Immobilization inside Langmuir−Blodgett Films of a Fluorescent Artificial Receptor for Zn(II) Recognition

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This work is focused on the study of the immobilization of a synthetic fluorescent macrocycloureide in a nanostructure obtained through Langmuir−Blodgett (LB) technique. Its interfacial behavior shows that it can take various orientations at the air/water interface. Mixed with behenic acid it is successfully transferred in Y-type onto hydrophobic substrates. The related LB films were controlled through Fourier transform IR spectroscopy and Nomarski microscopy. Fluorescence emission spectra were recorded in air, water, and phosphate buffer at pH 12. Three signals were emitted, corresponding to three different forms of the immobilized macrocycloureide: monomer, dimer, and molecule stacks. Micromolar concentrations of zinc could be detected through signal fluorescence enhancement of the dimer emission, giving evidence that the immobilized macrocycloureide keeps its fluorescence properties and its ability for molecular recognition of Zn(II) ions.

Introduction

The increasing interest in interfaces between biological elements and artificial nanostructures for the development of biosensors aims at the association of protein to biomimetic layers of nanometer scale like Langmuir−Blodgett (LB) films. Previous studies in our group have shown that behenic acid LB films were suitable to immobilize enzymes such as firefly luciferase[1] or glutamate dehydrogenase.[2]

Our group is involved in the design of stable artificial receptors for molecular recognition of analytes in aqueous solution which might be useful in the sensors area. For such a purpose, we have achieved the synthesis of a cyclophane macrocycle combining benzamide and urea.[3] The urea group has proven to be a powerful ligating moiety. The macrocycloureide dimerizes at alkaline pH. Both monomer and dimer have distinctive fluorescence emission spectra.[4] The homodimer is able to specifically complex Zn(II) ions, and the quantification is based on the linearity of the fluorescence signal increase of the dimer versus the ion concentration. The fluorescence signal change is actually the result of an induced shift of the equilibrium between monomer and dimer toward the dimer form. Among several analytical techniques, fluorescence-based detection is attractive owing to its high sensitivity and good selectivity. It has been developed over the last decade for several metal ions. Fluorometric determination of zinc based upon the formation of the fluorophore−Zn(II) complex[5−7] has been developed.

In the work presented here, we focused on the immobilization of the previously synthetized macrocycloureide inside behenic acid LB films. With this purpose, the interfacial behavior of the macrocycloureide alone or mixed with behenic acid was studied at the air/water interface before the build-up of LB films. The integrity of macrocycloureide/behenic acid LB films was controlled through Fourier transform infrared (FTIR) spectroscopy and Nomarski microscopy. Fluorescence studies were performed on the immobilized macrocycloureide, the ultimate goal being to detect zinc in aqueous media.

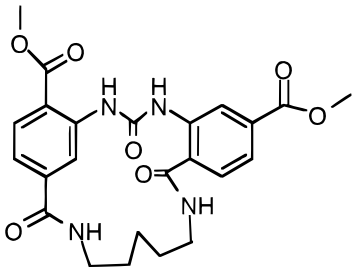

Figure 1. Structure of the macrocycloureide.

Experimental Section

Materials. Docosanoic acid (behenic acid, purity (99%) was purchased from Sigma-Chemical Co. (St Quentin-Fallavier, France). Behenic acid was dissolved in chloroform at 2×10^{-3} M. The macrocycloureide (13,21-bis(methoxycarbonyl)-1,9,17-trioxo-2,8,16,18-tetraaxa[9.3]metaorthocyclophane) is a urea macrocyclophane (Figure 1), synthesized and purified starting from tetramethyl-2,2′-ureylenediterephtalate, as previously described.[3] After purification it was dissolved in an aqueous solution at pH 12 where it forms a homodimer.[4] It was dried under vacuum and dissolved in chloroform. The chloroformic solutions were stored at 4 °C.

NaCl and $ZnCl_2$ of the highest grade available were obtained from Prolabo and Aldrich, respectively. Pure water (resistivity = 18.2 MΩ·cm) was obtained through a Milli-Q four-cartridge purification system (Millipore).

Calcium fluoride (CaF_2) substrates 35 × 9.5 × 2 mm (Sorem, France) and quartz substrates 35 × 9.5 × 1.25 mm (Thuet Biechelin, France) were cleaned as described elsewhere.[8]

Langmuir−Blodgett Film Deposition. Langmuir−Blodgett films were prepared through a computerized KSV 3000 Langmuir−Blodgett trough (Finland). The trough is enclosed in a

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filtered air flow cabinet to avoid dust deposition. A symmetric compression of the monolayer was achieved with two moving barriers made of Delrin. The surface pressure was measured with a platinum Wilhelmy plate attached to a sensitive balance. After each experiment, the plate was first soaked in aqueous solution with 10% ethanol and then rinsed in pure water.

The experiments were performed using either an aqueous subphase at pH 12, both with and without 0.1 M NaCl or a pure water subphase containing 0.1 M NaCl. The subphases were thermostated at 20 °C. Before experiments, the subphase surface was cleaned with a sucking system. The chloroformic spreading solutions, prepared with either macrocycloureide alone or mixed with behenic acid, were deposited on the subphase using a 500 μL SGE microsyringe. A 10-min period was observed between the molecules spreading and the beginning of the compression. The compression of the monolayer was carried out at a speed of 10 mm·min^{-1}, further referred to as standard conditions.

The substrates were clamped parallel to the barriers. For all these experiments, the surface pressure was maintained constant during the transfer and the dipping rate was 10 mm·min^{-1}.

For obtaining hydrophobic substrates, the calcium fluoride substrates were coated with seven layers of behenic acid, the quartz substrates were silanized by treatment with dichlorodimethylsilane (1% in $CHCl_3$).[8] Before use, the silanized substrates were rinsed with $CHCl_3$. After deposition, the LB films were placed in a filtered air flow prior to further treatments.

FTIR Studies. The transmission−absorption spectra were obtained at normal incidence and recorded with a 510 M FTIR interferometer (Nicolet Instruments, France) operating at 4 cm^{-1} resolution. Each spectrum resulted in the accumulation of 16 scans.

The area integration was calculated as proposed by Nicolet's software.

Nomarski Microscopy Observations. The coated substrates were observed through Nomarski differential interference contrast microscopy with a lateral resolution of 1 μm and a thickness difference of about 30 Å, at a magnification of 500.[9]

Fluorescence Studies. Fluorescence spectra were recorded through a Jobin Yvon (spectrofluo JY3D) spectrofluorometer using a 1 cm quartz cell, with the substrate inside, for measurements either in air or in 3 mL of aqueous solution. Due to its geometry, the substrate was placed in the cuvette forming an angle close to 40° with the excitation beam. The position of the substrate was adjusted in order to eliminate the peak of light scattering around 360 nm. The excitation wavelengths were 332 or 356 nm. The emission spectra were recorded within the range 360−500 nm, and the background emission was subtracted using a blank quartz slide. Aliquots of a 5 mM $ZnCl_2$ solution were added to the cell containing the substrate.

Results and Discussion

1. Interfacial Behavior of the Macrocycloureide Spread Alone at the Air/Water Interface. The macrocycloureide was spread on different aqueous subphases with and without 0.1 M NaCl. The pH of the subphase was adjusted to 12 (with KOH) since the macrocycloureide is insoluble in water at alkaline pH. Figure 2 shows isotherms reflecting the behavior of the macrocycloureide when the number of spread molecules was varied from 566 to 6000 nmol.

When 566 nmol of the macrocycloureide were spread on a water subphase, the $\pi-A$ isotherm was difficult to obtain and it was not reproducible (Figure 2a). To avoid this drawback, we used the salting-out phenomenon. When the same number of molecules were spread on a 0.1 M NaCl subphase, the $\pi-A$ isotherm could be effectively obtained up to 20 mN·m^{-1}; it exhibits a single liquid-expanded (L-E) phase (Figure 2b). If the number of deposited molecules was doubled (Figure 2c), the isotherm thus obtained paradoxically was shifted toward the lower molecular areas suggesting a sinking of some molecules. To check this hypothesis, after compression this monolayer was expanded, relaxed overnight, and then recompressed; the new isotherm was then shifted toward larger molecular areas (Figure 2d); it still exhibits a single L-E phase. This result proves that the macrocycloureide molecules did not sink into the subphase during the first compression. Table 1 shows the molecular areas deduced from the isotherms at 10 mN·m^{-1}. First, such results are not compatible with the steric hindrance of the macrocycle (around 140 Å^2 in the flat position). Second, it can be pointed out that the molecular areas are very close when deduced from the isotherms obtained directly either with a 566 nmol monolayer or with a 1132 nmol monolayer after compression, relaxation, and new compression. This result suggests that the relaxation step induced modifications in the orientation of the macrocycloureide molecules at the air/water interface leading roughly to a similar behavior as the one observed without relaxation with fewer molecules (566 nmol).

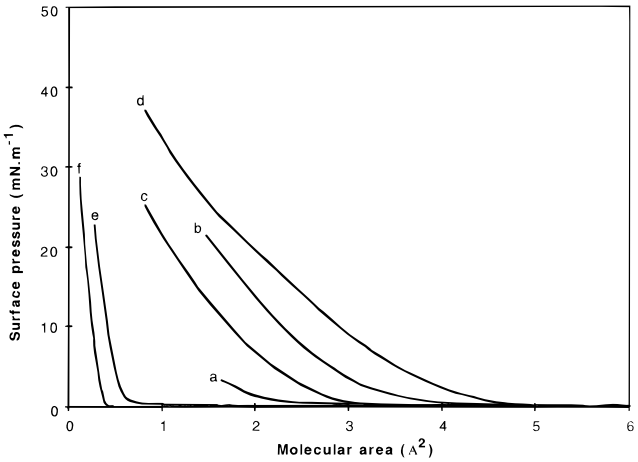

Figure 2. $\pi-A$ isotherms of macrocycloureide. The molecules were spread either on a water subphase at pH 12, 566 nmol (a), or on a 0.1 M NaCl aqueous solution at pH 12, 566 nmol (b), 1132 nmol (c), 1132 nmol after decompression and a night of relaxation (d), 3000 nmol (e), and 6000 nmol (f).

Table 1. Molecular Areas at 10 mN·m^{-1}

macrocycloureide[a] (nmol)	molecular area (Å^2)	macrocycloureide[a] (nmol)	molecular area (Å^2)
566	2.27	3000b	0.5
1132a	1.71	6000a	0.25
1132b	2.87	6000b	0.22
3000a	0.41		

[a] The molecules were spread on 0.1 M NaCl aqueous subphase at pH 12. Results are given after a first compression (a) after compression, decompression, and a second compression (b).

In order to obtain a larger monolayer area, 3000 and 6000 nmol of macrocycloureide were spread; the isotherm shapes changed dramatically yielding very low molecular areas (Figure 2e,f). Several phenomena may take place. First, at the moment the macrocycloureide molecules were spread at the interface, a solubilization may occur owing to a high concentration of the product in each deposited drop. However using fluorescence or absorption procedures, the macrocycloureide could not be detected in the subphase. Second, during the compression, some macrocycloureide molecules may be expulsed from the monolayer; a relaxation step after expansion followed by a new compression did not lead to larger molecular areas. Moreover, after two successive compression-decompression cycles up to 10 mN·m^{-1} performed with the 3000 nmol monolayer, the isotherms perfectly overlapped. This reveals that the monolayer integrity was not lost during the expansion step. Third, a stacking of molecules may take place during the compression. The interfacial

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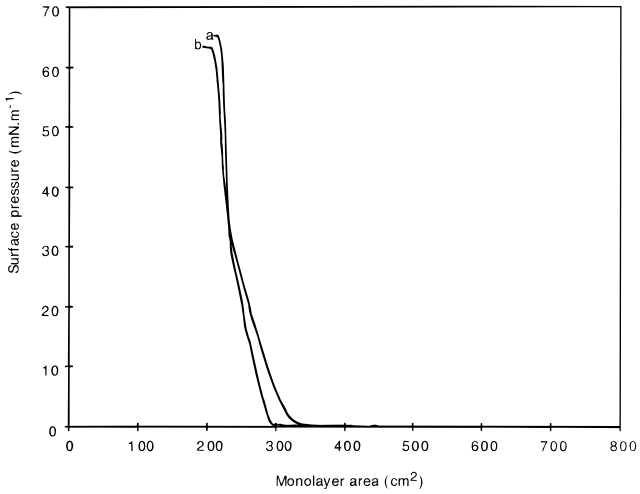

Figure 3. $\pi-A$ isotherms of 200 nmol of behenic acid (a) and 200 nmol of behenic acid mixed to 2000 nmol of macrocycloureide (b) spread on a 0.1 M NaCl subphase at 20 °C.

behavior of the macrocycloureide has to be related to its characteristics in solution;[10] the molecule is a strong base ($pK_a = 12.4$). Then, in the conditions used here (subphase at pH 12) it should significantly be in the deprotonated state. In this form, the carbonyl oxygen at the urea bridge is strongly basic with a large dipole moment. This allows long range interactions to occur between neighboring molecules giving rise, in addition to π-stacking, to homodimer formation. Such interactions can further lead to aggregate formation at a macrocycloureide concentration above 20 mM.

As pointed out above, the macrocycloureide is able to form a monolayer on a 0.1 M NaCl water subphase at pH 12, which reveals that the molecules are insoluble. On the comparison of the concentration of the solution to the molecular density of the floating monolayer, the compression of the layer may be considered as an increase in 2D concentration. Indeed, when the surface density became too large (for a 3000 nmol monolayer), the association of molecules could be irreversible, the molecules being not separated during expansion. Interactions between neighboring molecules could lead to stable stacks during compression.

2. Interfacial Behavior of Macrocycloureide/Behenic Acid Monolayers. The macrocycloureide mixed with behenic acid was spread on a 0.1 M NaCl aqueous subphase without adjusting the pH. Indeed, it has been previously shown[12] that a monolayer of behenic acid cannot be efficiently transferred when the pH of the subphase is above 8. Monolayers were prepared using three different macrocycloureide/behenic acid molar ratios. With ratios equal to 1 and 2, the isotherm shapes were similar to the pure behenic acid one (Figure 3a), whereas for 10, the first part of the isotherm was modified (Figure 3b). The stability of the monolayer was checked at 34 $mN \cdot m^{-1}$ using two different compression rates (10 and 7.5 $mm \cdot min^{-1}$). The isotherms so obtained were similar; when the compression was stopped, the shift of the barriers was 0.1 or 0.4 cm for 7.5 or 10 $mm \cdot min^{-1}$ compression rates, respectively, for 5 min. It can be noticed that these shifts occurred during the first 90 s; this is in favor of equilibrium in the monolayer. The macrocycloureide molecules induced either an expanding effect on the pure behenic acid monolayer up to 30 $mN \cdot m^{-1}$, or a small condensing effect above 30 $mN \cdot m^{-1}$. The area of the mixed monolayer was close to that of a pure behenic acid monolayer. In view of these data, we can infer that, during the compression step, the macrocycloureide is either expelled from the monolayer into the subphase or adsorbed on the hydrophilic carboxylic groups of behenic acid; it might also be inserted between the hydrophobic tails. This last hypothesis is confirmed by the compressibility modulus values[11] ($Cs^{-1} = 1/A\ d\pi/dA$). When the macrocycloureide was inserted in the film, the monolayer being in the solid state domain at 40 $mN \cdot m^{-1}$, the compressibility modulus shifted from 526 $mN \cdot m^{-1}$ (for the pure behenic acid monolayer) to 323 $mN \cdot m^{-1}$. With these experimental conditions, the molecular area of behenic acid deduced from the isotherm (19.2 $Å^2$) leads to an average distance of 4.5 Å between two hydrophobic tails. One can wonder whether the macrocycloureide could insert in such a narrow hydrophobic space. The deprotonated macrocycloureide was dissolved in a mixture of chloroform and behenic acid and spread onto the subphase. Upon evaporation of chloroform, the macrocycle remains solvated by the behenic acid. In the gaseous state, given the large macrocycloureide/behenic acid ratio (10), both the monomer and dimer can coexist in an equilibrium. When the film is compressed, the association of molecules is favored and the equilibrium is progressively shifted toward the dimer form. If we assume that the compression is similar to a concentration increase, it could ultimately lead to the formation of stacks.

3. Deposition of the Macrocycloureide/Behenic Acid Monolayers. Considering the isotherm shape, two transfer surface pressures were chosen: 18 $mN \cdot m^{-1}$ in the modified part of the isotherm and 34 $mN \cdot m^{-1}$ in the quasi-solid part. In the first case, with the ratio 10, four layers were transferred in Y-type with a transfer ratio close to 0.6−0.7; a slight collapse appeared during the deposition.

At 34 $mN \cdot m^{-1}$ whatever the ratio used (1 or 10), eight layers were deposited onto hydrophobic substrates (silanized quartz or behenic acid precoated CaF_2) in Y-type with a transfer ratio close to 0.9 ± 0.1. During the transfer, no collapse appeared.

To check the presence of the macrocycloureide in LB films and to control the homogeneity of the transfer, two techniques were used: FTIR spectroscopy and Nomarski microscopy. It must be pointed out that it was not possible to detect the specific bands of the macrocycloureide on the FTIR spectra.

Nomarski microscopy observations show that the macrocycloureide/behenic acid coated substrates were homogeneous (Figure 4). Moreover, when the substrates were immersed in pure water, no significant peeling-off occurred. In these conditions, the substrate surfaces remained homogeneous with hydrated macrocycloureide molecules even after drying in pure air flow for 3 h (Figure 4b,c).

4. Fluorescence of the Immobilized Macrocycloureide. Since the macrocycloureide is a fluorescent molecule,[4] it appeared valuable to investigate its fluorescent properties after immobilization. In aqueous solution, the monomer is predominant at acidic pH (below pH 6), whereas dimerization is significant at alkaline pH (above pH 11). Both the monomer and homodimer are fluorescent, but as previously shown,[4] their absorption and emission spectra are different. In Table 2, the excitation and emission wavelengths of the monomer and homodimer are given for the macrocycloureide in solution.

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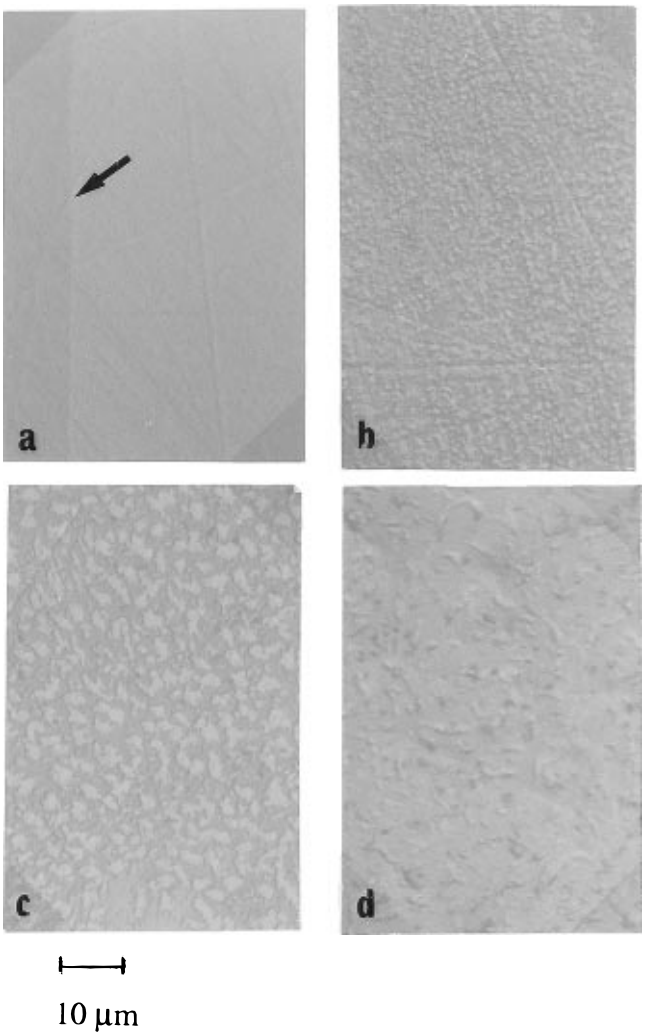

Figure 4. Photographs of macrocycloureide/behenic LB films ratio 10 (a), after 30 min of immersion in pure water (b), after 1 night of immersion in pure water (c), and after 5 min of immersion in solution at pH 12 (d). Arrow indicates the limit of coating. The stripes on the substrate are the consequence of the ultrasonic cleanup treatment. Magnification 625.

Table 2. Excitation and Emission Wavelengths for Both Monomer and Homodimer

forms of macrocycloureide	λ_{ex} (nm)	λ_{em} (nm)
monomer	332	380
homodimer	356	432

The following studies were performed with macrocycloureide/behenic acid LB films (ratio 10). With the emission wavelength set at 432 nm, the excitation spectrum of the immobilized molecules was recorded from 320 to 390 nm. Three maximum excitation wavelengths can be distinguished, at 332, 350, and 378 nm. The two first values are close to those of the macrocycloureide in aqueous solution and correspond to the wavelengths of the monomer and dimer, respectively. Hence, the fluorescent studies were performed using these two excitation wavelengths. The third excitation wavelength appears to be linked to the macrocycloureide in the immobilized state.

The emission spectra of macrocycloureide/behenic acid LB films were recorded from 370 to 500 nm with the excitation wavelength of the monomer at 332 nm (Figure 5a) and of the dimer at 356 nm (Figure 5b). Three signals are visible, two of them, around 400−410 nm and 445 nm, are due to the monomer and dimer, respectively. The emission signal of the monomer was surprisingly weak at

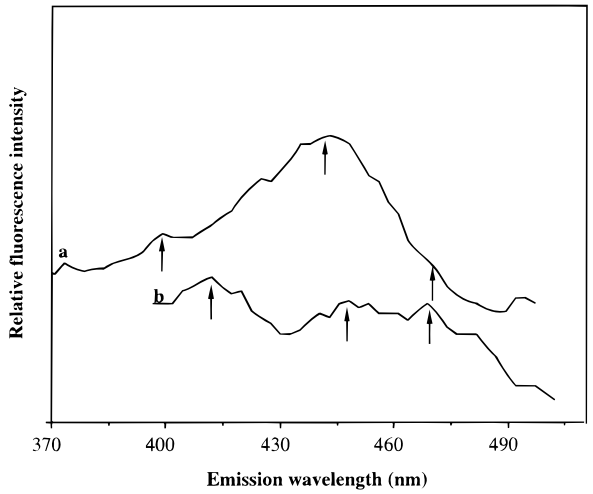

Figure 5. Emission spectra of macrocycloureide/behenic acid LB films: (a) $\lambda_{ex} = 332$ nm; (b) $\lambda_{ex} = 356$ nm. Arrows show the position of three signals.

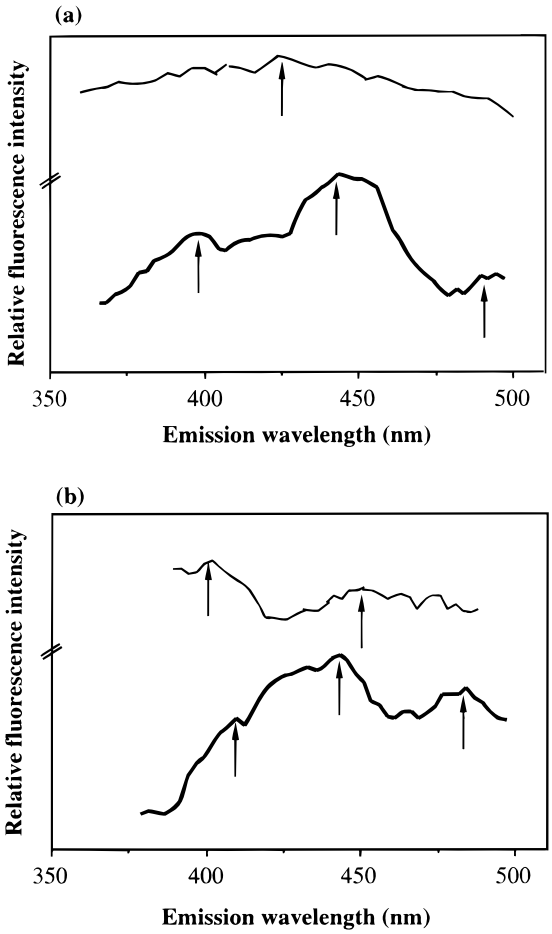

Figure 6. Emission spectra of macrocycloureide/behenic acid LB films when the substrates were immersed in pure water (bold line) or in 50 mM phosphate buffer pH 12 (thin line): (a) $\lambda_{ex} = 332$ nm; (b) $\lambda_{ex} = 356$ nm. Arrows show the position of different signals.

332 nm; more it merged in the dimer signal. The poor resolution of this peak could be due to the limits of the spectrofluorometer.

The emission spectra of the immobilized macrocycloureide were recorded with the substrate immersed either in pure water or in 50 mM phosphate buffer at pH 12 (Figure 6). Compared to spectra obtained in the air, they appear to be not significantly modified by the immersion in pure water whereas they are markedly flattened at pH

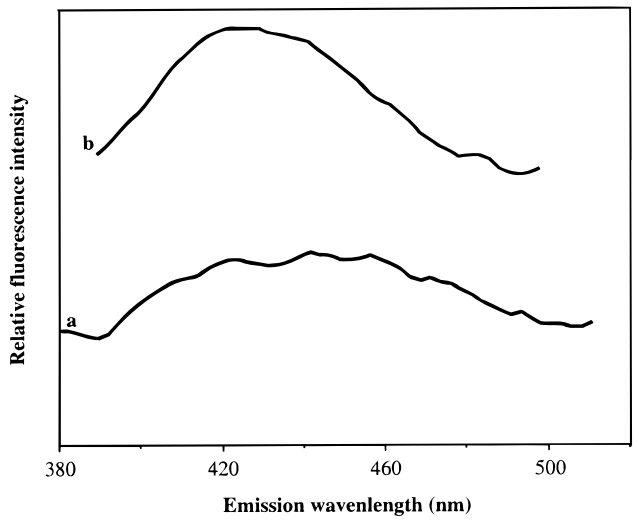

Figure 7. Emission spectra of macrocycloureide/behenic acid LB films when the substrates were again immersed in 50 mM phosphate buffer pH 12: (a) $\lambda_{ex} = 332$ nm; (b) $\lambda_{ex} = 356$ nm.

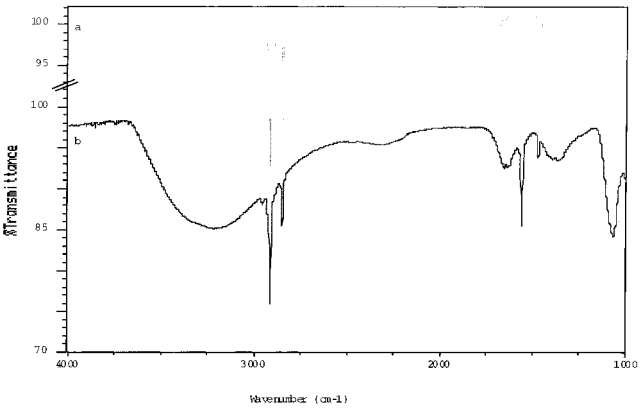

Figure 8. Transmission spectra of 15 layers of behenic acid deposited on each face of a CaF_2 substrate (a) and 8 layers of macrocycloureide/behenic acid (ratio 10) deposited on a CaF_2 substrate precoated with 7 layers of behenic acid (b).

12. After removal of the substrates, the spectra obtained on both aqueous solutions revealed that some macrocycloureide molecules were slightly released from the LB films (results not shown).

Furthermore, the emission spectrum recorded with the substrate when immersed again in the pH 12 solution shows a large dimer peak (Figure 7). This leads to the hypothesis that a second immersion could result in a restructuring of the layers with consequently an unmasking of the macrocycloureide molecules, enabling them to give a larger fluorescence signal. In order to check this hypothesis, FTIR studies and Nomarski microscopy observations were performed as described below.

5. FTIR and Nomarski Characterization of Macrocycloureide/Behenic Acid LB Films after Their Immersion in Phosphate Buffer at pH 12. Figure 8a shows FTIR transmission spectra of behenic acid LB films (15 behenic acid layers were deposited on each face of a CaF_2 substrate). The characteristic bands are the stretching vibrations of methyl groups (ν_s at 2952 cm^{-1}) and methylene groups (ν_{as} at 2918 cm^{-1}, ν_s at 2848 cm^{-1}), the vibrations of carboxylic groups (ν_{CO} at 1706 cm^{-1}, δ_{OH} at 1299 cm^{-1}), the split of scissoring vibrations δ_{CH_2} into two bands (δ' at 1460 cm^{-1} and δ'' at 1474 cm^{-1}), which is a criterium of highly crystallized state,[13] and the bending vibrations $\delta_{(CH_2)\alpha}$ at 1408 cm^{-1}. When eight layers of macrocycloureide/behenic acid (ratio 10) were deposited on a CaF_2 substrate precoated with seven layers of behenic acid, the spectrum obtained with such LB films was strictly identical to spectrum a. The specific bands of the macrocycloureide were not detectable: either its vibrations were hidden by the behenic acid bands or the amount contained in the LB films could be too low. When this substrate was immersed in pure water and dried under an air flow, there was no visible modification of this spectrum. On the contrary, after immersion of this substrate in aqueous solution at pH 12, the spectrum was dramatically modified (Figure 8b). The presence of the macrocycloureide was revealed by several peaks: the broad peak centered at 3240 cm^{-1}, which is attributed to the streching vibrations of the N−H urea moiety and of the peptidic bond (amide II), the indented peak from 1653 to 1628 cm^{-1} corresponding to the streching vibrations of carbonyl groups of urea and of amide I (with hydrogen bonding), the sharp peak at 1559 cm^{-1} attributed to the bending vibrations of N−H (amide II), and finally at 1068 cm^{-1} the peak corresponding to the bending vibrations of the C−H bonds of methylene groups. The peak of the stretching vibrations of the carboxylic groups of behenic acid (ν_{CO} at 1706 cm^{-1}) disappeared. The peak corresponding to the carboxylate groups was not distinguishable because it merged in the indented peak of carbonyl groups of urea and amide I. Moreover, the LB films have lost their crystallinity (there is no split of the δ_{CH_2} band at 1473 cm^{-1}). The integrated area of the stretching vibrations of methyl groups and methylene groups is known to be proportional to the number of deposited layers and to be a reliable test for controlling the thickness of the films.[14] In order to detect the influence of the immersion at pH 12 on the integrity of LB films, the peaks between 2807 and 2980 cm^{-1} in spectra a and b were integrated. The integration values were 2.4 and 1.4 au, respectively. These values prove a peeling-off of behenic acid molecules into the aqueous medium, which makes the macrocycloureide accessible to the infrared beam. This study shows that the immersion in an alkaline solution leads to an actual disorganization of the macrocycloureide/behenic acid LB films.

The photograph in Figure 4d confirms the disorganization of LB films after immersion of the substrate into an alkaline solution. The net of macrocycloureide molecules is visible and the underlying layers are greatly modified when comparing with Figure 4b.

6. Study of the Fluorescent Signal at 480 nm. The emission spectra of macrocycloureide/behenic acid LB films recorded in air and in water (Figures 5 and 6) show an additional signal at about 480 nm. A hypothesis to explain the presence of this signal is that, as pointed out previously, the compression of the monolayer could ultimately lead to the formation of stacks that would fluoresce at 480 nm. Thus, the LB films built up with such a monolayer could contain stacks of macrocycloureide beside monomer and dimer forms. The presence of these stacks could be due to the conditions chosen either for the formation of the monolayer or for the build-up of the LB films. It is well-known[15,16] that the monolayer integrity depends on the compression rate. Consequently, the mixed monolayer was compressed at a slower rate (7.5 instead of 10 $mm \cdot min^{-1}$). The emission spectra of the related LB films do not show any change, which leads to

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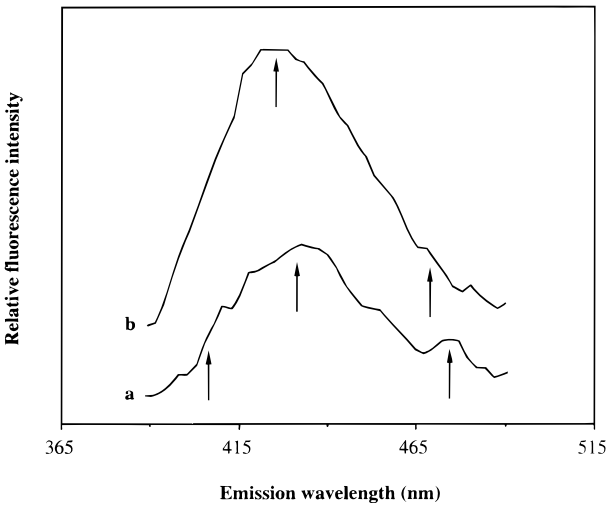

Figure 9. Emission spectra in the air of macrocycloureide/behenic acid LB films built up at a transfer pressure of 18 $mN \cdot m^{-1}$: (a) $\lambda_{ex} = 332$ nm; (b) $\lambda_{ex} = 356$ nm. Arrows show the position of peaks.

the conclusion that the orientation of molecules at the air/water interface was the same whatever the compression rate used.

The transfer surface pressure of the monolayer is also a key parameter of the LB films stacking. Figure 9 shows the emission spectra of the LB films transferred at the surface pressure of 18 $mN \cdot m^{-1}$ with a compression rate of 10 $mm \cdot min^{-1}$. The peak at 480 nm is clearly distinguishable when the excitation wavelength was 332 nm (Figure 9a), whereas at 356 nm (Figure 9b) it is hidden by the broad signal of the dimer. Besides, it must be noticed that the dimer emission is enhanced at this excitation wavelength when compared with the results obtained for the films transferred at 34 $mN \cdot m^{-1}$ (Figure 5b). This may be attributed to a higher mobility of the macrocycloureide inside the LB films, since the compressibility modulus is only 11 $mN \cdot m^{-1}$ at the transfer surface pressure of 18 $mN \cdot m^{-1}$ compared to 323 $mN \cdot m^{-1}$ at 34 $mN \cdot m^{-1}$. Even with this higher mobility, the stacks are still present and do not depend on the packing of the monolayer. These results suggest that the stacks of macrocycloureide are formed whatever the surface pressure chosen for the transfer.

The macrocycloureide/behenic acid ratio is another parameter which could rise stacks in the monolayer. When this ratio is lowered to 1, fluorescence studies on the related LB films show that stacks are also observed. The self-association giving rise to a stacking of the macrocycloureide molecules may thus take place even at low concentration in the mixed monolayer.

A slight shift can be noticed in the position of the peaks and shoulders in the different spectra. This can be due either to limited instrumental resolution or to the formation of stacks of various size during compression.

7. Zinc Recognition by the Immobilized Macrocycloureide. The macrocycloureide has been previously described as a good zinc receptor for quantification of this metal in alkaline aqueous solution.[4] Recognition of the metal is detected through enhancement of the dimer fluorescence owing to a metal-promoted shift of the equilibrium between the two forms of the macrocycloureide. It was interesting to check if the macrocycloureide immobilized inside LB films could detect the presence of zinc. It has been reported that specifically oriented molecules in a monolayer can selectively interact with ions in the subphase.[17]

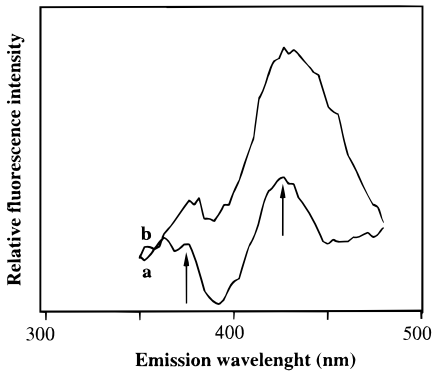

Figure 10. Emission spectra of macrocycloureide/behenic acid LB films when the substrate was immersed in pure water without Zn^{2+} (a) and with 10 μM Zn^{2+} (b), $\lambda_{ex} = 356$ nm.

The substrate coated with macrocycloureide/behenic acid LB films (prepared in standard conditions) was immersed either in phosphate buffer at pH 12 or in water. With phosphate buffer, there is no difference in the fluorescent signal before and after addition of zinc. This can be explained by competition between the macrocycloureide and the carboxylate groups of behenate for the complexation of zinc. In water, Figure 10 shows typical emission spectra obtained with the excitation wavelength at 356 nm before (a) and after (b) addition of 10 μM of $ZnCl_2$. The final concentration of zinc was chosen within the linear range determined for zinc quantification with the macrocycloureide in solution. Within spectra a and b, relative heights of peaks can be compared for both monomer and dimer (arrows). Clearly, the addition of zinc provokes a relative enhancement of the dimer fluorescence (spectrum b). Although this relative enhancement was rather weak, it was reproducible. However, it could not be obtained below 3.3 μM zinc concentration with enough confidence. The immobilized macrocycloureide inside LB films keeps its ability to both recognize zinc and deliver a signal upon complexation.

Conclusion

Taking into account the interaction properties of the macrocycloureide previously synthesized in our group, especially its ability to quantitatively complex zinc, our aim in the present work was to immobilize the macrocycloureide inside LB films. The interfacial behavior of the macrocycloureide was shown to be dependent on the number of molecules spread at the air/water interface. The results suggest that upon compression the macrocycloureide could take different orientations, by analogy with porphyrin derivatives which have been reported to orient gradually from flat to edge-on during the compression of the monolayer.[18]

It must be pointed out that the macrocycloureide alone can spread out to form a floating monolayer but it cannot be transferred onto a solid substrate. When a fatty acid with a long hydrophobic chain is mixed with the macrocycloureide, the isotherm shape is modified and does not depend on the amount of molecules spread at the interface. The use of this trigger molecule decreases the degree of freedom of the macrocycloureide at the air water/interface and allows the build-up of LB films. Recently it has been reported by other authors that a small amount of long chain n-alkane can drastically change the orientation of

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(18) Sartori, E.; Fontana, M. P.; Costa, M.; Dalcanale, E.; Paganuzzi, V. *Thin Solid Films* **1996**, *284−285*, 204.

porphyrin molecules in LB films.[19] In our case, in the presence of behenic acid, the macrocycloureide is successfully transferred in Y-type onto hydrophobic substrates. The immobilized macrocycloureide keeps its fluorescence properties. These results indicate that using a trigger molecule, we can maintain the macrocycloureide in a functional state in the LB films. When excited, the fluorescence emission spectra exhibit three signals which correspond to the monomer, dimer, and stack forms. This gives evidence for the presence of three arrangements of the macrocycloureide molecules in the monolayer prior to deposition. The macrocycloureide exists under monomer and dimer forms when it is spread at the interface, and the monolayer compression leads to a third form, *i.e.*, stacked structures. Moreover, the immobilized macrocycloureide gives the recognition signal with zinc at micromolar concentration; the dimer fluorescence is enhanced. These results are a first attempt with such a synthesized receptor in view of designing sensing nanolayers to quantify ions, for the development of selective sensors.

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