Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 205 (2018) 391–397

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

Polarized infrared spectroscopy imaging applied to structural analysis of bilirubin aggregate at liquid-liquid interface

Yanfei Lu [1], Mingyang Zhai [1], Yongkang Zhu, Xiao Wang, Jianhua Yin *

Department of Biomedical Engineering, Nanjing University of Aeronautics and Astronautics, Nanjing 210016, China

ARTICLE INFO

Article history:
Received 20 May 2018
Received in revised form 6 July 2018
Accepted 16 July 2018
Available online 17 July 2018

Keywords:
Bilirubin
Aggregate
Infrared spectroscopy imaging
Spectral analysis
Polarization analysis

ABSTRACT

Fourier transform infrared spectroscopy imaging (FTIRSI) combined with spectral analysis and polarization approach was creatively used to investigate both structures of bilirubin (BR) precipitate and BR aggregate at liquid-liquid interface. It was found by spectral analysis that the internal hydrogen bonds of BR molecules all broke and the dihedral angles increased during the formation of BR aggregate at liquid-liquid interface. And the BR molecule might be of layer assembly along the long axis direction of CD half-group to form J-type aggregates, which could be parallel to the direction of the transition dipole moment of BR aggregate. The further study of polarized imaging/anisotropy revealed that the absorbance of 1570 and 1703 cm^{-1} bands of BR aggregate changed periodically at intervals of 90°, which were not shown in BR precipitate case, indicating that the C═C of the corresponding lactam ring and the C═O of the adjacent carboxyl groups formed ordered arrangement in BR aggregate. It also suggested that the two positions might be the active sites which J-type aggregates assembled on. The combined technique was firstly applied in interfacial aggregate research, which was helpful for further understanding and controlling the aggregation as well as structural transformation of BR molecules so as to decrease physiological hazard and facilitate the wide spread application in biomedicine.

1. Introduction

Bilirubin (BR) is an orange-yellow product of heme going through normal catabolism in the body, as well as an antioxidant [1]. In clinical, BR plays a significant role in judging jaundice and indicating liver function. It can cause irreversible damage to the brain and nervous system due to its endogenous toxin in the human body [2].

Because of its toxicity, BR is transported to the liver through blood as a BR-albumin complex. Under normal conditions, BR is excreted by further metabolism after being combined with glucuronic acid. If the albumin produced in human body is not enough, unbound BR will accumulate under the skin, which increases its chance of crossing neuron membrane or penetrating into the cell [3]. However, it is still unknown how the BR penetrates the phospholipid membrane of neurons or cells. This complexity is not only related to the physical and chemical properties of the molecule, but is largely determined by the form of molecular aggregation [4]. Liquid-liquid interface is usually regarded as a specific reaction field and a simple model of biological membrane [5]. Therefore, the study on BR aggregation at interface is very helpful for understanding the interaction mechanism, transport process and physiological function of BR monomer and BR aggregate.

The molecular structure can be accurately expressed, as shown in Fig. 1. Individual BR molecule can be divided into AB half-group in upper plane and CD half-group in bottom plane. Each half-group has a lactam ring (L ring) and pyrrole ring (P ring) to make up two similar planar dipyrrolone chromophores with interplanar dihedral angles and small π-orbital overlap, resulting in optical chirality. But the structure and arrangement of BR aggregate have not yet been clearly understood since very limited report on them.

At present, BR has been studied by biochemistry and spectroscopic techniques [6–8]. Specifically, interfacial aggregation of BR molecule has been conveniently investigated by using spectroscopic techniques, such as Raman, UV–vis absorption, circular dichroism and fluorescence techniques [9–11], etc. However, it's still very hard to obtain the inner structure and molecular arrangement of BR aggregate at liquid-liquid interface because of the limitation of instrument. As a new spectroscopy and imaging technique, Fourier transform infrared spectroscopy imaging (FTIRSI) plays increasingly prominent roles in multi-fields, such as biomedical field [12–16] and bioanalytical chemistry [17], etc., by providing richer composition, structure and morphology information of biospecimen and rapider scanning than other spectroscopic techniques. In addition, one can select the regions of interest (ROI) through FTIRSI to achieve the visualization of the sample and high-precision and high-sensitive spectral analysis. When combined with polarization measurements, this spectroscopy method is capable to get special structural information on oriented molecules, such as collagen [12,15]. In this study,

* Corresponding author.
E-mail address: yin@nuaa.edu.cn (J. Yin).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.saa.2018.07.048
1386-1425/

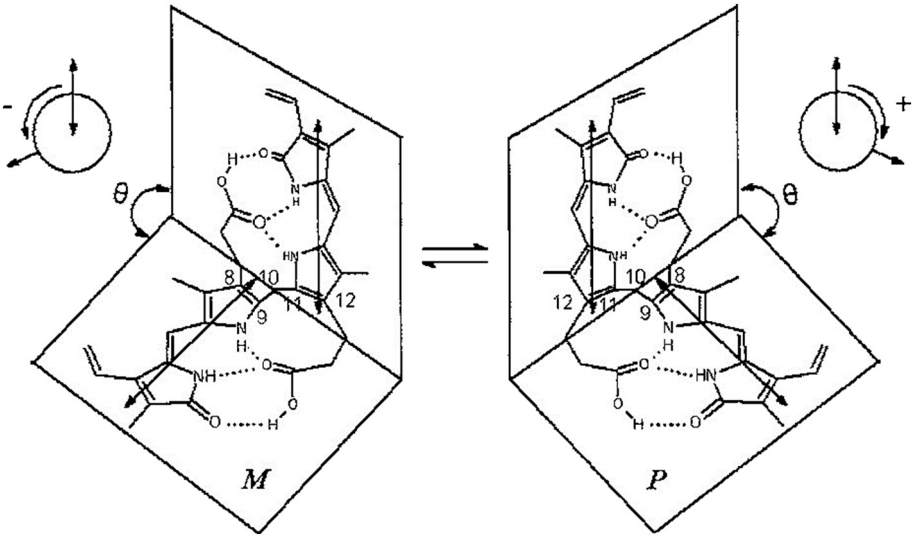

Fig. 1. The enantiomeric conformations of BR (*M* and *P*). The double-headed arrows indicate the approximate direction and intensity of the electric transition dipole moments and the angle of the two planar (θ) is ~100°.

therefore, FTIRSI combined with spectral analysis and polarization approach (polarized FTIRSI) was creatively used for structural analysis of BR precipitate and BR aggregate at liquid-liquid interface. To the best of our knowledge, no groups have yet investigated the oriented structure of BR aggregate at the interface. It's showing a novel combined spectroscopic technique and may help people easily understand BR aggregation, adjustment and roles in biomedicine.

2. Material and Methods

2.1. Chemicals and Sample Preparation

BR powder with purity of 99% was purchased from (Sigma-Aldrich, MO) and used as received. 0.25 mg BR powder was weighted and mixed into 100 mg dried-and-ground KBr powder to make a pellet with diameter of 10 mm.

BR stock solution with a concentration of 4.30×10^{-4} M was prepared by dissolving BR in chloroform. 15-μl BR stock solution was dropped onto the BaF_2 crystal and formed the BR precipitate sample after evaporation.

A special double-layer sample cell was home-made, whose lower layer was filled with water (pH 6.0) without adding any buffer. 150-μl heptane was added onto the aqueous layer to form a liquid-liquid interface. Then 15-μl BR stock solution was dropped in heptane solvent to produce BR aggregate at the liquid-liquid interface by extraction.

2.2. FTIRSI on BR Samples

FTIR spectra were measured on a PerkinElmer Spotlight-400 imaging system (Nanjing Forestry University), which consisted of a FTIR spectrometer and an infrared microscope. In the imaging mode, the analyte was mounted on the movable stage of the infrared microscope and fixed during the scanning under infrared radiation. The FTIR spectra were ranged in 4000–800 cm^{-1} with the spectral resolution of 4 cm^{-1}, while the pixel size was set as 6.25 μm × 6.25 μm. The CCD camera was fully integrated with the microscope stage motion and IR spectral data acquisition to produce a visible image under an internal coaxial light emitting diode with variable intensity. The desired areas on the visible image were identified for FTIRSI.

In addition, in order to obtain the anisotropy or orientation of the samples, an infrared wire grad analyzer was inserted between the sample and the detector. When the precipitate was measured, the analyzer/polarization angles were set at 0°, 45°, 90°, 135° and 180°, respectively, to obtain FTIR images at the corresponding analyzer angles. In each polarized FTIR image, three co-added spectra were extracted from 3 fixed ROI with the same length and width, and then were processed to get an averaged spectrum. Integration area of characteristic peak was regarded as the absorption intensity, absorbance.

Similarly, the FTIR images at different analyzer/polarized angles of 0°, 30°, 45°, 60°, 90°, 120°, 135°, 150° and 180° were obtained when the BR aggregate was scanned. In each FTIR image, 5 fixed ROI with same length and width were taken to get their average spectrum. The integration area of IR peak was used to characterize the absorption intensity.

2.3. Data Processing

In order to analyze the structural changes of BR in different states, we introduced the following relative intensity parameters and corresponding analytical method [18]. The 1188 cm^{-1} peak could be used as the internal standard among BR powder, BR precipitate and BR aggregate, and then other related characteristic peaks were normalized to 1188 cm^{-1}. The formula were shown below,

$$R_p = I_P / I_{1188} \tag{1}$$

$$R_c = I_c / I_{1185} \tag{2}$$

$$R_a = I_a / I_{1188} \tag{3}$$

$$\Delta R_a(\%) = \frac{(R_a - R_p)}{R_p} \times 100\% \tag{4}$$

$$\Delta R_c(\%) = \frac{(R_c - R_p)}{R_p} \times 100\%, \tag{5}$$

where I_P, I_c and I_a were the absorbance of the characteristic peaks in BR powder, BR precipitate and BR aggregate, respectively. R_p, R_c and R_a represented the normalized intensity of the specific band of the BR powder, BR precipitate and BR aggregate relative to the 1188 cm^{-1} band in the respective spectra. ΔR_a and ΔR_c represented the relative intensity changes of each band of the BR aggregate and BR precipitate with respect to that of the BR powder. When the ΔR was more than 20%, it indicated that spectral intensity change of BR aggregate or BR precipitate to BR powder was noticeable.