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Quartz crystal microbalance studies on bilirubin adsorption on self-assembled phospholipid bilayers

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Abstract

Bilirubin adsorption on self-assembled phospholipid bilayers was studied using quartz crystal microbalance, and factors influencing its adsorption such as pH, temperature, and solution ionic strength were discussed in detail. The results show the amount of adsorbed bilirubin on self-assembled phospholipid bilayers is small at higher temperature and large at higher pH and solution ionic strength, and the adsorption kinetic parameter estimated from the in situ frequency measurement is $(1.8 \pm 0.27) \times 10^6\ M^{-1}$ (mean ± S.D.). With the present method, the desorption of adsorbed bilirubin caused by human serum albumin and the photoinduced decomposition of adsorbed bilirubin under light illumination were also examined. QCM measurement provides a useful method for monitoring the adsorption/desorption process of bilirubin on self-assembled phospholipid bilayers.

Keywords: Phospholipid bilayer; Bilirubin; Adsorption; Quartz crystal microbalance

1. Introduction

Bilirubin is the main bile pigment that is formed from the breakdown of heme in red blood cells [1,2]. Normally, it is conjugated with albumin to form a water-soluble complex and excreted from hepatocytes into bile mainly as bilirubin glucuronides [3–5]. However, it has been suggested that it is toxic when the amount of bilirubin in body exceeds the binding capacity of albumin, extra free bilirubin binds and deposits to various tissues, especially in the brain. Its deposition and accumulation in tissues will cause disorders in the metabolism of bilirubin and lead to jaundice or kernicterus [6,7]. This may cause cell death in various tissues, mental retardation, celebral palsy or death [8,9]. To prevent this from happening, many techniques have been developed for the removal of bilirubin directly from plasma of patients suffering from hyperbilirubinemia [10–12]. However, it has not still been reported in literatures about the behavior of bilirubin deposition on various cell types whose surfaces are mostly composed of phospholipid, and the actual mechanisms of the interaction between bilirubin and phospholipid membranes are still poorly understood. It has been demonstrated that mimic phospholipid bilayer membranes surrounding living cells are chemically relatively passive and become biologically active primarily through membrane-bound molecules [13,14]. Thus, it would become possible to investigate the adsorption of bilirubin on mimic phospholipid bilayers. The study of bilirubin adsorption on phospholipid bilayer membranes would be helpful in the development of various preventive measures against jaundice and kernicterus. At present, quartz crystal microbalance (QCM) technology may be a comparatively good method for directly detecting the bilirubin adsorption on phospholipid bilayers.

QCM is an extremely sensitive surface sensor which is capable of measuring the nanogram level of mass change on the surface. Its uses in biochemistry, environment, food, and clinical analysis are very attractive because the technology provides a label-less method for the direct study of biospecific interaction process [15–17]. With immobilized antibodies on the surface of crystal, some QCM immunosensors have been used for the detection of viruses [18,19], proteins [20,21], bacteria [22,23]. In addition, it has also been demonstrated that it is useful for the

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study of adsorption/desorption process at solid/solution interface [24,25].

In the present work, a QCM device has been employed to study the adsorption behavior of bilirubin on self-assembled phospholipid bilayer. The desorption of adsorbed bilirubin caused by human serum albumin and photoinduced decomposition of adsorbed bilirubin during light illumination have also been investigated.

2. Materials and methods

2.1. Materials

Bilirubin IX-α (93%) was purchased from Sigma Chemical Co. The egg yolk phosphatidylcholine (egg PC) was obtained from Sigma-Aldrich (Sweden). Benzoquinone (BQ) and Human serum albumin (HSA) were obtained from Sigma, USA. All other chemicals were of analytical grade purchased from Merck. Highly pure water was obtained from a Milli-Q system (Millipore Inc.) and was used for rinsing and for the preparation of all aqueous solutions.

Bilirubin stock solution (1 mM) was prepared daily by dissolving 5.8 mg of bilirubin in 10 ml of 50 mM NaOH solution. Standard solutions of lower concentrations were prepared by further dilution of the bilirubin stock solution. All solutions were stored in amber glass vials wrapped with aluminum foil and placed in the dark to prevent light-initiated bilirubin oxidation. The pH of the solutions was adjusted by adding an adequate amount of dilute HCl and buffered at the desired pH by the phosphate buffer solution, unless otherwise stated.

2.2. Apparatus

QCM measurements were performed using a Q-sense instrument (Gothenburg, Sweden). Mechanically polished AT-cut 9-MHz quartz crystals (diameter of 12 mm, International Crystal Manufacturing Co. Inc., Oklahoma City, OK) were vacuum-deposited with gold electrodes (6-mm diameter) on both sides of the surface. To reduce background frequency drift, one electrode was completely insulated from the test liquid. This was accomplished by mounting a glass cover slip over one side of the quartz crystal, which was held in place by two silicon o-rings. The o-rings were large enough (10 mm) so that they could make contact only with the exposed quartz peripheral to the centrally located electrode and not with the electrode itself. Thus, the covered electrode was in contact only with air. This covering assembly was held in place using silicone glue. The electrical contacts were insulated from the test solution using silicon tubing and silicon glue, and only one electrode was exposed to the test solution. The working crystal was connected to an IC-TTL oscillator. Both the detector cell and the oscillator were put in a copper Faradaic cabin to remove the surrounding electromagnetic noise. The frequency was monitored by a frequency counter (Iwatsu, Model SC-7201), and the recorded data were stored in a computer.

The curves of current versus potential were recorded between -500 and $+200$ mV, at 100 mV s^{-1} scan rate. The cyclic voltammograms were measured by the CHI660 electrochemical station (CH Corporation, USA).

2.3. Preparation of phospholipid bilayers on the surface of QCM

Prior to use, quartz crystals with gold electrode were rinsed with ethanol and then immersed in 1:3 (v/v) H_2O_2/H_2SO_4 (piranha solution) for at least 5 min at room temperature. After that they were repeatedly rinsed with water and ethanol, then sonicated for a few seconds in ethanol, and finally dried with pure nitrogen gas.

The clean crystals were incubated for 3 h in 1.0 wt% egg PC dissolved in ethanol and then washed with water. The obtained crystals with phospholipid bilayer were stored in water before use.

2.4. Adsorption of bilirubin on phospholipid bilayers

To monitor the in situ adsorption process of bilirubin on *phospholipid bilayers*, the following procedure was adopted. After mounting the crystal in the cell, a small amount of the phosphate buffer saline (PBS) was introduced into the cell. When the frequency became stable, the bilirubin solution was introduced into the cell within 5 s. Time-dependent change in the frequency was recorded continuously by a frequency counter and stored in a microcomputer during the adsorption process of bilirubin.

3. Results and discussion

3.1. Formation of self-assembled phospholipid bilayer on the surface of QCM

The phospholipid bilayers, which were rigid, stable and as smooth as the original gold surfaces, were prepared on gold surfaces as detailed elsewhere [26]. The formation process of self-assembled phospholipid bilayers on gold electrode could be described as follows: when egg PC vesicles came in contact with the hydrophilic gold surfaces, they first adsorbed to gold surface as intact vesicles and then they ruptured and transformed spontaneously to a bilayer with polar head group outside (see Fig. 1). Fig. 2 presents cyclic voltammograms of 0.5 mM BQ in 0.1 M KCl aqueous solution on phospholipid bilayers/Au

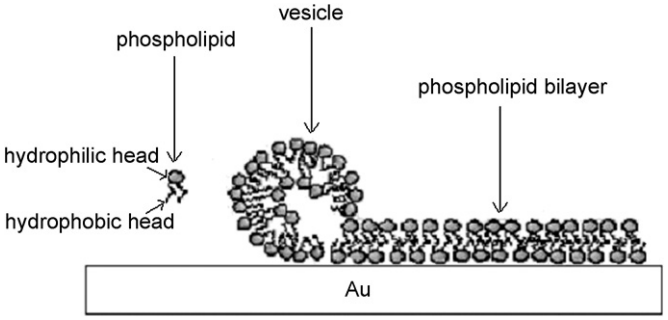

Fig. 1. Schematic of phospholipid bilayer formation.