Molecular Recognition between 2,4,6-Triaminopyrimidine Lipid Monolayers and Complementary Barbituric Molecules at the Air/Water Interface: Effects of Hydrophilic Spacer, Ionic Strength, and pH

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Previously it was shown that molecular recognition occurs between complementary vesicles containing either a lipid with a 2,4,6-triaminopyrimidine (TAP) or with a barbituric acid (BAR) head group separated from the alkyl chains by an tetraoxyethylenic spacer. In view of better understanding the nature of the interaction, Langmuir monolayers and LB films of these complementary lipids are investigated. The selective binding of the TAP−Lipid with various barbituric acids is studied by π/A isotherms, Fourier transform infrared spectroscopy, X-ray photoelectron spectroscopy, atomic force microscopy, and fluorescence microscopy. The effects of the subphase pH and ionic strength on this molecular recognition point out an electrostatic contribution due to the acido−basic properties of TAP and BAR in addition to an hydrogen bonding interaction. The comparison with a melamine compound (diC_{12}mela) shows that this recognition is still efficient in the presence of the tetraoxyethylenic spacer.

I. Introduction

Molecular recognition in artificial systems provides a basis for understanding the mechanisms of the specific recognition between membranes in living organisms. Besides its biological relevance as a model, the elaboration of artificial membrane systems and the control of interactions should allow for the generation of well-defined molecular systems possessing suitable recognition features.[1−4]

Langmuir monolayers have been successfully used as a simple model of lipidic cell membranes and tend to mimick biological phenomena such as enzymatic action,[5,6] crystallization,[7] and molecular recognition.[8−11] In the solid state, 2,4,6-triaminopyrimidine (TAP) and melamine derivatives have been found to bind complementary barbituric acid (BAR) compounds forming an organized ribbon-helix superstructure in which each unit is associated with the two neighborning ones through two arrays of three hydrogen bonds.[12,13] One of our groups has shown previously that melamine monolayers can bind barbituric guest molecules at the air/water interface and that the preoriented monolayer could play an important role in the formation of an H-bond network at the air/water interface.[14] Furthermore, it was shown that TAP guest molecules are selectively bound to BAR lipid monolayer by inducing a chemical reaction at the interface in some cases according to their molecular structure.[15] The molecular structure and organization at the interface resulting from the molecular recognition are currently in question.

We have focused our interest on complementary molecular systems involving electrostatic or hydrogen bonding interactions in aqueous solution.[16] In one of our groups, it was shown that complementary vesicles containing either TAP or BAR lipids interact selectively by

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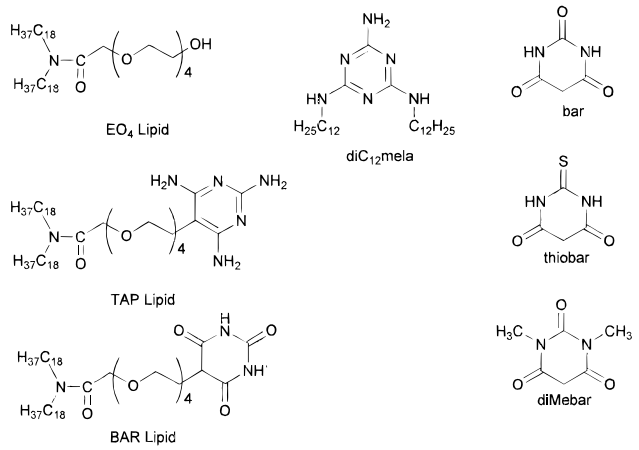

Figure 1. Chemical structures of the TAP, BAR, and EO_4 lipids and of the barbituric acid molecules used.

inducing aggregation and fusion.[16b] In view of a better understanding of the nature of the interaction involved in these observed phenomena, we have chosen to study the monolayer properties of the TAP and BAR synthesized lipids and the ability of a TAP lipid monolayer to bind complementary BAR derivatives from the subphase. We report here the study of the monolayer behavior of the TAP, BAR, and EO_4 lipids (see Figure 1). Furthermore, the nature of the interaction between a TAP monolayer and BAR derivatives in the subphase was investigated through several complementary techniques. Because of the acido−basic properties of the 2,4,6-triaminopyrimidine, melamine, or barbituric acid groups,[17] an electrostatic contribution is expected to play a role in the interaction. For this reason, we have investigated the effect of pH and ionic strength on the molecular recognition properties (see Figure 2).[18] The behavior of the TAP lipid monolayer, bearing a hydrophilic spacer between the recognition group and the alkyl chains, was compared with that of a melamine compound (diC_{12}mela) already known.[14]

II. Experimental Section

A. Materials. All the barbituric derivative guest molecules were commercially supplied (Wako Pure Chem) and used without further purification: barbituric acid (bar), 2-thiobarbituric acid (thiobar), *N*,*N*′-dimethylbarbituric acid (diMebar). Octadecyl rhodamine B (Rh-C_{18}), used in the experiment as a fluorescence probe was purchased from Molecular Probes. DiC_{12}mela compound was prepared by following the procedure described in the literature.[14] The preparation method of the synthetic TAP, BAR, and EO_4 lipids (see Figure 1) is briefly reported in our previous paper[16b] and will be described in detail elsewhere. Their structures and purities were confirmed by ^{1}H and ^{13}C NMR, elementary analyses, and mass spectroscopies. The Britton−Robinson buffers at pH 4, 5, 7, and 9 and at a 0.1 M ionic strength were prepared according to the literature.[19] The water used for the subphase was deionized and doubly distillated by a Nanopure II-4P abd Glass Still D44 system (Barnstead). Spectroscopic-grade benzene and ethanol (Wako) were used as spreading solvents. Gold (99.999%) and chromium (99.99%), used for coating the surface of substrates, were purchased from Soekawa Chemicals.

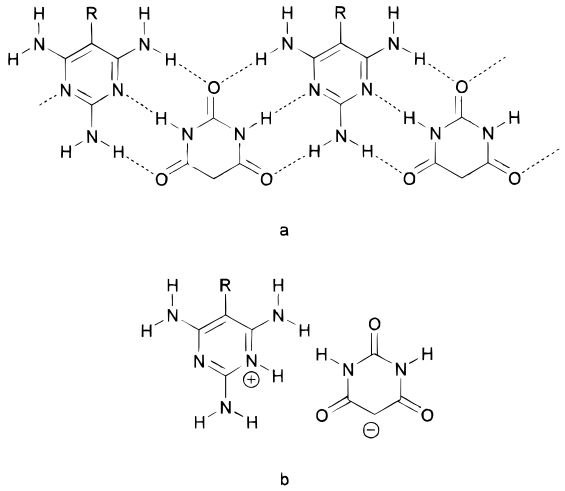

Figure 2. Possible TAP/BAR binding via (a) hydrogen bonding and (b) electrostatic interaction.

B. π−*A* Isotherms Measurements and LB Transfer. π−*A* isotherms were measured with a computer-controlled film balance system FSD-50 (USI System, Fukuoka). The reproducibility was checked in each case. A mixture of benzene/ethanol (8/2) was used as a spreading solvent. Compression was started 10 min after spreading at the rate of 55 $mm^2 \cdot s^{-1}$. The subphase temperature was maintained at 20 ± 0.2 °C. The surface pressure was measured by a Wilhelmy plate, which was calibrated with the transition pressure of a stearic acid monolayer.

LB films were transferred onto gold-deposited glass plates for reflection−absorption Fourier transform infrared (FT-IR) spectroscopy. The substrate was prepared as follows: a slide glass (precleaned, 176 × 26 × 1 mm, Iwaki Glass) was immersed in a detergent solution (Dsn 90, Bokusui Brown Co.), overnight. The glass was then washed with a large excess of ion-exchanged water to remove the detergent completely and subjected to sonication in fresh ion-exchanged water several times. After the glass was dried under a vacuum for over 1 h, thin layers of chromium and gold were consecutively formed by a vapor-deposition method (1000 Å Au/50 Å Cr/slide glass) with a vapor-deposition apparatus VPC-260 (ULVAC Kyushu). Langmuir−Blodgett (LB) transfer of 10 monolayers was carried out with a FSD-21 instrument (USI System, Fukuoka) by the vertical dipping method. Monolayers were transferred on Au-coated glass plates at 50 $mN \cdot m^{-1}$ (for TAP, BAR, and EO_4 lipids) and at 15 $mN \cdot m^{-1}$ (for diC_{12}mela) with dipping speeds of 100 $mm \cdot min^{-1}$ (down stroke) and 20 $mm \cdot min^{-1}$ (up stroke). Transfer ratios were almost equal to 1, except in the case of diC_{12}mela compound particularly in the presence of a buffer as subphase. These monolayers were not stable against collapse and/or dissolution during the LB transfer. The transferred films were returned to water in the subsequent downstroke process (usually the third transfer process).

C. Characterization of LB Films. FT-IR spectra (reflection−absorption spectrum (RAS) mode) were measured with LB films (10 layers) transferred on gold-deposited glass plates with a Nicolet 710 FT-IR spectrometer.

X-ray photoelectron spectra (XPS) were measured for the LB films (10 layers) on Au/Cr/glass with a Perkin-Elmer PHI 5300 ESCA. The X-ray source was Mg Kα (300 W). Repeated scans over the same surface region at a takeoff angle of 45° gave reproducible spectra. The elemental composition was obtained by dividing the observed peak area by the intrinsic sensitivity factor of each element.

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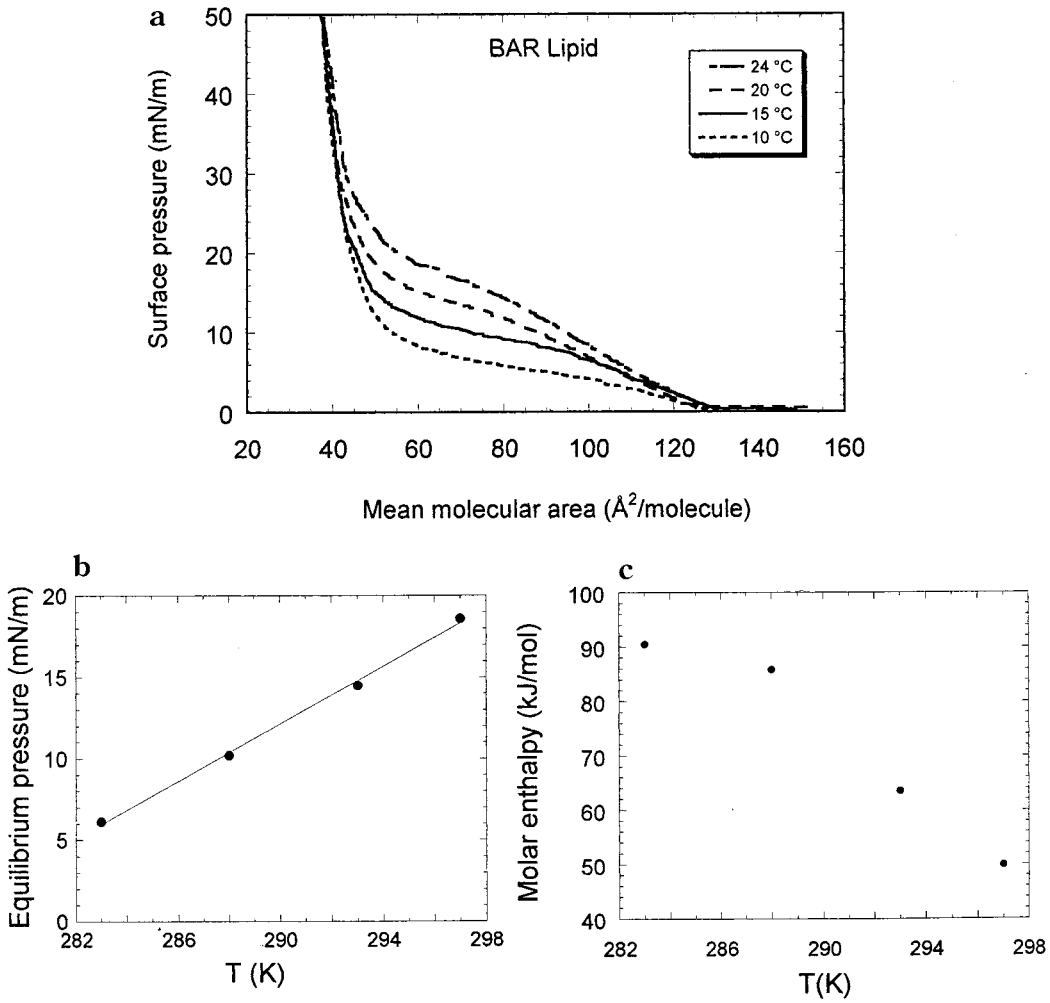

Figure 3. (a) Surface pressure−area ($\pi-A$) isotherms of a BAR monolayer on pure water and at various temperatures: 10 °C; 15 °C; 20 °C; 24 °C. (b) Equilibrium surface pressure $\pi_t(T)$ of the LE−LC transition against the temperature, fit: $\pi_t(T) = -244 + 0.88$ T. (c) Molar enthalpy $\Delta H_t(T)$ of the LE−LC transition against the temperature.

D. Fluorescence Microscopy and AFM Measurements. Measurements of the $\pi-A$ isotherms and fluorescence imaging in situ were carried out at 20 °C by using a microprocessor-controlled Langmuir film balance (USI System, FSD-50) equipped with an epifluorescence microscope (Olympus, BHS−RFK), SIT camera (Hamamatsu, C2741), and a film lift.[20] Langmuir−Schäffer transfers were carried out by horizontally attaching the monolayer at 14 mN·m^{-1} onto a micro coverglass slide (Matsunami, Japan) hydrophobized by trimethylchlorosilane. Fluorescence and atomic force microscopy (AFM) imaging of the transferred films were carried out by using a fluorescence microscope (Olympus NV2100) equipped with a AFM cantilever (Point probes, Germany), with a spring constant of around 3.5 N·m^{-1} and a resonance frequency of around 70 kHz in ac mode and in the air.

III. Results and Discussion

A. Thermodynamic Properties and Charge Effect of the Monolayers. 1. $\pi-A$ Isotherms at Different Temperatures. $\pi-A$ isotherms of TAP, BAR, and EO_4 lipids monolayers, the chemical structures of which are shown in Figure 1, were measured at various temperatures on pure water. The EO_4 lipid was also prepared to dilute the TAP or BAR lipids in an electrically neutral membrane and to show the specificity of the headgroup interaction.[16b,21] The case of BAR lipid is shown in Figure 3a. The curves obtained exhibited similar shapes, and the behavior according to the temperature is the same for all the lipids. One can observe an almost horizontal plateau corresponding to the transition from a liquid expanded (LE) phase to a liquid condensed (LC) phase, characterized respectively by a weak then a strong compressibility modulus. The collapse pressures of these lipids are very high varying from 60 to 70 mN·m^{-1}, which reflects the good stability of the monolayers. From these curves, one can deduce the equilibrium pressure π_t at the LE−LC transition corresponding to the coexistence of the LC and LE phases for each temperature. Assuming the Clapeyron relation in two dimensions,[22] the LE−LC transition entropy $\Delta S_t(T)$ can be calculated from the slope of the plot of the equilibrium pressure π_t against the temperature (see Figure 3b)

$$\Delta S_t(T) = \Delta a(T) \left(\frac{d\pi_t}{dT}\right)(T) \qquad (1)$$

where $\Delta a(T) = a_{LC} - a_{LE}$ and a_{LC} and a_{LE} are the mean molecular areas respectively in the LC and LE phases.[23] One can then deduce the LE−LC transition enthalpy $\Delta H_t(T)$ from the following relation at the equilibrium of

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Table 1. Enthalpies ΔH_t(20 °C) of the Transition LE−LC or Lipid Suspension

lipid	EO_4	TAP	BAR
T_t (°C)[a] ± 0.2	43.8	37.0	39.2
ΔH_t (kJ·mol^{-1})[a] ± 2	45	38	40
ΔH_t (kJ·mol^{-1})[b] ± 10	30	55	65

[a] Evaluated from DSC measurements on aqueous lipid suspensions. [b] Evaluated from the $\pi-A$ isotherms of the monolayers at 20 °C.

the LE and LC phases (see Figure 3c)

$$\Delta H_t(T) = T\,\Delta S_t(T) \qquad (2)$$

The enthalpy of the LE−LC transition at 20 °C is found to be around 30 to 65 kJ·mol^{-1}. Furthermore, a differential scanning calorimetry (DSC) study has been carried out on aqueous suspensions of each pure lipid. The corresponding DSC diagrams exhibit a transition whose temperature and enthalpy were determined for each lipid (see Table 1). Both techniques show a transition of the chains. The values obtained for the transition enthalpies are in the same range order as those obtained from the Langmuir monolayers. The previous results show that these synthesized amphiphiles present a transition from a LE phase to a LC phase in which associated entropy and enthalpy appear to depend weakly on the nature of the headgroup, particularly in the case of the aqueous suspensions.

These observations indicate that the packing of the alkyl chains controls the transition observed by DSC and in Langmuir films. Furthermore, the headgroups are separated from the hydrophobic chains by a flexible spacer and appear to be decorrelated from the alkyl chains. Investigations of phosphocholine where the headgroups are decoupled from the chains by EO-spacers of different length have been previously done. It has been shown that the longer the EO-spacer is, the less the chain lattice is influenced by the headgroup.[24] Whereas the TAP lipid shows a LE−LC transition, the diC_{12}mela lipid is crystalline at room temperature.[14] These observations indicate that the tetraoxyethylenic spacer fluidizes the monolayer.

2. pH Effect on the $\pi-A$ Isotherms and the LB Films. In view of the acido−basic properties of BAR, TAP lipids, and melamine derivatives (see Figure 2),[17] the observed $\pi-A$ isotherms of the BAR, TAP, and diC_{12}-mela monolayers are expected to vary with the subphase pH and the ionic strength that control the charge of the headgroups. At a 0.1 M ionic strength, the surface charges are strongly screened. So the pH near the surface is the same as that in bulk. Under such conditions, one can study the only pH effect onto the monolayer. The $\pi-A$ isotherms of the diC_{12}mela monolayer at different pH in the presence of various buffers and at a 0.1 M ionic strength are shown in Figure 4a. One can observe an expansion of the mean molecular area when pH decreases. Indeed, by decreasing the pH, the protonation of the melamine group occurs (melamine, pK_a = 5) and the electrostatic repulsion between the charged headgroups within the monolayer causes an increase in the mean molecular area, as observed for example in the case of phosphatidic acid.[25] The Debye length is around 1 nm at a 0.1 M ionic strength, so that the repulsion between two neighboring headgroup within the monolayer is still effective. Conversely, in the case of TAP and BAR lipids, no monotonic change in mean molecular area is observed according to the subphase pH as shown respectively in parts b and c of Figure 4. This observation indicates that there is a loss of couplage between the headgroups and the chains. That is the reason we have studied the pH effect also onto the LB films by FT-IR.

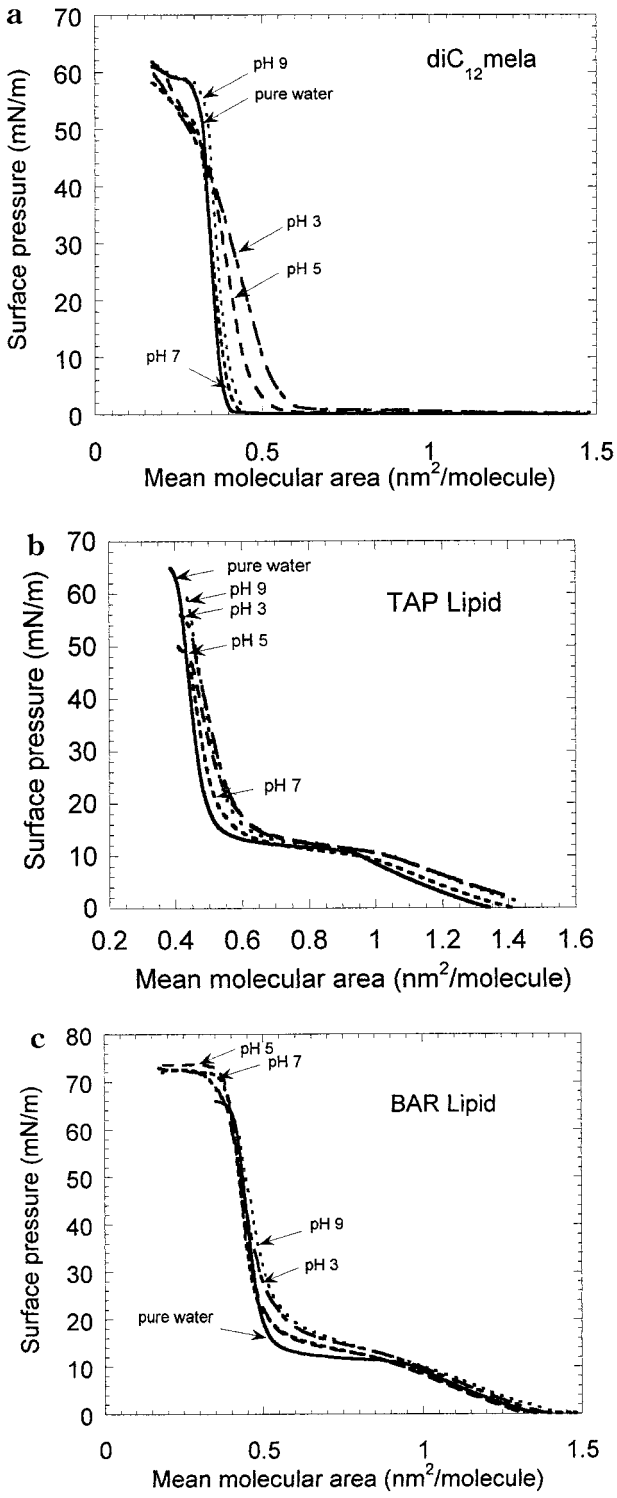

Figure 4. $\pi-A$ isotherms at 20 °C on buffers of various pH 3, 5, 7, 9 and of a 0.1 M ionic strength of the following monolayers: (a) diC_{12}mela; (b) TAP; (c) BAR.

From FT-IR spectra of the corresponding LB films (see Figure 5), one can observe some significant changes in agreement with protonation of the TAP lipid (2,4,6-triaminopyrimidine, pK_a = 6.8) at pH 3 and the deprotonation of barbituric acid (barbituric acid, pK_a = 4.1) at

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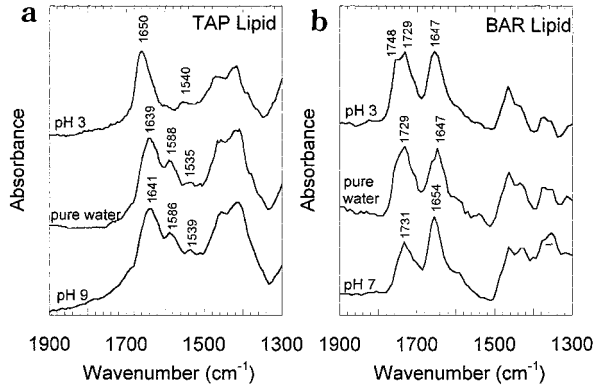

Figure 5. FT-IR spectra of the LB films transferred from buffers of various pH and of a 0.1 M ionic strength of the following lipids: (a) TAP; (b) BAR.

pH 7.[17] The FT-IR data show that TAP and BAR lipids are charged depending on the pH. The IR spectra of TAP and BAR lipids transferred from pure water or various pH buffers show one peak around 1650 cm^{-1}, which is attribuated to the tertiary amide C═O itself. In the case of the TAP lipid, one observes a peak at 1588 cm^{-1}, which may be assigned to the C═N stretching bond of pyrimidine ring and which is not visible at pH 3, where the C═N group is protonated as shown in Figure 2.[26] In the case of the BAR lipid, a band is found around 1730 cm^{-1} whatever the pH, which is assigned to the C═O of the BAR ring. At pH 7 or in water, where the BAR lipid is partially deprotonated, the barbiturate form does not exhibit two distinguable peaks for the C═O stretching bond of the BAR ring. At pH 3, where the barbituric acid is not deprotonated, one additional peak at 1748 cm^{-1} is observed, due to the carbonyl C═O in positions 4 and 6.[27]

In conclusion the pH study shows that these compounds may be partially charged depending on the pH. The mean molecular area is influenced more strongly by the repulsion between the charges within the monolayer when no spacer is present. Indeed the spacer appears to decouple the headgroups from the alkyl chains so that the headgroup charge is not the main factor controlling the mean molecular area in the case of the TAP and BAR lipid monolayers.

B. Molecular Recognition between Monolayer and Solutes in the Subphase. 1. Morphological Changes of a TAP Monolayer Investigated by Fluorescence Microscopy and AFM. To examine the TAP/BAR complex resulting from the interaction of a TAP lipid monolayer and complementary barbituric acid molecules in situ, we have studied the morphological changes in TAP monolayer induced by the addition of barbituric acid in the subphase.

In the first step, we obtained a direct picture of the phase diagram of the TAP monolayer by epifluorescence microscopy at the air/water interface. A fluorescent dye probe (Rh-C_{18}) was incorporated into the TAP lipid monolayer and the lateral distribution was measured from the analysis of the fluorescence micrographs. A high contrast was obtained due to the different dye solubilities in the coexisting phases. Indeed, by fluorescent microscopy at the air−water interface, one can observe the appearance of dark areas ascribed to crystallized domains with a low dye solubility in the plateau region. Similar textures were obtained from horizontally transferred monolayer of TAP lipid (by the LS technique) at the range of the plateau as shown in Figure 6a. This technique tends to confirm clearly the existence of a transition from a LE to a LC phase observed during the compression of the Langmuir TAP monolayer and that no large reorganization occurs during the transfer.

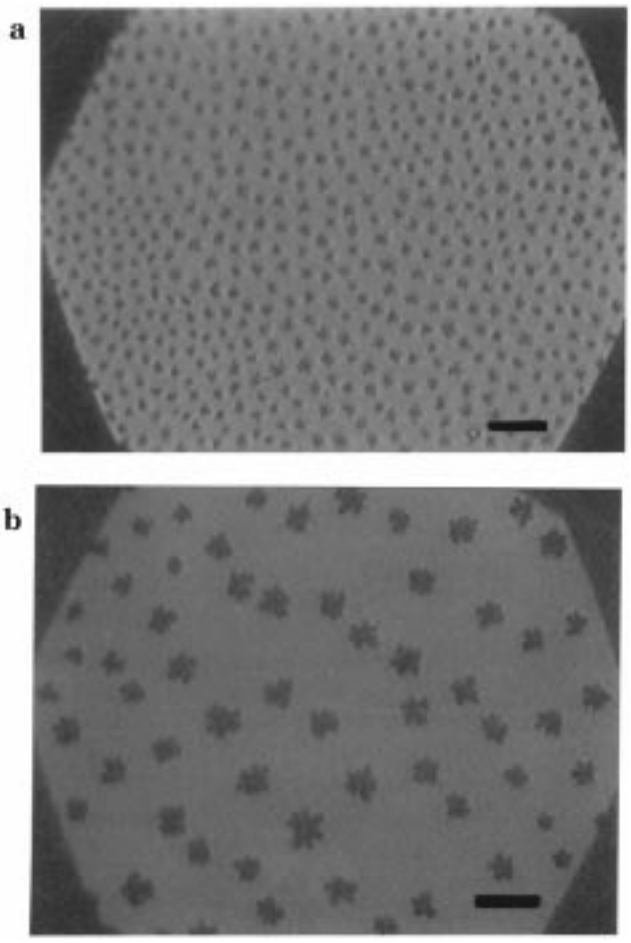

Figure 6. Fluorescence microscopy pictures of the transferred TAP lipid Langmuir−Schäffer (LS) film containing 1% of Rh-C_{18} at 14 $mN \cdot m^{-1}$: (a) from pure water; (b) from a 10 mM barbituric acid solution. The bar represents 40 μm.

The addition of barbituric acid in the subphase causes an interesting morphological change in the shape of the crystallized domains. Whereas structures such as small rough disks were observed in the case of TAP only, the addition of barbituric acid in the subphase changes the dark area in clover leaves as shown in Figure 6b. This morphological change is ascribed to result from the competition between nucleation and crystallization processes.[31] Figure 7 presents the corresponding AFM pictures of these domains measured in ac mode after a LS transfer onto hydrophobized glass. The crystallized domains are shown to be flat and two-dimensionnal even if barbituric acid is present in subphase. A thickness difference of 9 Å between the crystallized and liquid domains was determined whether or not bar is present in the subphase. This difference can be attributed solely

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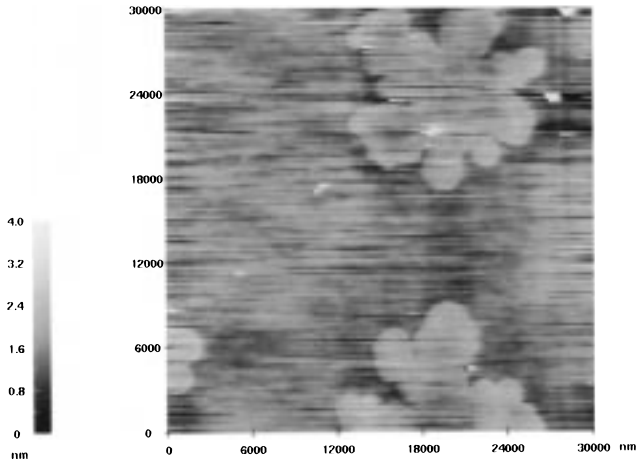

Figure 7. AFM images (in top view) of the transferred TAP lipid LS film containing 1% of Rh-C_{18} at 14 mN·m^{-1} from a 10 mM barbituric acid solution, corresponding to Figure 6b. It was obtained in air and in ac mode. The bar represents 2.7 μm.

to chain effects and tend to indicate that bar can be adsorbed in both LE or LC phases. Recently it was shown that the interaction between a monolayer of barbituric derivatives bearing no spacer and 2,4,6-triaminopyrimidine in the subphase occurs only if the monolayer is in the crystalline phase.[15c]

These experiments have shown structural changes resulting in the formation of two-dimensionnal large crystals, which could be attributed to the TAP lipid/bar complexation.

2. Change in $\pi-A$ Isotherms of the TAP Monolayer. We have studied the effect of various barbituric molecules on a TAP lipid monolayer. As shown in Figure 8a, the presence of barbituric molecules induces an expansion of the mean molecular area within a TAP lipid monolayer. The same observation can be made in the case of *N*,*N*′-dimethylbarbituric acid (diMebar), although it possesses only hydrogen bond acceptor C═O groups. One cannot exclude that the tetraoxyethylenic spacer creates a more hydrophobic region near the surface, with a lower ϵ_r than water, promoting the nonspecific adsorption of guest molecules such as diMebar, which is more hydrophobic than bar. It is difficult to form a conclusion about the guest molecules binding from the shape change. Indeed the addition of guest molecules causes a dramatic change in pH. For example the measured pH of a 1 mM barbituric acid solution is 3.5, so that the TAP monolayer can become positively charged. Because of the acido−basic properties of TAP and BAR lipids, pH and ionic strength can also induce an additional change in the shape of the $\pi-A$ isotherms and therefore it is necessary to use other techniques to prove the presence of guest molecules at the air/water interface and the complex formation.

3. FT-IR Investigation and XPS Analyses on LB Films. To confirm the adsorption of the barbituric guest molecules onto the TAP monolayer and analyze the nature of the resulting association, we have studied the corresponding LB films by FT-IR and XPS techniques.

As shown in Figure 8b, IR spectra of TAP lipid films transferred from pure water exhibit a C═N stretching peak of the triaminopyrimidine ring at 1588 cm^{-1} and the NC═O peak at 1639 cm^{-1}. In case of a diMebar subphase, no large difference in the spectrum appears. This observation indicates that diMebar is not present in the TAP LB film. The spectrum with barbituric acid in the subphase presents an additional peak at 1697 cm^{-1} corresponding to the C═O carbonyl group in position 2 of

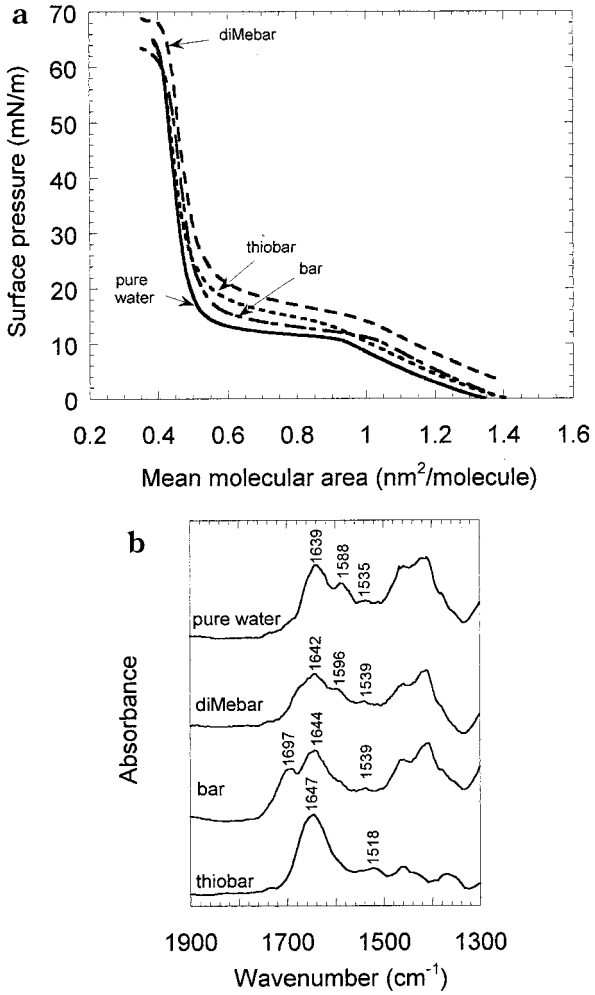

Figure 8. (a) $\pi-A$ isotherms of a TAP lipid monolayer in the presence of various 1 mM solutions of complementary molecules in a pure water subphase and at 20 °C. (b) FT-IR spectra of the corresponding LB films transferred at 50 mN·m^{-1}.

the barbituric acid ring. This result clearly shows the adsorption of barbituric acid onto the TAP monolayer. One can observe the disappearance of the C═N stretching peak at 1588 cm^{-1}. Concerning the C═O group in position 2 of the barbituric acid ring, a shift from 1730 to 1697 cm^{-1} was observed in our case (see also Figure 5). Previously, it was shown that the peak of this C═O group of monomeric bar is observed at 1730 cm^{-1} in Ar matrix.[27] The bonding of barbituric acid to a melamine compound such as diC_{12}mela induces a shift of this peak to 1715 cm^{-1}.[14] The study of the 2,4,6-triaminopyrimidine/bar interaction in aqueous solution[15b] or the binding of 2,4,6-triaminopyrimidine to a barbituric amphiphile monolayer[15c] also show shifts to lower wavenumber. We observe the same tendency (with a larger shift) in the case of TAP lipid and barbituric acid. Nevertheless, the peak positions are not the same in all of these studies. This is due probably to nonidentical local structures of the complexes. In the presence of 2-thiobarbituric acid, one can observe the same behavior concerning the C═N stretching peak at 1588 cm^{-1} and moreover a broad peak was observed at 1647 cm^{-1} attributed to the amide and the C═S stretching vibrations together. These results show clearly a selective bonding of bar and thiobar onto the TAP LB film and are in agreement with the formation of TAP/bar or TAP/thiobar complexes via hydrogen bonding.

To define the stoichiometry between barbituric derivatives and the TAP monolayer, the transferred LB films on

Table 2. Elemental Composition of the Transfered LB Films As Determined by XPS Measurements

template	C (%)[a]	N (%)	O (%)	S (%)	TAP/thiobar ratio[b]
TAP LB film in water					
none	88.50	6.20	5.20	0	
barbituric acid, 1 mM	83.10	7.60	9.00	0	
10 mM	82.90	7.50	9.60	0	
thiobarbituric, 1 mM	85.10	7.00	7.00	0.85	
10 mM	84.20	7.35	7.35	1.10	0.95
TAP LB film in pH 7 buffer					
none	86.40	6.00	7.30	0	
barbituric acid, 1 mM	86.20	6.60	7.15	0	
10 mM	85.20	6.50	7.90	0	
thiobarbituric, 1 mM	87.35	6.00	6.50	0.05	
10 mM	82.30	6.40	8.75	0.60	

[a] Errors on the values is less than ±0.05. [b] Deduced from the S/N ratio.

gold-deposited glass plates were characterized by an XPS analysis. The obtained results are summarized in Table 2. The observed signal results from the average of elements located at a certain distance from the interface. Elements near the outermost surface give stronger signals than those coming from elements located deeper in the sample so that it sometimes requires depth corrections.[32] In contrast to melamine derivatives, the presence of oxygen coming from the tetraoxyethylenic spacer of the TAP monolayer makes it difficult to extract the stoichiometry of the TAP/bar complex from the O/N ratio. Indeed the O and N elements contributing to the signal are located at a depth of 25 Å. Nevertheless, one can notice the increase of the O and N ratios by the addition of barbituric acid in the subphase, in agreement with the association of barbituric acid to the TAP monolayer. In the other hand, one can extract the stoichiometry of the TAP/thiobar complex from the S/N ratio. The S and N atoms contributing to the signal are all located at a short distance in the recognition group. So said, it appears that thiobarbituric groups form a *n*:*n* complex with the TAP lipid.

XPS measurements confirm the presence of bar and thiobar in LB films and permit determination of a *n*:*n* stoichiometry for the TAP/thiobar complex.

4. Molecular Recognition Investigated at Different pH and a Constant Ionic Strength. To investigate a possible contribution of electrostatic interaction between barbituric guest molecules and a TAP or a diC_{12}mela monolayer, we have studied the molecular recognition between diC_{12}mela compound or TAP lipid and barbituric acid molecules in the presence of a buffer at pH 7 and at an ionic strength of 0.1 M.

Figure 9a presents the $\pi-A$ isotherms of a diC_{12}mela monolayer in the presence of various guest molecules in a subphase buffered at pH 7 and at a 0.1 M ionic strength. The observed expansion of the mean molecular area is smaller than that observed in pure water. Nevertheless, the same behavior is found as previously observed in pure water.[14] Particularly, one can observe a higher area expansion for thiobar than for bar as observed in pure water. At pH 7, diC_{12}mela is very weakly protonated. Moreover, the strong ionic strength induces a large charge screening by decreasing the Debye length to around 1 nm.[33] In Table 3, the results concerning the FT-IR of the corresponding LB films are summarized. The presence of bar and thiobar in diC_{12}mela LB films is confirmed by the appearance of the characteristic C=O peak. The

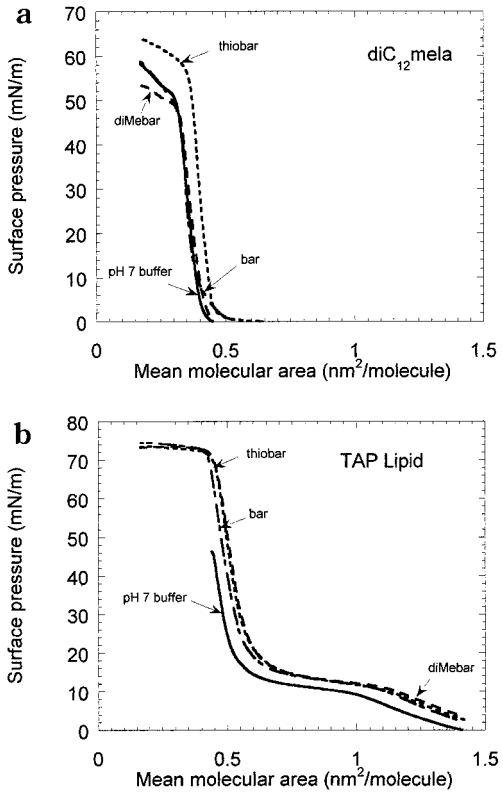

Figure 9. $\pi-A$ isotherms of the lipid monolayers in the presence of various 1 mM guest molecules in a pH 7 buffer of a 0.1 M ionic strength at 20 °C: (a) diC_{12}mela; (b) TAP.

Table 3. Peak Positions in FT-IR Spectra of the Transfered LB Films of DiC_{12}mela in Presence of pH 7 Buffer (Ionic Strength 0.1 M) in the Range of 1800−1500 cm^{-1}

template (in pH 7 buffer)	peak position (cm^{-1})		
none			
barbituric acid, 1 mM	1711	1655 (weak)	1609 (weak)
10 mM	1709		1607(weak)

change in $\pi-A$ isotherms observed in the presence of bar or thiobar in the subphase are attributed to the binding of guest molecules onto the diC_{12}mela monolayer, which is confirmed by the FT-IR study of the corresponding LB films. Therefore, the molecular recognition is still efficient under these conditions of strong charge screening by the ionic strength.

Under the same conditions of subphase, we have investigated the molecular recognition between TAP lipid monolayer and barbituric guest molecules. Figure 9b presents the $\pi-A$ isotherms of a TAP lipid monolayer in the presence of various guest molecules at a 1 mM

(32) Kurihara, K.; Kawahara, T.; Sasaki, D. Y.; Ohto, K.; Kunitake, T. *Langmuir* **1995**, *11*, 1408.

(33) The Debye screening length range was calculated from the following relation: $\kappa^{-1} = 0.308/\sqrt{c}$ (nm) where c is the 1:1 electrolyte concentration.

Table 4. Peak Positions in FT-IR Spectra of the Transfered LB Films of TAP Lipid in Presence of pH 7 Buffer (Ionic Strength 0.1 M) in the Range of 1800−1500 cm^{-1}

template (in pH 7 buffer)	peak position (cm^{-1})				
none			1641	1588	
barbituric acid,					
1 mM	1686 (weak)		1643	1588	
10 mM	1685		1647		
thiobarbituric acid,					
1 mM		1653 (broad)		1588	
10 mM		1657			1557

concentration and at pH 7 and a 0.1 M ionic strength. Contrary to the case of diC_{12}mela, the expansion of the mean molecular area is the same, whatever the chemical nature of the guest molecule. No apparent selectivity occurs in such conditions, which could be due to the decoupling effect of the spacer. The FT-IR spectra of the corresponding transferred TAP films have been measured and the results are shown in Table 4. The effect of higher concentrations in bar and thiobar was also studied. No large changes were observed in the presence of barbituric derivatives at a 1 mM concentration. The shift and broadening of the C═N peak at 1588 cm^{-1} and the appearance of the C═O peak characteristic of bar binding were observed at a concentration in guest molecules of 10 mM, whereas it was observed at 1 mM in pure water. The same behavior was observed in the case of thiobar. In conclusion, the interaction between the TAP lipid monolayer and barbituric guest molecules appears to be weaker in the presence of a pH 7 buffer and at high ionic strength of 0.1 M.

Concerning the nature of the interaction taking place between TAP and bar at the air/water interface, one can envision that TAP and BAR groups either interact via hydrogen bonding or form an electrostatic pair (see Figure 2). The dependence on the ionic strength indicates that electrostatic interactions contribute to the binding of barbituric guest molecules onto a complementary TAP monolayer which is partially charged at pH 7. In the case of a purely electrostatic interaction, the bar, thiobar, and diMebar are expected to have the same behavior in water according to their pK_a,[17] which is not the case. We observed a selective binding of bar and thiobar in comparaison to diMebar: the addition of diMebar does not induce any significant change in the FT-IR spectrum of TAP LB film, contrary to the bar and thiobar compounds. The IR results are in agreement with the formation of a TAP/bar complex via hydrogen bonding. Furthermore, the XPS measurement indicates that the stoichiometry of the TAP lipid/thiobar complex is *n*:*n*. Such a stoichiometry is in agreement with the formation of a complex via hydrogen bonding rather than via a purely electrostatic interaction, in which the guest molecule would play the role of a counterion. All these results show that electrostatic interactions contribute to adsorb guest molecules onto the monolayer. But near the air/water interface, the relative permittivity ϵ_r decreases dramatically so that ionic TAP/bar pairing is less favored than pairing via hydrogen bonding.

IV. Conclusion

The results of this study provide a description of the properties of the TAP, BAR, and EO_4 lipids designed for molecular recognition between artificial membranes.[16b,21] To this end, the molecular recognition by hydrogen bonding between a TAP monolayer and barbituric molecules at the air/water interface has been investigated by several techniques. The effects of pH and ionic strength have been studied. The data point to an electrostatic contribution in the TAP lipid/bar interaction in addition to hydrogen bonding. The comparison of the TAP lipid, bearing a flexible hydrophilic tetraoxyethylenic spacer, with those previously studied,[14,15c] provides further information. Despite the flexibility and the more hydrophilic environment of the recognition group induced by the spacer, TAP lipid/bar association occurs. The spacer decorrelates the molecular recognition features of the headgroups from the hydrophobic part of the monolayer. Further investigations of its effect onto molecular recognition between artificial membranes could be carried out.

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