield difference of about 20% found for the two latices is not related to a corresponding trend in the TMB^+–water interactions or to the particle surface charge. This result suggests that the interfacial structure of the latices have a role in the photocation generation, even if secondary to that of the TMB^+–water interactions.

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Registry No. TMB, 366-29-0; TMB^+, 21296-82-2; SDS, 151-21-3; butadiene–acrylonitrile–methacrylic acid polymer, 9010-81-5; butadiene–styrene–acrylic acid polymer, 25085-39-6.

Electron Spin Echo Modulation Studies of Doxylstearic Acid Spin Probes in Frozen Vesicles: Interaction of the Spin Probe with D_2O and Effects of Cholesterol Addition

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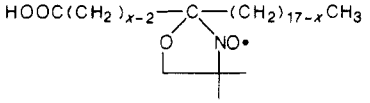
Electron spin echo studies have been carried out for a series of x-doxylstearic acid (x = 5, 7, 10, 12, and 16) spin probes in frozen deuteriated aqueous solutions of phospholipid vesicles and cationic dioctadecyldimethylammonium chloride (DODAC) vesicles. Modulation effects due to interactions of the nitroxide group of the spin probes with D_2O give information about the conformations of the probes and the degree of hydration of the surfactant headgroups as well as about the degree of packing of the alkyl chain. We show that DODAC headgroups are more hydrated than choline headgroups and that the doxylstearic acid probes show a larger tendency for bending in DODAC vesicles than in phospholipid vesicles. Upon addition of cholesterol into phospholipid vesicles, the headgroups are separated and their degree of hydration increases.

Introduction

Photoinduced charge separation of photosensitive solutes and the subsequent charge transport in organized molecular assemblies are typical models for artificial photosynthetic reactions.[1-3] Among other colloidal systems, vesicles have been widely used to mimic natural membranes.[4-6] Photoionization and net charge separation may be markedly affected by the structural parameters of the vesicle, such as counterions,[7,8] headgroups,[9,10] and hydrocarbon tail length[11,12] of the amphiphiles, or by adding salts[13,14] or slightly water soluble alcohols or cholesterol.[15]

Stable nitroxide radicals have been widely used as spin probes in studies of biological membranes and membrane mimetic systems.[16-18] Among others, x-doxylstearic acids of the formula



have turned out to be particularly useful, for they are sparingly soluble in water but can be readily incorporated into heterogeneous

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aqueous systems containing amphiphilic molecules.[19]

Electron spin echo modulation (ESEM) spectroscopic methods developed in our laboratory[20] have already led to investigations of the structural parameters of micelles[7,8,21,22] and have led to a correlation of the photoionization yield of *N,N,N',N'*-tetramethylbenzidine (TMB) with increasing interaction between the photoproduced TMB^+ cation and D_2O of the bulk water phase.[23,24] Previous studies also aimed at determining the location of doxyl spin probes as well as medium chain length alcohols in micellar structures differing by their charge and counterions.[25,26]

In this work, we have studied systematically with ESEM the locations of doxyl spin probes in zwitterionic phospholipid surfactant vesicles, dipalmitoylphosphatidylcholine (DPPC), and others containing either a cis double bond at the C9 position of the alkyl chain like dioleoylphosphatidylcholine (DOPC) or a trans double bond at the same position in dielaidoylphosphatidylcholine (DEPC), as well as in the cationic surfactant dioctadecyldimethylammonium chloride (DODAC). We have also studied the effect of cholesterol addition to DPPC vesicles which was shown recently to modify the vesicle structure.[15] These results are discussed in terms of hydration of the headgroups and ordering of surfactant aggregates and are compared to analogous results obtained with micelles.

Experimental Section

DL-α-Dipalmitoylphosphatidylcholine (DPPC), L-α-dioleoylphosphatidylcholine (DOPC), and L-α-dielaidoylphosphatidylcholine (DEPC) were purchased from Sigma Chemical Co. and used without further purification. Cholesterol from Sigma was recrystallized from ethanol. Dioctadecyldimethylammonium chloride (DODAC) was obtained after exchanging a methanol/chloroform (70:30 v/v) solution of dioctadecyldimethylammonium bromide (DODAB) from Eastman Chemicals through an ion-exchange resin type AG2-X (20–50 mesh) from Biorad Laboratories. A 98% DODAC purity was confirmed by elemental analysis for chlorides by Galbraith Laboratories, Knoxville, TN. The spin-labels 5-, 7-, 10-, 12-, and 16-doxylstearic acids from Molecular Probes, Inc., were stored at −10 °C upon arrival and used as received.

A deuteriated buffer solution was prepared with D_2O from Aldrich Chemical Co., containing 0.1 M sodium phosphate from Fisher Scientific Co., 0.1 M sodium pyrophosphate from Aldrich Chemical Co., and 1 mM sodium ethylenediamine tetraacetate (EDTA) from Alfa Products. The pH was adjusted to 7.0 with hydrochloric acid.

Further operations were carried out in nitrogen atmosphere. Vesicle solutions were prepared by the method described by Huang[27] and modified by Norris and others.[28,29] Chloroform surfactant solutions were evaporated at 60 °C for 3 h. The resulting films were sonicated in the aqueous buffer solution, which had been deaerated beforehand by N_2 bubbling for 15 min, with a Fisher 300 sonic dismembrator operated at 30 W with a 4-mm-o.d. microtip for 1 h, at 55 °C for DPPC and DODAC and 0 °C for DOPC and DEPC. (These temperatures are slightly above the respective gel–liquid transition temperatures of the surfactants.) DODAC vesicles were prepared in pure D_2O.

Cholesterol, if added, was added before evaporation to the chloroform surfactant solution. However, it could also be added to pure phospholipid vesicle solutions provided we allowed these mixtures to stand for 24 h.

Final solutions were added to previously evaporated spin probe films and were allowed to stand for 24 h. This method solubilized the probes specifically into the outer part of the bilayer of the vesicles. The respective concentrations of probes and surfactants were 0.2 and 45 mM, which amounts to a ratio of 1 probe per 225 surfactant molecules. The samples were prepared by introducing the vesicle solution into 2 × 3 mm Suprasil quartz tubes and rapid freezing to 77 K in liquid nitrogen.

Electron spin resonance (ESR) spectra were obtained at X band with a Bruker ER300 spectrometer with 100-kHz modulation frequency. Two-pulse electron spin echo (ESE) spectra were recorded at 4.2 K with a home-built spectrometer,[30] for the $M_I = 0$ ^{14}N hyperfine line of the powder spectrum of our samples.

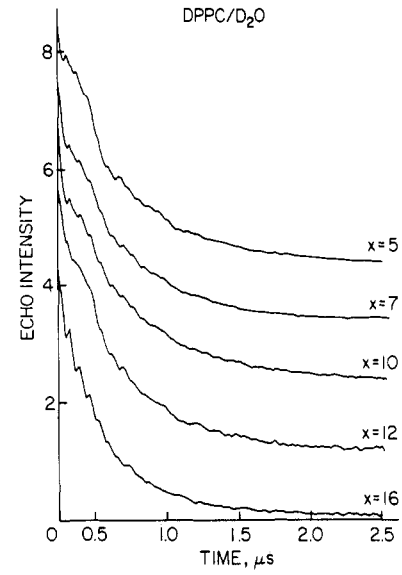

Figure 1. Two-pulse electron spin echo spectra recorded at 4.2 K for *x*-doxylstearic acid spin probes in DPPC vesicle solutions. The base lines have been offset vertically to avoid overlap.

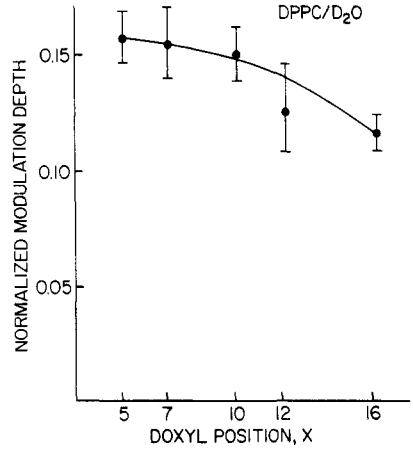

Figure 2. Dependence of deuterium ESE normalized modulation depth on the position of the doxyl group in *x*-doxylstearic acid spin probes in DPPC vesicle solutions.

Results

Two-pulse electron spin echo envelopes were recorded from the $M_I = 0$ ^{14}N hyperfine transition of the nitroxide electron spin resonance spectrum. The spectra as a function of the *x*-doxyl probe in DPPC vesicles are shown in Figure 1. All the echo spectra exhibit modulation with a period of 0.08 μs that is related to weak hyperfine interactions of the nitroxide groups with protons

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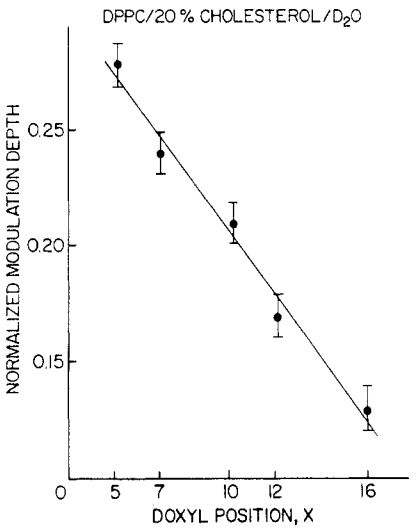

Figure 3. Dependence of deuterium ESE normalized modulation depth on the position of the doxyl group in x-doxylstearic acid spin probes in DPPC vesicle solutions containing a 20% mole ratio of cholesterol.

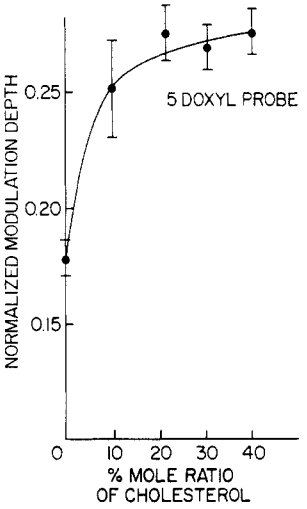

Figure 4. Dependence of deuterium ESE normalized modulation depth on the molar ratio of cholesterol incorporated into DPPC vesicles for 5-doxylstearic acid spin probe.

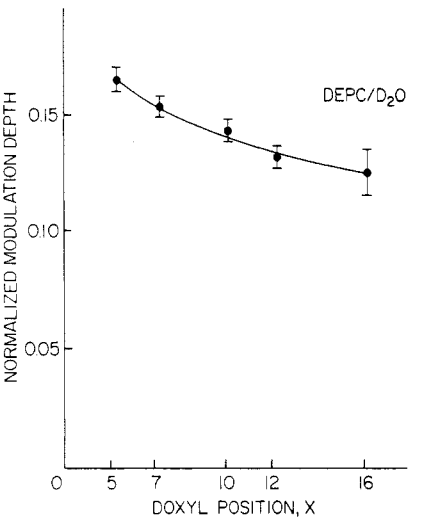

Figure 5. Dependence of deuterium ESE normalized modulation depth on the position of the doxyl group in x-doxylstearic acid spin probes in DEPC vesicle solutions.

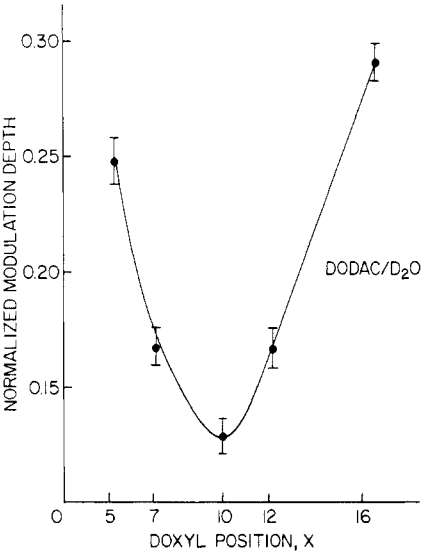

Figure 6. Dependence of deuterium ESE normalized modulation depth on the position of the doxyl group in x-doxylstearic acid spin probes in DODAC vesicle solutions.

from the surfactant alkyl chains and a second modulation with a period of 0.55 μs resulting from interactions of the probe with deuterium in D_2O located at the vesicle interface. Figure 2 shows the respective normalized deuterium modulation depths, computed by the graphic method.[21] Figure 3 shows the variation of modulation depth versus x-doxyl for DPPC vesicles with a 20% mole ratio of cholesterol incorporated. Figure 4 shows the exploration of the headgroup regions of such vesicles with the 5-doxyl probe as a function of the incorporated mole ratio of cholesterol.

Figure 5 shows the modulation depth dependence versus x-doxyl for DEPC. Note that no or only weak modulation is observed with DOPC vesicles. Figure 6 illustrates the same dependence for the cationic DODAC vesicles.

Discussion

When the ESEM experiments are conducted in frozen vesicle solutions, molecular averaging of the anisotropic electron nuclear dipolar interactions responsible for the modulation effects can be avoided and at 4.2 K sensitivity is enhanced and relaxation rates are slower.[20] Moreover, evidence has been presented for the retention of micellar structures in frozen solutions.[31,32] The normalized modulation depth is dependent on the number of deuterium nuclei surrounding the unpaired electron and their distances.[20] For realistic numbers of interacting nearest-neighbor deuterium nuclei modulation is only detectable for those within about 0.6 nm from the unpaired spin.[20]

DPPC Vesicles. Figure 2 indicates that the normalized modulation depth decreases monotonically as the doxyl group is incorporated further from the carboxylic acid moiety in the stearic acid alkyl chain. Some nitroxide groups are still interacting with D_2O for large x values since finite modulation depths are observed. Interaction with D_2O in the inner water pool is improbable. We selectively solubilized the spin probes in the outer part of the bilayer which is typically 50 Å thick.[33] The stearic acid probes are about 20 Å long in their extended conformation, and the acid group has been shown to be solubilized near the headgroups at the interface in micellar systems with the carbon chain in the lipophilic core.[34,35] Therefore, the observed tendencies have to be discussed in terms of probabilities of solubilization sites and conformations of the spin probes.

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The observed tendency in DPPC vesicles indicates that the probability of interactions between the probe and D_2O decreases as x increases. This observation seems consistent with a majority of probes in largely extended conformations parallel to the phospholipids and thus a low tendency for probe bending. Another noteworthy feature concerns the amplitude of the normalized modulation depth, particularly for the 5-doxyl probe. In DPPC vesicles, the maximum value is about 0.17, which we can compare to a reported value of 0.25 for sodium dodecyl sulfate (SDS) micelles.[7] We therefore have to conclude that DPPC vesicles are less hydrated than micelles, which supports the fact that vesicles are better organized aggregates than micelles. It is known that the vesicle bilayer thickness is comparable to micellar diameters and that the aggregation number is typically 20 times greater for vesicles than for micelles. Most of the surfactant alkyl chains are in an extended, mostly trans configuration.[33]

Effect of Cholesterol Addition. In Figure 3, we see a linear decrease of the normalized modulation depth as x increases, for DPPC vesicles containing a 20% mole ratio of cholesterol. For $x \leq 10$, the normalized modulation depth is greater than the one found for pure DPPC vesicles. For $x > 10$, we have comparable results with Figure 2. We can account for the higher values for $x \leq 10$ by the fact that cholesterol is known to increase the size of the vesicles by up to 40% and to separate the headgroups of DPPC vesicles, thus increasing their degree of hydration.[36,37] This conclusion is supported by Hiromitsu and Kevan,[15] who found a decrease of vectorial electron transfer of chlorophyll *a* to ferricyanide in frozen vesicles containing cholesterol.[15] Thus, it is reasonable to think that the probes with lower x will show larger modulation depth if they are mostly parallely packed. Since cholesterol can be incorporated in DPPC vesicles up to 50% mole ratio[15,38] and since 5-doxylstearic acid ideally probes the headgroup region,[34,35] we show in Figure 4 that the modulation depth of that probe increases with increasing amounts of cholesterol. This confirms an increasing degree of hydration of the vesicle headgroups with increasing amounts of cholesterol. This increase is dramatic with low amounts of cholesterol.

Effect of Unsaturation in the Alkyl Chain. Figure 5 shows that for DEPC, containing a trans double bond at the C9 position, the modulation depth versus x-doxyl is comparable to that for DPPC. Theoretical predictions by Israelachvili et al.[39] suggest that a trans double bond should hardly affect the structure of a vesicle and only slightly shorten the length of the surfactant molecule. Spin probes should be therefore solubilized in the same way as they are in pure DPPC vesicles. Samples prepared with DOPC containing a cis double bond at the C9 position showed echos that were not modulated at all. Theoretical studies[33] indicate that cis double bonds should perturb the packing more than trans double bonds. Experiments by nuclear magnetic resonance have shown a chain stiffening effect of cis double bonds.[40] Our data suggest a solubilization site of the doxyl probes inside the bilayer more than 0.6 nm from the surface, possibly in channels created by the cis double bonds.

DODAC Vesicles. DODAC vesicles are cationic vesicles. Their sizes are comparable to DPPC vesicles.[41] In Figure 6, one can see that the tendency of modulation depth versus x-doxyl is quite different from what is observed with the other systems studied above. The curve shows a minimum at $x = 10$, and the 16-doxylstearic acid spin probe has a strong interaction with D_2O. These data can be compared to what has been observed[21] for cationic micelles. Doxyl spin probe interactions with D_2O in cationic hexadecyltrimethylammonium bromide (HTAB) and dodecyltrimethylammonium bromide (DTAB) micelles and with deuterium in fully deuteriated trimethylammonium headgroups were studied. Comparison can be made since those deuteriums label the interface. Plots of normalized modulation depths versus x-doxyl also showed a minimum at $x = 10$ and a high value at $x = 16$.

The rationale for explaining such data still holds for DODAC vesicles. It was suggested that the spin probes show a great tendency for bending with the end of the alkyl chain of the stearic acid close to the surface of the vesicle. This tendency for bending can be explained by an enhanced degree of packing of DODAC surfactant molecules in the bilayer that prevents the weakly polar and bulky heterocyclic probe from penetrating too deep inside the bilayer.

Conclusion

The present ESE results support the fact that vesicles are more ordered organized molecular assemblies than micelles and swell if cholesterol is added. The degree of packing seems less perturbed by a trans double bond relative to a cis double bond. Cationic vesicles are more hydrated and solubilize spin probes in more folded conformations than do zwitterionic phospholipid vesicles.

Acknowledgment. This research was supported by a grant from the Division of Chemical Sciences, Office of Basic Energy Sciences, Office of Energy Research, U.S. Department of Energy.

Registry No. DODAC, 107-64-2; DPPC, 2797-68-4; DOPC, 4235-95-4; DEPC, 56782-46-8; 5-doxylstearic acid, 29545-48-0; 7-doxylstearic acid, 40951-82-4; 10-doxylstearic acid, 50613-98-4; 12-doxylstearic acid, 29545-47-9; 16-doxylstearic acid, 53034-38-1; cholesterol, 57-88-5.

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