

# Ordered Molecular Films of Bilirubin and Its Amphiphilic Derivatives†

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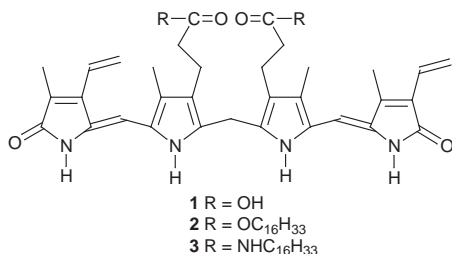
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The packing density, molecular orientation and calcium ion complexation of bilirubin and its two amphiphilic derivatives in ordered molecular films were investigated; the coordination in ordered molecular films is very different from that in bulk solution.

Recently, a great deal of attention has been given to the study of bilirubin and other bile pigments, because of their importance in physiological systems such as calcium bilirubinate, the main component of black-pigment gallstones, and copper bilirubinate, a free-radical scavenger in bile, and in protecting phospholipids from peroxidation.<sup>1</sup> But so far there has been little attempt at ordering or assembling their structures. The Langmuir–Blodgett (LB) technique makes it possible to prepare organic functional ultrathin films at a molecular size and with well-defined molecular orientation; if bilirubin ( $H_2BR$ , **1**) or its derivatives can be incorporated in LB films these films may have potential applications in mimicking the mineralization processes,<sup>2</sup> such as the formation of gallstones and, furthermore to investigate the structure of bilirubin, which is believed to play a very important role in its chemistry and that of its salts.

The amphiphilic derivatives of bilirubins, bilirubin dihexadecyl ester [ $B(CO_2C_{16}H_{33})_2$ , **2**] and dioctadecyl bilirubinamide [ $B(CONHC_{16}H_{33})_2$ , **3**] were synthesized by classical procedures.<sup>3</sup> The formation of air–water monolayers and the deposition of the LB films were carried out according to a previous paper.<sup>3</sup> The transfer ratios of these LB films were *ca.* 0.90–1.0 and 1.0–1.10 for down- and upstrokes, respectively. This indicates that the LB films were head-to-head bilayer films (Y-type). The number of layers of the LB films was 21 layers.



Both bilirubin **1** itself and its amphiphilic derivatives **2** and **3** can form stable monolayers with collapse pressures of *ca.* 45, 42 and 43  $mN m^{-1}$  on pure water subphase (pH 5.6). The respective limiting areas of about 0.70, 0.86 and 0.68  $nm^2$  per molecule were obtained by extrapolating the surface pressure to zero. According to a space-filling molecular (C.P.K.) model, bilirubin can be approximated as a rectangular block with dimensions of about  $1.95 \times 0.77 \times 0.36 nm^3$  in linear conformation and about  $1.40 \times 1.15 \times 0.36 nm^3$  in a porphyrin-like conformation.<sup>3,4</sup> The area values of 0.70, 0.86 and 0.68  $nm^2$  per molecule therefore correspond to the side area ( $1.95 \times 0.36 nm$ ) of bilirubin. That is, the monolayers of **1**, **2** and **3** on pure water subphase have a close-packed structure.

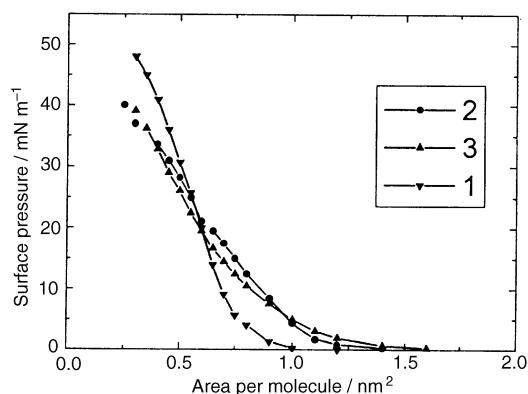


Fig. 1 Surface pressure–area isotherms of **1**, **2** and **3** on pH 3.0 subphase

The acidity of the aqueous subphases have a marked effect on the  $\pi$ – $A$  isotherms of these molecules. Fig. 1 shows the isotherms of monolayers of **1**, **2** and **3** on an acidic subphase (pH 3.0). All the three molecules can form expanded monolayers. The expansibility of the monolayers increase in the order:  $H_2BR$  **1** <  $B(CONHR)_2$  **3** <  $B(CO_2R)_2$  **2**.

The isotherms of both **2** and **3** show similar features over the pH range of 2–5. Only a slight increase in the expansibility was observed with increasing acidity of subphases.

However, on basic subsolution, the isotherms of **1**, **2** and **3** show a different dependence on the pH value of the subphase. Fig. 2 shows the  $\pi$ – $A$  isotherms of monolayers of **1**, **2** and **3** on pH 10.0 subphase. **3** can still form an expanded monolayer with a collapse pressure of 43  $mN m^{-1}$ . But **1** and **2** can not form stable monolayers, especially in strong basic subsolution. For example, the isotherms of **1** and **2** monolayers hardly have a condensed region at pH 12.0. It can be seen that the amide derivative of bilirubin (**3**) has an usual stability.

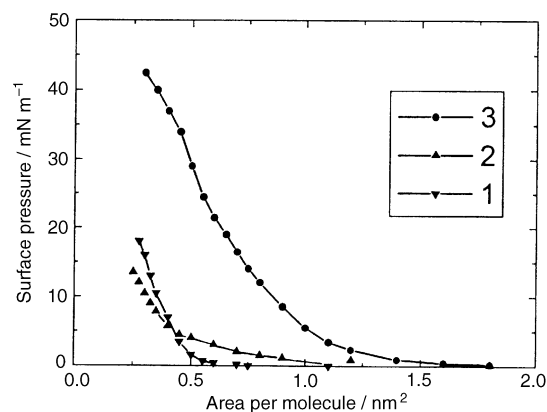
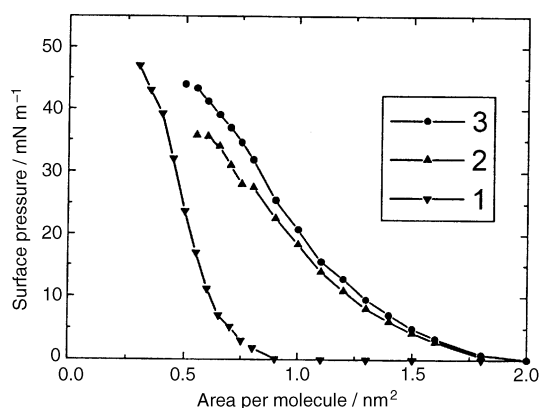


Fig. 2 Surface pressure–area isotherms of **1**, **2** and **3** on pH 10.0 subphase

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**Fig. 3** Surface pressure–area isotherms of **1**, **2** and **3** on a subphase containing  $\text{Ca}^{2+}$  ions.

Metal ions, including alkaline earth metal (such as  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ ) and transition metal ions (such as  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Pb}^{2+}$ ), contained in subphases have a striking effect on the monolayer behaviour, especially for monolayers of amphiphilic bilirubin molecules **2** and **3**. To a particular ligand, different metal ions show similar characteristics. The  $\pi$ - $A$  isotherms of **1**, **2** and **3** monolayers in subphases containing  $\text{Ca}^{2+}$  ions are shown in Fig. 3. Compared with pure water subphase, both the shape of the figures and the values of limiting areas of **1**, **2** and **3** changed in varying degrees, indicating that coordination between these ligands and  $\text{Ca}^{2+}$  ions have occurred at the air–water interface. The areas per **1**, **2** and **3** molecular unit on  $\text{Ca}^{2+}$  ion subphase are 0.65, 1.52 and 1.38  $\text{nm}^2$ , respectively. The latter two areas correspond to the face area of bilirubin, but the former (**1**) is still consistent with the side area of bilirubin.

The coordination of **1**, **2** and **3** with calcium ion at the air–water interface can be confirmed by X-ray photoelectron spectroscopy (XPS) composition measurements. The XPS spectra present the core level peaks of all the elements of the ligands (*i.e.* carbon, oxygen and nitrogen) as well as the calcium-containing subphase. Compared with the binding energy of N1s and O1s (399.6, 532.3 eV for **1**, 399.8, 532.2 eV for **2**; 399.7, 532.4 eV for **3**) in the ligands, N1s increased to 400.3 eV in the 1-M ( $\text{CaBR}$ ), 400.9 eV in the 2-M [ $\text{CaB}(\text{CO}_2\text{R})_2$ ] and 401.0 eV in the 3-M [ $\text{CaB}(\text{CONHR})_2$ ] LB films. The binding energy of O1s hardly changed and remains at 532.2–532.4 eV in the complexes of **1**, **2** and **3**. It is clear that the lone pair of electrons from nitrogen are shifted into the metal atoms and thereby a strong complexation of N atoms with  $\text{Ca}^{2+}$  occurred. Oxygen atoms do not take part in coordination. The charge-transfer from the N atoms of ligands to metal ions leads to a decrease of charge density and an increase of binding energy of N atoms. All of **1**, **2** and **3** are coordinated to calcium ions in the subphase through the pyrrole N atoms and the lactam N atoms.

Low-angle X-ray diffraction measurements were used to obtain some useful structure information on the 21-layer LB films of **1**, **2** and **3** deposited from subphases containing  $\text{CaCl}_2$ . There are two peaks ( $2\theta = 0.855$  and  $1.735^\circ$ ) for the LB film of the complex of compound **2** [ $\text{CaB}(\text{CO}_2\text{R})_2$ ], but only one peak ( $4.20$  and  $1.635^\circ$ ) for the complexes of compound **1** ( $\text{CaBR}$ ) and **3** [ $\text{CaB}(\text{CONHR})_2$ ], respectively. These peaks indicate that the deposited LB films have a good layered structure. The derived bilayer spacings are about 2.10, 5.15 and 5.40 nm, for **1**, **2** and **3** respectively. The former

(2.1 nm) is near the double height ( $2 \times 1.15$  nm) of **1** oriented in a porphyrin-like conformation.<sup>4</sup> The latter are near the double thickness of the long side-chain ( $2 \times 2.5$  nm). It is clear that a Y-type bilayer structure was formed during the deposition process of **1**, **2** and **3** from subphases containing  $\text{Ca}^{2+}$  ions. Since the thickness of one single monolayer of  $\text{B}(\text{CO}_2\text{R})_2$  or  $\text{B}(\text{CONHR})_2$  estimated by the C.P.K. model is *ca.* 2.35 and 3.25 nm, assuming orientation with the face and the side in touch with the water, respectively,<sup>3,5</sup> we can deduce that the orientation of molecules of  $\text{B}(\text{CO}_2\text{R})_2$  or  $\text{B}(\text{CONHR})_2$  in LB films deposited from subphases containing metal ions is such that the metal ions present in the subphase diffuse to the monolayer–water interface and form complexes with a metalloporphyrin-like structure. The faces of the complexes lie flat on the air–water interface, while the hydrophobic aliphatic chains of the  $\text{H}_2\text{BR}$  are localized above the air–water interface. This result is inconsistent with that obtained from the  $\pi$ - $A$  isotherms.

In the light of the results of  $\pi$ - $A$  isotherms, X-ray diffraction and XPS measurements, we deduced that all the three ligands **1**, **2** and **3** coordinate  $\text{Ca}^{2+}$  ion with four N atoms (two pyrrole N and two lactam N atoms), then a 1:1 complex with a ring structure analogous to metalloporphyrin complexes is formed. This suggests that, for complexes of **2** and **3**,  $\text{Ca}^{2+}$  ion present in the subphase diffuse to the monolayer–water interface and coordinate with the head groups (bilirubin group) of **2** or **3**; the obtained complexes  $\text{CaB}(\text{CO}_2\text{R})_2$  or  $\text{CaB}(\text{CONHR})_2$  lie flat on the air–water interface with the hydrophobic aliphatic chain [ $\text{CH}_2\text{CH}_2\text{CO}_2\text{C}_{16}\text{H}_{33}$  or  $\text{CH}_2\text{CH}_2\text{CONHC}_{16}\text{H}_{33}$ ] localized above the air–water interface. However,  $\text{CaBR}$  forms a monolayer with two  $\text{CO}_2\text{H}$  groups oriented into water and the pyrrole rings plane oriented in the air at a tilting angle with respect to the normal of the interface. Other metal ions, such as  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Zn}^{2+}$ , show similar characteristics.

The 1:1 coordination ratios of **1**, **2** and **3** with calcium ions are different from that of bilirubin in organic and aqueous basic solution. Bilirubin can form 1:1 and 2:1 complexes with  $\text{Ca}^{2+}$  ions and 1:1, 1:2 or 2:1 complexes with other metal ions such as  $\text{Zn}^{2+}$ ,  $\text{Cd}^{2+}$  and  $\text{Cu}^{2+}$  in bulk solution.<sup>3,6,7</sup> This difference was recognized as arising from the difference between the heterogeneous air–water interfacial environment and bulk solution. For the complexes of **2** and **3** the steric hindrance caused by the two long aliphatic chains might have an effect on the coordination.

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