NMR Study of Micelles Formed by Monoalkylphosphoryl Nucleosides

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An NMR investigation of aqueous micelles obtained from surfactants bearing a nucleotide head attached to a linear hexadecyl hydrocarbon chain is presented. In particular, hexadecylphosphoryl-adenosine (C16-AMP) and hexadecylphosphoryl-uridine (C16-UMP) are studied by a combination of ^{1}H-NMR techniques such as NOESY, ROESY, and spin-lattice relaxation times. Both the intramolecular (*i.e.*, within one surfactant monomer) and the intermolecular interactions (*i.e.*, between neighboring surfactant molecules) are investigated. Relaxation measurements show that different groups of the surfactant molecule have distinct dynamic properties, the internal mobility decreases starting from the head group towards the Me terminal, while protons belonging to the base (which should be exposed to water) enjoy considerable freedom. The large upfield shift of the resonance of the terminal Me groups is evidence of a collective property of the micelle, an effect that, to the best of our knowledge, has not been reported so far. The micelles are studied both in water and salt solution, and the noticeable difference between the two cases is interpreted as a salt-induced stiffening effect. By mixing C16-AMP with C16-UMP, mixed micelles are obtained, *i.e.*, micelles that contain both surfactant monomers in each aggregate; our analysis shows that a significant interaction between the two complementary aromatic bases is present. All these results allow us to draw a picture of the surfactant in the micelle in which the plane of the aromatic ring lies parallel to the surface of the micelle and towards the aqueous medium. There are no basic structural differences between C16-AMP and C16-UMP micelles or C16-AMP/C16-UMP mixed micelles.

Introduction. – Surfactant aggregates such as micelles and vesicles, in addition to their technical relevance, are important as models for biological structures, especially when the surfactant in question is a lipid. Vesicles have been considered for a long time as valuable models for biological cells, as they have also a water compartment separated from the external aqueous medium by means of a bilayer. Spherical micelles with a simple monolayer structure have the advantage of a greater structural simplicity.

To understand the properties of complementary binding and recognition in a compartmentalized system, our groups have carried out an investigation of phosphatidyl nucleosides obtained from complementary nucleo-bases such as 5′-(1,2-dialkyl-*sn*-glycero(3)phospho)adenosine and 5′-(1,2-dialkyl-*sn*-glycero(3)phospho)uridine [1], which form liposomes, as well as single-chain lipids such as hexadecylphosphoryl-adenosine, which form micelles in H_2O [2]. These compounds can be readily synthesized by a biphasic enzymatic reaction originally developed by *Shuto et al.* [3][4].

Thus, 'complementary' micelles were prepared, *i.e.*, micelles containing complementary bases such as adenosine and uridine head groups. It was previously shown that complementary liposomes, derived from 5′-(1-palmitoyl-2-oleyl-*sn*-glycero(3)phospho)adenine and from 5′-(1-palmitoyl-2-oleyl-*sn*-glycero(3)phospho)uridine mixed with each other, give rise to a small hypochromic effect [1]. Micelles from hexadecyl phosphoryl adenosine (C16-AMP) and from hexadecylphosphoryl-uridine (C16-UMP) were also studied; again, a modest hypochromic effect was observed.

In this study, the C16-AMP and C16-UMP spherical micelles were analyzed by a combination of 1D and 2D ^{1}H-NMR techniques. Special care was taken to compare the structural properties of these micelles in H_2O and in a buffered solution (presence of salt). In spherical micelles, the polar head can be orientated toward the water pool, toward the inner part of the micelles, or can be randomly directed. In C16-AMP and C16-UMP micelles, we can show that the orientation of the polar head is that perpendicular to the micellar radius. In the presence of salt, a noticeable stiffness is induced, thus providing evidence that, in mixed micelles, the distribution of both types of polar heads on the micellar surface is far from random.

Results and Discussion. – *Assignment and Overall Structural Properties.* The structures of the monomeric surfactants is shown in *Fig. 1*.

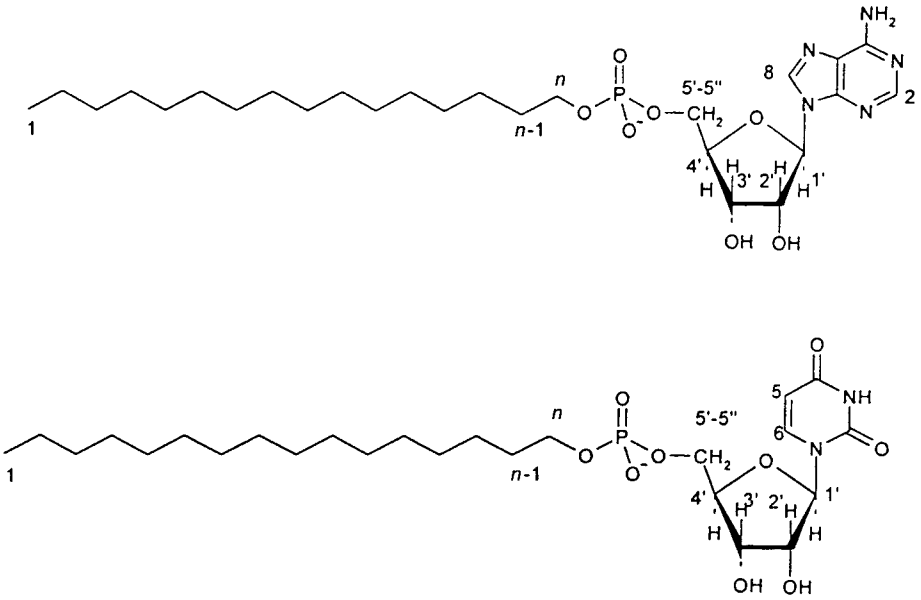

Fig. 1. *Structural formulae of C16-AMP* (top) *and C16-UMP* (bottom) *monomeric surfactants*

In the ^{1}H-NMR spectrum of C16-AMP in D_2O at 300 K, all peaks appear extremely well-resolved, allowing the full assignment reported in *Table 1*, obtained from a combination of 2D experiments (TOCSY, COSY, data not shown). However, some superimposition with the H_2O resonance is present. A better resolution is obtained on

the same sample at 285 K; here, the H_2O resonance is fully separated from the resonances of the sugar moiety: the assignment of ^{1}H-NMR spectrum at 285 K is reported also in *Table 1*. Both the aromatic as well as the aliphatic proton signals can be investigated. The most relevant observation on this ^{1}H-NMR spectrum is the strong upfield shift of the resonances of the Me group and more generally of the fatty chain as a whole, compared to the spectra obtained in organic solvents as observed already in our preliminary study [2].

Table 1. *^{1}H-NMR Assignments* (at 300 and 285 K) *and Spin-Lattice Relaxation Time* (at 285 K) *of Selected Resonances of C16-AMP Micelles in D_2O.* Chemical shifts [ppm] measured from an internal trace of DSS.

	$T = 300$ K ^{1}H [ppm]	$T = 285$ K ^{1}H [ppm]	T_1 [s]	Standard deviation [s]
H–C(1′)	6.134	6.146	1.663	0.038
H–C(2′)	4.622	4.618		
H–C(3′)	4.586	4.618		
H–C(4′)	4.398	4.421		
H–C(5′), H–C(5″)	4.175; 4.256	4.190; 4.283		
CH_2 (n)	3.911; 3.962	3.932; 3.994	0.626	0.033
CH_2 ($n-1$)	1.580	1.597	0.614	0.012
CH_2 ($n-2$)	1.211	1.224	0.576	0.016
CH_2 ($n-3$)	1.050	1.059		
$(CH_2)_{11}$	0.86–1.00	0.77–1.00	0.569	0.010
Me	0.561	0.554	0.830	0.005
H–C(2)	8.218	8.226	1.626	0.004
H–C(8)	8.562	8.589	1.475	0.036

To fully appreciate this effect, it would be necessary to make a comparison with the spectrum of the monomeric surfactant, *i.e.*, in a condition of nonaggregation. The system forms micelles in D_2O and, since its CMC is extremely low [2], *i.e.*, in the range of 20–35 μM, the best way to study the demicellization process is to warm the sample, thus inducing melting of the micelles.

In *Fig. 2*, for the system C16-AMP in D_2O, the plots of chemical-shift values of few selected resonances are shown at increasing temperatures. In agreement with the extremely low CMC value, we note that, even at 365 K, the demicellization process is incomplete, as clearly shown by the lack of a final plateau in all curves.

For the different chemical positions, for each molecular species, the chemical-shift dependence on the temperature is extremely different. Note, in particular, the large chemical-shift dependence of the Me group (>0.3 ppm), the $CH_2(11)$ (>0.3 ppm), and H–C(8) (>0.2 ppm), while the resonances due to H–C(2) and to the anomeric proton show a very modest temperature dependence (<0.05 ppm). Thus, different chemical groups are affected by a different local microenvironment. The observed shifts do not seem to depend on a variation of the dielectric constant, as usually observed in micellar systems [5]; in fact, in H_2O at high temperature or in an organic solvent, *i.e.*, in absence of micelles, the chemical shift of the terminal Me group is about the same.

Tentatively, we may attribute the upfield shift of terminal CH_2 protons of the aliphatic chain, observed at room temperature in the pure micellar form, as due to the presence of a large magnetic anisotropy on the aromatic polar head. This effect is

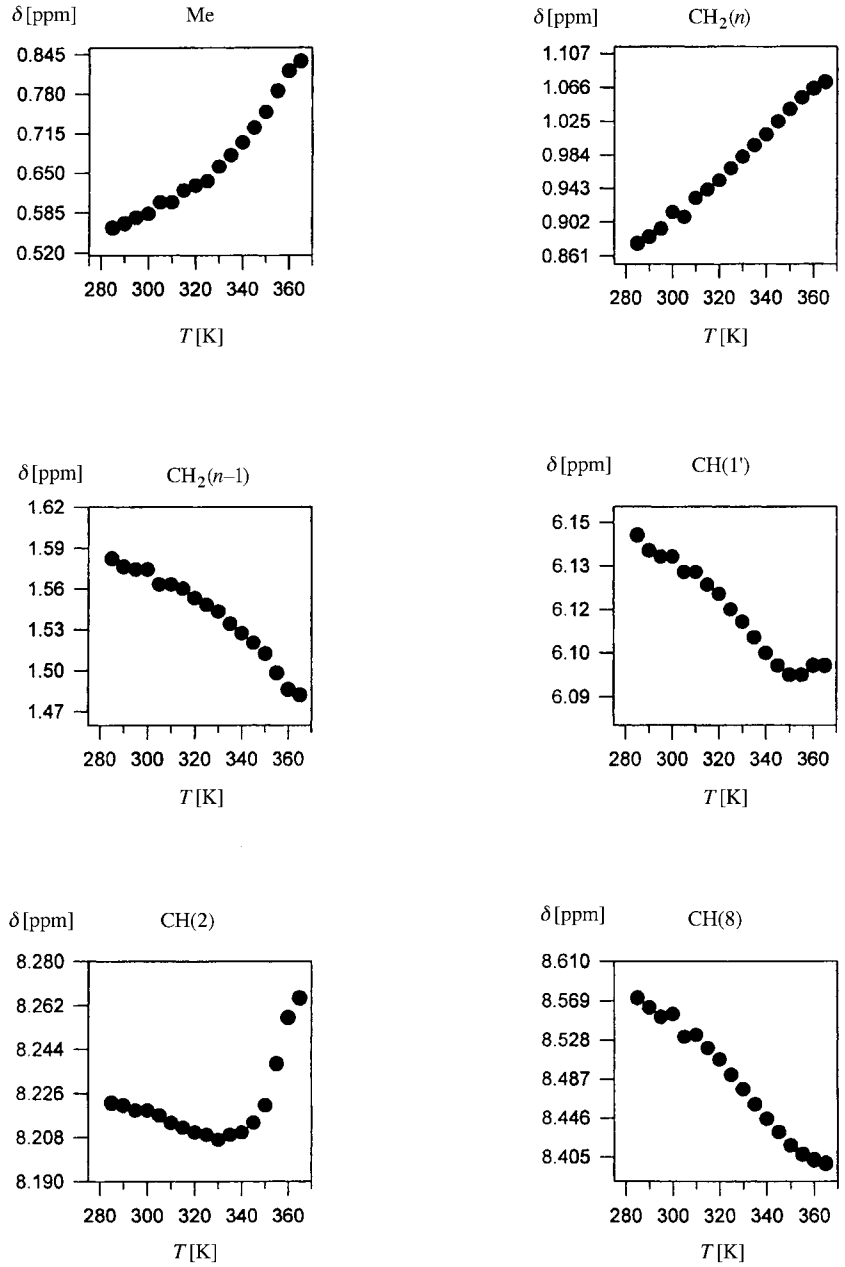

Fig. 2. *Dependence of chemical shift, δ, of selected resonances of C16-AMP micelles in D_2O as function of temperature,* T [K]

extended down to the Me group, which is more than 20 bonds away from the aromatic moiety. A possible interpretation of this effect can be sought in the relative position of

the aromatic group with respect to the surfactant chain: most likely the aromatic moiety lies parallel to the surface of the micelles, thus inducing an upfield shift on chain CH_2 groups. This upfield contribution should be very modest in view of the large distances we are dealing with (the radius of the micelles (see below) is in the range of 0.5–2 nm, which is a large distance on the NMR scale). However, in spherical micelles the effect is additive, *i.e.*, proportional to the aggregation number. Thus, if the aromatic ring is perpendicular to the micellar radius, the effect on the Me group may be considered a collective one, as indicated in *Fig. 3*.

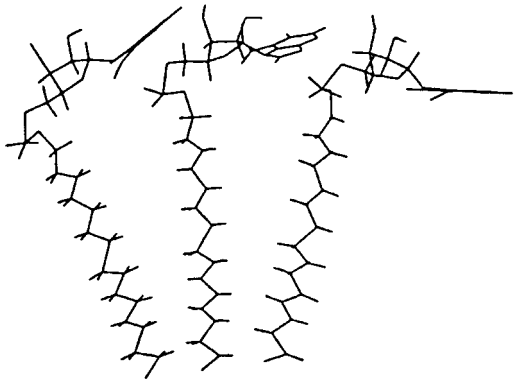

Fig. 3. *Molecular packing of C16-AMP with the adenosine group parallel to micellar surface*

In the case of C16-UMP, all the above-mentioned effects are also present. The assignment of C16-UMP spectrum in D_2O at 300 and 285 K is reported in *Table 2*. With respect to the adenine moiety, all induced shifts appear quite reduced. This effect may be viewed as a consequence of the minor magnetic anisotropy present in the uridine ring [6][7].

Table 2. *^{1}H-NMR Assignments* (at 300 and 285 K) *and Spin-Lattice Relaxation Time* (at 285 K) *of UMP Micelles in D_2O.* Chemical shifts [ppm] measured from an internal trace of DSS.

	$T = 300$ K ^{1}H [ppm]	$T = 285$ K ^{1}H [ppm]	T_1 [s]	Standard deviation [s]
H–C(1′)	5.984	6.002	1.469	0.043
H–C(2′)	4.339	4.349		
H–(3′)	4.382	4.401		
H–C(4′)	4.276	4.300		
H–C(5′), H–C(5″)	4.191; 4.096	4.098; 4.207		
CH_2 (n)	3.890; 3.934	3.899; 3.949	0.419	0.075
CH_2 ($n-1$)	1.635	1.642	0.668	0.015
CH_2 ($n-2$)	1.337	1.346	0.650	0.015
$(CH_2)_{12}$	1.197	1.190	0.598	0.010
Me	0.805	0.804	0.808	0.007
H–C(5)	5.949	5.972	1.591	0.048
H–C(6)	8.002	8.028	1.175	0.037

A 2D NOESY map relative to C16-AMP in D_2O, obtained with a very short mixing time, 15 ms, shows only extremely weak cross-peaks. Better results were obtained with a ROESY experiment performed again with 15-ms mixing time (see *Fig. 4,a*).

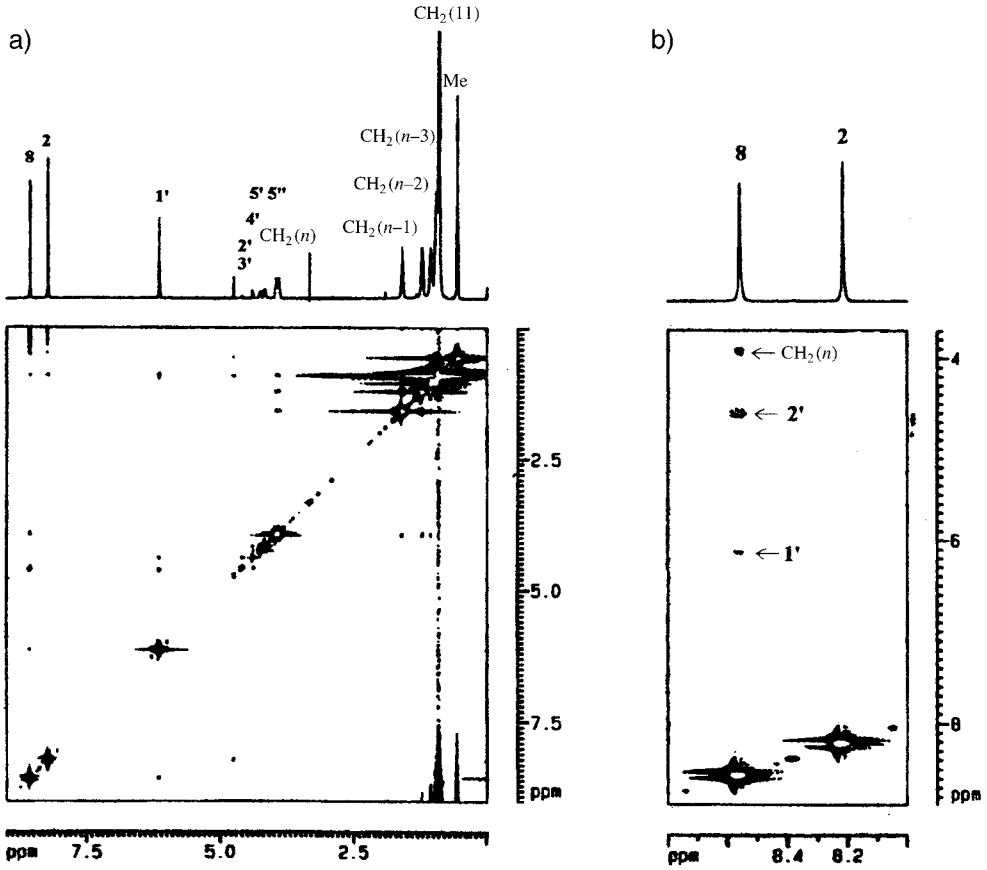

Fig. 4. a) *2D ROESY Spectrum* (mixing time 15 ms) *of C16-AMP in D_2O.* b) *A slice of the same experiment relative to the range 8.0–8.8 ppm/3.5–9.0 ppm is shown.*

First of all, let us consider a particular slice of the ROESY map as shown in *Fig. 4,b*. Strong ROE contacts can be observed between H–C(8) and the following protons: H–C(2′), H–C(1′) (see *Fig. 1* for the formulas), and, surprisingly, the first $CH_2(n)$ of the aliphatic chain. Note that no contacts are present between H–C(2) and any protons of the pentose ring (see *Fig. 5*).

These observations allow us to sketch a model of the single adenine polar head in the micellar moiety: the six-membered aromatic ring should lie on the external part of the micelle, perpendicular to the micellar radius as indicated in *Fig. 3*.

About the same type of cross-peaks are observable in the ROESY 2D map (15-ms contact time) of micelles formed by C16-UMP in D_2O. This similarity suggests that both types of micelles most likely have similar structures. Possible structures that account for the observed cross-peaks will be discussed later. All NOE contacts previously discussed do not allow a distinction between inter- and intramolecular contributions.

Relaxation Times. To gain information about the structure and the internal mobility of C16-AMP and C16-UMP micelles in D_2O, a full ^{1}H-NMR spin-lattice-relaxation

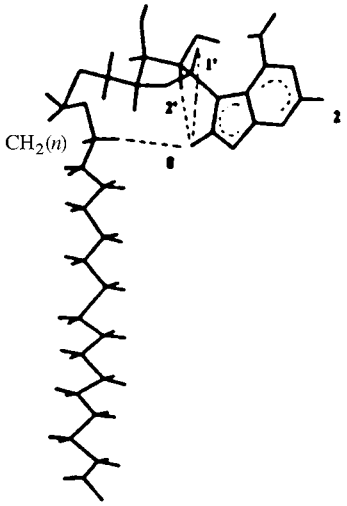

Fig. 5. *Model of a C16AMP monomeric surfactant.* Significant NOE contacts are indicated.

study was performed at 285 K. Experimental T_1 values relative to selected resonances of C16-AMP and C16-UMP have been reported in *Tables 1* and *2*, respectively. Here, the relaxation values and the modest line-broadening point to data lying in the extreme narrowing region of relaxation [8], in which the faster the motion, the longer is its spin-lattice relaxation time.

In agreement with a normal micellar structure with external polar heads, moving from the Me group toward the polar head, an increase of mobility is apparent. With respect to monomeric systems, a noticeable reduction of the overall mobility is present everywhere in the aliphatic chain, which appears strongly hindered [9]. The measured spin-lattice relaxation times are consistent with the presence of a reduced overall mobility of the polar head, *i.e.*, with the proposed molecular assembly (see *Fig. 3*).

Comparison between Water and Salt Solution. Preliminary NMR observations on these micelles had given evidence of a surprising difference between H_2O and a particular buffered solution (borate buffer). This salt effect is partly known in the literature [10][11] and may potentially provide important information on the structure of the micelles. For this reason, we thought it worthwhile to carry out a systematic investigation in the two solvent systems.

Let us look at the NMR data in the two cases, *i.e.*, in the presence of salts (*Fig. 6*,*b*) or in its absence (*Fig. 6*,*a*).

The addition of the salt (borate buffer) modifies deeply the spectrum of the micellar system, introducing a large signal multiplicity and broadening some of the resonances. This is true both for C16-AMP and C16-UMP micelles.

To assign the rather complex ^{1}H-NMR spectrum of buffered samples, 2D *J*-correlated experiments were performed, namely COSY and TOCSY (data not shown). From these experiments, a full assignment of all observed resonances is obtained (data not reported).

It is apparent that, in the buffered solutions of both C16-AMP and C16-UMP, at a concentration much higher than CMC, high-field ^{1}H-NMR spectra show the presence

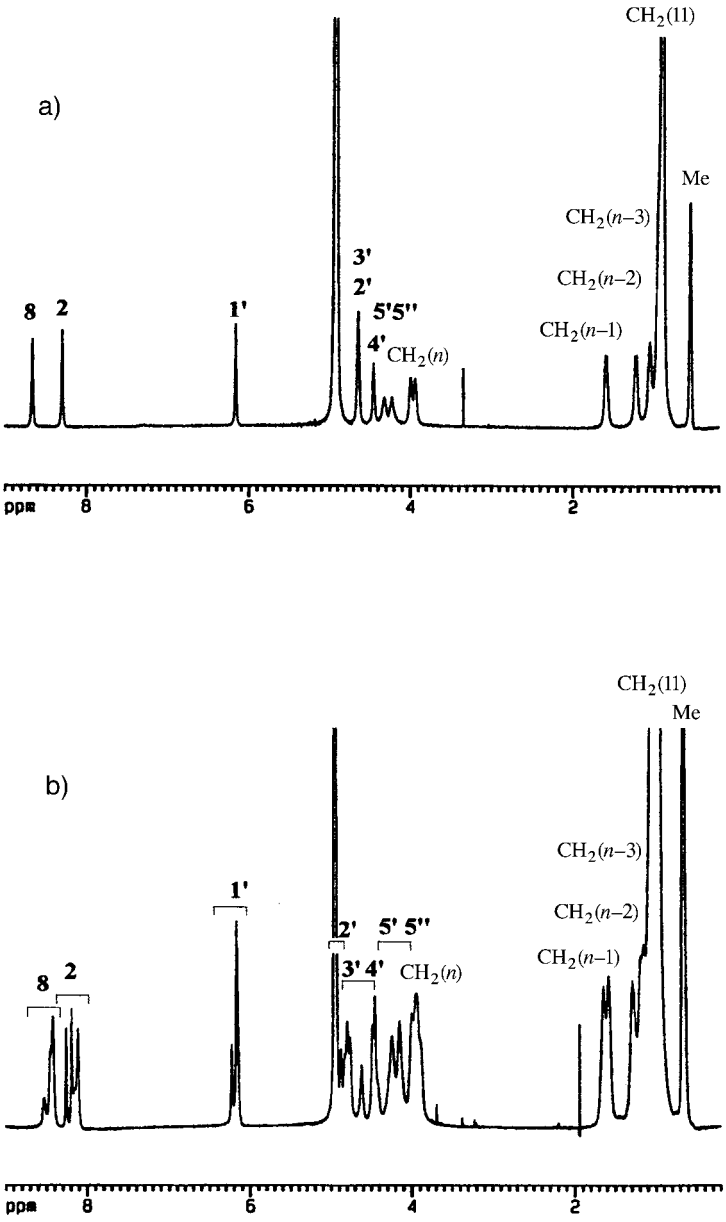

Fig. 6. a) *600.13-MHz ^{1}H-NMR Spectrum of C16-AMP micelles in D_2O at 285 K.* b) *600.13-MHz ^{1}H-NMR spectrum of C16-AMP micelles in buffered solution at 285 K*

of different forms in a slow exchange with respect to the NMR observation time [12]. In fact, a large number of resonances characterized by different line-widths are present. Moreover, a 2D NOESY experiment (data not shown) shows a large number of strong

cross-peaks due to chemical exchange [13]. We also note that different parts of the spectrum, corresponding to different parts of the molecule, show different multiplicities.

To further confirm the presence of many forms in slow exchange, we carried out several experiments at different temperatures. By slightly increasing the temperature (from 300 to 305 K; spectra not shown), some peaks tend to collapse with a significant loss of resolution. It is apparent that 285 K is an optimal temperature, since it engenders good peak separation and no superimposition with the H_2O resonance. Moreover, no precipitation occurs. Hence, as mentioned in the *Exper. Part*, all spectra of micelles in buffered solution were run at 285 K unless stated otherwise.

The most important characteristic of the ^{1}H-NMR spectra in the buffered solution, both of C16-AMP and C16-UMP, compared with the spectra of micelles in D_2O, is the resonance multiplicity of aromatic protons. The most likely explanation for the observed multiplicity can be sought in the different configurations of cluster of the aromatic rings when packed together in the micellar surface. In particular, consider that two vicinal adenine rings may give rise to different geometrical couplings as illustrated below.

Clearly, the signal arising from the A–A coupling will be different from that arising from the A–A′ coupling. Since the micellar surface may have clusters involving several interacting rings, a very large number of different signals may arise. For example, if we consider planar clusters of five adenine rings (a given ring being surrounded by four other rings), we can have 16 different stereochemical arrangements.

Note that this steric situation is similar to that originated by a two-dimensional polymeric [14] chain. In this case, the large magnetic anisotropy [6] of the aromatic rings causes dramatic chemical-shift variations in systems differing only in the molecular packing[1]). This suggests that the molecular packing present in the local environment within a single micelle is able to cause a strong chemical-shift variation in proximity of the aromatic systems (see *Fig. 3*). For instance, for different orientations, at a molecular distance of *ca.* 5 Å, chemical shifts as large as -0.1 ppm can be induced. Due to the r^{-3} dependence, this effect is very weak at large distances from the aromatic

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[1]) By progressive dilution of the micellar solutions, a strong enlargement and a collapse of resonances can be observed (data not shown); this is due to the equilibrium between monomeric species and micelles. To observe the multiplicity, one must operate at a rather high concentration of *ca.* 10^2 higher than the CMC. This effect can be quantitatively evaluated considering that a simplified expression of the anisotropy effect is given by:

$$\Delta\sigma = 1/3\ (r)^{-3}(\chi_{\parallel} - \chi_{\perp})(1 - 3\cos^2\theta)/4\pi$$

where: $\Delta\sigma$ is the induced chemical shift; r is the distance between the observed nucleus and the center of the electronic group inducing the anisotropy; θ is the angle between the bond axis and r; $\chi_{\parallel} - \chi_{\perp}$ are the longitudinal and transverse susceptibilities, respectively. Thus, in a benzene ring even at a 15-Å distance, an effect of the order of 2 Hz is present [6][7].

moiety. In fact, the signal multiplicity is still present in the furanose-ring resonances and still observable on the CH_2 resonances of the aliphatic chain in position n and $n-1$ (see *Fig. 6*). However, it is not observable in all the other resonances of the aliphatic chain.

As in the case of C16-AMP, resonances due to aromatic protons of C16-UMP in buffered solution show the presence of four different forms (see *Fig. 6,b*). However, in the case of C16-UMP, the spectral resolution is worse than that of C16-AMP. Moreover, some of the spectral multiplicity seems lost. In fact, the CH_2 furanose-ring resonance appears only broadened, while no splitting or broadening can be observed on resonances due to the aliphatic chain. These effects could be due to the minor magnetic anisotropy of the uridine aromatic ring with respect to that of the condensed rings of the adenine [6][7].

All above observations are consistent with a micellar geometry in which the plane of the aromatic head is almost parallel to the micellar surface. In fact, if the polar head is directed toward the solvent, a stiff molecular packing on a rather rigid surface would be impossible, and, nevertheless, downfield shifts would be induced on the aliphatic chain.

The NOESY map of micelles in buffered solution is shown in *Fig. 7*. The same cross-peaks are observable in the ROESY 2D map (15-ms contact time) of micelles formed by C16-UMP in D_2O. Thus, in H_2O and in the buffered solution, the same type of structure is present for both micelles. The presence of a large magnetic anisotropy on the aromatic polar head causes the observed upfield shift of the resonances of the terminal CH_2 groups of the aliphatic chain and of the Me group more than 20 bonds away from the aromatic group. This effect is about the same in the presence or absence of salt. All previous observations indicate a structure in which the polar heads lie on the micellar surface bent in such a way to be almost perpendicular to the long axis of the aliphatic chains. Even at a distance of *ca.* 15 Å, the overall effect of the magnetic anisotropy of a uridine ring is not null [6], causing an upfield shift of *ca.* 2 Hz, while that of an adenine ring can be quite large. It is also worth noting that an aromatic group with an orientation parallel to the direction of the aliphatic chain would cause a small downfield shift on neighboring polar-head protons, and that this effect could also reach CH_2 groups in positions n and $n-1$ if the aromatic head is slightly bent toward the hydrophobic core.

Assuming an aggregation number of 70 [2], the presence of an upfield shift would be 70×2 Hz (single average upfield shift), *i.e.*, *ca.* 140 Hz. Extremely small upfield shifts have been previously reported on terminal Me groups of aggregated systems [15]. The strong upfield shift observed in C16-UMP and C16-AMP is a true collective effect.

The Case of Mixed Micelles. One of the aims of the study of micelles from phosphatidyl-nucleotides is to detect whether, and to what extent, there is chemical recognition between the 'complementary' micelles with adenine and uridine. By mixing one type of micelles with the other, mixed micelles can be formed, *i.e.*, micelles that would contain in each aggregate both adenine and uridine in proportion to their relative quantities. In any mixture, two possible extreme cases can be expected: the ^{1}H-NMR spectra are additive, or novel peaks arise. In the first case, no interaction between the complementary bases is present, while, in the second one, an interaction is present.

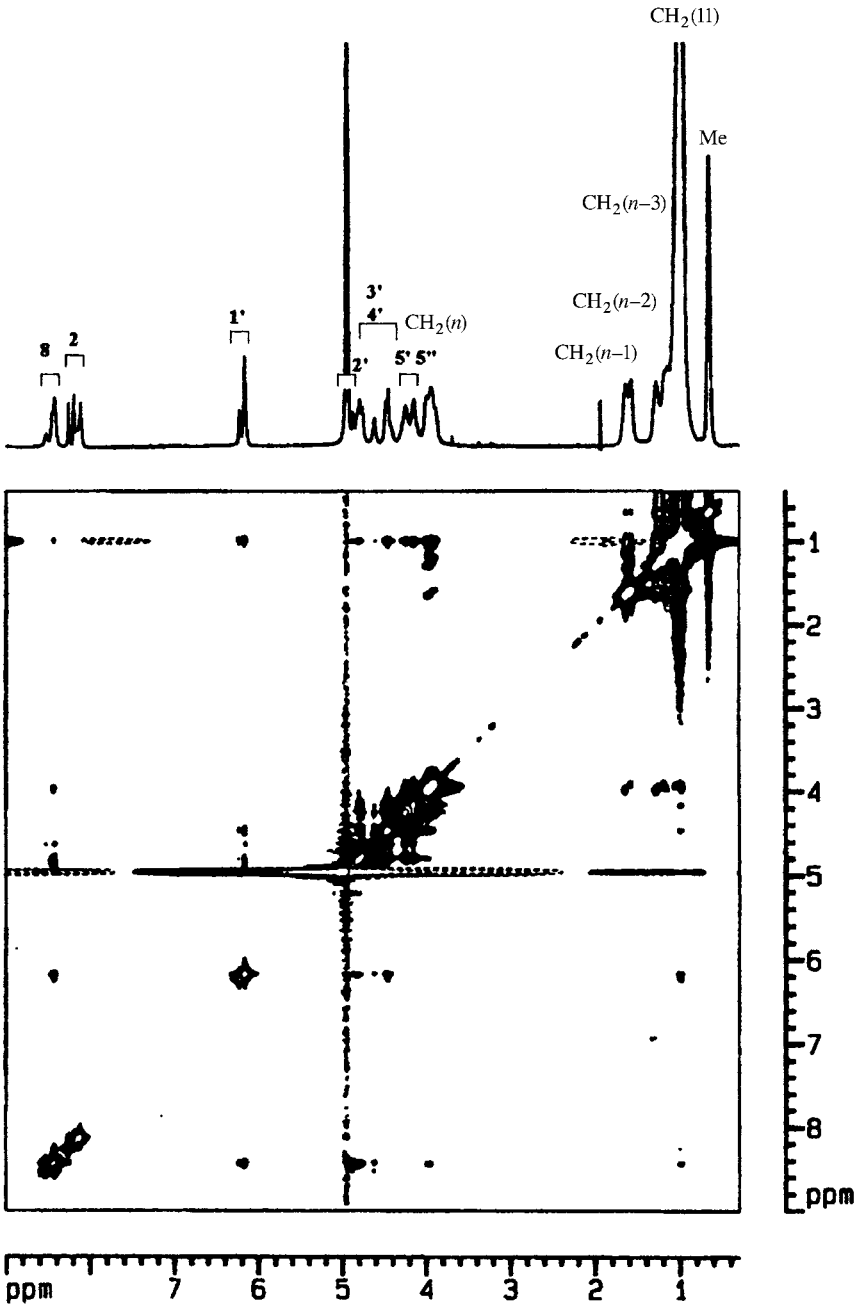


Fig. 7. *2D NOESY Spectrum of C16-AMP in buffered solution at 285 K*

Consider a D_2O solution containing both C16-AMP and C16-UMP in a *ca.* 1:1 ratio with a total concentration of *ca.* 10 mM. By comparing the ^1H spectrum of this sample with those of each component (see *Fig. 8*), it is obvious that it is rather different from the ^1H-NMR spectra of each component.

In particular, the chemical shift of the Me group (see *Fig. 8,c*, left) is between the value observed in C16-AMP (see *Fig. 8,c*) and that observed in C16-UMP (see *Fig. 8,b*). In accordance with the previous interpretation, the upfield shift of the Me resonance is due to the magnetic anisotropy of the polar head. Thus, in the case of mixed micelles, the effect should be proportional to the number of species present, *i.e.*, additive. This is exactly the situation found in *Fig. 8,c*. Thus, the observed chemical shift proves, once more, that the previously proposed structure is present also in the case of mixed micelles.

The resonances due to H–C(8) and H–C(2) of C16-AMP, and those due to H–C(5) and H–C(6) of C16-UMP can be quite easily recognized in the spectral region between 5.7–6.3 ppm and 7.8–8.6 ppm (see *Fig. 8*; middle and right). However, in the mixture, these resonances are shifted with respect to the corresponding ones present in the spectra of each component. Thus, mixed micelles are indeed present. A 2D ROESY experiment (data not shown) on this sample shows a large number of cross-peaks.

Mixed micelles were also studied in buffer (see *Fig. 9,c*; left, middle, and right). The restricted mobility induced by the buffer leads to a spectrum in which a minor chemical exchange is present.

The downfield spectral range between 7.80 and 8.65 ppm relative to C16-AMP, C16-UMP, and to C16-MIX, shows that the spectrum of *Fig. 9,c* does not represent the superimposition of *Fig. 9,a* and *b*. Thus, mixed micelles are formed with an observable interaction between the complementary moieties.

In particular, the interaction between the adenine and uridine aromatic rings leads to new resonances in the spectral range due to H–C(5) of the uridine moiety. Moreover, one of the resonances due to H–C(6) of the uridine moiety undergoes a noticeable upfield shift, and the intensity distribution of H–C(2) resonances due to adenine is strongly perturbed. A full assignment of all resonances regarding the involved species was also performed (data not shown).

2D NOESY Experiments relative to C16-AMP, C16-UMP, and C16-MIX were obtained with a very short mixing time of 15 ms (data not shown). In the buffer solution, strong NOE contacts can be observed between H–C(8) and the following protons: H–C(2′), H–C(1′), and, surprisingly, the first CH_2 of the aliphatic chain (data not shown). This observation sets stringent constraints on the geometry of the adenine polar head in the micelle. No contacts are present between H–C(2) and any protons of the pentose ring so that the six-membered aromatic ring should lie on the external part of the micelle towards the water pool.

In the case of the C16-UMP micelles, the situation is less clear due to the partial overlap between the H–C(5) and the H–C(1′) resonances at *ca.* 6 ppm. A strong contact is present between H–C(6) and H–C(2′), confirming the same kind of structure that is present in the C16-AMP micelles. An extremely weak contact between H–C(6) and the CH_2 in position *n* of the aliphatic chain is also observable. Thus, most likely, the same bent structure of C16-AMP micelles is present, but the weakness of the

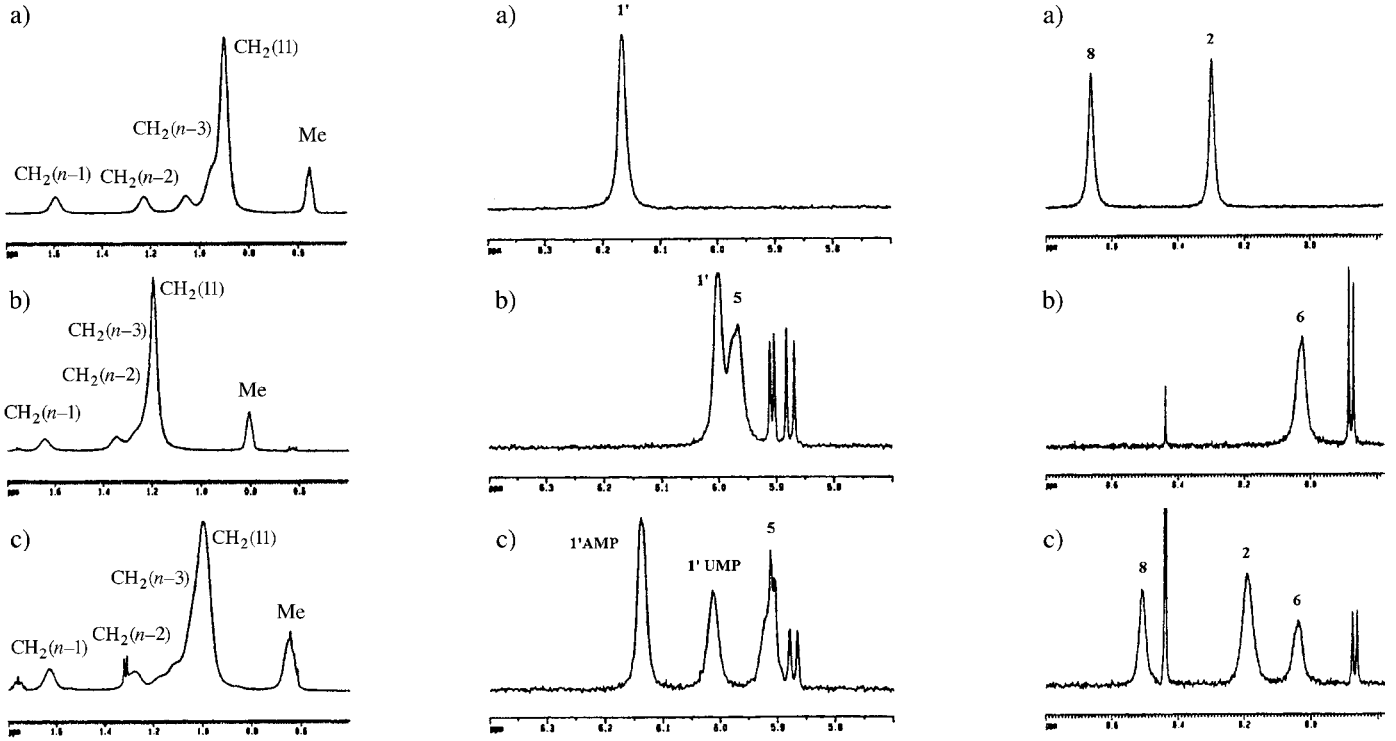


Fig. 8. Left: *expansion of the 0.4 – 1.8-ppm region in the 600.13-MHz ^{1}H-NMR spectrum of* a) *C16-AMP micelles in D_2O at 285 K,* b) *C16-UMP micelles in D_2O at 285 K,* c) *1 : 1 mixture of C16-AMP and C16-UMP in D_2O.* Middle: *expansion of the 5.6 – 6.5-ppm region in the 600.13-MHz ^{1}H-NMR spectrum of* a) *C16-AMP micelles in D_2O at 285 K,* b) *C16-UMP micelles in D_2O at 285 K,* c) *1 : 1 mixture of C16-AMP and C16-UMP in D_2O at 285 K.* Right: *expansion of the 0.55 – 1.80-ppm region in the 600.13-MHz ^{1}H-NMR spectrum of* a) *C16-AMP micelles in D_2O at 285 K,* b) *C16-UMP micelles in D_2O at 285 K,* c) *1 : 1 mixture of C16-AMP and C16-UMP in D_2O at 285 K.*

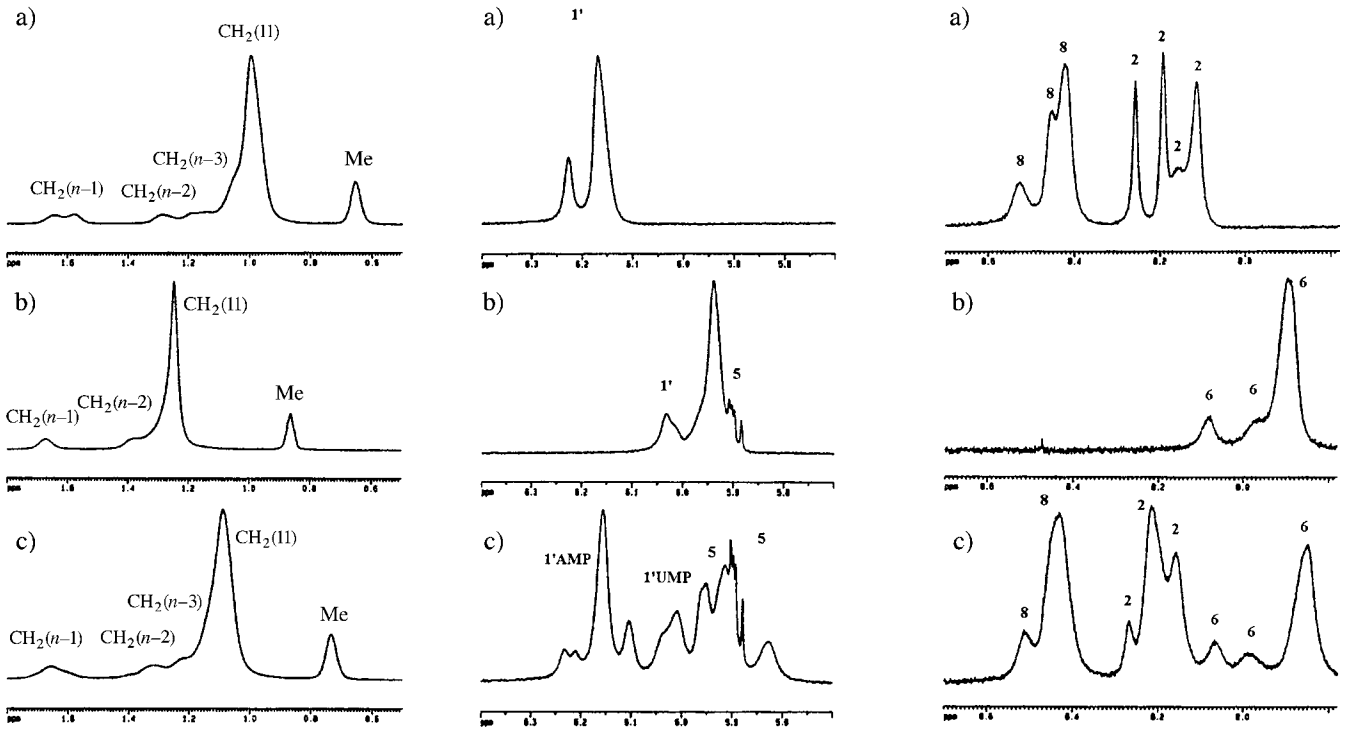
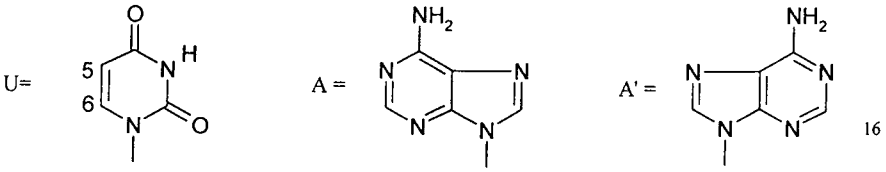


Fig. 9. Left: *expansion of the 0.4–1.8-ppm region in the 600.13-MHz ^{1}H-NMR spectrum of* a) *C16-AMP micelles in buffered solution at 285 K,* b) *C16-UMP micelles in buffered solution at 285 K,* c) *1:1 mixture of C16-AMP and C16-UMP.* Middle: *expansion of the 5.6–6.5-ppm region in the 600.13-MHz ^{1}H-NMR spectrum of* a) *C16-AMP micelles in buffered solution at 285 K,* b) *C16-UMP micelles in buffered solution at 285 K,* c) *1:1 mixture of C16-AMP and C16-UMP in buffered solution.* Right: *expansion of the 7.80–8.65-ppm region in the 600.13-MHz ^{1}H-NMR spectrum of* a) *C16-AMP micelles in buffered solution at 285 K,* b) *C16-UMP micelles in buffered solution at 285 K,* c) *1:1 mixture of C16-AMP and C16-UMP in buffered solution.*

NOE cross-peak and the lack of a cross-peak with the $CH_2(n-1)$ indicates that, as a whole, the structure is more mobile, and that other conformers are present. This is confirmed by the observation of a NOE cross-peak between H–C(5) and the CH_2 in position *n* of the aliphatic chain.



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In the case of C16-MIX, NOE contacts can also be observed between the H–C(2) of C16-AMP and H–C(6) of C16-UMP (data not shown). Note that no cross-peak is present between H–C(8) of AMP and H–C(6) of UMP. These observations could be explained on the basis of the different stereochemical couplings discussed before. In particular, in the case of uridine, all couplings A–U, A′–U, and U–U are possible, whereas, in the case of adenine, only the U–A coupling is possible.

Notwithstanding the large multiplicity of signals that affect the interpretation of NOE cross-peaks, a clear cross-peak occurs between the anomeric protons of the two chemically different species, thus confirming the evidence of the mixed nature of these micelles.

Conclusion. – The main result of this NMR study is that polar heads lie almost perpendicular to the micellar radius both in C16-AMP and C16-UMP micelles. This particular conformation is present both in H_2O and in buffer.

The presence of buffer induces a remarkable stiffening [16] of the whole system and is in agreement with previous data on salt-induced stiffening effects. In mixed micelles, NMR data show that a significant interaction is present between the two complementary aromatic bases.

The distribution of bases on the micellar surface is far from random; there is precise evidence that some particular couplings among the bases are favored, and others are completely absent. A full study of these systems by molecular dynamics is in progress.

Experimental Part

Materials. C16-AMP and C16-UMP were synthesized from hexadecylphosphorylcholine and the corresponding nucleoside with the help of phospholipase D as described in [2]. Phospholipase D from *Streptomyces sp.* AA 586 (PLD) was purchased from *Genzyme Diagnostic* (West Malling, Kent, UK). D_2O and DCl were purchased from *Cambridge Isotope Laboratories*, water was purified with a *MilliRo/Milliq* apparatus. All the other reagents were from *Fluka*, *Sigma*, or *Merck* and of the highest-purity grade.

Sample Preparation. Borate buffer (0.1M in D_2O, pD 8.8) was prepared from sodium tetraborate, correcting the pD by adding DCl. The micellar solns. were prepared by simple shaking of the weighted amount of phosphoryl-nucleosides.

NMR Experiments. ^{1}H-NMR Experiments were performed at 600.13 MHz on a *Bruker AMX-600* spectrometer. Unless otherwise stated, all NMR experiments were run at 285 K. This is the lowest possible temp. obtainable without the presence of demixing due to surfactant insolubility [17].

Sample concentrations were kept at 10 mM unless otherwise stated. Samples were not degassed. The good quality of D_2O ensured the proper receiver gain without H_2O suppression. Chemical shifts are reported with respect to 2,2-dimethyl-2-silapentane-5-sulfonate sodium salt (DSS), used as internal standard.

To ensure proper experimental conditions in all 2D experiments, a careful measurement of spin-lattice (T_1) relaxation times was performed. Spin-lattice relaxation times were measured with the standard inversion recovery sequence, $(180 - \tau - 180)$ with at least 20 τ intervals, and calculated by standard *Bruker* program (see *Tables 1* and *2*).

The following 2D experiments were performed [18]: ^{1}H,^{1}H COSY; ^{1}H,^{1}H TOCSY, contact time 80 ms; ^{1}H,^{1}H NOESY, contact time 15 ms; ^{1}H,^{1}H ROESY, contact time 15 ms. All 2D experiments were obtained with a z-gradient selection and a matrix 1 K $\times$ 512 words and were acquired and processed in the phase-sensitive mode (TPPI).

In micellar systems or in liposomes, NOESY experiments at different mixing times show the presence of strong spin-diffusion effects that completely hide the NOESY information [19]. To bypass this problem, extremely short mixing times have to be used; this, in turn, reduces the intensity of the cross-peaks, which does not allow the measurement of NOE buildup.

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