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Thermodynamic adsorption properties of bovine serum albumin and lysozyme on the bubble surface from the binary solution

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ABSTRACT

In this work, the thermodynamic adsorption properties of bovine serum albumin (BSA) and lysozyme (LZM) on the bubble surface from the binary solution were studied to promote the application of foam fractionation in the selective separation and purification of proteins. The thermodynamic adsorption properties included the adsorption isotherm, the distribution coefficient and the separation factor. Redlich–Peterson equation could be effectively used to describe the equilibrium adsorption of BSA and LZM on the bubble surface from the binary solution. Under the conditions of temperature 30 °C and pH 4.6, the adsorption isotherms of BSA and LZM were $\Gamma_1 = 3.12 \times 10^{-1} C_1/(1 + 61.70 C_1^{0.75} + 0.18 C_2^{0.93})$ and $\Gamma_2 = 5.30 \times 10^{-2} C_2/(1 + 61.70 C_1^{0.75} + 0.18 C_2^{0.93})$, respectively. According to the adsorption isotherms, the BSA concentration had significant effects on the distribution coefficient of LZM, but the LZM concentration had almost no effects on the distribution coefficient of BSA. The separation factor of BSA to LZM was a constant, 5.89, and was irrelative to the BSA and LZM concentrations. The absorption of BSA and LZM on the bubble surface was heterogeneous because of their different molecular sizes and charges.

1. Introduction

In recent years, foam fractionation has attracted more people's attention and got a rapid development in biochemical engineering (Sarachat et al., 2010; Suzuki et al., 2008) because of its advantages of low energy consumption, non-pollution and operation at room temperature and atmospheric pressure. There have been not only many references of lab-scale researches (Aksay and Mazza, 2007; Brown et al., 1999a; Liu et al., 2013; Maruyama et al., 2000; Sarkar et al., 1987) but also a few commercial reports (Burghoff, 2012) on separating proteins using foam fractionation. Through the efforts of many years, we have successfully applied foam fractionation to the separation of nisin from its fermentation broth and realized the large-scale commercial production (Burghoff, 2012; Wang et al., 2012; Zhang et al., 2011). At present, we are developing the technology of recovering whey soy protein from its wastewater using foam fractionation (Jiang et al., 2011; Wang et al., 2013).

To promote the application of foam fractionation in biochemical engineering, it is very important to study the selective separation of biosurfactants, especially proteins, and make them purified with each other. However, there have been only a few references on the separation of different biosurfactants, especially proteins, with each other and of course, there are not any such commercial reports. Suzuki et al. (2002) showed that in foam fractionation, the addition of sodium dodecyl sulfate (SDS) in an aqueous solution of lysozyme (LZM) and ovalbumin (OA) could greatly increase the percentage of LZM of the total proteins in the foamate to 85% when the

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initial molar concentration ratio of SDS:OA:LZM in the feed solution was 10:1:1. In their study, the combination of LZM and SDS was enhanced by adjusting their ratio in the SDS–OA–LZM system. The surface excess of LZM was also improved at the gas–liquid interface. The increase in the surface excess of LZM at the gas–liquid interface was conducive to its purification. So their study showed a method for purifying a protein from the binary protein solution. However, the added SDS in their study became an impurity which hindered the purification of LZM or OA. Brown et al. (1999b) and Nakabayashi et al. (2011) increased the enrichment ratio of a protein from the binary protein solution by adjusting the solution's pH in foam fractionation. These experiments showed that the difference of surface excess among biosurfactants could be increased by changing their physical properties due to the change of the operation conditions. So, it is feasible for a biosurfactant to be selectively separated and purified from other biosurfactants by foam fractionation.

However, there have been not any more references on studying the selective separation and purification of biosurfactants, especially proteins, from their multi-component solutions. At present, all the references only show how to improve the enrichment ratio and the recovery percentage of proteins because single-stage foam fractionation cannot meet the requirements on the purification of proteins. A multi-stage foam fractionation technology should be used for the purification of proteins. Then, the thermodynamic adsorption properties of proteins on the bubble surface from their multi-component protein solutions should first be studied. Anand and Damodaran (1995) studied the interaction relations between bovine serum albumin (BSA) and LZM adsorbed at the plane gas–liquid interfaces from the binary solution. They pointed out that the adsorption of the proteins did not follow the Langmuir-type competitive adsorption model but they did not give any adsorption isotherms of the proteins. Up to now, no references are available for the thermodynamic adsorption properties of proteins on the bubble surface from the binary solution in foam fractionation. These properties include the adsorption isotherm, the distribution coefficient and the separation factor. It is necessary to study the thermodynamic adsorption properties of proteins on the bubble surface for purifying them by foam fractionation.

In this work, an aqueous solution with BSA and LZM was used as the simulation system. In biochemical engineering, many production processes involve the mixture of proteins and enzymes, for example, the production of enzymes by microbial fermentation. Lots of proteins come from their medium or metabolites in microbial fermentation. So, there are mixtures of proteins and enzymes. BSA and LZM are often used as a simulation protein and a simulation enzyme, respectively in bioseparation, especially in foam fractionation (Barackov et al., 2012; James et al., 1991; Mitropoulos et al., 2014). In our work, BSA represented a protein and LZM represented an enzyme. The mixture of BSA and LZM was used as the simulation system to study the thermodynamic adsorption properties of the mixture of a protein and an enzyme.

First, the effect of the liquid phase height on the surface excess of BSA or LZM on the bubble surface was studied to ensure that the surface excess of BSA or LZM reached its equilibrium with the concentration of BSA or LZM in the liquid phase. Second, the Redlich–Peterson equation was used to determine the adsorption isotherms of BSA and LZM on the bubble surface from the binary solution. Then the corresponding distribution coefficients and the separation factor were determined on the basis of their adsorption isotherms. Lastly, the absorption mechanisms of BSA and LZM were shown for explaining their thermodynamic adsorption properties on the bubble surface.

2. Material and methods

2.1. Experimental materials

BSA (purity > 99%) and LZM (purity > 98%) were purchased from Tianjin Ding Guo Biotechnology Co. Ltd., China and used without further purification. Citric acid and sodium hydroxide were purchased from Tianjin Fengchuan Chemical Reagent Science and Technology Co. Ltd., China. Sodium hydrogen phosphate was purchased from Tianjin Bo Di Chemical Ind. Co. Ltd., China. Citric acid and sodium hydrogen phosphate were used to obtain buffers at required pHs. Sodium hydroxide was used to adjust the pHs of samples to 7.0. All above chemicals were of analytical grade except BSA and LZM.

2.2. Measurement of BSA and LZM concentrations

The concentrations of BSA and LZM were measured by using gel permeation chromatography (GPC) with a Viscotek 270 SEC system (Malvern Instruments Ltd., England). BSA and LZM were firstly separated by a P3000 protein SEC column (8.0 mm × 300 mm) using a citrate-phosphate buffer (with 0.1 M citric acid and 0.2 M Na_2HPO_4 for adjusting its pH to 7.0) as the mobile phase at a flow rate of 0.8 mL/min and a column temperature of 35.0 ± 0.1 °C. Then the separated BSA and LZM were detected by a UV detector. The measurement wavelength was 280 nm. The OmniSEC 4.7 software was used for the on-line recording of the signals from the UV detector. All buffers and samples were filtered with a polytetrafluoroethylene (PTFE) filter of 0.22 μm in pore diameter and the pHs of samples were adjusted to 7.0 using 2 M NaOH before they went in to the column. Purified water from a UPR-II-10T water purification system (Chengdu Ultrapure Technology Co. Ltd., China) with a resistivity of 18.2 mΩ cm was used in all measurements.

BSA solution (0.60 g/L), LZM solution (0.60 g/L), and their binary solution (BSA 0.60 g/L and LZM 0.60 g/L) were prepared by using citrate–phosphate buffer (pH 7.0). The UV absorption chromatograms at 280 nm of these three solutions are presented in Figs. 1–3.

From Fig. 1, the two peaks at retention volumes of 8.3 and 9.4 mL could be attributed to BSA aggregate and BSA monomer

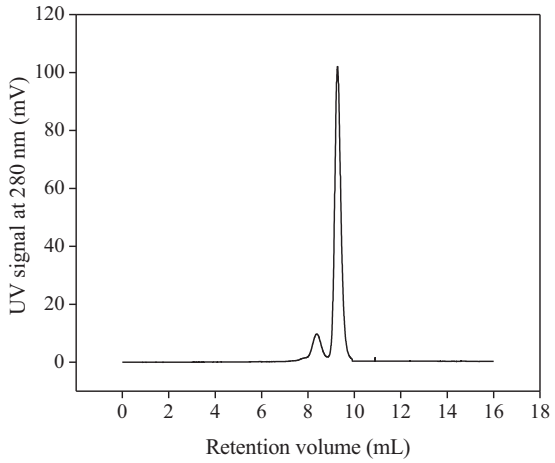

Fig. 1 – Chromatogram of BSA solution.