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Interaction of a digestive protease, *Candida rugosa* lipase, with three surfactants investigated by spectroscopy, molecular docking and enzyme activity assay

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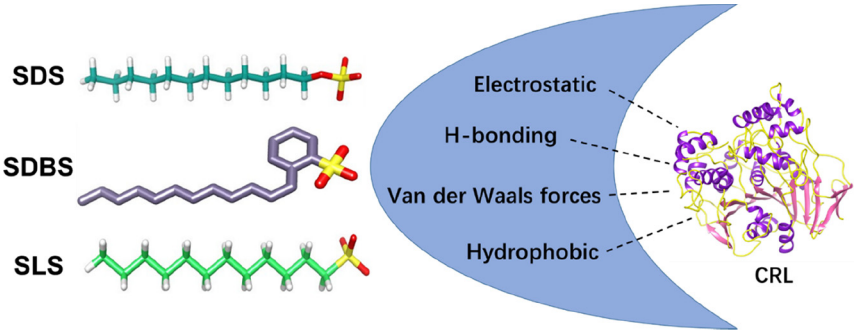
HIGHLIGHTS

- Binding interactions of surfactants with CRL were systematically investigated.
- The fact that SDS, SDBS and SLS are not a perfect emulsifiers in industrial production was proved.
- All the three surfactants cause negative effects to lipase but from different angles.
- The work establishes a molecular toxicity evaluation method.

GRAPHICAL ABSTRACT

There are four main drive forces that may exist between CRL and these three surfactants: electrostatic, H-bonding, Van der Waals forces and Hydrophobic. In the structure image of CRL, the helix of CRL was colored as purple, the coil structure of CRL was colored as yellow and the strand was colored as pink.



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ABSTRACT

The extensive use of surfactants in food, laundry products and agriculture has caused concern about their biosafety. However, few studies have been done on their potential effect on the lipase which has always been used with surfactants in food and laundry industry. Herein, we investigated the interaction of three surfactants (sodium dodecyl sulfate (SDS), sodium dodecyl benzene sulfonate (SDBS), sodium lauryl sulfonate (SLS)) with *Candida rugosa* lipase (CRL), which is a popular biocatalyst used regularly with surfactants. The effect of the three surfactants on the conformation and activity of CRL was evaluated by using multiple spectral methods, enzyme activity assay and molecular docking modeling. The results demonstrated that CRL interacted with SDS, SDBS and SLS primarily through hydrophobic forces, H-bonding and electrostatic forces, respectively. The binding constants (K_A) of SDBS with CRL varied with temperature: 1.99×10^3 mol/L at 298 K and 4.13×10^3 mol/L at 318 K. SDS and SDBS affected the secondary structure and skeleton of CRL, which changed the polarity of CRL and enhanced its activity. SLS also changed the secondary structure and activity of CRL moderately, but had little effect on its polarity and chromophore microenvironment. Accordingly, all three surfactants exhibited effect to CRL on the molecular level calling for more attention to pay on their biosafety.

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Spectroscopy
Binding interaction

The work demonstrates that SDS, SDBS and SLS could cause negative effects to CRL from different angles and therefore are not bio-friendly detergents.

1. Introduction

Ionic and non-ionic surfactants have been widely used in laundry detergents, agriculture and other domestic/industrial applications as wetting agents and emulsifiers (Lentz, 2003; Marcomini et al., 1989; Lee et al., 2011). As amphiphilic molecules and surface-active compounds, surfactants consist of hydrophilic and hydrophobic domains. The hydrophobic part of the surfactant is a non-polar hydrocarbon chain whereas the polar, hydrophilic component varies dependently (Banat et al., 2000). The enormous market demand for surfactants is stimulating the worldwide production of them. However, surfactants are usually toxic to the organisms and have poor biodegradability (Azizullah et al., 2011; Rebello et al., 2014). Thus, their potential effect may cause serious problems in their application in industry. Massive studies on their effect are imperative to conduct. To date, Tardioli et al. investigated the interaction of bovine serum albumin with gemini surfactants and found the hydrophobic and electrostatic force dominate in their interaction (Tardioli et al., 2010). Gull et al. proved the cetyltrimethylammonium bromide (CTAB) caused slighter effects on serum albumin than its gemini counterpart (Gull et al., 2009). Based on the similarity in their chemical and physical properties, the detergents used in our work most likely have a mode of action similar to that of the gemini surfactants and CTAB. In this paper, we investigated the interaction of CRL and SDS, SDBS and SLS to explore their denaturation effect to an enzyme.

Lipase is a kind of biomacromolecules called as "most pliable biocatalyst" in industrial production, and could cause a series of bioconversion reactions, such as esterification, hydrolysis and transesterification (Rodriguez-Nogales et al., 2005). These typical reactions of lipase are widely used in the oil, baking, dairy and pharmaceutical industries (Li et al., 2012; Casas-Godoy et al., 2012). Various substrates including synthetic triglycerides, natural oils and esters of fatty acids can be catalyzed by lipase. Interestingly, lipase could transform fats into glycerol and fatty acids at the water-lipid interface, while the reaction process could be reversed in non-aqueous media (Aravindan et al., 2007). So, lipase could be used in laundry detergents in aqueous media. When we use the detergent remove glycerides from the fabric, the surfactant component could only be used to remove the fatty acids, while triglycerides could not be eliminated from the fabric. After the addition of lipases, the protease-containing detergents gain a higher washing capacity and their ability to remove fatty food stains and sebum from fabrics is improved by a large margin (Hemachander and Puvanakrishnan, 2000).

In summary, lipase has been widely used simultaneously with surfactant in the laundry detergent industry and it is common for them to interact with each other in practical applications. Therefore, it is of great significance to understand how surfactants influence lipase structure and activity. To elucidate this problem, the mechanism of the interaction between lipase and three surfactants — sodium dodecyl sulfate (SDS), sodium dodecyl benzene sulfonate (SDBS), sodium lauryl sulfonate (SLS) — was investigated by multiple spectroscopic methods, molecular modeling and enzyme activity assay.

2. Materials and methods

2.1. Materials

SDS, SDBS and SLS were purchased from Aladdin (Shanghai, China). *Candida rugosa* lipase Type VII (CRL) was obtained from Sigma -Aldrich (St. Louis, MO) and directly dissolved in distilled water to prepare the stock solution (5×10^{-5} mol/L). A Lipase Activity Kit (conventional turbidimetric method, Nanjing Jiancheng Bioengineering Institute, Jiangsu, China) was purchased from Nanjing Jiancheng Bioengineering Institute (conventional turbidimetric method, Jiangsu, China). $NaH_2PO_4 \cdot 2H_2O$ and $Na_2HPO_4 \cdot 12H_2O$ were bought from Tianjin Damao Chemical Reagent Factory (Tianjin, China). A phosphate buffer (pH = 7.4) containing 0.02 M phosphoric acid was used.

2.2. CRL activity assay

CRL activity experiments were performed as reported before (Zhang et al., 2016a). In short, a Lipase Activity Kit was employed and the activity of CRL (A_{CRL}) was calculated using the following equation: $A_{CRL} = [(A_1 - A_2) / A_S] \times 136$, where A_1 is the initial absorbance of the sample, A_2 refers to the absorbance of the sample after reacting with the substrate for 10 min at 37 °C, and A_s is the absorbance of the substrate.

2.3. Fluorescence spectra measurements

All fluorescence spectra were measured on a Hitachi F-4600 fluorescence spectrophotometer (Hitachi, Japan) with a 10-mm quartz cell and a 150 W Xenon lamp. The photomultiplier tube voltage, excitation/emission slit widths and scanning speed were set at 750 V, 5 nm and 1200 nm min^{-1}, respectively. The interior fluorescence emission spectra of CRL was scanned over the wavelength range of 290–450 nm and the excitation wavelength was fixed at 280 nm. Synchronous fluorescence spectra were acquired by setting the difference of excitation and emission wavelength at $\Delta\lambda = 15$ nm and $\Delta\lambda = 60$ nm. The excitation wavelength scans ranged from 250 to 350 nm. The three–dimensional fluorescence measurement was carried out under the following conditions: the emission and excitation wavelength ranged from 290 to 450 nm and 200 to 400 nm, respectively. The concentration of CRL used in this section is 1.0×10^{-5} mol/L.

2.4. UV–visible absorption measurement

UV–visible absorption spectra were measured on a UV-2450 spectrophotometer (Shimadzu, Kyoto, Japan) equipped with 1.0 cm quartz cells with the wavelength scan ranged from 190 nm to 450 nm.

2.5. Circular dichroism (CD) measurement

CD spectra were recorded on a J-810 CD spectrometer (Jasco, Tokyo, Japan) over a wavelength range of 190–260 nm in a 1.0 cm quartz cell. Each spectrum was the average of three successive scans. The scanning speed was set at 200 nm min^{-1}. The scans of phosphate buffer solution used as a blank were recorded under the same conditions and subtracted from the experimental spectra. The CD spectra results were expressed as mean residue ellipticity (MRE) in deg cm^2 $dmol^{-1}$ which is defined as: $MRE = \theta_{obs} / (10 \times n \times l \times C_p)$, where θ_{obs} refers to the CD in millidegrees, n is the number of amino acid residues of CRL (534), l is the path length of the cell, and Cp is the mole fraction. CDpro software (available at http://lamar.colostate.edu/~sreeram/CDPro/) was employed to calculate the secondary structure of CRL. The concentration of CRL used in this section is 1.0×10^{-5} mol/L.