Biophysical Journal Volume 68 May 1995 1960–1965

Membrane Hydration and Structure on a Subnanometer Scale as Seen by High Resolution Solid State Nuclear Magnetic Resonance: POPC and POPC/$C_{12}EO_4$ Model Membranes

Frank Volke and André Pampel
University of Leipzig, Faculty of Physics and Geosciences, Physics of Biomembranes, D-04103 Leipzig, Germany

ABSTRACT The position on a subnanometer scale and the dynamics of structurally important water in model membranes was determined using a combination of proton magic-angle spinning NMR (MAS) with two- dimensional NOESY NMR techniques. Here, we report studies on phosphocholine lipid bilayers that were then modified by the addition of a nonionic surfactant that is shown to dehydrate the lipid. These studies are supplemented by ^{13}C magic-angle spinning NMR investigations to get information on the dynamics of segmental motions of the membrane molecules. It can be shown that the hydrophilic chain of the surfactant is positioned at least partially within the hydrophobic core of the lipid bilayer. With the above NMR approach, we are able to establish molecular contacts between water and the lipid headgroup as well as with certain groups of the hydrocarbon chains and the glycerol backbone. This is possible because high resolution proton and ^{13}C-NMR spectra of multilamellar bilayer membranes are obtained using MAS. A phase-sensitive NOESY must also be applied to distinguish positive and negative cross-peaks in the two-dimensional plot. These studies have high potential to investigate membrane proteins hydration and structural organization in a natural lipid bilayer surrounding.

INTRODUCTION

Water is essential for the structure and dynamics of biological and model membranes formed, e.g., by phospholipids and is capable of influencing function (Crowe and Crowe, 1984). There is an increasing research effort to characterize those properties of the membrane surface that establish hydration (e.g., Gawrisch et al., 1992; Israelachvili and Wennerström, 1992; McIntosh and Simon, 1993; Volke et al. 1994a, b). There is no doubt that surfaces like lipid bilayer surfaces influence the physical properties of nearby water molecules, which differ from those of bulk water (Woessner, 1980). Progressive hydration of bilayer-forming phospholipids allows greater motional freedom of the lipid molecules in the polar headgroups (Bechinger and Seelig, 1991; Ulrich and Watts, 1994) as well as in the hydrophobic fatty acid chains (Volke et al. 1982) and, therefore, changes occur on both structural and dynamic properties of the lipids as well as of the interacting water (Volke et al., 1994a). A large quantity of data on structural and dynamic properties of water interacting with membranes were obtained using NMR techniques. Nevertheless, there is a need to observe the position of structurally important water on an atomic scale level and changes of this position due to the interaction of the lipids with biological active molecules like membrane proteins, pharmaca, and, e.g., amphiphilic surfactants that are used for membrane protein crystallization and reconstitution (Moller et al., 1986).

Received for publication 6 December 1994 and in final form 9 February 1995.

Address reprint requests to Dr. Frank Volke, University of Leipzig, Physics, Physics of Biomembranes, Linnestr. 5, D-04103 Leipzig, Germany. Tel.: 49-341-973-2478; Fax: 49-341-973-2497; E-mail: fvolke@mp.physik.uni-leipzig.d400.de.

0006-3495/95/05/1960/06 $2.00

Recently, the power of sophisticated NMR techniques was used to study the position and kinetic of bound water of proteins in solution (Otting et al., 1991). To apply techniques of high resolution proton NMR to membranes is difficult because the lipids form extended bilayers where residual static dipolar interactions between different proton pairs dominate the NMR lineshape up to several kHz (Bloom et al., 1977). Nevertheless, the NMR technique of magic-angle spinning at high magnetic fields was shown recently to yield ^1H-NMR spectra where the isotropic chemical shifts of all proton groups in the polar headgroup and the glycerol backbone of phosphocholines are well resolved (Forbes et al., 1988; Halladay et al., 1990). In the case of a bilayer-forming lipid POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine, Avanti Polar Lipids, Birmingham, AL), which is used in this study, further proton groups close to the chain's double bond are resolved as well (see Fig. 1). A sufficient spinning rate must be selected to remove spinning sidebands from the observed ^1H-NMR spectral region. It should be noted that for certain bilayer-forming molecules like the nonionic surfactant dodecyl-tetraethyleneoxide-ether ($C_{12}EO_4$, Nikko, Japan) even the J-coupling between adjacent proton groups is resolved by MAS (Volke and Pampel, 1995), which gives additional means for structural analysis. Having obtained high resolution ^1H-NMR spectra of the membrane, the homonuclear two-dimensional NOE spectroscopy (NOESY) is used to establish molecular connectivity between communicating proton groups by observing the cross-peaks (Otting et al., 1991; Bodenhausen et al., 1987). NOESY spectroscopy was done recently using ultrasonicated lipid vesicles (Xu and Cafiso, 1986). The combination of MAS and NOESY on lipids to establish molecular contacts in lipid model membranes was shown by Forbes et al. (1988) and Halladay et al. (1990). In this article, we focus special attention to the communication (dipolar

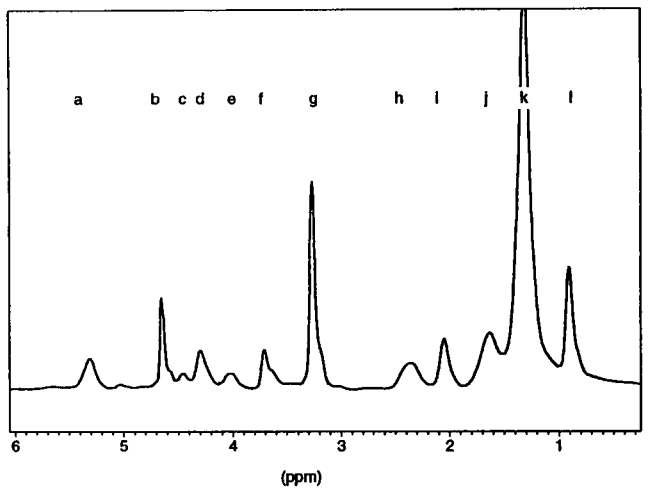

FIGURE 1 One-dimensional proton NMR MAS spectrum of a lamellar, liquid crystalline POPC water dispersion (9 mole water/mole lipid) measured at 303 K. The spinning rate was 4000 Hz, proton frequency 300 MHz (BRUKER MSL 300, Bruker, Karlsruhe, Germany), 32 accumulations. Resonances assignment as indicated in the top trace (compare with Table 1).

cross-relaxation) of water with potential hydration sites of POPC. Then the nonionic surfactant $C_{12}EO_4$ (Mitchell et al., 1983; Mädler et al., 1994) was intercalated into the POPC matrix, which competes for the hydration water of the lipid. The cross-peaks of the ethyleneoxide units with proton groups of POPC and water are of intermolecular origin and allow (i) characterization of the position of $C_{12}EO_4$ within the lipid matrix and (ii) observation of the changes of water position in comparison with the pure lipid matrix. The implications of the above approach for the study of other biological relevant systems like membrane proteins are discussed.

MATERIALS AND METHODS

POPC, chain-perdeuterated DMPC (dipalmitoylphosphocholine), and headgroup-deuterated DMPC-d13 were purchased from Avanti Polar Lipids, and $C_{12}EO_4$ was from Nikko. 9 mole water/mole POPC was added to the dried lipid, and the sample was homogenized as described in Volke et al. (1994a). 80 vol% of the water was deuterated to allow for i) good shimming of the magnetic field and ii) frequency stabilization during the long run (about 10 h) of a two-dimensional NMR experiment. POPC and $C_{12}EO_4$ were co-dissolved in deuterated methanol, dried, and water was added as for the pure POPC sample. The lamellar structure of the dispersions was checked using ^{31}P-NMR (e.g., Cullis et al., 1976). The samples were transferred into air-tight Teflon inserts (Bruker, Karlsruhe, Germany) for MAS-ZrO_2 rotors (7-mm diameter; Cerobear, Wemhöner and Popp, Herzogenrath, Germany). This allows for safe spinning at high rotor frequencies. All measurements were done using a Bruker MSL 300 NMR spectrometer (Bruker, Karlsruhe, Germany) operating at 300 MHz for protons. The NMR frequency was locked on deuterium during the MAS-NOESY experiments. To study the segmental mobility of the lipid and surfactant, ^{13}C-MAS NMR was done using a Bruker MSL 500 spectrometer and high power broad band decoupling. The rotation frequency was 4000 Hz all samples and stabilized to within 1 Hz. A standard three-pulse NOESY, but in a phase-sensitive mode, was used (TPPI, 16-phase cycle (Bodenhausen et al., 1984). We used 256 time increments in the t1 time domain, zero-filled to 2k and 2k data points for t2. 16 accumulations were collected for each time increment. Mixing times were selected between 50 and 500 ms to follow the cross-peak intensity as a function of "communication" time. A $\cos^2$ appodization function was applied, along with an automatic baseline correction. We have symmetrized the two-dimensional result after checking cross-peaks in the nonsymmetrized plot, which is important to detect real cross-peaks.

RESULTS AND DISCUSSION

In Fig. 1, a one-dimensional proton MAS spectrum is presented for POPC. The resolution is slightly better than in ultrasonicated lipid-water dispersions (Xu and Cafiso, 1986), and in the multilamellar liposome proton MAS spectra of DMPC published by Forbes et al. (1988). The latter should be due mainly to the unsaturation of the oleoyl-chain, which gives i) additional resolved resonances and ii) makes the hydrophobic core more fluid, which may reduce the individual linewidth of proton resonances. The resonance assignment is given in Table 1 together with the additional resolved resonances if $C_{12}EO_4$ is added to the POPC matrix.

The NOEs that give rise to cross-peaks are due to time-dependent dipolar interactions of adjacent protons. It is usually accepted that NOEs can be observed for proton distances <0.4 nm because of their r_{ij}^{-6} dependence (r is distance between protons i and j). Furthermore, they are related to a correlation function that describes the motion of the r_{ij}-vector (Otting et al., 1991).

Fig. 2 shows the $\omega 1$-$\omega 2$ cross-plane through a homonuclear ^{1}H-NOESY of a lamellar, liquid-crystalline POPC water dispersion with 9 mole water/mole POPC for an MAS experiment with 4000-Hz spinning rate, and a cross section through the two-dimensional NOESY showing the *negative* cross-peaks between water and essentially all proton groups of the polar headgroup, the glycerol backbone, and fatty acid chain protons close to the carbonyl group of POPC and the methylene groups of the chains (the complete two-dimensional plot is shown in Fig. 2 *B*, a section without t1 noise in Fig. 2 *A*). Fig. 3 shows the two-dimensional NOESY cross-plane of the POPC/$C_{12}EO_4$ water dispersion in the lamellar, liquid-crystalline water dispersion. No *negative*

TABLE 1 Assignment of proton resonances of POPC and $C_{12}EO_4$ obtained from ^{1}H-NMR-MAS measurements

Peak	Chemical shift (ppm from TMS)	Group assignment
POPC		
a	5.3	—CH=CH and CH
b	4.7	HDO
c	4.5	Glyc.-CH_2
d	4.3	α-CH_2
e	4.0	Glyc.-CH_2
f	3.7	β-CH_2
g	3.3	$N(CH_3)_3$
h	2.4	$CH_2\underline{CH_2}COO$
i	2.1	$\underline{CH_2}CH{=}CH\underline{CH_2}$
j	1.6	$\underline{CH_2}CH_2COO$
k	1.3	$(\underline{CH_2})_n$
l	0.9	CH_3
$C_{12}EO_4$		
	3.4	$(CH_2)\underline{CH_2}CO$
	3.7	$—(O\underline{CH_2}CH_2)_4$

Compare with Fig. 1.

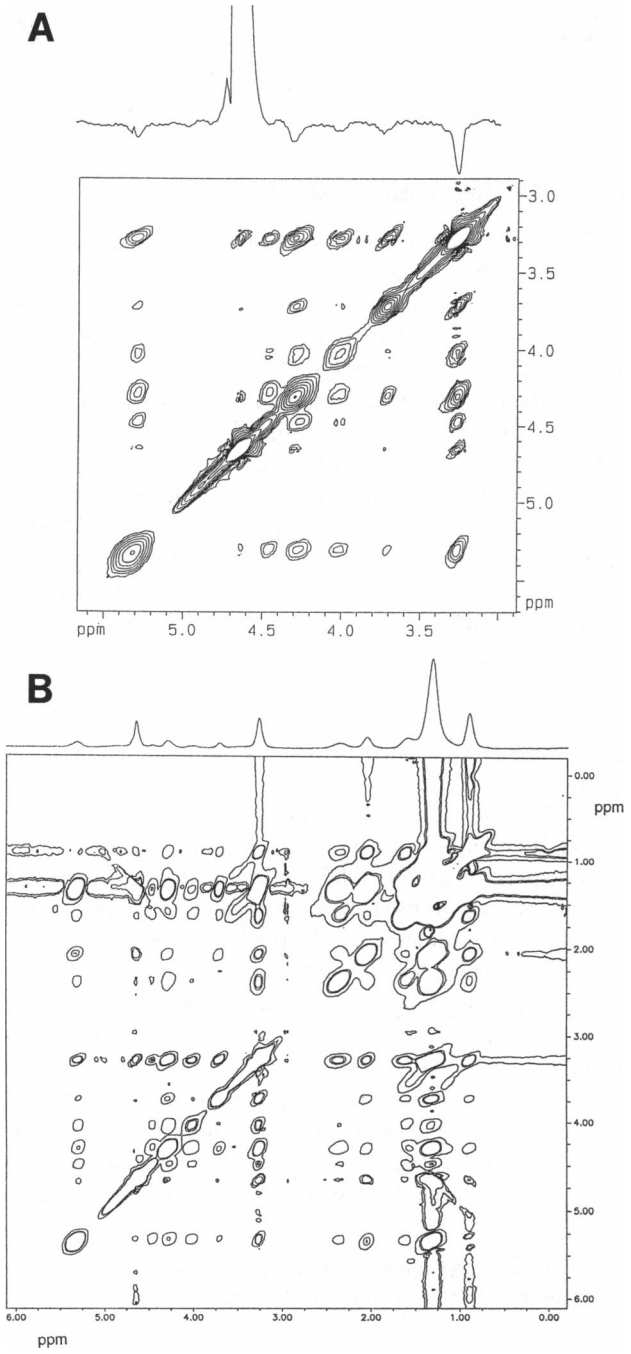

FIGURE 2 Two dimensional, expanded plot (*A*) of the system shown in Fig. 1 using a 16- phase cycle NOESY with 256 time increments (spectral width 2500 Hz). Mixing time was 400 ms, 32 accumulations per time increment, phase-sensitive recording. A 1024x1024 data matrix was two-dimensional Fourier-transformed by zero filling 256 data points of the $\omega 1$-domain. The spinning rate was 4000 Hz, and the temperature of the sample was 303 K. Negative cross-peaks of water with POPC segments are shown in the top trace, which is a cross section through the $\omega 1$-resonance of the water peak. The region of the hydrocarbon chain methylene-groups and methyl-group is not shown here because of t1-noise of the strong signals. Nevertheless, a cross-peak between water and these groups may indicate a substantial amount of water in the chain region, as seen in the complete plot in *B*, with a $\omega 2$-projection in the top trace. Water cross-peaks were observed with the lipid chain region but without their sign (Forbes et al., 1988; Xu and Cafiso, 1986).

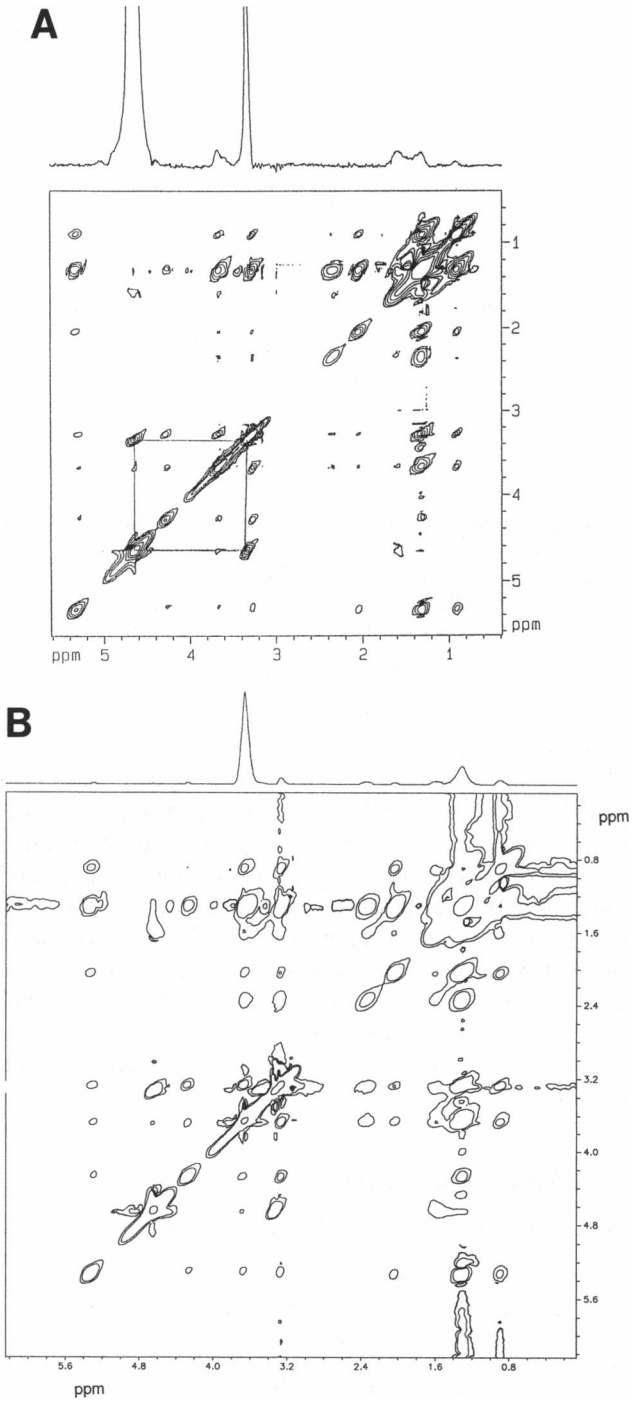

FIGURE 3 (*A*) Two-dimensional plot of POPC/$C_{12}EO_4$ (2:1). Experimental conditions as given in Fig. 2. The ethyleneoxide resonances are at 3.7 and 3.4 ppm for the ethyleneoxide and $(CH_2)CH_2O$ group of the surfactant, respectively. Water (4.7 ppm) cross-peaks are shown in the top trace. (*B*) Same as *A*, but with a different selection of intensity levels. The top trace shows the cross-peaks of the ethylene moiety with different resonances of the lipid.

cross-peaks could be detected between the hydration water site and any peak of the membrane matrix. The water concentration with respect to the lipid was the same in both systems. To understand the *negative* cross-peaks between water and some lipid segments, we have to discuss the cross-

relaxation rate σ^{NOE}, which is positive. For protein hydration in aqueous solution, Otting et al. (1991) have proposed recently three models to calculate the dipolar cross-relaxation rates between different polypeptide protons and water. One of their models involves independent translation and rotation of the protein and the water molecule and yields positive σ^{NOE} values with the assumption of short residence times of the hydration water. The diffusion coefficient of the water was estimated; the residence times of the hydration water molecules were found to be shorter than 500 ps, a value shorter than the lifetime of a proton in water. It was concluded that complete water molecules exchange rapidly. Their model fits (compare Fig. 2 of Otting et al., 1991) excellently with our data for hydrated *lipid membranes* and is supported by our ^{2}H-NMR results of deuterated water published recently (Volke et al., 1994a). In that work, the diffusion coefficient of water at low hydration was measured with pulsed field gradient NMR to be about 2×10^{-10} m^2/s. On the other hand, using spin-lattice relaxation measurements on progressively hydrated POPC, the lifetime of water at a hydration site was determined to be about 100 ps. These values yield positive σ^{NOE} and, therefore, negative cross-peaks as observed in Fig. 2 *A*. In a first approximation, the model of protein hydration is working well for membrane hydration, which opens a new way to study hydration properties of membranes on an atomic level. It is important to do the NOESY experiment in a phase-sensitive way to differentiate positive and negative cross-peak intensities. In a stimulating recent paper, cross-peaks between water and the $N(CH_3)_3$ group of dimyristoylphosphocholine and the hydrocarbon chain protons were reported, but without giving the sign of the intensities (Forbes et al., 1988). Therefore, water molecules are in spatial proximity (<0.4 nm) to all segments of the lipid headgroup, the glycerol backbone, and even some protons of the hydrocarbon chain.

With $C_{12}EO_4$ inserted into hydrated POPC bilayers, *only positive* cross-peaks are observed (Fig. 3 *A*). Intermolecular cross-peaks between $C_{12}EO_4$ and POPC are detected between the ethyleneoxide protons and the $(CH_2)CH_2COO$ groups of POPC, with lipid methylene groups close to the double bond. A relatively strong cross-peak of the ethyleneoxide moiety and the methylene resonance is seen. Further relatively strong cross-peaks are observed between the ethyleneoxide groups and the $N(CH_3)_3$ group, the α-group, and the glycerol —CH group of the lipid.

The water peak of the $C_{12}EO_4$/POPC system has only positive cross-peaks with the ethyleneoxide moiety and a strong interaction with the $(CH_2)_n$ CH_2O group of the hydrocarbon chain of the surfactant (Fig. 3 *B*). No magnetic dipolar communication of water with the lipid headgroup could be detected in this case. These results imply that the ethyleneoxide chain is positioned with a part of the ethyleneoxide groups, and the hydrocarbon chain within the hydrophobic part of the lipid bilayer and takes up the water of hydration, especially at the ether bond. The lifetime of this water must be an order of magnitude larger than for water in pure POPC systems to explain the positive cross-peaks. The surfactant seems to dehydrate the lipid. There are several results that support this model. (i) In a one-dimensional ^{13}C-MAS experiment of stepwise-hydrated POPC, the isotropic chemical shift of the POPC carbonyl group, which is a hydration site of the lipid, was measured as a function of hydration. The progressive hydration leads to a downfield shift of this resonance as expected (Fig. 4), up to a certain limit of hydration of about 12–14 water molecules per lipid. Adding the surfactant at a comparable hydration level, the carbonyl group resonance is shifted to the value of dehydrated POPC. (ii) The ^{13}C spin lattice relaxation times (T_1) of headgroup segments were selectively measured with MAS for different lipid hydration. With the surfactant intercalated into the hydrated POPC bilayer, T_1 values of these segments decrease remarkably, reaching values measured for low POPC hydration numbers (see Table 2). One may speculate that the terminal OH group of the surfactant is close to the positively charged $N(CH_3)_3$ group of the lipid so that there is no space for water. There is no water —$N(CH_3)_3$ cross-peak in the POPC/$C_{12}EO_4$ system (Fig. 3 *B*). Because the positive water cross-peaks observed in this system indicate a reduced water mobility (Fig. 2 of Otting et al., 1991) as compared with the pure lipid system (negative peaks there), we do not expect that a particular correlation time of the water is responsible for zero cross-peak intensity. This would lead to roughly two types of water species, one with a longer effective correlation time, where we see the positive cross-peaks, and one with a short correlation time with almost zero cross-relaxation rate (Otting et al., 1991). In the unlikely case of a particular correlation time of about 5×10^{-10} s, where the cross-peaks change from positive to negative sign according to the Otting et al. model (Otting et al., 1991), there also would be zero cross-peak intensity. Nevertheless, we expect that the water with the longest correlation time is that which is associated with the lipid/surfactant bilayer (Volke et al., 1994a) and, therefore, in close distance to the matrix molecules.

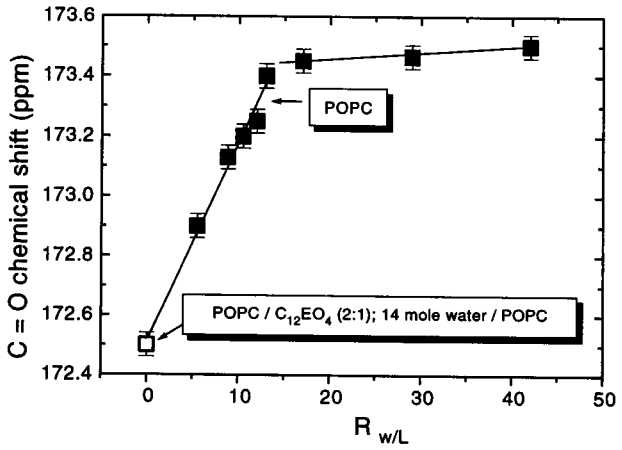

FIGURE 4 Isotropic chemical shift of the carbonyl groups of POPC water dispersions measured as a function of water concentration ($R_{w/l}$: mole water/mole lipid) as obtained from a ^{13}C-NMR MAS experiment (^{13}C-NMR frequency: 125.76 MHz, BRUKER, MSL 500). The open square indicates the chemical shift of the carbonyl group for a POPC/$C_{12}EO_4$/water dispersion (14 mole water/mole lipid). Temperature: 298 K.

TABLE 2 ^{13}C-MAS-NMR spin-lattice relaxation time T_1

T_1 (ms)	α-CH_2	β-CH_2	$N(CH_3)_3$
20 mol water/mol POPC	314	308	487
5 mol water/mol POPC	215	191	220
POPC/$C_{12}EO_4$ (2:1); 15 mol water/mol POPC	235	155	183

See text.

Summarizing, the above NMR approach indicates the positional vicinity of water protons to all protons of the polar headgroup of POPC as well as to the glycerol backbone, the carbonyl group, and the beginning of the hydrocarbon chains. The lifetime of such water at a hydration site is on the order of 100 ps, and there is a fast exchange of water molecules between the sites as stated recently (Volke et al., 1994a).

The presence of the nonionic surfactant $C_{12}EO_4$ dehydrates the POPC hydration sites and produces a slowdown of segmental mobility (rate of motion) within the lipids headgroup, possibly because of close contact of the terminal ethyleneoxide-OH group with the $N(CH_3)_3$ group. Only the ethyleneoxide groups and the methylene group adjacent to the ether bond have positive cross-peaks with water. The lifetime of this water must be longer than or on the order of 10 ns. On the other hand, it could be shown that there is a proximity of the ethyleneoxide protons to protons of the hydrocarbon chains closed to the glycerol backbone of the lipid down to the chain's double bond.

There is a large quantity of structural information in the positive cross-peaks of the lipid headgroup segments with the glycerol backbone and the hydrocarbon chain, as well as in the cross-peaks of the surfactant with the lipid. Particularly interesting is the spatial proximity of some chain protons to protons of the headgroup in the lipid water systems, and this was observed previously (Xu and Cafiso, 1986; Forbes et al., 1988). Therefore, we are currently testing and developing software (Molecular Simulations, Cambridge, U.K.; Biosym, San Diego, CA) to get three-dimensional images of lipids that fit the NOE distance constrains (Wüthrich, 1986). The aim is to analyze data sets calculated by molecular dynamics simulations of hydrated lipid bilayers.

Nevertheless, there is still a strong need to get detailed understanding how spin diffusion influences the cross-peak intensities and the cross-peak built-up curves as a function of mixing time (Xu and Cafiso, 1986; Halladay et al., 1990).

We can exclude intramolecular spin diffusion between the headgroup and the terminal CH_3 groups of the fatty acid chains. Hydrated $C_{12}EO_4$ itself forms lamellar liquid crystalline bilayer phases over a wide concentration and temperature range (Mitchell et al., 1983). In a proton MAS-NOESY experiment, we could show that no cross-peaks are formed between the hydrated ethyleneoxide chain and the hydrocarbon chain of the surfactant (Volke and Pampel, 1995). Furthermore, using an equimolar mixture of headgroup- and chain-deuterated DMPC in ^2H_2O in the L_α-phase, it can be seen that the positive cross-peaks of the protonated chain lipid methylene and methyl groups with the

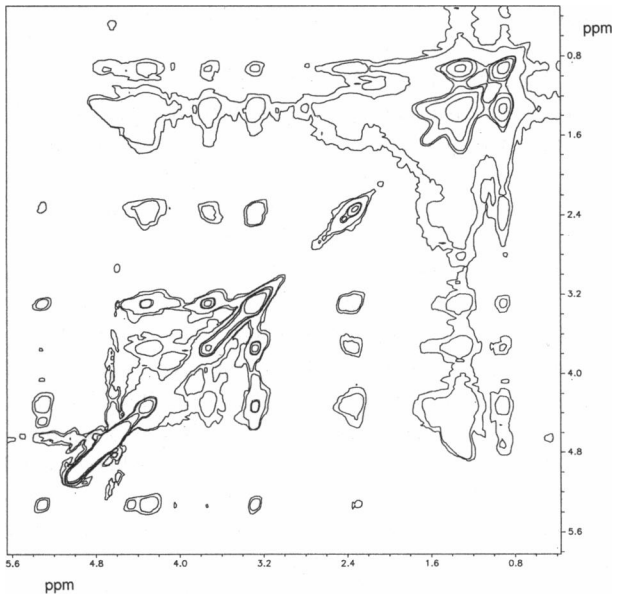

FIGURE 5 Proton NMR NOESY of an equimolar mixture of chain perdeuterated DMPC and headgroup-deuterated DMPC-d13 in the liquid-crystalline L_α phase state hydrated with pure ^2H_2O. Temperature: 303 K.

$N(CH_3)_3$ group of the perdeuterated chain lipid observed in a MAS proton NOESY experiment are mainly of intermolecular origin (Fig. 5). In a proton spin echo experiment, we have shown previously (Volke et al., 1982) that there is no spin diffusion between the headgroup proton reservoir and the chain protons in DPPC.

In general, it is important to monitor such hydration/dehydration processes to understand the action of biological active molecules in membranes. One may get a better knowledge of why such nonionic surfactants are suitable for membrane protein crystallization (dehydration). The combination of MAS and cross-relaxation NOESY spectroscopy turned out to be a powerful tool for investigating hydration and structural properties of multicomponent membrane systems. In the light of increasing interest in studying physical properties of membrane proteins, the above approach is promising to get detailed insight into hydration properties and molecular organization of such systems where the function may be triggered through local hydration/dehydration. Such studies using selected polypeptides are in progress in our laboratory.

This work was supported by the Deutsche Forschungsgemeinschaft (Vo 526/1-1 and SFB 294).

REFERENCES

Bechinger, B., and J. Seelig. 1991. Conformational change of the phosphatidylcholine head group due to membrane dehydration. *Chem. Phys. Lipids.* 58:1–5.

Bloom, M., E. E. Burnell, S. B. W. Roeder, and M. Valic. 1977. Nuclear magnetic resonance line shapes in lyotropic liquid crystals and related systems. *J. Chem. Phys.* 66:3012–3021.

Bodenhausen, G., H. Kogler, and R. R. Ernst. 1984. Selection of coherence-transfer pathways in NMR pulse experiments. *J. Magn. Reson.* 58:370–388.

Crowe, J. H., and L. M. Crowe. 1984. Biological Membranes, Vol. 5. D. Chapman, editor. Academic Press, London. 58–103.

Cullis, P. R., B. De Kruyff, and R. E. Richards. 1976. Factors affecting the motion of the polar headgroup in phospholipid bilayers. A ^{31}P NMR study of unsonicated phosphatidylcholine liposomes. *Biochim. Biophys. Acta.* 426:433–446.

Forbes, J., C. Husted, and E. Oldfield. 1988. High-field, high-resolution proton "magic-angle" sample-spinning nuclear magnetic resonance spectroscopic studies of gel and liquid crystalline lipid bilayers and the effects of cholesterol. *J. Am. Chem. Soc.* 110:1059–1065.

Gawrisch, K., J. Ruston, J. Zimmerberg, V. A. Parsegian, R. P. Rand, and N. Ful ler. 1992. Membrane dipole potentials, hydration forces, and the ordering of water at membrane interfaces. *Biophys. J.* 61:1213–1223.

Halladay, H. N., H. E. Stark, S. Ali, and R. Bittman. 1990. Magic-angle spinning NMR studies of molecular organization in multibilayers formed by 1-octadecanoyl-2-decanoyl-*sn*-glycero-3-phosphocholine. *Biophys. J.* 58:1449–1461.

Israelachvili, J. N., and H. Wennerström. 1992. Entropic forces between amphiphilic surfaces in liquids. *J. Chem. Phys.* 96:520–531.

Mädler, B., G. Klose, A. Möps, W. Richter, and C. Tschierske. 1994. Thermotropic phase behaviour of the pseudobinary mixture DPPC/$C_{12}E_4$ at excess water. *Chem. Phys. Lipids.* 71:1–12.

McIntosh, T. J., and S. A. Simon. 1993. Contributions of hydration and steric (entropic) pressures to the interactions between phosphatidylcholine bilayers: experiments with the subgel phase. *Biochemistry.* 32:8374–8384.

Mitchell, D. J., G. J. T. Tiddy, L. Waring, Th. Bostock, and M. P. McDonald. 1983. Phase behaviour of polyoxyethylene surfactants with water. *J. Chem. Soc. Faraday Trans.* 1:975–1000.

Moller, J. V., LeMaire, M., and J. P. Andersen. 1986. Progress in Protein-Lipid-Interaction, Vol. 2. A. Watts and J. J. H. H. M. dePond, editors. Elsevier/North Holland, Amsterdam. 147–196.

Otting, G., E. Liepinsk, and K. Wüthrich. 1991. Protein hydration in aqueous solution. *Science.* 245:974–980.

Ulrich, A. S., and A. Watts. 1994. Molecular response of the lipid headgroup to bilayer hydration monitored by ^2H-NMR. *Biophys. J.* 66:1441–1444.

Volke, F., K. Gawrisch, and K. Arnold. 1982. The effect of hydration on the mobility of phospholipids in the gel state. A proton NMR spin echo study. *Chem. Phys. Lipids.* 31:179–189.

Volke, F., S. Eisenblätter, J. Galle, and G. Klose. 1994a. Dynamic properties of water at phosphatidylcholine lipid bilayer surfaces as seen by deuterium and pulsed field gradient proton NMR. *Chem. Phys. Lipids.* 70: 121–131.

Volke, F., S. Eisenblätter, and G. Klose. 1994b. Hydration force parameters of phosphatidylcholine lipid bilayers as determined from ^2H-NMR studies of deuterated water. *Biophys. J.* 67:1882–1887.

Volke, F., and A. Pampel. 1995. Membrane hydration and structure on an atomic level as seen by two-dimensional NMR in combination with high-speed sample spinning. Biophysical Society Meeting 1995, San Francisco, CA, Tu-Pos444, and *Biophys. J.* 68:214*a*. (Abstr.)

Woessner, D. E. 1980. An NMR investigation into the range of the surface effect on the rotation of water molecules. *J. Magn. Reson.* 39:297–308.

Wüthrich, K. 1986. NMR of proteins and Nucleic Acids. A Wiley-Interscience Publication, John Wiley & Sons, New York.

Xu, Z. Ch., and D. S. Cafiso. 1986. Phospholipid packing and conformation in small vesicles revealed by two-dimensional ^1H nuclear magnetic resonance cross-relaxation spectroscopy. *Biophys. J.* 49:779–783.