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Influence of alkaline phosphatase on phase state of the SM monolayers at the air-water interface

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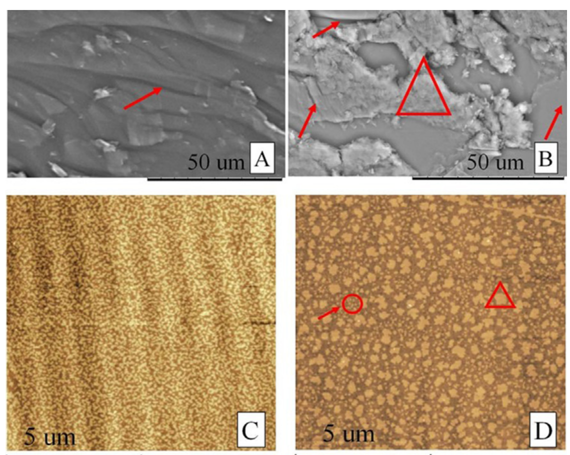
HIGHLIGHTS

- The different amount of alkaline phosphatase have an influence on the phase transition of SM monolayer at the air–water interface.
- The interfacial mixing ratio of protein/lipid has been calculated by the mass conservation plots.
- The images of AFM and SEM shown the microstructure of the monolayer at 5 and7.8 mN/m.

GRAPHICAL ABSTRACT



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ABSTRACT

The Langmuir monolayer is a most suitable model membrane system to study the interfacial interaction between protein and lipid. The surface pressure-area per molecule isotherms of the SM monolayer in the presence of different amount of alkaline phosphatase have been studied in this work. The compression modulus and the interfacial mixing ratio of protein/lipid have been calculated. The different amount of alkaline phosphatase has an influence on the phase transition of SM monolayer at the air–water interface. A coexist state of liquid expanded and liquid condensed is due to the presence of alkaline phosphatase. The process of phase transition from liquid expanded state to coexist state is longer in the presence of more amount of protein. The emergence of coexistence phase state reflects the interaction of the protein and the SM monolayer. The interfacial mixing ratio at the liquid expanded state is higher than that of the coexist state of liquid expanded and liquid condensed. The images of SEM and AFM also shown the microstructure of SM monolayers at two phase states in the presence of alkaline phosphatase.

1. Introduction

Membrane models are often used to study biophysical and biochemical phenomena involved in the interaction between molecules and cellular membranes [1,2]. Lipid monolayer is a most

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commonly used system [3,4]. The Langmuir technique is based on the property of amphiphilic molecules to form a monolayer film when spread at the air-water interface and subsequently subjected to compression [5]. The surface pressure isotherm studies can provide the information on the phase transition of monolayers. And the interfacial interaction ratio of protein/lipid can be calculated by data of the surface pressure-area per molecule curves.

The enzyme of alkaline phosphatase has a role in the biomineralization process [6]. Alkaline phosphatases from several sources have been intensively studied at the air–water interface [7,8]. The orientation and distribution of the enzyme on the lipid monolayer were studied frequently, while papers about the influence of the amount of alkaline phosphatase to the lipid monolayer are rarely reported. Alkaline phosphatase, as a medical check index, is used to help with the disease diagnosis of bone, liver and gallbladder system [9,10].

High content of alkaline phosphatase is relative to the diseases. So, the influence of different amount of the enzyme to the lipid monolayer model is worth being studied. Sphingomyelin (SM), containing saturated fatty acids, was used to as model membrane in this work. The effect of different amount of alkaline phosphatase on the phase of SM monolayer at the air–water interface was investigated.

2. Materials and methods

Sphingomyelin, alkaline phosphatase from bovine intestinal mucosa, Tris, Chloroform, methanol, ethanol absolute, potassium chloride, sodium chloride, hydrochloric acid were obtained from Sigma Chemical Co. (St.Louis, MO). All experiments were carried out at 25 ± 1 °C and all solutions were prepared using ultrapure water.

2.1. Preparation

5 mM Tris–HCl buffer (pH 7.5, containing 150 mmol NaCl, 2 mmol $MgCl_2$ and 0.4 mol KCl) was used as a subphase solution under the air–water interface. Alkaline phosphatase was dissolved in Tri–HCl buffer with the concentration of 0.05 μmol/ml. Sphingomyelin(SM) solution was prepared in chloroform/methanol (proportion 3:1 in volume). The concentration was 0.5 μmol/ml.

2.2. Langmuir setup

The SM monolayer at the air-buffer interface was run using a Teflon Langmuir trough (KSV, Finland). The surface pressure was measured by the Wilhelmy method, using a very thin plate of filter paper [11–13]. Phospholipids were spread at the air–water interface and different amount of alkaline phosphatase were injected under the interface. After 20–min for solvent evaporation, the monolayer was compressed to obtain a surface pressure-area per molecule ($\pi - A$) isotherm. Monolayer compression was performed at a compression rate of 0.5 cm^2 s^{-1}.

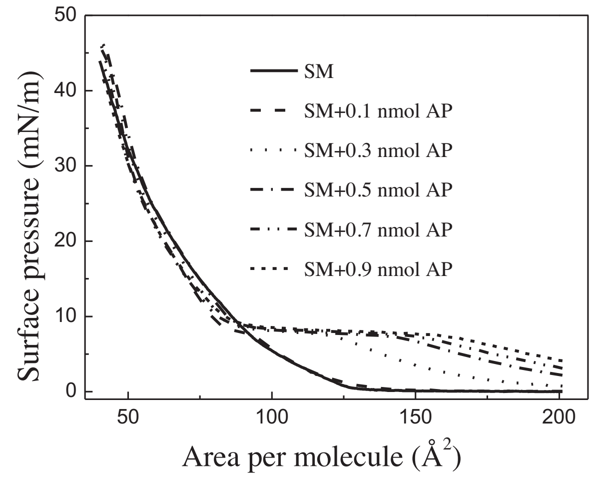

Fig. 1. The surface pressure-area per molecule isotherms of SM monolayers in the presence of different amount of alkaline phosphatase.

2.3. SEM and AFM images

Transfer the Langmuir monolayers onto the fresh mica at a certain surface pressure, forming Z-Langmuir film. The dipping rate for transfer was 5 mm/s. Observe their microstructure characterization by scanning electron microscope (Hitachi, Japan) and atomic force microscopy (Shimadzu, Japan). SEM samples were coated with gold before examination. AFM images can be obtained in the contacting mode using a silicon nitride pyramidal tip mounted on a 100 um long cantilever with a force constant of 0.1 N/m.

3. Results and discussion

3.1. phase transitions

For the pure SM monolayer, the isotherm has no clear inflection point. But after the injection of alkaline phosphatase under the air–water interface, a flat region is shown (Fig. 1). The flat region changes longer with the increase of the amount of alkaline phos-

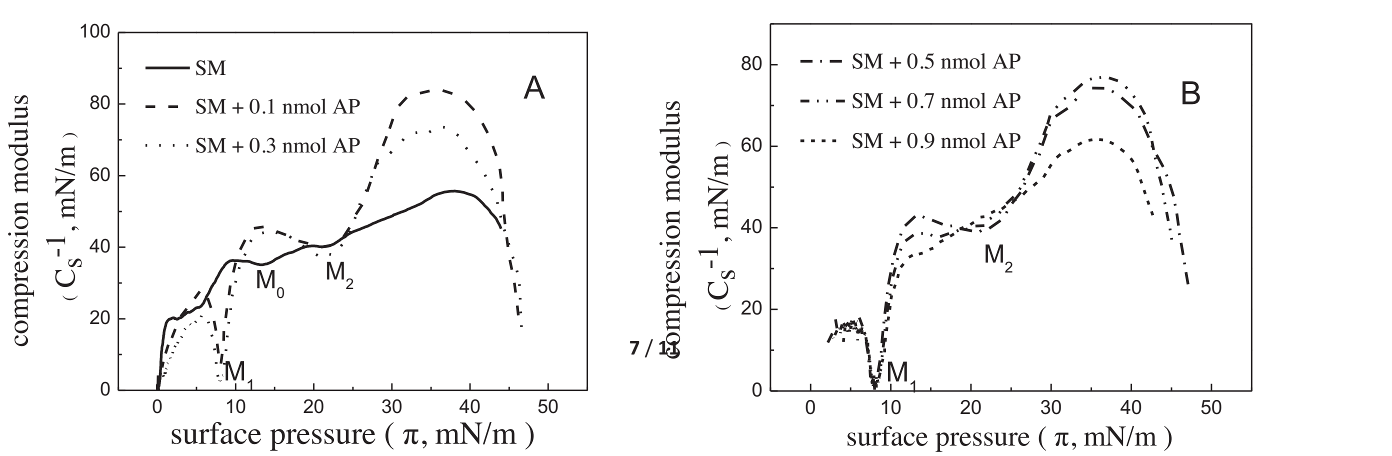

Fig. 2. The compression modulus-surface pressure curves of SM monolayers in the presence of different amount of alkaline phosphatase.