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Electron spin–echo modulation studies of ionic and nonionic micelle structure via stearic acid nitroxide probes

Larry Kevan[+] and Piero Baglioni[++]

Department of Chemistry, University of Houston[+], Houston, Texas 77204-5641, USA and University of Udine[++], 33100 Udine, Italy

ABSTRACT - Electron spin-echo modulation is used to study the nuclear environment around a nitroxide moiety located at different positions on a stearic acid alkyl chain embedded in ionic and nonionic micelles. By using deuterium as nuclear labels the interface and internal structure of micelles can be probed. The conformation of the nitroxide probe and surfactant molecules is determined as is the degree of water penetration into the micelle.

INTRODUCTION

Nitroxide moieties attached to surfactant molecules at different positions along the alkyl chain have been used to probe the internal and interface structure of organized molecular assemblies such as micelles, vesicles and bilayer membranes. In the majority of these studies the electron spin resonance spectrum of the nitroxide has been analyzed in terms of its correlation time and its lineshape in order to say something about the local dynamics of the environment of the nitroxide probe. A different method which is somewhat complimentary and which gives direct structural information is to measure weak dipolar hyperfine interactions between the unpaired electron of the nitroxide and nuclei at the interface and/or at specifically labelled parts of the surfactant molecules forming such organized assemblies. To measure such weak nuclear interactions electron spin-echo modulation (ESEM) studies have proved to be particularly useful.

ESEM

Electron spin echoes are generated in pulsed ESR (electron spin resonance) in response to suitable resonant pulse sequences (ref. 1). The pulse sequences reorient the magnetic dipoles such that they dephase and then rephase in response to all time-dependent magnetic interactions in the system; the rephasing of the magnetic dipoles to reform the macroscopic magnetic moment constitutes the echo. The echo thus measures the net magnetization of the spin system at a given time. The simplest type of pulse sequence consists of a 90° pulse followed by a 180° pulse. The 90° pulse nearly instantaneously orients the various electron spins including those in different nuclear environments along one axis in the xy plane perpendicular to the externally applied magnetic field direction along the z-axis. These various spins then precess apart in the xy plane at slightly different rates depending on their various nuclear environments or other magnetic interactions. After a time τ, a 180° pulse is applied which reorients the spins in the xy plane so that they now precess toward one another. After a second time τ, they rephase and coalesce to form a burst of microwave energy called an echo. An actual experiment is done by varying the time between the pulses to produce successive echoes. The echo amplitude decreases with increasing interpulse time because of transverse relaxation processes. The echo intensity is measured as a function of interpulse time and if magnetic nuclei are within about 0.2 - 0.6 nm from the unpaired electron the echo intensity will typically be modulated. This modulation is primarily a function of the number and distance of the closest surrounding magnetic nuclei.

To apply ESEM to micelle systems which have a complex structure the modulation depth is analyzed semiquantitatively. Typically, deuterium modulation is observed from deuterium localized at some position in the surfactant system. The normalized deuterium modulation depth is defined as the depth of the first modulation minimum relative to the unmodulated echo intensity divided by the depth to the baseline. This normalized modulation depth is a very reproducible parameter for comparing the extent of interaction with deuteriums at various locations within surfactant systems. Since the number of interacting deuteriums in such complex systems is typically large, changes in the modulation depth are primarily interpreted as changes in the distance to the interacting deuteriums.

Since the ESE modulation arises from a dipolar interaction it is averaged out by rapid molecular tumbling in liquid solution and consequently can only be observed in rapidly frozen solutions. Many ion and atom solvation studies have been carried out in rapidly frozen aqueous solutions and have shown consistently the retention of a solvation shell in rapidly frozen solutions (ref. 2). In micellar systems several specific experiments indicate that the micellar structure is retained in rapidly frozen aqueous solutions. (1) One evidence is the lifetime of photogenerated tetramethylbenzidine cation (ref. 3). In bulk solution the lifetime of this cation is a few microseconds whereas in micellar liquid solutions it is of the order of minutes. If the cation is generated in liquid micellar solution and that is rapidly frozen and then thawed, the cation is still visible by electron spin resonance indicating that the micellar structure has been retained in the freezing and thawing process. (2) Analogous results are obtained for tetramethylbenzidine cation photogenerated in micelles and vesicles (ref. 4). Since vesicles are much less dynamic and are more ordered structures than are micelles, one would expect that the vesicular structure would be retained on rapid freezing to a greater degree than the micellar structure. Since analogous and reproducible results are obtained for experiments in both media this suggests that the micellar structure is retained upon rapid freezing of aqueous solutions. (3) A third indication is that similar aggregation numbers for sodium dodecyl sulfate micelles are measured in liquid and frozen solutions by luminescence quenching (ref. 5). This is a direct measure of the size of the micelle that is retained in both liquid and frozen solutions. (4) A fourth indication is that Baglioni et al. (refs. 6-8) have measured the partition coefficient for several alcohols and crown ethers in frozen sodium dodecylsulfate micellar solutions and have found values in good agreement with those obtained by NMR at room temperature (ref. 9). (5) Further evidence that the structure of micelles, vesicles and microemulsions is retained upon freezing comes from freeze fracture electron microscopy and from the method of direct imaging (refs. 10-15).

IONIC MICELLES

The x-doxylstearic acids have turned out to be useful spin probes in micellar systems. These probes have the following structures, $HOOC(CH_2)_{x-2}$–(doxyl)–$(CH_2)_{17-x}CH_3$ where the doxyl group is a five membered ring > $\overline{C\text{-}NO\text{-}C(CH_3)_2\text{-}CH_2O}$, where the position of the doxyl group can be varied along the stearic acid chain and is denoted by x. Figure 1 shows the type of ESEM data that are obtained in micellar systems by using a 5-doxylstearic acid probe in tetramethylammonium dodecylsulfate (TMADS) micelles. In the protiated system, only shallow proton modulation is observed but when the tetramethylammonium chloride counterion is deuterated (TMADS-d_{12}) deeper modulation at the frequency characteristic of deuterium interactions is clearly observed. The normalized deuterium modulation depth is defined as a/b as shown in Fig. 1. Figure 2 shows the normalized modulation depth in the TMADS-d_{12} system,

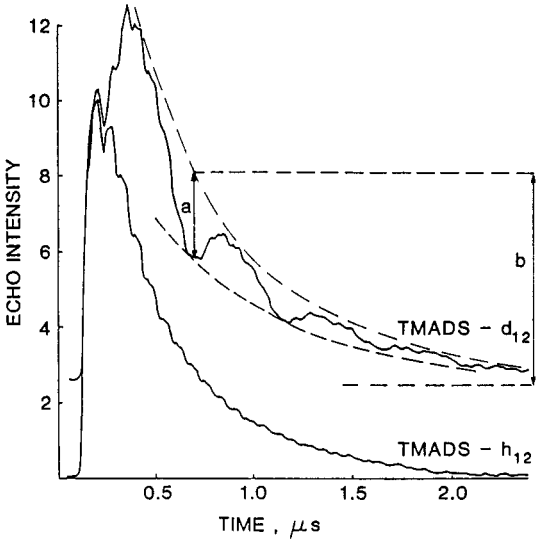

Fig. 1. Two pulse electron spin echo decay envelopes at 4.2 K of 5-doxylstearic acid probe in TMADS micellar solutions. The baselines have been offset vertically to avoid overlap. The normalized modulation depth is a/b. From ref. 16.

where the tetramethylammonium group has been deuterated, as a function of the doxyl position x (ref. 16). As x increases the doxyl group is moved further from the polar headgroup of the spin probe and would be expected to correspond to a decrease in the modulation depth for a counterion located at the surface of the micelle. This is indeed observed from x = 5 to

10 but at higher x values the modulation depth increases again. This indicates that the doxyl probe bends near x = 10 to 12 on the average and that its tail can probe the micellar surface. This implies that the doxyl probe has some gauche links and is roughly U-shaped in some cases. Very similar results are obtained for TMADS in D_2O which indicates that the deuterated water is localized at the micellar interface and does not penetrate significantly into the micelle.

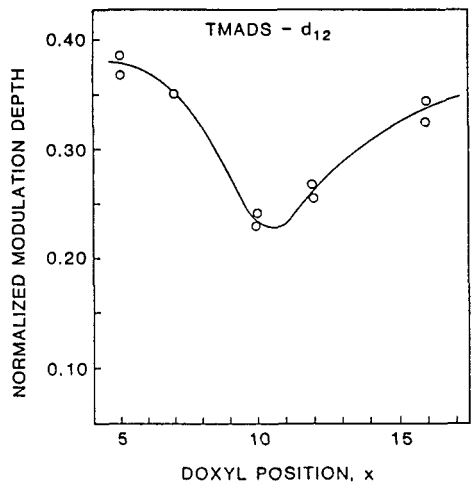

Fig. 2. The dependence of the normalized deuterium modulation depth on the doxyl group position in stearic acid spin probes for TMADS-d_{12} micellar solution. From ref. 16.

By specifically deuterating the terminal methyl group of the dodecylsulfate alkyl chain and using a series of x-doxylstearic acid probes it is possible to determine information about the average conformation of the surfactant chains of the surfactant within the micellar structure (refs. 17,18). It is found that the counterion has a strong effect on the micellar interface and internal structure. Comparison of sodium dodecylsulfate micelles with lithium dodecylsulfate micelles shows that the surfactant molecules are much more extended in the sodium dodecylsulfate micelles. Also water penetrates more significantly into the interface region in lithium but not in sodium dodecylsulfate micelles. The TMADS micelles behave very similarly to the lithium dodecylsulfate micelles and have a quite distinctly different interface and internal structure than the sodium dodecylsulfate.

The effects of added cosurfactants such as alcohols with varying alkyl chain lengths (refs. 6,19) and of crown ethers to complex the counterion associated with micelles (refs. 7,8) can also be studied by ESEM and nitroxide probe methods to explore how these factors influence the interface structure of such organized molecular assemblies.

NONIONIC MICELLES

An important class of nonionic micelles is composed of oligo(oxyethylene) alkyl surfactants which are more commonly called polyethylene oxide surfactants. These are composed of an alkyl chain typically 8 - 12 carbons in length attached to a more polar ethylene oxide chain typically 6 - 8 carbons in length. Thus the polar interface region is quite extensive if one considers it to include all of the ethylene oxide linkages. The nitroxide probe method combined with ESEM can be used to explore the extent of water penetration into this ethylene oxide region as well as to reveal other aspects of the interface and internal structure of nonionic micelles.

The structure of nonionic micelles was explored by localizing deuterium in three different regions within the micellar structure (ref. 20). One region has deuterium localized in the alkyl core region by deuterating the alkyl chain. The second region has deuterium in the ethylene oxide region by deuterating the carbons in the ethylene oxide chain and the final region is the external interface which is "labeled" with deuterated water. Figure 3 shows the echo intensity versus interpulse time for 5-doxylstearic acid probes in nonionic micelles composed of hexakis (ethyleneglycol)monododecyl ether ($C_{12}E_6$) in which

deuterium is localized in water, in the ethylene oxide chain ($C_{12}D_6$) and in the alkyl chain [$(CD)_{12}E_6$]

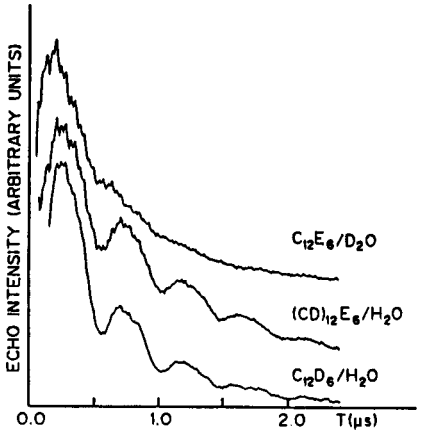

Fig. 3. Two-pulse electron spin echo decay envelopes at 4.2 K for 5-doxylstearic acid in $C_{12}D_6/H_2O$, $(CD)_{12}E_6/H_2O$, and $C_{12}E_6/D_2O$. The spectral base lines have been offset vertically to avoid overlap. From ref. 20.

It can be readily seen that the degree of deuterium modulation is quite different for deuterium localized in these three different positions. It is most obvious that the deuterium modulation is quite weak when the deuterium is located in the water which indicates that the water does not penetrate very significantly even into the ethylene oxide region of the nonionic micelle. Figure 4 shows the normalized deuterium modulation depth as a function of the x-doxyl position for deuterium located in the ethylene oxide region and for deuterium located in the alkyl region of the surfactant molecules. The shapes of these curves

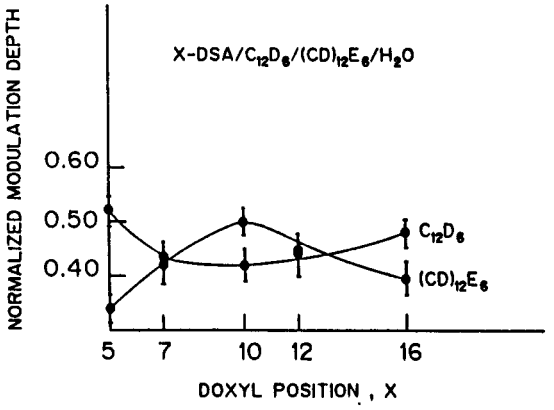

Fig. 4. Dependence of the normalized deuterium modulation depth with the position of the doxyl group, x, in x-doxylstearic acid spin probes in $(CD)_{12}E_6/H_2O$ and $C_{12}D_6/H_2O$ micelles. From ref. 20.

indicate that the x-doxylstearic acids are bent in these nonionic micelles as they are in the ionic dodecylsulfate micelles with lithium and tetramethylammonium as counterions. However the bending is not nearly as dramatic as in the ionic micelles. Secondly, it is clear that the x-doxylstearic acids "see" deuterium in both the alkyl and ethylene oxide regions. Model simulations of the deuterium modulation show that little if any modulation would be observed for distances between the unpaired electron on nitroxide and the deuterium of greater than 0.6 nanometers so this gives some distance scale for the regions that are being investigated. The symmetrically inverse shapes in Figure 4 for the deuterated alkyl versus the deuterated ethylene oxide curves are also consistent with the different deuterated portions of the surfactant molecules being separated as expected from small angle neutron scattering experiments (refs. 21 - 23).

An analysis of the deuterium modulation depth with 7-doxylstearic acid in $C_{12}D_6$ and $(CD)_{12}E_6$ allows determination of the average probe location in the micelle. The deuterium modulation is slightly greater for the probe in $C_{12}D_6$ than in $(CD)_{12}E_6$. This indicates that the probe is located in the ethylene oxide region. Such there is evidence that the surfactant alkyl chains do not penetrate into the ethylene oxide region (refs. 21-23), the values

of the deuterium modulation found for 7-doxylstearic acid in $(CD)_{12}E_6$ and $C_{12}D_6$ means that the probe sees a slightly greater number of deuteriums in $C_{12}D_6$ than in $(CD)_{12}E_6$ micelles. This implies that the nitroxide is located in the ethylene oxide region very near to the border between the alkyl core and the ethylene oxide region. However, these two regions have a different deuterium density, with an average deuterium volume of about 0.014 nm^3 in the alkyl core and 0.017 nm^3 in the ethylene oxide region (refs. 22,23). It follows from simple geometry that to obtain the same deuterium modulation from the core and the outer shell a probe must be located about 0.1 nm into the ethylene oxide region from the alkyl core. Since the 7-doxyl probe experiences slightly higher deuterium modulation for $C_{12}D_6$ with respect to $(CD)_{12}E_6$ micelles we can conclude that the 7-doxyl probe is located about 0.1 - 0.2 nm from the alkyl core into the ethylene oxide region. With similar reasoning together with the geometry of the stearic acid chain a schematic drawing of a cross section of a $C_{12}E_6$ micelle showing a probable conformation and location of an x-doxylstearic acid molecule is shown in Figure 5.

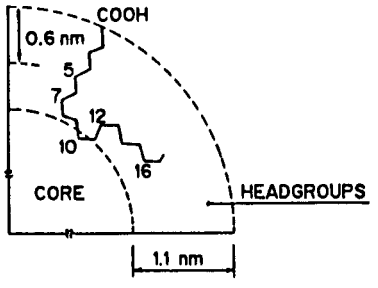

Fig. 5. Schematic drawing of a cross section of a $C_{12}E_6$ micelle showing a probable conformation of an x-doxylstearic acid molecule and its average location within the micelle. The doxyl group is not shown, and its position along the stearic acid chain is indicated by the corresponding number. From ref. 20.

VESICLES

Similar nitroxide probe experiments combined with ESEM have been carried out with vesicles (ref. 24). Phospholipid and dioctadecyldimethylammonium chloride (DODAC) vesicles have been investigated. In dipalmitoylphosphatidylcholine (DPPC) vesicles the doxylstearic acids have an extended probe conformation similar to what is seen in sodium dodecylsulfate micelles. The effect of added cholesterol to DPPC vesicles increases the hydration of the interface (ref. 19). This is shown in Figure 6 where the deuterium modulation depth of the 5-doxylstearic acid probe increases significantly with the mole ratio of cholesterol. In cationic DODAC vesicles the normalized deuterium modulation depth is greater than in DPPC vesicles indicating greater interface hydration than in zwitteronic DPPC vesicles. Also the x-doxylstearic acid probes show a deuterium modulation depth trend as in Figure 2 indicating that they are bent in DODAC in contrast to DPPC vesicles. As stated above, added cholesterol to DPPC vesicles increases their interface hydration. With 20 mole percent cholesterol the interface hydration of DPPC vesicles is similar to that of DODAC vesicles. However this seems

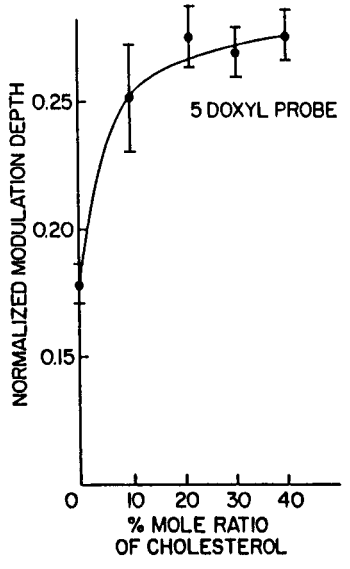

Fig. 6. Dependence of the normalized deuterium modulation depth on the molar ratio of cholesterol incorporated into DPPC vesicles for 5-doxylstearic acid spin probe. From ref. 24.

to be primarily an interface effect and not an internal effect on the vesicle because the doxylstearic acid probes still show no bending in DPPC vesicles as they do in DODAC vesicles. This behavior is a contrast to what is observed in micellar systems.

The experiments summarized here show the ESEM spectroscopy coupled with nitroxide probes can effectively probe the internal and interface structure of surfactant assemblies like micelles and vesicles. This experimental approach is particularly sensitive to changes in the degree of water interaction or penetration into surfactant assembly interfaces.

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