Molecular Recognition through H-Bonding in Micelles Formed by Dioctylphosphatidyl Nucleosides†

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Short-chain phospholiponucleosides, namely diC_8P-adenosine and diC_8P-uridine have been, for the first time, synthesized through an enzymatic pathway that allows transphosphatidylation of phosphatidylcholines. Phospholiponucleosides, which have a number of potential applications in several areas such as anticancer therapy, are able to give in water self-organized aggregates. diC_8P-adenosine and diC_8P-uridine phosphatidylnucleosides form micelles in water solution with critical micellar concentrations around 10^{-3} M. Mixed micelles, formed from equimolar mixture of phosphatidylnucleosides, show nonideal mixing, suggesting specific interactions between the polar heads of the nucleolipids. We show through NMR, UV−vis, and CD spectroscopies that in mixed micelles formed from diC_8P-adenosine and diC_8P-uridine phosphatidylnucleosides, both stacking and hydrogen-bonding interactions are present between the bases at the micellar surface. NMR indicates that a H-bonded Watson−Crick adduct is formed despite the exposure of the bases to the highly competitive aqueous environment. This suggests a specific molecular recognition pattern between the complementary bases adenosine and uridine that resembles polynucleotides' behavior.

Introduction

DNA-linked nucleobases are able to undergo molecular recognition through H-bonding in living systems, while isolated nucleosides simply stack in water solutions.[1−3] The pattern of intermolecular interaction is driven by the organization of complementary bases on a covalent skeleton. Bases interact mainly via H-bond with their Watson−Crick partner on the complementary strand, and each nucleic acid strand is greatly stabilized by stacking interactions between neighboring bases.[1,4−6]

A great deal of experimental and theoretical work has been performed in the past decade to model and mimic biological complexity through supramolecular devices.[7−17] Tailoring of supramolecular architecture to display particular assembling properties, functionalities, or/and molecular recognition capabilities is one of the major tasks of soft matter science.[18,19]

Following the enzymatic pathway proposed by Shuto and coworkers,[20−22] we synthesized several phospholiponucleosides varying both the alkyl chain length and the polar headgroup. These new compounds differ from the original lecithin by the presence of a nucleotide moiety that meaningfully alters the thermodynamic aggregation properties with respect to the corresponding lecithins.[23]

The modification of the polar head modulates the recognition properties, while the length and degree of unsaturation of the hydrophobic segment drives the aggregation. We have recently reported the molecular recognition properties of some nucleoside-functionalized phospholipids in monolayers[24] and vesicular aggregates.[25]

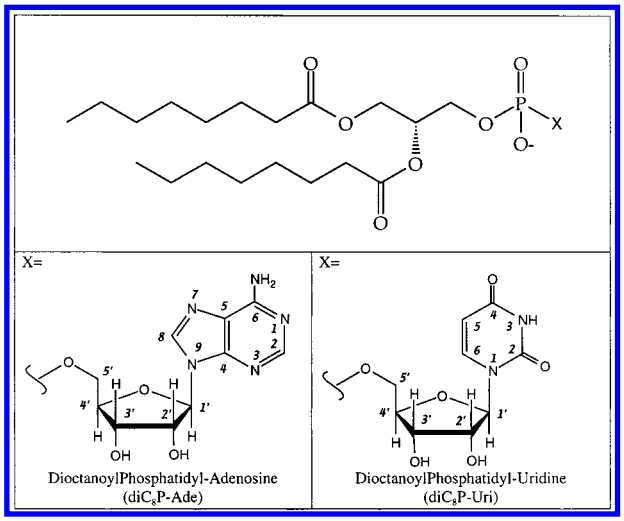

Figure 1. Schematic drawing of the chemical structures of dioctanoylphosphatidyl nucleosides. X can be either the adenosine or uridine complementary bases in ribonucleic acid.

Dioleoylphosphatidyl nucleoside derivatives give stable insoluble monolayer at the air/water interface and prefer to self-aggregate in bilayered structures in aqueous solutions,[25] while short-chain derivatives C_8−C_{12} form direct micellar solutions.[23]

The properties of micellar solutions of diC_8P-adenosine and diC_8P-uridine (the chemical structure is reported in Figure 1) have been studied by small-angle neutron scattering [23] as a function of concentration, pH, and ionic strength (concentration range 3.5×10^{-2} to 1×10^{-2} M; pH range 3.5−7.5; ionic strength range 0−0.25 M). SANS results highlight the presence

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of quasi-spherical micellar structures, whose surface charge depends on the medium pH and salt concentration. The aggregation numbers range from 35 to 45, according to ionic strength and pH. An interesting observation concerns the behavior of the 1:1 mixture of the phospholiponucleosides. The surface charge and the aggregation numbers do not show appreciable deviations from those observed for pure component micelles. However, the average area per polar head is meaningfully decreased for the mixture, indicating that some stabilizing factor must take part in the mixing process, and that specific interactions among the nucleoside polar headgroup are present.

Micelles greatly differ from bilayers or air/water monolayers for their structural and dynamical properties, such as the bigger area per polar head occupied by the surfactant in the micelle, and the fast monomer−micellar aggregate exchange rate (10^{-6} s).[26] However, this exchange does not prevent specific interactions between polar heads.[27,28]

Base−base interactions at the micellar surface might be driven by stacking and/or H-bonding, both necessary for nucleic acid stabilization. It is worthwhile to recall that hydrogen bonding does not provide significant driving force for molecular recognition in water.[29−31] Nowick et al.[32−34] investigated molecular recognition between adenine and thymine derivatives in sodium dodecyl sulfate micellar solutions showing that a H-bonded adduct between the complementary bases is observed within the hydrophobic environment of SDS core, where water solvent cannot penetrate.

In this study, phospholiponucleoside bases, which are located in the hydrophilic corona layer of the micelle, are exposed to water interaction. The increased local concentration due to self-aggregation might favor interactions between bases providing the appropriate environment for molecular recognition, as evidenced from SANS, monolayer, and vesicle investigations for short- and long-chain nucleoside derivatives. We report NMR, UV, and CD findings supporting the presence of specific stacking and H-bonding interactions between adenosine and uridine phospholipo-derivatives in water solution.

Experimental Section

Materials. Dioctanoylphosphatidylcholine (diC_8PC) for phospholiponucleoside synthesis was obtained from Avanti Polar Lipids (USA) and used without further purification. Adenosine and uridine were purchased from Fluka, Milan, Italy. All the reagents used for phospholiponucleoside synthesis and purification were of 99+% purity grade. Phospholipase D-P *ex Streptomyces sp* (E.C.3.1.4.4.) was provided by Genzyme (UK).

NaH_2PO_4 and Na_2HPO_4, were purchased from Fluka. Bidistilled water was purified with a MilliQ water system supplied by Millipore. Adenosine monophospate (AMP) and uridine monophosphate (UMP) were obtained from Sigma Chemicals.

Synthesis of diC_8P-Nucleosides. Short-chain phospholiponucleosides, dioctanoylphosphatidyl-adenosine (diC_8P-Ade) and dioctanoylphosphatidyl-uridine (diC_8P-Uri), were synthesized starting from the corresponding lecithins in a two-phase system, according to the method proposed by Shuto and co-workers for long-chain compounds, and obtained as ammonium salts.[20−22] Separation from the byproducts was achieved by silica gel flash chromatography, as previously reported for DOP-derivatives.[24,25] See also the Appendix.

NMR Spectra of Micellar Solutions. ^{1}H NMR spectra were recorded at 294 K on a Bruker Avance DRX-500 spectrometer operating at 500.132 MHz and equipped with a variable temperature control unit accurate to ±0.1 °C. Chemical shifts are relative to 5×10^{-2} M DSS solution in D_2O as external reference. Samples were prepared by dissolving the compound in 0.50 mL of phosphate buffer 0.1 M in H_2O (pH 7.5). The final lipid or nucleoside−monophosphate concentration was 3.5×10^{-2} M for each sample. The solutions were placed in an Evan's NMR tube, whose inner tube was filled with D_2O to provide a lock signal. ^{1}H NMR spectra were acquired using a Watergate sequence for the minimization of water signal.[35,36] Thirty-two scans of 32 K size were recorded covering the full range (5500 Hz) with a relaxation delay of 1.5 s.

^{1}H NOESY measurements were carried out on the "mixed" 1:1 micellar solution using Watergate minimization, with 1024 increments of size 2 K, mixing times 0.1, 0.2, 0.5, and 1.0 s.

The NMR spectra were processed on a Bruker data station, equipped with an INDY-Silicon Graphics computer using the Xwin NMR Ver. 1.3 software.

UV and CD Absorption. UV−vis spectra were recorded on a double beam Perkin-Elmer Lambda 5 spectrometer, while circular dichroism spectra were recorded on a Jasco J500 spectrometer. Since both base stacking and micellar aggregation are typical cooperative effects, sample dilution has to be avoided, since it deeply affects micellar solutions, eventually leading to the disappearing of micellar aggregates. For both micellar and nucleosides solutions, to avoid signal saturation, 121.000 cylindrical Hellma cells with a 0.005 cm path were used for UV and for CD measurements.

Light Scattering. Critical micellar concentrations were determined monitoring the discontinuity in the intensity of the light scattered at 90° with respect to the incident beam as a function of concentration. The light source was the second harmonic (532 nm) of a Nd:YAG diode pumped laser (Compass 315M diode pump laser, Coherent). The laser power before the sample was about 50 mW, with a power stability better than ±0.5%. The scattered light was filtered and then collected by a Thorn-Emi 96350 photomultiplier. For each sample at least five separated experiments were performed. The error bars are reported on the graphics.

Results and Discussion

Determination of the critical micellar concentration. Critical micellar concentrations have been determined monitoring the intensity of the light scattered by the surfactant solution as a function of the concentration. The intensity of the light scattered at 90° shows two linear regions separated by a discontinuity, corresponding to the formation of supramolecular aggregates.[37]

Figure 2a and b reports the light scattering curves for diC_8P-adenosine and diC_8P-uridine, respectively, in a pH 7.5, 0.1 M phosphate-buffered medium.

The estimated critical micellar concentrations from these data are respectively 5.1×10^{-4} for diC_8P-adenosine and 1.0×10^{-3} for diC_8P-uridine. These values are higher than that of the diC_8-PC in phosphate buffer,[38] 2.4×10^{-4}, as should be expected from a charged surfactant with respect to a zwitterionic one possessing the same hydrophobic portion. In phosphate buffer (pH = 7.5) diC_8P-nucleosides form quasi-spherical micelles with an aggregation number ranging from 35 to 45 in the concentration interval 1×10^{-2} to 3.5×10^{-2} M, as indicated by SANS experiments.[23]

We are interested in comparing the micellization properties of the 1:1 mixed systems with those of the pure components. In fact, the micellar aggregation being an equilibrium process, the presence of preferential interactions can be deduced from the cmc value analysis of mixed systems.[39,40]

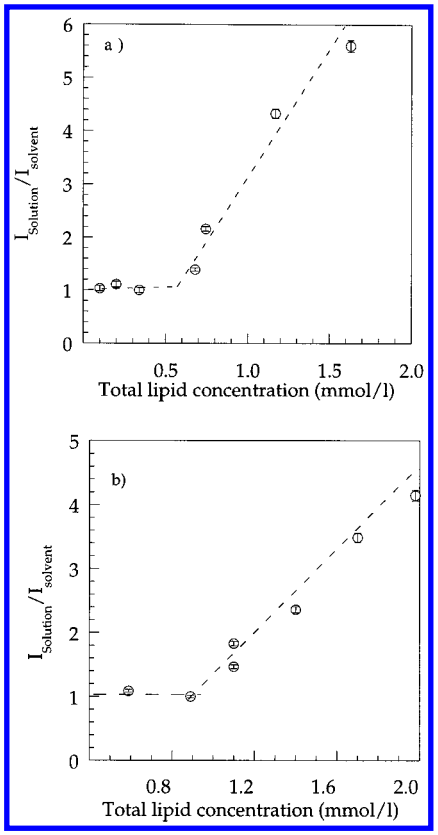

Figure 2. Critical micellar concentrations for diC_8P-adenosine (a) and diC_8P-uridine (b) as obtained from the discontinuity of the two linear regions of the intensity of the light scattered as a function of the surfactant concentration in 0.1 M phosphate buffer solution (pH = 7.5).

According to the simplified pseudophase model for mixed aggregated systems the cmc of the mixed system, C^*_{mix}, is given by

$$\frac{1}{C^*_{mix}} = \sum_{i=1}^{n} \frac{\alpha_i}{f_i C^*_{mix}} \qquad (1)$$

where α_i is the surfactant mole fraction of each component, C_i are the critical micellar concentrations of the pure components, and f_i are the activity coefficients in the mixed micelle. For ideally mixing systems, the activity coefficients are unity, and the cmc of the mixed systems are predictable.

In mixed micelles formed by phospholiponucleoside derivatives with the same chain length, the presence of deviations from ideal behavior can be attributed to specific interactions between polar head.[40]

The cmc of the 1:1 mixture is 5.3×10^{-4} (see Figure 3), which is about 20% lower than that expected from an ideal behavior. This clearly indicates that some attractive mechanism is operating between the adenosine and uridine polar headgroups.

UV and CD Spectra. In Figures 4 and 5 the UV and CD spectra of a 3.5×10^{-2} M (total lipid concentration) 1:1 mixture of diC_8P-nucleosides are shown and compared to the corresponding spectra of the 1:1 mononucleotides. The UV spectrum of bases is sensitive to stacking interactions,[1,3] since two or more neighboring stacking bases give rise to the well-known hypochromic effect. This effect is pronounced in DNA, where the double helix shows an absorption intensity considerably lower than that of the corresponding mixture of isolated while no spectral shift or band broadening with respect to momomer spectra occurs.

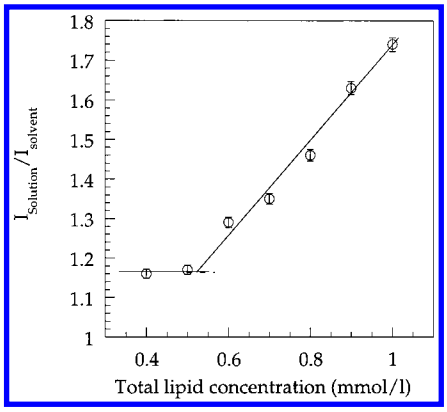

Figure 3. Critical micellar concentration for a 1:1 mixture of diC_8P-adenosine and diC_8P-uridine in 0.1 M phosphate buffer solution (pH = 7.5).

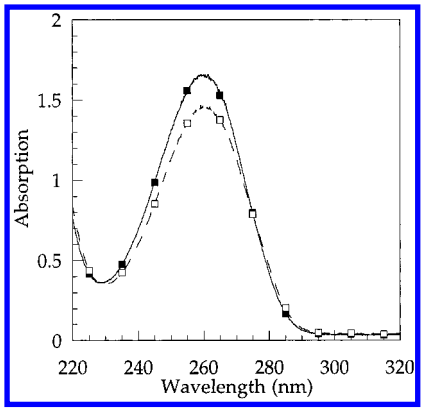

Figure 4. UV spectrum of a 3.5×10^{-2} M diC_8P-nucleoside micellar solution containing an equimolar mixture of diC_8P-adenosine and diC_8P-uridine (open squares), compared to the spectrum of an equimolar mixture of AMP and UMP at the same bulk total concentration (filled squares) in a phosphate buffer 0.1 M (pH = 7.5).

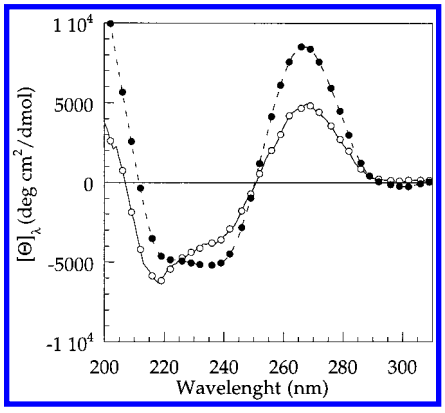

Figure 5. CD spectrum of a 3.5×10^{-2} M diC_8P-nucleoside micellar solution containing an equimolar mixture of diC_8P-adenosine and diC_8P-uridine (filled circles), compared to the spectrum of an equimolar mixture of AMP and UMP at the same bulk total concentration (open circles) in a phosphate buffer 0.1 M (pH = 7.5).

Bases in water are known to stack rather than to hydrogen bond, and even at low concentration a certain degree of base stacking might be present. In fact, the intrinsic molar extinction coefficient for AMP determined by us in 10^{-1} M phosphate buffer is about 10% higher than that relative to the 3.5×10^{-2} concentration under investigation,[41] indicating that part of the bases are in a stacked configuration.

The choice to refer the absorption spectrum of the micellized mixture to the mononucleotide mixture at the same chromophore

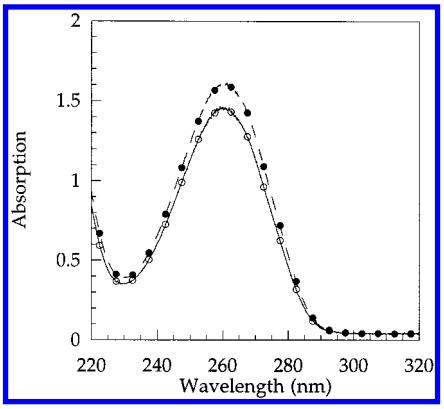

Figure 6. UV spectrum of a 3.5×10^{-2} M diC_8P-nucleoside micellar solution containing an equimolar mixture of diC_8P-adenosine and diC_8P-uridine (open circles), compared to the spectrum obtained averaging single component spectrum of each lipid at the same bulk concentration (filled circles).

concentration is aimed to extract the information about the excess effect of base−base interactions induced by the arrangement in a supramolecular aggregate, at the same pH and ionic strength.

The spectrum of diC_8P-adenosine in micelles shows an 18% hypochromicity with respect to the free nucleotide, indicating stacking among adenosine polar headgroups. This behavior is not displayed by diC_8P-uridine, which presents an UV spectrum identical to the UMP one within the experimental error. It is interesting to note that the UMP absorption coefficient is apparently constant in this concentration range. We have previously reported a similar behavior for DOP-uridine and DOP-adenosine vesicles which, notwithstanding the structural and dynamical differences from their micellar counterparts, show hypochromic segregation for the adenosine derivative, while uridine absorption was not affected by the anchoring to a vesicular aggregate.[25]

The spectrum of the mixture of the two nucleotides (AMP−UMP) is virtually not distinguishable from the spectrum obtained averaging single-component spectra. In the mixed micellar system there is evidence of excess stacking (hypochromic effect) either with respect to the average of the micellar system of the pure phospholiponucleobase (see Figure 6) and to the mixture of nucleoside monophosphates at the same bulk concentration. This effect is particularly remarkable with respect to the monophosphates, while it is slightly smaller with respect to the average of the phospholiponucleobase surfactant.

It is important to notice that the UV molar extinction coefficients of the micellized bases do not change in the surfactant concentration range upon investigation (3.5×10^{-2} M/1.75×10^{-2} M for each component in the mixture) while at lower concentrations (below 1×10^{-3}), the monomer contribution and probably a looser packing constraint start to emerge.

In the concentration range of interest we can predict similar packing conditions for the polar head region, that is, a substantially unvaried local concentration of the bases, as already deduced by us from neutron scattering data.[23]

CD spectra support the pattern deduced by UV absorption. In fact, when an UV hypochromic effect is present, this is accompanied by an increase of the molar ellipticities.[42,43] Again, while the free mononucleotides at 3.5×10^{-2} M do not show any synergic effect when mixed, the micellized bases display an excess of stacking in the mixed surfactant system both with respect to the mononucleotides and with respect to the pure phospholiponucleoside system. A detailed description and interpretation of CD spectra is well beyond the aim of this work, but evidence of stacking even for the mononucleotides, particularly for AMP, is worthwhile to be stressed.[44]

TABLE 1: ^{1}H NMR Chemical Shifts of Base Moiety Protons in Micellar Solutions[a,b]

	diC_8P-Ade		diC_8P-Uri		diC_8P-Ade + diC_8P-Uri[c]	
proton	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$
H_8^A	8.38	−65.0			8.41	−64.8
H_2^A	8.05	−42.5			8.14	−17.3
NH_2^A	6.88	+61.6			6.92	+68.3
H_6^U			7.92	−71.8	7.90	−76.0
H_5^U			5.89	−21.8	5.85	−37.6

[a] In ppm. 500.132 MHz, 294 K, phosphate-buffered H_2O solutions pH = 7.5, total concentration 3.50×10^{-2} M. [b] $\Delta\delta = \delta$(micelle) − δ(monophosphate) = chemical shift difference from the corresponding monophosphate in Hz. Downfield shifts reported as positive. [c] 1:1 mixture.

NMR Monodimensional ^{1}H Spectra. ^{1}H NMR is one of the techniques most frequently used to investigate molecular recognition between biologically relevant molecules, particularly nucleobases.[45−51] A great deal of experimental and theoretical work has been performed to elucidate base−base interactions by this technique and to discriminate between stacking and H-bonding interactions. Nowadays the effect of base pairing on the chemical shifts of the base protons is fully assessed. Stacking causes an upfield shift of the resonances of the base protons, while H-bonding causes a downfield shift.[2,52−54]

In this work the ^{1}H NMR properties of diC_8P-uridine and diC_8P-adenosine micelles have been investigated in order to determine whether molecular recognition in these systems may be evidenced by this technique.

Nondeuterated water was employed to allow the detection of H-bond participating base protons that are inherently acidic.[51,53,55] It is worth recalling that, as a rule of thumb, non-H-bonding solvents allow monomer−monomer recognition, while H-bonding solvents are strong competitors for the bases, which predominantly interact by a π-stack mechanism in these media.[1,2] The ^{1}H NMR spectra of the "micellized" base derivatives have been compared to those of the corresponding monomer monophosphate in the same experimental conditions. This interpretative approach allows to directly infer if the micellar supraorganization of phospholiponucleosides leads to base−base interaction patterns different from bulk water. The effect of micellization in this case would not be merely that of providing a local excess concentration, because this condition might not be sufficient to justify base−base attractive interactions such those observed in nucleic acids. Stacked adducts between monomers determine in fact an increase of local concentration, but water is still a too strong competitor for H-bonding.

The ^{1}H NMR chemical shifts for the base protons of diC_8P-uridine and diC_8P-adenosine micellar solutions and their 1:1 mixture are reported in Table 1. The numbering scheme adopted is that reported in Figure 1 and commonly accepted for nucleosides.[1] The assignment of the resonances of the micellar solutions is straightforward from the comparison with the spectra of the corresponding monomers in DMSO-d_6 (Appendix) and with those of the corresponding monophosphates in water.

The H_8 and H_2 adenine protons resonate at 8.38 and 8.05 ppm, respectively, while the protons belonging to the amino group appear at 6.88 ppm. The uracil H_6 and H_5 protons resonate at 7.92 and ca. 5.89 ppm, respectively.[56]

A significant upfield shift of the resonances of the base protons is displayed comparing the spectra of the micellar solutions with those of the corresponding monophosphate

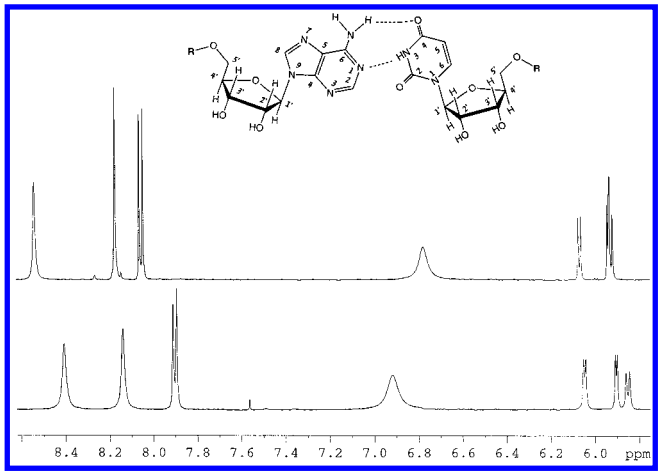

Figure 7. ^{1}H NMR spectra (500.132 MHz, 294 K, H_2O, pH = 7.5, 3.5×10^{-2} M) of AMP/UMP = 1/1 mixture (upper) and diC_8P-adenosine/diC_8P-uridine = 1/1 micellar solution (lower). In the inset Watson−Crick H-bonding for adenosine and uridine is reported.

derivatives at the same concentration (Table 1, columns 3 and 5). The H_8 and H_2 protons of adenine show a $\Delta\delta$ shift of −65.0 and −42.5 Hz, respectively, on passing from AMP to diC_8P-adenosine micelles, whereas the H_6 and H_5 protons of uracil are shifted upfield by −71.8 and −21.8 Hz, respectively, comparing diC_8P-uridine micelles to UMP. A very interesting observation concerns the NH_2 amino protons that experience a remarkable downfield shift, $\Delta\delta$ = +61.6 Hz, upon micellization. These data clearly show that strong base−base interactions occur as a direct consequence of the supraorganization due to the micellization process. Both stacking and H-bonding interactions are active.[57] The chemical shift values observed in the micellar solutions will therefore be due to a balance of the contribution arising from both mechanisms.[58] Interestingly, as for DNA/RNA linked nucleobases, stacking is prevailing on the H-bonding contribution for the ring protons, whereas it is comparatively negligible for the protons directly involved in the hydrogen bond.[59]

An analogous behavior is displayed by the comparison of the 1:1 mixed micellar solution diC_8P-adenosine/diC_8P-uridine with the 1:1 mixture AMP−UMP at the same total concentration (Table 1, column 7, and Figure 7). Particularly, the NH_2 amino proton chemical shift difference ($\Delta\delta$ = +68.3 Hz) indicates that strong H-bonding interactions still occur as a consequence of the micellization process. To ascertain a real Watson−Crick molecular recognition, one should be able to discriminate whether this interaction preferably takes place at an hetero or at an homo base pair, since both these adducts have been reported for nucleosides in solid state.[1] A simple balance criterion may address this point. It is a well-recognized feature that the relative strength of the base-pair hydrogen bonds decrease in the order adenine-uracil > adenine−adenine ∼ uracil−uracil.[49,60−65] On the other hand, evidence for base−base stacking in our concentration range has already been reported, and the tendency is purine−purine > pyrimidine−purine > pyrimidine−pyrimidine.[66−68] Thus, the increase of the $\Delta\delta$ value for the amino protons on passing from the pure diC_8P-adenosine to the "mixed" micellar solution is ascribable to the complementary base-pairing which carries a favorable H-bonding contribution, even if a weak destacking contribution due to adenosine moiety dilution might be expected.

Watson−Crick and Hoogsten pairs are formed with more or less equal frequency. The NMR results reported in this work do not give a definite indication on which pair is preferred in micelles. We cannot exclude a priori the presence of both interaction modes (Watson−Crick and Hoogsten). We recall that an adenosine molecule can form an 1:2 adduct with uridine where both H-bonding patterns are displayed.[23,69] However, the lower upfield shift observed for $H_2{}^A$ with respect to $H_8{}^A$ supports a Watson−Crick base pairing mechanism, since it might result from simultaneous upfield shift derived from stacking and downfield shift deriving from H-bonding. If the geometry of the base pair is of Watson−Crick type, it is clearly the H_2 proton the most affected by the shielding effect.

We are addressing this point using 2D-NMR (see also below) and ^{13}C relaxation experiments.

Similarly, for the ring protons the increased downfield and upfield shifts observed for adenosine and uridine phospholipids, respectively, passing from the pure components to the mixed micellar solution are ascribable to unfavorable and favorable stacking contributions, respectively.

A very interesting feature is a consistent line broadening for diC_8P-adenosine ring protons observed in passing from "pure" micelles to the 1:1 mixture. Since a line broadening is not expected to be due to self-stacking,[1] which is actually present in the homopurine micelles, this effect might be ascribable to the presence of H-bond adducts with a slow breakdown rate with respect to the NMR time scale.[70]

Preliminary ^{1}H NOESY measurements[71] carried out on the "mixed" 1:1 micellar solution show that under our experimental conditions the micellar system falls in the *slow tumbling* motional regime ($\tau_c \gg 1/\omega_L$) and only negative NOEs are observed.[72−76] Well-resolved intramolecular cross peaks are displayed which accounts for a preferred time-averaged anti conformation of both adenosine and uridine moieties.[77] For example, medium-intensity NOE of the adenine H_8 proton are displayed with both H_1' and H_2' ribose protons whereas H_2 shows only a weak NOE interaction with H_1'. The H_6 proton of uracil show medium-intensity NOE with both H_1' and H_2' protons of ribose.[78] The lack of the detection of the uracil N_3−H proton at the pH investigated prevented us to draw out further conclusions on the nature of the base−base coupling pathway.

Concluding Remarks

The system under investigation has been approached by a number of different experimental techniques, namely light scattering, CD and UV absorption, and NMR spectroscopy. The results have to be compared in order to give an overall picture, since each technique is able to give information on different mechanisms that may be acting on the micellar system.

First of all, it is worthwhile recalling that both CD and UV absorption of nucleobases are directly related to stacking and usually give no information about H-bonding that might well be present, but not detected. Our experimental data indicate excess stacking for the micellized chromophore, particularly for diC_8P-Ade and, more important, excess stacking for the 1:1 surfactant mixture either with respect to the mononucleotide mixture or with respect to the averaged pure component micellar systems.

This observation is very interesting for the interpretation of NMR data. In fact, if we keep in mind that the mixture presents excess stacking with respect to the mononucleotide spectra, the downfield shifts observed can only be attributed to H-bonding, since a stacking deficiency due to base dilution can be ruled out.

Cmc values for the mixed and the pure micellar systems reveal the presence of specific interactions between polar heads in the 1:1 micellar system. The characterization of the micellar

solutions, performed by small-angle neutron scattering and reported elsewhere, indicates that mixed micellar solutions are very similar to pure component ones, since the aggregation number and the surface charge do not change to an appreciable extent. A careful analysis reveals higher packing constraints for the polar heads in the mixture at pH 7.5.

All this experimental evidence definitely points toward the presence of base−base interactions through stacking and H-bonding at micellar surface.

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Appendix

Synthesis of diC$_8$P-adenosine. 1.5 mg of PLD-P (300 units) was dissolved in 5 mL of acetic buffer 0.2 M (pH 5.5), containing 0.25 M $CaCl_2$, where 3.4 mmol of adenosine had previously been dissolved. 15 mL of $CHCl_3$ containing 0.7 mmol of diC$_8$PC was then added and the mixture was stirred at 40 °C under gentle Ar flow. After 5 h, 5 mL of 2 M HCl, 20 mL of $CHCl_3$, and 10 mL of MeOH were added, the mixture was shaken and the organic phase collected and washed with 5 mL of water and then evaporated to dryness. Silica gel flash chromatography ($CHCl_3$:MeOH:NH_4OH = 120:30:1 → 120:60:2.5) afforded the desired product in acidic form. The collected fractions were evaporated to dryness and washed with $CHCl_3$ (20 mL), MeOH (10 mL), and HCl 0.5 N (5 mL). The organic phase was washed with 5 mL of water, an excess of NH_4OH was added, and the solvent was evaporated. The product was obtained with a 60% yield.

^{1}H NMR (DMSO-d_6): δ = 0.95 (t, J = 8.9, 6 H, CH_3), 1.32 (m, 16 H, CH_2 aliphatic), 1.58 (m, 4 H, CH_2−CH_2−COO), 2.33 ÷ 2.37 (m, 4 H, CH_2−COO), 3.84 ÷ 3.95 (4 H, *sn*-3-CH_2, H5′/5″), 4.10 ÷ 4.20 (2 H, H4′, *sn*-1-CH_2), 4.29 (t, 1 H, H3′), 4.37 (dd, J_1 = 11.0, J_2 = 3.3, 1 H, *sn*-1-CH_2), 4.69 (t, 1 H, H2′), 5.15 (m, 1 H, *sn*-2-CH), 5.4 ÷ 5.7 (bs, 2 H, 2′ OH e 3′ OH), 6.1 (d, J = 5.7, 1 H, H1′), 7.0 ÷ 7.3 (bm, 6 H, NH_4^+, NH_2), 8.2 (s, 1 H, H2), 8.5 (s, 1 H, H8).

^{31}P NMR (DMSO-d_6): δ = − 0.54.

Synthesis of diC$_8$P-uridine. 1.5 mg of PLD-P (300 units) was dissolved in 5 mL of acetic buffer 0.2 M (pH 5.5), containing 0.25 M $CaCl_2$, where 16 mmol of uridine had previously been dissolved. 15 mL of $CHCl_3$ containing 0.8 mmol of diC$_8$PC was then added and the mixture was stirred at 40 °C under gentle Ar flow. After 6 h, 5 mL of HCl 2 M, 20 mL $CHCl_3$, and 10 mL of MeOH were added, the mixture was shaken, and the organic phase collected and washed with 5 mL of water and then evaporated to dryness. Silica gel flash chromatography ($CHCl_3$:MeOH:NH_4OH = 120:30:1 → 120:60:2.5) afforded the desired product in acidic form. The collected fractions were evaporated to dryness and washed with $CHCl_3$ (20 mL), MeOH (10 mL), and HCl 0.5 N (5 mL). The organic phase was washed with 5 mL of water, an excess of NH_4OH was added, and the solvent was evaporated. The product was obtained with a 79% yield.

^{1}H NMR (DMSO-d_6): δ = 0.97 (t, J = 6.6, 6 H, CH_3), 1.35 (m, 44 H, CH_2 aliphatic), 1.62 (m, 4 H, CH_2−CH_2−COO), 2.35 ÷ 2.40 (m, 4 H, CH_2−COO), 3.82 ÷ 3.89 (4 H, *sn*-3-CH_2, H5′/5″), 4.02 (t, 1 H, H2′), 4.10 (t, 1 H, H3′), 4.17 ÷ 421 (2 H, H4′, *sn*-1-CH_2), 4.39 (dd, J_1 = 14.0, J_2 = 3.2, 1 H, *sn*-1-CH_2), 5.16 (m, 1 H, *sn*-2-CH), 5.48 (bs, 2 H, 2′ OH e 3′ OH), 5.67 (d, J = 8.1, 1 H, H5), 5.90 (d, J = 6.4, 1 H, H1′), 7.20 (b, 4 H, NH_4^+), 8.04 (d, J = 8.1, 1 H, H6), 8.5 (bs, 1 H, NH).

^{31}P NMR (DMSO-d_6): δ = − 0.09.

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(56) The uracil N_3−H imino proton is not detected in our experimental conditions in the pH range 7.5−6.0, as well as in the temperature range 274−304 K.

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(58) The chemical shifts of the ribose protons are not reported in Table 1 as they show a modest dependence on micellization ($\Delta\delta$ less than −10 Hz) This is consistent with the fact that they are not directly involved in stacking or H-bonding processes. The H_1' sugar proton can be observed at 5.91 and 6.03 ppm, respectively, in diC_8P-uridine and diC_8P-adenosine micelles.

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