Chinese Chemical Letters Vol. 12, No. 5, pp. 457 – 460, 2001

Langmuir-Blodgett Films and Calcium Ion Coordination of Biliverdin and Its Amphiphilic Derivatives

Jian Ming OUYANG

Department of Chemistry, Jinan University, Guangzhou 510632

Abstract: Monolayer formation and LB film fabrication of amphiphilic derivative of biliverdin **1**, diododecyl biliverdinamide [$B(CONHC_{12}H_{25})_2$, **2**] at an air-water interface on pure water subphase and subphase containing calcium ion were investigated and compared with **1**. The coordination in ordered molecular films is much different from that in bulk solution. The formation of ligand-calcium complex was confirmed by X-ray photoelectron spectroscopy.

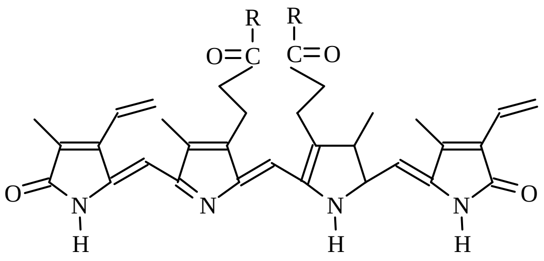
Keywords: Monolayer, Langmuir-Blodgett films, biliverdin, calcium ion.

Up to now, the investigations of the chemistry of biliverdin and its analogs has been limited in organic solvents[1-4]. There is no report on organized molecular films of biliverdin and its derivatives. The structural organization and biophysical properties of biliverdin molecules in ordered molecular assemblies might be different from those in organic solvents, but similar to those in biological membranes in mammals. Therefore, biliverdin or its derivatives incorporated in ordered molecular films may have potential applications in mimicking the mineralization processes[4,5], and to explore the mechanism of the formation of gallstones[6,7].

Both biliverdin **1** itself and its amphiphilic derivative **2** can form stable monolayers with collapse pressures of *ca*. 29 and 33 mN m^{-1} on pure water subphase (pH 5.6). As shown in **Figure 1**, the limiting areas of about 0.58 and 0.63 nm^2 per molecule were obtained by extrapolating the surface pressure to zero. According to a space-filling molecular (C.P.K.) model, biliverdin can be approximated as a rectan-gular 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 porphyrin-like conformation[8-10]. Therefore, the area values of 0.58 and 0.63 nm^2 per molecule correspond to the side area of biliverdin. It means the monolayers of **1** and **2** on pure water subphase have close-packed structure in porphyrin-like conformation.

The Ca^{2+} ion contained in subphase has a striking effect on the monolayer behavior for the monolayer of amphiphilic biliverdin molecule **2**. The π-A isotherms of **1** and **2** monolayers in subphases containing Ca^{2+} ions are also shown in **Figure 1**. Compared with pure water subphase, both the shape of the figures and the values of limiting areas of **1** and **2** changed in different degree, indicating the coordinations between these ligands and Ca^{2+} ions have occurred at the air-water interface.

Figure 1 Surface pressure-area isotherms of **1** and **2** on aqueous subphase with and without Ca^{2+} ion (pH = 5.6, 25℃)



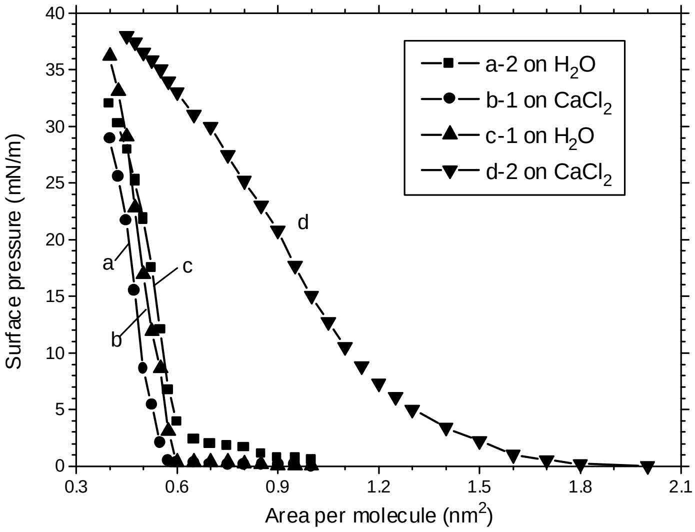
1 R = H
2 R = $NHC_{12}H_{25}$

The areas per **1** and **2** molecular unit and the collapse pressures on Ca^{2+} ion subphase are 0.60, 1.30 nm^2 and 37, 38 mN/m, respectively. The latter area (1.30 nm^2) corresponds to the face area of biliverdin, but the former (0.60 nm^2) is still consistent with the side area of biliverdin.

The coordinations of **1** and **2** with calcium ion at air-water interface have been confirmed by X-ray photoelectron spectroscopy (XPS) measurements. Two-layer LB films on hydrophilic quartz plates were made at 20 mN m^{-1}. An Al K_{α} X-ray radiation source (1486.6 eV) under a vacuum of 10^{-7} Pa. The XPS spectra present the core level peaks of all the elements of the ligands (*i.e.* carbon, oxygen, and nitrogen) as well as metal calcium containing subphase (**Figure 2**). Compared with the binding energy of N1s and O1s (399.85, 532.6 eV for **1**, 399.8, 532.8 eV for **2**) in ligands, N 1s increased to 400.3 eV in the **1**-M (CaBV), 400.9 eV in the **2**-M [$CaB(CONHR)_2$] LB films, respectively. But the binding energy of O1s hardly changed. It is clear that a lone pair electrons from the nitrogen atoms are shifted into the metal atoms and thereby a strong complexation of N atoms with Ca^{2+} was formed. Oxygen atoms do not take part in coordination. The charge-transfer from the N atoms of ligands to metal ions leads to decrease of charge density and an increase of binding energy of N atoms. That is, both **1** and **2** are coordinated to calcium ion in subphase through the pyrrole N atoms and the lactam N atoms.

The 1:1 coordination ratios of **1** and **2** with calcium ion at the air-water interface are different from that of biliverdin in organic and aqueous basic solution. Biliverdin can form 1:1 and 2:1 complexes with Ca^{2+} ion, and 1:1, 1:2 or 2:1 complexes with the other metal ions such as Zn^{2+}, Cd^{2+} and Cu^{2+} ions in bulk solutions[4,11,12]. This difference was recognized as the difference between the heterogeneous air-water interfacial environment and bulk solution. To the complex of **2**, the steric hindrance caused by the two long aliphatic chain might have an effect to the coordination.

Figure 2 XPS survey scan for **2** LB film deposited from subphase containing $CaCl_2$



Since black pigment gallstones which produced from the surface of biological membrane are the products of abnormal biomineralization in mammals bodies, so the investigation in the coordination of biliverdin and its derivatives with metal ions, especially with Ca^{2+} ion, at the air-water interface will be helpful to understand the formation mechanism of gallstones in molecular level.

Acknowldgment

This research work was granted by a Key Project from the National Natural Science Foundation of China and Key Project of Science and Technology from the Education Department of China.

References

1. K .P. Heirwegh, N. Blankaert, G. V. Hees, *Anal. Biochem.*, **1991**, *195*, 273.
2. S. Modi, *J. Inorg. Biocchem.*, **1993**, *52*, 297.
3. I. Sovago, B. Harman, *Inorg. Chim. Acta*, **1985**, *106*, 181.
4. T. K. With, *Bile Pigments. Chemical, Biological and Clinical Aspects*, 1st Ed., Academic Press, New York, **1968**, 1.
5. J. M. Ouyang, W. J. Zheng, C. Li, *Mater. Sci. Eng. C*, **1999**, *10*, 115.
6. R. V. Person, B. R. Peterson, D. A. Lightner, *J. Am. Chem. Soc.*, **1994**, *116*, 42.
7. S. E. Boiadjiev, R. V. Person, D. A. Lightner, *et al*, *J. Am. Chem. Soc.*, **1992**, *114*, 10123.

8. J. M. Ouyang, C. Li, *Thin Solid Films*, **1999**, *348*, 242.
9. D. Kaplan, G. Navan, *Israel J. Chem*., **1983**, *23*, 177.
10. W. S. Sheldrick, *Israel J. Chem.*, **1983,** *23*, 155.
11. J. D. Van Norman, M. M. Humens, *Anal.Chem*., **1974**, *46*, 926.

Received 23 May, 2000
Revised 20 November, 2000