

Metal Ion Complexation of Bilirubin Monolayer

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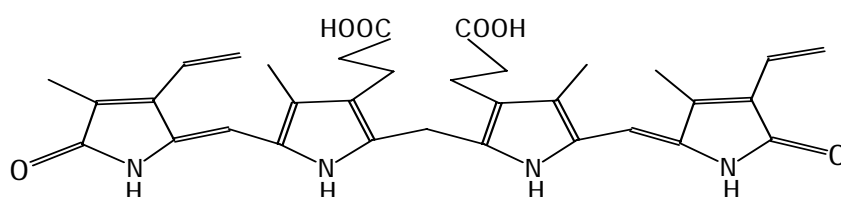
Abstract: The surface pressure-area isotherms of bilirubin (BR) monolayers at an air-water interface on subphases with and without metal ions were investigated. The formation of BR-metal complexes leads to changes in the shape of isotherms of BR and changes in UV-visible absorption spectra of the monolayer assemblies. In XPS spectra new XPS peaks assigned to the metal ions containing subphases appeared. Low-angle X-ray diffraction indicates this Y-type LB films of MBR were formed with bilayer spacing of *ca.* 2.1 nm.

Keywords: Monolayer, bilirubin, complexation.

Recently, biomimetic strategies have focused on the application of bioinorganic nano-composites and shaped hybrid materials with microscale organization¹. Because of the importance of bile pigments in physiological system such as calcium bilirubinates, the main component of black-pigment gallstones, an abnormal product of biomineralization in mammal bodies and copper bilirubinate, a free-radical scavenger in bile and protecting phospholipids from peroxidation², a great deal of attention has already been given to the study of bilirubin (BR) and other bile pigments³. Excellent reviews of these studies have been made many years ago⁴. But so far there is little attempt at organized molecular films of bilirubin. LB technique makes it possible to prepare organic functional ultrathin films with a thickness at a molecular size and with well defined molecular orientation. Since the structural organization and biophysical properties of bilirubin molecules in ordered molecular assemblies might be different from those in solution, therefore, if bilirubin or its derivatives can be incorporated in ordered molecular films, these ordered systems may have potential applications in mimicking the mineralization processes, such as the prevention and cure of black pigment gallstones in human body and the promotion in animal body, especially in cattle and monkey bodies, as well as to investigate the structure of bilirubin, which is believed to play a very important role in the chemistry of bilirubin and its salts.

Figure 1 shows the π -A isotherms of BR monolayers in subphases with and without metal ions. Compared with pure water subphase, the π -A isotherms of BR monolayers in metal ions containing subphases are more condensed. The area per BR molecule is *ca.* 0.63~0.72 nm².

The molecular structure of bilirubin 1



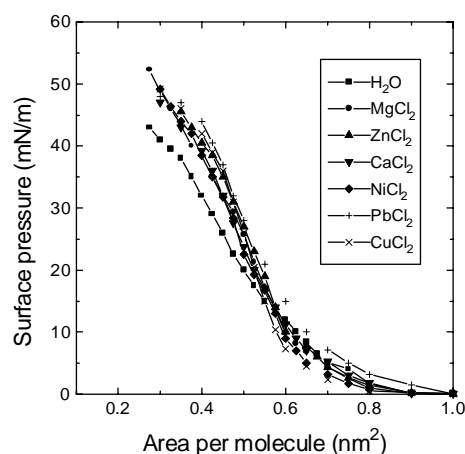
The coordination of BR with metal ions at air-water interface were confirmed by UV-visible spectra of the LB film of BR deposited in subphases containing these metal ions. In the absorption spectra of an 11-layer LB film on a quartz substrate on pure water subphase and on subphases containing Ni²⁺ and Cu²⁺ ions, respectively, the appearance of two new absorption bands centered at about 610 and 660 nm (602 and 660 nm for Ni²⁺; 608 and 658 nm for Cu²⁺) in the LB films deposited from Ni²⁺, or Cu²⁺ ion containing subphases respectively, indicate that interfacial coordination occurs immediately after spreading the BR molecules. These new bands are assigned to an electron transition from the pyrrole rings of bilirubin to the hole in the 3d shell of metallic ions. In contrast to the well documented instability of BR in solution, BR confers an unusual stability on the monolayers. The UV-visible spectra of BR and its amphiphilic complexes MBR in organized molecular films can be stabilized for two weeks without apparent change, showing distinct difference between ordered molecular films and the bulk solution.

The coordination of BR with metal ions was confirmed by X-ray photoelectron spectroscopy (XPS). In the XPS survey scans of 2-layer LB films of BR deposited from subphases with Ca²⁺, Cu²⁺, Mg²⁺, Zn²⁺ ions, in addition to the constituents of BR, the O1s, N1s and C1s core level peaks common to all the spectra, the spectra of the BR LB films deposited from metal ions containing subphases are marked by the presence of the corresponding metal core level peaks, Ca2p, Cu2p, and Zn2p, indicating the presence of metal ion in the LB films. That is, a complexation of BR with metal ions has occurred at the air-water interface and the BR-M complexes monolayers were transferred onto quartz plates.

Compared with the binding energy of N1s (399.6 eV) and O1s (532.3 eV) in BR LB films, N1s increased to 400.2~400.6 eV in the BR-M LB films, while O1s remains nearly unchanged (532.2~532.4 eV). It is clear that the lone pair electrons from the nitrogen atoms are shifted into the metal atoms and thereby a complexation took place. BR is coordinated to the metal ions in subphase through the pyrrole N atoms and the

lactam N atoms, but the oxygen atoms in BR do not take part in coordination. The charge-transfer from the N atoms of BR to metal ions leads to a decrease of charge density and an increase of binding energy of N atoms. That is, BR coordinates metal ion with four N atoms, then a ring structure complex (MBR) analogous to metalloporphyrin formed. MBR forms monolayer with two COOH groups oriented into water and the pyrrole rings oriented in the air, at an tilting angle perpendicular to the interface.

Figure 1 π -A isotherms of BR monolayers in subphases containing metal ions



This 1:1 coordination ratio of BR with divalent metal ions M^{2+} is unique as compared with that of bilirubin in organic and aqueous basic solution. Bilirubin can form 1:1, 1:2, and sometimes 2:1 complexes with metal ions such as Ca^{2+} , Zn^{2+} , Cd^{2+} , and Cu^{2+} ion. This difference was recognized as the difference between the heterogeneous air-water interfacial environment and bulk solution.

Acknowledgment

This research work was granted by a Key Project of Science and Technology from the Education Department of China and Natural Science Foundation of Guangdong Province (980898, 970635).

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Received 5 January 1999