

Molecular Ordering of Interfacially Localized Tryptophan Analogs in Ester- and Ether-Lipid Bilayers Studied by ^2H -NMR

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ABSTRACT Perdeuterated indole- d_6 and *N*-methylated indole- d_6 were solubilized in lamellar liquid crystalline phases composed of either 1,2-diacyl-glycero-3-phosphocholine(14:0)/water or 1,2-dialkyl-glycero-3-phosphocholine(14:0)/water. The molecular ordering of the tryptophan analogs was determined from deuteron quadrupole splittings observed in ^2H -NMR spectra on macroscopically aligned lipid bilayers. NMR spectra were recorded with the bilayers oriented perpendicular to or parallel with the external magnetic field, and the values of the splittings differed by a factor of 2 between these distinct orientations, indicating fast rotational motion of the molecules about an axis parallel to the bilayer normal. In all cases the splittings were found to decrease with increasing temperature. Relatively large splittings were observed in all systems, demonstrating that the tryptophans partition into a highly anisotropic environment. Solubilization most likely occurs at the lipid/water interface, as indicated by ^1H -NMR chemical shift studies. The ^2H -NMR spectra obtained for each analog were found to be rather similar in ester and ether lipids, but with smaller splittings in the ether lipid under similar conditions. The difference was slightly less for the indole molecule. Furthermore, in both lipid systems the positions of the splittings from indole were different from those of *N*-methyl indole. The results suggest that 1) the tryptophan analogs are solubilized in the interfacial region of the lipid bilayer, 2) the behavior may be modulated by hydrogen bonding in the case of indole, and 3) hydrogen bonding with the lipid carbonyl groups is not likely to play a major role in the solubilization of single indole molecules in the ester lipid bilayer interface.

INTRODUCTION

Partitioning of aromatic amino acids into lipid bilayers reveals many interesting physicochemical features that may be important for understanding processes like the insertion and integration of proteins in membranes, protein binding, and stabilization of membrane protein structure. The lipid-water interfacial region is a complex environment in which the lipid headgroup dipolar moments and hydrogen bonding sites offer several possibilities for interaction with aromatic side chains.

The aromatic amino acids Phe, Tyr, and Trp are abundant in many membrane-associated proteins (Schiffer et al., 1992). It is found that the tryptophans are preferentially located at the regions where the protein intersects the membrane interface region (Ippolito et al., 1990). It is believed that the aromatic amino acids can assist in stabilizing the protein in the membrane (Durkin et al., 1992; O'Connell et al., 1990; Schiffer et al., 1992) and that they are essential for the proper function of membrane proteins (Becker et al., 1991; Hu and Cross, 1995). It is further proposed that tryptophans are involved in the ability of transmembrane α -helical peptides to induce nonbilayer structures in model membrane systems under conditions of hydrophobic mismatch (Killian et al., 1996; Morein et al., 1997). And for membrane-active water-soluble proteins, tryptophans are

likely to play an important role in membrane interaction. Examples are prostaglandin H_2 synthase-1 (Picot et al., 1994), substance P antagonists (Seelig, 1992), and indolicidin (Ladokhin et al., 1997).

Tryptophan has the largest nonpolar surface of all of the naturally abundant amino acids. However, its side chain is also capable of forming hydrogen bonds through its NH group. This gives rise to the ambivalent behavior of the tryptophan, as its hydrophobicity is ranked very differently on different hydrophobicity scales that are based on the partitioning of single tryptophan molecules into polar and nonpolar solvents (Fauchere and Pliska, 1983; Radzika and Wolfenden, 1988).

Although aromatic molecules like benzene and indole have a relatively high solubility in water, in fact it was observed as early as 30 years ago by NMR spectroscopy that aromatic molecules are solubilized in the interfacial region of amphiphilic aggregates (Eriksson and Gillberg, 1966; Lindblom et al., 1973). More recently, it was also found by other techniques that tryptophan (analogs) partition into the interface of the bilayer (White and Wimley, 1994; Kachel et al., 1995; Wimley and White, 1996; Jacobs and White, 1989). Besides the polarity of the molecule, one can think of several other reasons for this preferred localization, such as favorable dipolar interactions between the aromatic amino acid side chain and the lipid bilayer, and/or hydrogen bonding with the headgroups or carbonyl groups of the lipids or with water molecules in the headgroup region.

We study here the interaction of indole and *N*-methyl indole with lipid bilayers consisting of either 1,2-diacyl-

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glycero-3-phosphocholine(14:0) (DMPC) or 1,2-dialkylglycero-3-phosphocholine(14:0) (DTPC). Indole was used instead of tryptophan because it is uncharged and therefore better resembles the tryptophan side chain in a membrane-associated protein. *N*-Methyl indole was used as a non-hydrogen-bonding analog to indole. The ether-linked lipid analog DTPC was used to remove a possible site for hydrogen bonding. Ether lipids, in addition, have a smaller dipole moment across the interface than ester lipids (Gawrisch et al., 1992), although other physicochemical properties of the two lipid bilayers are the same.

To observe the behavior of the perdeuterated tryptophan analogs, the method of ^2H -NMR spectroscopy was employed on macroscopically aligned lipid bilayers. This method has proved to be sensitive to the molecular ordering in the system, and information thereof is readily obtained from the recorded spectra (see information on NMR spectroscopy in Materials and Methods). In addition, one-dimensional proton NMR chemical shift experiments were conducted on 1-acyl-2-hydroxy-glycero-3-phosphocholine(14:0) (lyso-PC) micellar suspensions with solubilized tryptophan analogs to gain insight into the localization of the tryptophan analogs with respect to the lipid/water interface.

By a systematic comparison of systems that each comprise different combinations of a tryptophan analog and a lipid analog, we present here a qualitative insight into how hydrogen bonding and dipolar interactions influence the molecular ordering of tryptophan analogs in lipid bilayer systems.

MATERIALS AND METHODS

Perdeuterated indole and DTPC were obtained from Larodan Fine Chemicals (Malmö, Sweden). The degree of perdeuteration for the indole was given as 78%. DMPC and lyso-PC were bought from Avanti Polar Lipids (Alabaster, AL). Deuterium-depleted water was obtained from Isotech (Horseham, PA). All chemicals were used without further purification.

N-Methyl indole was synthesized from indole with methyl iodide as the methylating agent, as described by Barco et al. (1976). Perdeuterated (ring- d_6) indole and protonated methyl iodide were used to obtain *N*-methyl indole ring- d_6 , as depicted in Fig. 1. The product was purified with column chromatography, and the purity was checked by thin-layer chromatography and one-dimensional ^1H -NMR on a Bruker AMX2-500 spectrometer.

Sample preparation

Macroscopically aligned lipid bilayer samples were prepared as follows. Indole and the lipid were dissolved in chloroform/methanol, and the solution was distributed on glass plates under a gentle flow of gaseous nitrogen. The plates were stored overnight under vacuum and then transferred to cuvettes. Deuterium-depleted water was added, and the cuvettes were sealed with glue and allowed to equilibrate for several days at 37°C. Because of its higher volatility, a slightly modified scheme was used for the samples containing methyl indole: the methyl indole and lipid were codissolved in chloroform only, which was removed from the sample under a flow of nitrogen. These samples were not stored under vacuum.

Micellar systems were prepared by mixing appropriate amounts of stock solutions of lyso-PC and of the tryptophan analogs in D_2O . The final amount of lyso-PC was equal to 0.3% (w/w), and the molar ratio of analog to lyso-PC was 1:4.

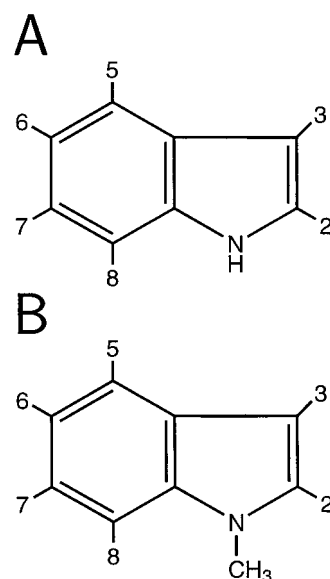


FIGURE 1 (A) Structure of the perdeuterated indole ring- d_6 . The molecule is deuterated in the positions labeled with arabic numerals. The permanent dipole moment of the molecule is ~ 1.9 D; it is roughly parallel to the NH bond and is directed toward the hydrogen. (B) Structure of the perdeuterated *N*-methyl indole ring- d_6 . It has a permanent dipole moment of 2.1 D in a direction similar to that in A. Note that the nitrogen position in A and the methyl group in B are not deuterated.

NMR spectroscopy

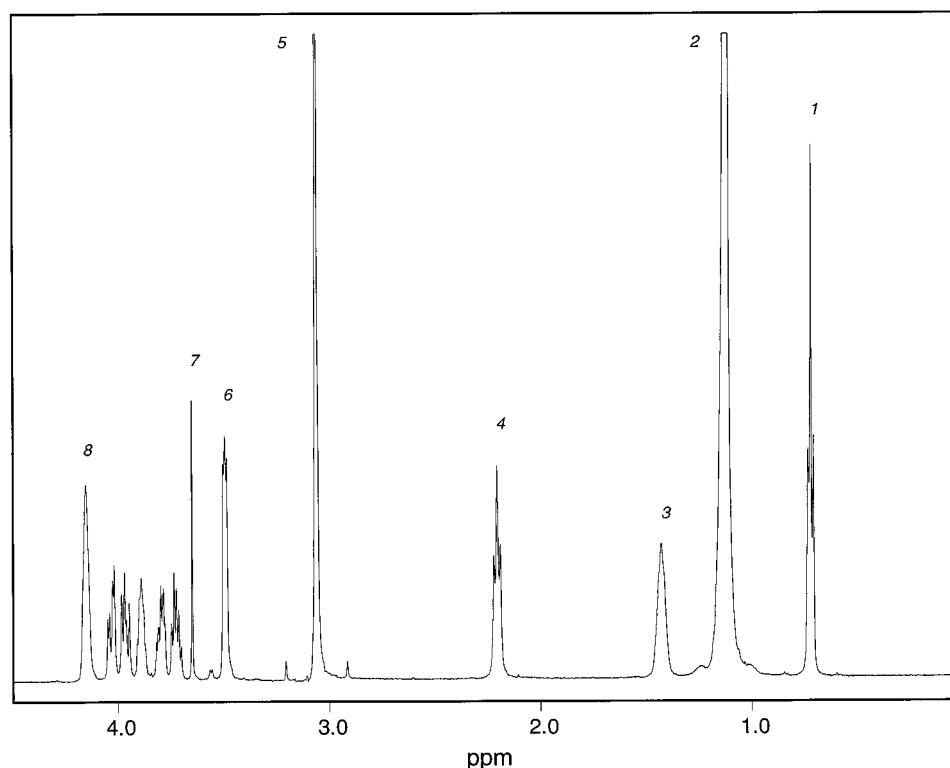
High-resolution ^1H -NMR spectra were recorded on an AMX2-500 spectrometer at 500.13 MHz with a phase-cycled one-pulse experiment. The pulse width was 6 μs , and a total of 8 free induction delays (FIDs) were acquired with an interpulse time of 10 s. The data was transferred to an SGI workstation and processed with the Felix 2.3.0 software package. An exponential line broadening of 0.3 Hz and zero filling up to double the size of the FID were carried out before Fourier transformation. The tryptophan analogs are aromatic molecules that have delocalized π -electron systems. Electric currents are induced in their ring systems as these molecules are placed in an external magnetic field. The resulting modulation of the local magnetic field results in a shift of the resonance frequency of any nucleus that is sufficiently close, provided that the aromatic systems have an anisotropic angular distribution with respect to the nucleus during the time scale of the NMR experiment.

^2H -NMR spectra were recorded on a Bruker MSL300 spectrometer at 46 MHz by quadrupolar echo techniques (Davis et al., 1976), with a 90° pulse width of 5 μs and a spectral width of 400 kHz. The interpulse time was 60 μs , and the delay time between pulse sequences was 250 ms. Typically 300,000 FIDs were accumulated for each spectrum.

^2H -NMR is a well-established tool for measuring the degree of order in lipid systems (for a thorough review see Lindblom, 1996). ^2H nuclei possess a quadrupolar electric moment, which interacts with a local electric field gradient. This interaction can be treated as a perturbation to the Zeeman energy when the nuclei are placed in a strong external magnetic field. The local electric field gradient defines the molecular frame, and the external magnetic field defines the laboratory frame; hence the derived expression for the total energy will contain information about how these frames are oriented with respect to each other. In NMR, the transitions that the nuclei make between these energy levels are observed. One observes two transitions in ^2H -NMR, and the distance between the resonances (in frequency units) for $\text{C}-^2\text{H}$ bonds that undergo fast reorientation about the bilayer normal is given by the expression

$$\Delta\nu_Q = \nu_Q S_{\text{C-H}} (3 \cos^2 \Theta_{\text{LD}} - 1),$$

FIGURE 2 The figure shows a typical high-resolution proton NMR spectrum of a micellar system recorded at 30°C. The system consists of lysoPC(14:0)/D₂O/*N*-methyl indole. The amount of lyso-PC is 0.3% (w/w), and the molar ratio of *N*-methyl indole to lyso-PC in the solution is 1:4. The chemical shift of residual protons on D₂O was used as the reference and was set to 4.6 ppm. The assignment of the proton resonances from the lipids is as follows (based on Plesniak et al., 1995): 1) acyl-CH₃, 2) acyl-CH₂, 3) acyl-β-CH₂, 4) acyl-α-CH₂, 5) choline-(CH₃)₃, 6) CH₂N, 8) PO₃CH₂, 7) is the methyl group on *N*-methyl indole.



where ν_Q is 3/4 times the quadrupole coupling constant, which is ~ 180 kHz (Goldfarb et al., 1985). Θ_{LD} is the angle between the director (i.e., the normal to the macroscopically aligned lipid bilayers) and the direction of the external magnetic field. S_{C-H} is the order parameter, and it characterizes the degree of ordering of the C-H bond with respect to the director,

$$S_{C-H} = \frac{1}{2} (3 \overline{\cos^2 \Theta_{DM}} - 1),$$

where Θ_{DM} is the angle between the C-H bond direction and the director, and the bar denotes a time average. A necessary condition for splittings to be observed is that the molecules undergo anisotropic motion. No splittings are obtained when the C-H bond undergoes fast isotropic motion or if Θ_{DM} is equal to 54.7°. Hence, a large splitting indicates a large ordering of the C-H bond. The main advantages of using macroscopically aligned bilayers in this study are the increased signal-to-noise ratio and the much better resolution obtained compared to the nonoriented case. This is accomplished by the accumulation of intensities from many nuclei in bilayer fragments, which all have the same Θ_{LD} because of the macroscopic orientation, into a single pair of resonances.

RESULTS

The behavior of indole and *N*-methyl indole molecules in the lamellar liquid crystalline (L_α) phase consisting of lipids with ester or ether linked acyl chains was investigated by ²H-NMR on the perdeuterated aromatic molecules. Furthermore, to verify that the tryptophan analogs have a preference for the lipid/water interface, high-resolution one-dimensional proton NMR spectra were obtained from three micellar systems: one system consisting of pure lyso-PC micelles, one system in which indole was added, and one system in which *N*-methyl indole was added. A typical spectrum obtained from a system in which *N*-methyl indole has been added to the lyso-PC micelles is shown in Fig. 2,

together with the assignments for the lipids. Several resonances are shifted upfield as compared to the spectrum of pure lyso-PC. As shown in Fig. 3, the resonances of protons near the surface of the micelle are more influenced by the tryptophan analogs than the methyl protons on the acyl chains that reside mainly in the micelle interior. This indicates that both analogs adopt a localization at the lipid/water interface. The results further suggest that methylindole may be buried slightly deeper in the micelle than indole.

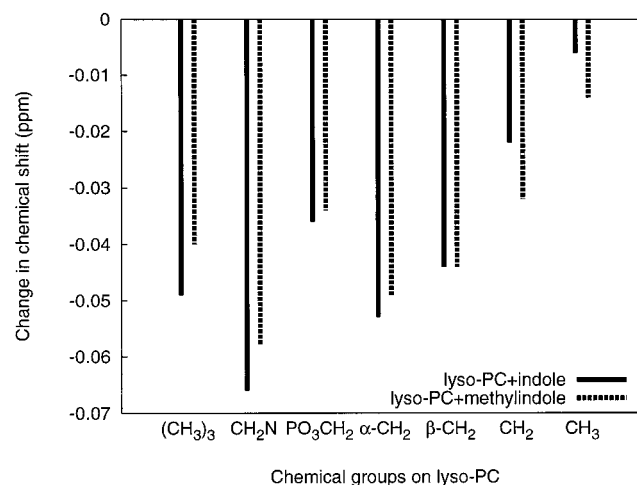


FIGURE 3 The diagram shows the change in proton chemical shifts of different groups on the lyso-PC lipid when indole or *N*-methyl indole has been added to the system. The shifts are obtained from a micellar system of 0.3% (w/w) lyso-PC(14:0) in D₂O at 30°C, and the molar ratio of tryptophan analog to lyso-PC was equal to 1:4.

Fig. 4 shows two ^2H -NMR spectra of an oriented L_α phase containing 64.5% (w/w) DMPC, 35.0% (w/w) water, and 0.461% (w/w) indole. The amount of indole equals one solubilized indole per 25 lipids. The spectra were recorded with the bilayer normal parallel (Fig. 4 *A*) and perpendicular (Fig. 4 *B*) to the external magnetic field. The spectra show relatively large quadrupolar splittings of tens of kilohertz, indicating that they are associated with the lipids and are consistent with solubilization at the lipid/water interface, where the molecules are expected to exhibit a relatively high degree of order. All six pairs of resonances, one from each deuteron, are well resolved in the spectra. It is easy to identify a one-to-one correspondence between resonances in the two spectra, and one can see that the quadrupolar splittings change by a factor of 2 when the orientation of the aligned sample is changed by 90° . This implies that the indole molecules undergo fast motional averaging about an axis parallel to the director. Although it is not possible to assign all of the splittings, the two quadrupolar splittings having almost the same magnitude of ~ 37 kHz most likely represent the deuterons in positions 5 and 8 on the indole (cf. Fig. 3 *A*), because they are on the same molecular axis (Koeppel et al., 1994; Hu et al., 1993).

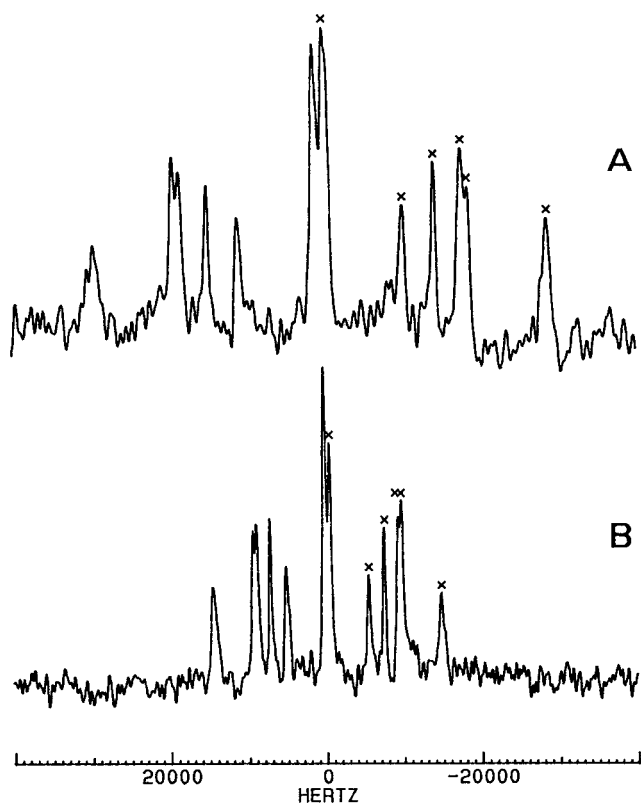


FIGURE 4 ^2H -NMR of indole- d_6 in an L_α phase consisting of indole/DMPC/ H_2O . The water content was 35% (w/w), the indole/lipid molar ratio was equal to 1:25, and the temperature was 30°C . The L_α phase was macroscopically aligned between glass plates with the bilayer normal parallel (*A*) and perpendicular (*B*) to the external magnetic field. One member of each pair of resonances is marked with an \times .

To obtain information about a possible hydrogen bonding to the carbonyl groups of the ester-linked fatty acid chains, samples of indole- d_6 /DMPC/water were compared with samples consisting of indole- d_6 /DTPC/water. The ^2H -NMR spectrum of a macroscopically aligned system prepared with DTPC and recorded at 30°C is shown in Fig. 5 *A*. Fig. 5 *B* shows a ^2H -NMR spectrum of a system prepared with DMPC recorded at 50°C . There is a striking similarity between these two spectra, suggesting that the assignment of the indole quadrupolar splittings is the same in both spectra. The reason for the low intensity of the central resonances in the DTPC sample is not known. In Table 1 it can be seen that the indole quadrupolar splittings decrease with increasing temperature for all systems, which can be explained by a decrease in the molecular ordering with temperature. Table 1 also shows that all indole quadrupolar splittings, except the smallest one, are larger for the samples with ester lipids than those with the ether lipids at the same composition and temperature. Hence one can conclude that indole molecules in ester lipid bilayers are more ordered than indole molecules in ether lipid bilayers. Furthermore, Fig. 5 shows an attempt to compensate for the greater degree of ordering of the indole in the ester lipid bilayer (Fig. 5 *B*) by increasing the temperature until the NMR

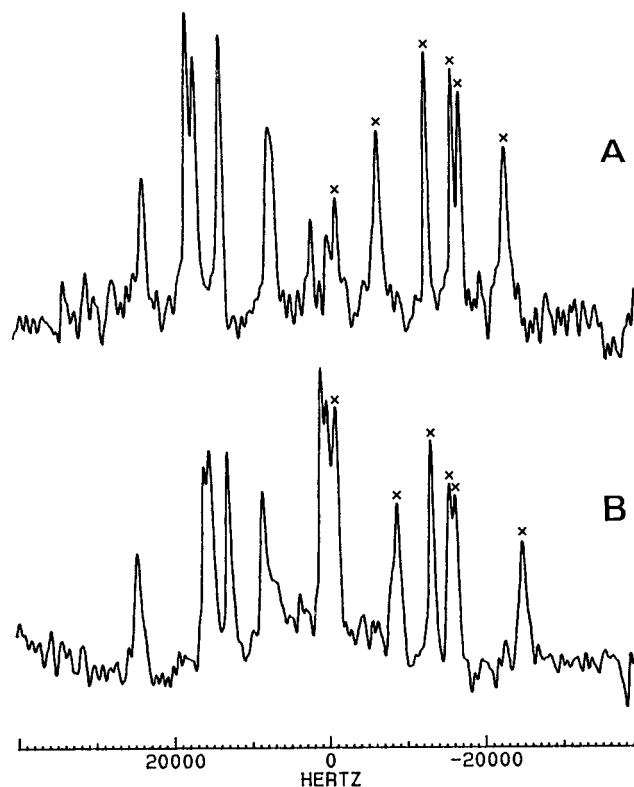


FIGURE 5 ^2H -NMR spectra of a L_α phase consisting of indole- d_6 /DTPC/ H_2O at 30°C (*A*) and indole- d_6 /DMPC/ H_2O at 50°C (*B*). The water content was 35% (w/w), and the indole/lipid molar ratio was 1:25. The L_α phase was oriented between glass plates with the bilayer normal parallel to the external magnetic field. One member of each pair of resonances is marked with an \times .

TABLE 1 Quadrupolar splittings from the perdeuterated tryptophan analogs in macroscopically oriented lipid bilayers with the bilayer normal parallel to the direction of the external magnetic field

		Indole quadrupolar splittings $\Delta\nu_Q$ (kHz)		<i>N</i> -methyl indole quadrupolar splittings $\Delta\nu_Q$ (kHz)	
30°C		DMPC	DTPC	DMPC	DTPC
		58.5	45.8	60.2	38.2
		38.5	34.8	48.5	33.7
		35.9	32.9	14.5	11.3
		29.3	26.1	9.7	7.7
		21.0	13.5	2.0	3.4
		2.3	3.1		
50°C		DMPC	DTPC	DMPC	DTPC
		49.4	39.8	45.0	30.2
		32.2	30.4	36.6	26.4
		30.7	28.6	12.6	9.6
		26.1	22.8	8.2	7.4
		17.2	11.4	~1.4	~3
		2.0	~3		

Some of the values are obtained from measurements where the bilayer normal was oriented perpendicular to the external magnetic field, and the magnitudes of those splittings were multiplied by a factor of 2 to account for the different geometry.

spectrum resembles the one for indole in ether lipid bilayers at 30°C. As can be seen, the resemblance is good but not perfect, suggesting that there is a difference in the mean orientation of the molecule. The higher molecular ordering of the indole molecules in the ester lipid bilayer system might be interpreted to be due to either hydrogen bonding interactions with the ester carbonyl or differences in dipolar interactions. Insight into this question can be obtained by performing the same experiment on a similar molecule that does not hydrogen bond.

Fig. 6 *A* shows a spectrum of *N*-methyl indole in macroscopically aligned ester lipid bilayers. This tryptophan analog is not able to hydrogen bond. Still, its quadrupolar splittings are of the same magnitude as those of indole, suggesting that it experiences considerable motional restriction as well. However, the positions of the resonances are very different compared to those of indole, and furthermore, only five splittings are resolved. The magnitudes of the *N*-methyl indole quadrupolar splittings at 30°C and 50°C are listed in Table 1.

From this table it can be inferred that also for *N*-methyl indole, the degree of ordering decreases with increasing temperature and that the splittings in ester lipids are larger than those obtained in ether bilayers. This suggests that the *N*-methyl indole molecule, like indole, has a higher ordering in the ester lipids. Because ester lipid bilayers have a larger dipole moment across the interface than their ether analogs, whereas the structural parameters are the same (Gawrisch et al., 1992), and because hydrogen bonding is not involved in either case, it is likely that it is the dipolar interaction between the bilayer and the *N*-methyl indole that is respon-

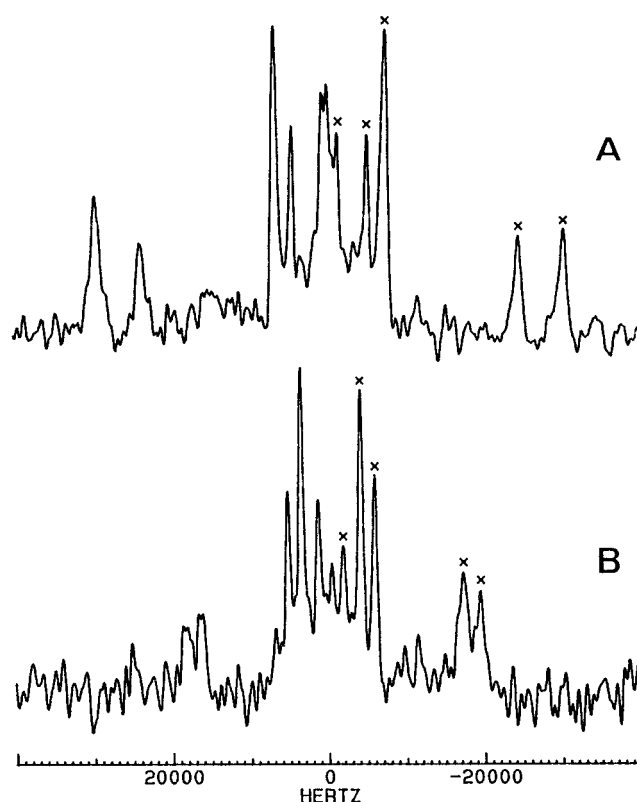


FIGURE 6 ^2H -NMR spectra of a L_α phase consisting of *N*-methyl indole- d_6 /DMPC/ H_2O (*A*) and *N*-methyl indole- d_6 /DTPC/ H_2O (*B*). The water content was 35% (w/w), and the indole/lipid molar ratio was 1:25. The L_α phase was oriented with the bilayer normal parallel to the external magnetic field. One member of each pair of resonances is marked with an \times .

sible for the observed differences in the ordering of this molecule in DMPC and DTPC bilayers.

DISCUSSION

Our main objective in this study was to obtain information about the molecular mechanisms behind the preference of tryptophan for membrane interfaces. We studied the interaction of indole and *N*-methyl indole with lipid bilayers consisting of either DMPC or DTPC. First it was established by ^1H -NMR experiments that the tryptophan analogs have a preference for the lipid/water interface. In agreement with this, the ^2H -NMR experiments indicated that both indole and *N*-methyl indole partition into a highly anisotropic environment in the lipid membrane. Moreover, the apparent sensitivity toward electrostatic interactions and hydrogen bonding are consistent with a partitioning of the molecules into the headgroup region, in agreement with the work of Wimley and White (1993). Furthermore, Kachel et al. (1995) found that molecules that share some of their properties with indole partition into the bilayer at a very shallow depth. We believe that the tryptophan side chains have properties when incorporated as an amino acid residue in a

water-soluble protein similar to those they have when they are free molecules.

We will now consider the possible interactions that are responsible for the preferred localization of indole and *N*-methyl indole near the lipid/water interface.

Dipolar interactions

The tryptophan analogs may carry a considerable dipole moment. The permanent dipole moment of the analogs was estimated using the program library MOPAC, and was found to be equal to 1.9 D and 2.1 D for indole and *N*-methyl indole, respectively. The directions are roughly parallel to the bond between the nitrogen and the proton in the NH group of indole (Fig. 1). Furthermore, the polarizability does not contribute significantly to the total dipole moment. The isotropically averaged polarizability was estimated using MOPAC, and it was found to be on the order of 10 \AA^3 in units of polarizability volume. The magnitude of the electric field may be up to 10^8 V/m at the membrane interface (Wimley and White, 1993). However, such an electric field will only induce a dipole moment of $\sim 0.004 \text{ D}$ in the indole molecules. The clearest picture of the importance of rather long-range electrostatic interactions arises from a comparison of *N*-methyl indole solubilized in ester and ether lipid bilayers. We speculate that this long-range electrostatic interaction is dominated by dipolar interactions. The molecular ordering of this tryptophan analog is lower in the ether lipid system than in the ester lipid system, as can be seen from the magnitude of the splittings in Table 1. Dipolar interactions provide the most plausible explanation for this behavior because this molecule is unable to hydrogen bond. Because the ether lipid matrix has to undergo a considerably smaller change in the dipolar potential across the interface than the ester lipid matrix (Gawrisch et al., 1992), the interaction between the ether lipids and the *N*-methyl indole will be much weaker, and this can account for the lower ordering. It is not due to differences in packing properties between ester and ether lipids, because the ether lipid system has a slightly higher main transition temperature (23.1°C and 26.2°C , respectively; McKeone et al., 1986), which suggests a somewhat greater degree of order. This would favor a slightly greater degree of order of the associated tryptophan analogs, contrary to the observed decrease. Although the choline headgroups of the lipids have a considerable dipole moment, they are probably not responsible for the observed difference in the ordering of the tryptophan analogs between ester and ether lipid bilayers, because the headgroups are similar in all of our systems.

Hydrogen bonds

Possible candidates with which indole can form hydrogen bonds are the water molecules, the lipid headgroups, and the carbonyl groups that link the acyl chains to the glycerol backbone of the ester lipids. When comparing the spectrum

obtained from indole with that of *N*-methyl indole in ester lipid bilayers, one finds splittings of similar magnitude, suggesting a similar degree of ordering for the two indoles. However, the values of the individual splittings are different. This suggests that hydrogen bonding makes only a minor contribution, if any, to the molecular ordering, but it may alter the mean orientation. The same remarks apply to the ether lipid system.

From measurements of the free energy of transfer of tryptophan analogs from water into cyclohexane, it was concluded that the NH group in tryptophan is considerably less polar than expected, which implies that the hydrogen bonds it forms are particularly weak (Wimley and White, 1992). This would be in agreement with a relatively small contribution of hydrogen bonding for molecular ordering of indole, as indicated in the present study. Another possible explanation for the different behavior of indole and *N*-methyl indole is that the difference in hydrophobicity of the analogs and/or the small difference in dipole moment influences their localization. Indeed, our studies of tryptophan analogs in micellar systems suggests that *N*-methylation of the indole may slightly affect the analogs' preference for the lipid/water interface.

If hydrogen bonding to lipid carbonyls is important for the ordering of the solubilized tryptophan analogs, one is to expect a large decrease in the ordering of indole and no appreciable effects on *N*-methyl indole when the ester lipids are substituted for ether lipids. However, the experiments show a similar change in the ordering of indole and *N*-methyl indole when the lipid type is altered. Most likely both the indole NH and lipid carbonyl groups hydrogen bond to water molecules present in the bilayer interface.

Cation- π interactions

The indole molecule may participate in cation- π binding with positively charged residues of the lipids because it has an aromatic ring system. Model gas-phase studies have shown that such bonds can be very strong, and a number of possible applications to biological systems have already been suggested (Dougherty, 1996). The distinction between strict dipole and cation- π interactions is not clear. The main contribution to cation- π binding is the interaction between the quadrupole moment of the aromatic molecule rings and an external charge distribution. However, to quantitatively model cation- π interactions one needs to take into account induced dipoles, polarizabilities, dispersion forces, and charge transfer (Caldwell and Kollman, 1995). The cation- π interaction has contributed to the understanding of fields such as molecular recognition, but its implications for protein-lipid interactions still remain to be investigated (Ma and Dougherty, 1997).

CONCLUSIONS

In this study a qualitative insight into the interactions between tryptophan analogs and lipid bilayers was obtained.

Given the three above considerations of possible interactions at the interface, we speculate that dipolar interactions may be dominant for the molecular ordering of the tryptophan analogs. Hydrogen bonding between indole and the lipid carbonyl appears to play an insignificant role in the solubilization of the molecule in the bilayer. However, the situation may be different for transmembrane proteins with interfacially localized tryptophans, because then the indole ring may be situated closer to the hydrocarbon interior, where it is less exposed to water. This can be investigated by performing similar experiments on transmembrane peptides with ^2H -labeled tryptophan side chains.

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