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# Study on the interaction between surfactin and alkaline protease in aqueous solution☆

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ABSTRACT

The interaction between surfactin and alkaline protease in aqueous solution has been studied. Ultraviolet visible absorption spectra (UV–vis) show that surfactin causes the extension of peptide chain of the alkaline protease resulting in the weakening of hydrophobic interaction between the hydrophobic groups. Fluorescence spectra indicate that the interaction of surfactin with the tryptophan and tyrosine residues led to a change of conformation of the alkaline protease. Fourier transform infrared spectroscopy (FTIR) proves complex weak interactions between surfactin and alkaline protease, especially hydrogen bonds. Enzyme activity measurements demonstrate that low concentration of surfactin can increase the enzyme activity of alkaline protease, while high concentrations inhibit it. The particle size and Zeta potential measurements confirm that the system particle size and Zeta potential are dependent on the concentration of surfactin, in addition, there is the electrostatic interaction between surfactin and alkaline protease. Surface tension measurements indicate that the binds of surfactin and alkaline protease molecules are spontaneous. Based on experimental results, the composite model of surfactin and alkaline protease in aqueous solution is proposed.

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## 1. Introduction

Surfactin, based on renewable energy such as hydrocarbons as raw materials, is a natural secondary metabolite produced by the fermentation of *Bacillus subtilis* [1]. It belongs to the extracellular product and has a crystalline morphology. It is one of the most surface-active biosurfactant species reported so far known [2]. Its basic structure is a cyclic lipopeptide which is composed by a C13–C15 β-hydroxy hydrophobic fatty acid chain and seven amino acid peptides. The most common sequence of peptides is L-Glu → L-Leu → D-Leu → L-Val → L-Asp → D-Leu → L-Leu [3, 4]. It is an amphiphilic material with both hydrophilicity and hydrophobicity [5]. The Glu residue and Asp residue of the surfactin molecule provide hydrophilic domains, while other amino acid residues and fatty acid chain provide hydrophobic domains. Surfactin has characteristics that are strong surface activity (for example, the critical micelle concentration (CMC) of surfactin in water is 25 mg/L and lowers the surface tension to 27 mN/m. The minimum interfacial tension against hexadecane is 1 mN/m [6]), good emulsifying and foaming properties [7], low toxicity, easy biodegradation [8] and no environmental pollution, etc. It also has biological activity such as antifungal and bacteria, dissolve red blood cells and protoplasts as well as spheroplasts [9, 10]. Surfactin has a wide range of uses in the environmental protection industry, oil extraction industry, high-end cosmetics industry, pharmaceutical industry, and food processing as well as other industries [11–13]. In recent years, there is a major breakthrough about application research of surfactin in antiviral preparations [14], antitumor preparations [15], thrombolytic agents [16], oral immunoadjuvants [17], hepatitis B vaccine [18], and diabetes treatment [19].

As a biosurfactant, surfactin can mildly interact with biological macromolecules and there are related studies. Zou Aihua et al. [20] studied the interaction between surfactin-C15 and bovine hemoglobin in aqueous solution. It found that surfactin-C15 could induce the conversion of hydrated hemoglobin to methemoglobin, and the effect of surfactin-C15 on bovine hemoglobin was related to its concentration. It proved that there was electrostatic repulsion between surfactin-C15 and bovine hemoglobin, as well as surfactin-C15 micelle binds to the surface of bovine hemoglobin through hydrophobic interaction and intramolecular hydrogen bonding; Hsin-Hui Shen et al. [21] studied the destruction and dissolution of the phospholipid bilayer membrane by surfactin. It found that the surfactin gradually penetrated into the outer layer of the phospholipid bilayer membrane until the threshold concentration was reached. (The threshold concentration is the critical micelle concentration of the surfactin, which is $5 \times 10^{-5}$ M). If the concentration of the surfactin exceeds the threshold during this process, the phospholipid bilayer film is gradually removed. At the same time, it found that surfactin formed micelles or micropores in phospholipid bilayer membrane vesicles, which indicated that the desorption process involved the rearrangement of surfactin in the bilayer membrane.

☆ Electronic supplementary information (ESI) available: molecular weight measurements and enzyme activity measurements of surfactin-alkaline protease system.

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It is worth noting that the interaction of surfactant-protein systems has become a research hotspot in the fields of medicine, chemistry, biology, cosmetics and drug delivery [22]. In addition, relevant papers have reported that the biological activity of surfactin is closely related to the interaction with biological membranes [13, 23], and biological membranes contain most biologically active enzymes. For promoting the exertion of the biological activity of surfactin, and promoting its application in the drug delivery system, protein and cell membrane solubilization, as well as the life system and synthetic biology, the interaction between surfactin and biologically active enzymes should be studied. Alkaline protease belongs to a group of biologically active enzymes and it mainly produced by the microbial *Bacillus* secretion [24]. Alkaline protease is a class of the proteases that can catalyze the hydrolysis of partial peptide bonds in proteins into peptides or amino acids under alkaline conditions [25]. Alkaline protease has a wide range of applications [26–28], so it was selected as a research object. In order to understand the interaction between surfactin and alkaline protease in aqueous solution, the ultraviolet, fluorescence, infrared, enzyme activity, particle size and Zeta potential, as well as surface tension of the surfactin-alkaline protease system was performed in this paper.

## 2. Experimental

### 2.1. Materials

Surfactin was purchased from Wako Pure Chemical Industries Ltd. (Japan), which molecular formula is $C_{53}H_{93}N_7O_{13}$ and molecular weight is 1036.34. The liquid alkaline protease was purchased from Yong forever Co., Ltd. (China) produced by the fermentation of *Bacillus subtilis*, the molecular weight is 27 kDa approximately, which determined by gel electrophoresis and described in detail in the Supplementary materials (seen first section and Fig. S1). Anhydrous sodium carbonate (AR) was purchased from Tianjin Guangfu Technology Development Co., Ltd. (China). Trichloroacetic acid (AR) was purchased from Tianjin Damao Chemical Reagent Factory (China). Tetraborate (AR) was purchased from Tianjin Tianli Chemical Reagent Co., Ltd. (China). Sodium hydroxide (AR) was purchased from Tianjin Hengxing Chemical Reagent Co., Ltd. (China). And casein (BR) was purchased from Sinopharm Group Chemical Reagent Co., Ltd. (China).

Surfactin was dissolved in borate buffer (pH 10.5). Liquid alkaline protease was diluted by borate buffer solution (pH 10.5). Putting surfactin solution adds in the alkaline protease solution. Then, the volume was adjusted by borate buffer. All solutions were prepared using deionized water. Seven kinds of surfactin-alkaline protease system samples with surfactin content of 0 mg/L, 5 mg/L, 10 mg/L, 20 mg/L, 40 mg/L, 60 mg/L, 80 mg/L and alkaline protease with volume fraction of 1% were obtained. Surfactin-alkaline protease system samples stand for 35 min after shake well.

### 2.2. Experimental methods

#### 2.2.1. UV–vis spectra measurements

The UV–vis spectra of the sample after standing for 35 min were measured at 20 °C using the UV–vis spectrophotometer (UH5300, Hitachi High-tech Company, China). Borate buffer solution with a sample-free (pH 10.5) was used as a blank control. Scanning range was 240 nm–360 nm.

#### 2.2.2. Fluorescence spectra measurements

The fluorescence spectrum of the sample after standing for 35 min was measured at 20 °C using the Fluorescence spectrophotometer (F-4600, Hitachi High-tech Company, China). The fixed excitation wavelength was 280 nm. The excitation slit was 2.5 nm and emission slit was 5 nm. The scanning speed was 1200 nm × $min^{-1}$. The response time was 0.004 s. The working voltage was 620v. And the scanning range was 300 nm to 440 nm.

#### 2.2.3. FTIR spectra measurements

Took 4 mL of each sample and dried it in the electric blast oven (DHG-9140, Shanghai Qixin Scientific Instrument Co., Ltd., China) at 40 °C. The FTIR spectrum of the sample was measured at 20 °C using the Fourier Transform Attenuated Total Reflection Infrared Spectrometer (ATR-FTIR, Nicolet Nexus FTIR, USA). The scanning range was from 700 nm to 4000 nm. The number of scans was 64, and the resolution was 4 $cm^{-1}$.

#### 2.2.4. Enzyme activity measurements of surfactin-alkaline protease system

Folin-phenol method [29] was used to measure the enzyme activity of 7 different concentrations of surfactin-alkaline protease system samples. The progress was described in detail in the Supplementary materials (seen second section). The enzyme activity formula is as follows:

$$X = A \times K \times \frac{4}{10} \times n$$

where X represents the alkaline protease activity (U/mL); A represents the average of the absorbance measured in parallel experiments; K represents the light absorption constant (this experiment K is 100); 4 is the total volume of the reaction reagent (mL); 10 represents the reaction time; n is the total fold dilution.

The enzyme activity of each sample concentration was measured 3 times for average, and the error was guaranteed within 10%.

#### 2.2.5. Particle size and Zeta potential measurements

Particle size and Zeta potential of surfactin-alkaline protease system in bulk solution was obtained by Zetasizer (Malvern, Nano ZS ZEN3600, USA). In this method, the light source is 633 nm red lasers, the measurement angle is 173°. Each sample concentration was measured 3 times for average.

#### 2.2.6. Surface tension measurements

Surface tension of surfactin-alkaline protease system was obtained by Contact Angle System OCA (Germany). The method was a video-enhanced drop tensiometer to record the data of dynamic surface tension. The solutions were delivered from a gas tight plastic syringe by a manual drive through a 0.91 mm diameters needles to form a rising droplet with a diameter of approximately 1.5 mm into a cuvette containing air. Regard the value of surface tension changes <0.05 mN/m during 5 min as the equilibrium interfacial tension value. All experiments were carried out at 20 ± 1 °C. The surface tension of each sample concentration was measured 3 times for average, and the error was guaranteed within 3%.

## 3. Results and discussion

### 3.1. UV–vis spectra measurements

Because the phenyl ring of tyrosine and tryptophan residues in the protein molecule contains conjugated double bonds, the Π-Π* transition and the n-Π* transition can occur, making the protein UV-absorbing and the absorption peak at 280 nm [30]. Alkaline protease belongs to the protein and has an absorption peak at 280 nm. In addition, the surfactin molecule itself does not have UV–vis absorption at 280 nm. Fig. 1 shows the UV–vis spectra of the surfactin-alkaline protease system with different concentrations of surfactin, which have been baseline calibrated.

It can be seen from Fig. 1, after adding surfactin increased the intensity of the absorption peak of the UV spectrum in surfactin-alkaline protease system. The increase in the intensity of UV absorption peaks at other concentrations of surfactin is almost identical, except that the adding of surfactin is 80 mg/L. There was no red shift or blue shift. This indicates that interaction occurs between surfactin molecules and alkaline protease molecules, but the adding surfactin does not affect the configuration of the α-C atom of the peptide bond between the amino acids in the alkaline protease [31]. This also shows that the adding surfactin molecules causes the extension of the peptide chain of alkaline protease [32], which

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