International Journal of Biological Macromolecules 81 (2015) 560–567

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Role of spacer length in interaction between novel gemini imidazolium surfactants and *Rhizopus oryzae* lipase

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ARTICLE INFO

Article history:
Received 19 May 2015
Received in revised form 6 August 2015
Accepted 23 August 2015
Available online 28 August 2015

Keywords:
Lipase
Gemini imidazolium surfactant
Activity
Stability
Structural study
Interactions

ABSTRACT

An insight into the effects of new ionic liquid-type gemini imidazolium cationic surfactants on the structure and function of the lipases is of prime importance for their potential application. Changes in the activity, stability and structure of *Rhizopus oryzae* lipase in the presence of novel gemini surfactants, [C_{16}-3-C_{16}im]Br_2 and [C_{16}-12-C_{16}im]Br_2 were probed in the present study. Surfactant with shorter spacer length, [C_{16}-3-C_{16}im]Br_2 was found to be better in improving the hydrolytic activity and thermal stability of the lipase. For both the surfactants, activation was concentration dependent. CD spectroscopy results showed a decrease in α-helix and an increase in β-sheet content in the presence of these surfactants. A higher structural change observed in presence of [C_{16}-12-C_{16}im]Br_2 correlated with lower enzyme activity. Isothermal titration calorimetric studies showed the binding to be spontaneous in nature based on sequential two site binding model. The forces involved in binding were found to differ for the two surfactants proving that the spacer length is an important factor which governs the interaction. These surfactants could be used as promising components both in enzyme modification and media engineering for attaining the desired goals in biocatalytic reactions.

1. Introduction

Surfactants find their application in diverse fields like food, pharmaceutical, cosmetics, detergents and biotechnology. The association of surfactants and proteins has been observed to play an important role, where the associations are very much surfactant/protein dependent. Surfactants affect the protein structure, conformational stability, polarity, solubility and catalytic activity of enzymes [1–4]. Among enzymes, extensive study and application of surfactants has been carried out with lipases. Surfactants change the rate of catalysis, the interfacial properties and enhance substrate availability [5–9]. An insight into the mechanism involved in lipase–surfactant interactions could provide the basis for enzyme activation, stabilization, media designing and help in better utilization of surfactants.

In order to better understand the interaction between the lipase and surfactants, numerous studies have been undertaken. Association between lipase and surfactants has been observed at interfaces and bulk, with subsequent effect on the enzyme structure and biological activity [10,11]. These interactions have been found to be dependent on the type of lipase and molecular structure of surfactants [1]. *Rhizomucor miehei* lipase was found to bind with cationic surfactants whereas *Humicola lanuginose* lipase formed complex with anionic surfactant [12]. *Thermomyces lanuginosus* lipase was well activated by ionic and non-ionic surfactants alike, at different range of concentrations exclusive of the classical interfacial activation phenomenon [13]. Both positive and negative effects of surfactants on activity of *Rhizopus* sp. lipase have been reported along with changes in the structural organization [14,15].

Although most of the studies have focussed on conventional surfactants, interaction with gemini surfactants has shown far better results [16,17]. Gemini surfactants are made up of two hydrophilic head groups linked by a spacer and two hydrophobic alkyl tails. Ionic liquid-type gemini cationic imidazolium surfactants are new generation of amphiphilic molecules and are superior to conventional surfactants. These novel gemini surfactants with imidazolium head group possess special properties and find potential application in the field of separation science, electrochemistry, material science, petrochemistry, organic synthesis and biocatalysis [18–20]. Apart from tail length, spacer length also plays an important role in modifying the polarity as well as aggregation properties of these surfactants [21]. Gemini surfactants with

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http://dx.doi.org/10.1016/j.ijbiomac.2015.08.051
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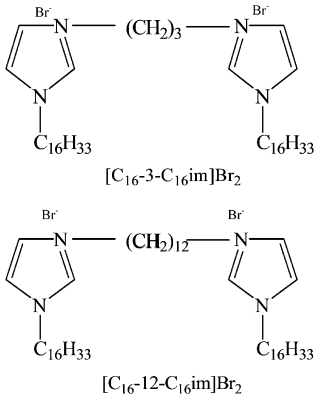

Fig. 1. Structure of gemini surfactants.

shorter spacer have lower critical micelle concentration (CMC) value [21–23] depicting better surface active property than geminis with longer spacer. The spacer length also influences the shape of the micelles and nanoparticles formed as observed by Pal et al. [22] and Datta et al. [23]. A transition in micelle shape from ellipsoid to spheroid has been reported with increasing spacer length. Both short and long chain imidazolium cationic ionic liquids have shown the ability to enhance the catalytic activity of lipases in various biotransformation reactions and in some cases improved stability as well regioselectivity of the enzyme [24–26]. However, the effect of ionic liquid-type gemini cationic imidazolium surfactants on lipases along with its structure–function relationship has not been probed yet.

In the present study, the authors have attempted for the first time to find out the effect of novel ionic liquid-type gemini cationic imidazolium surfactants on *Rhizopus oryzae* lipase (rhL). These surfactants possess two 16 carbon (C_{16}) long alkyl tails, each attached to two separate imidazolium rings linked by an alkyl chain spacer. The spacer lengths in [C_{16}-3-C_{16}im]Br_2 and [C_{16}-12-C_{16}im]Br_2 are 3 and 12 respectively (Fig. 1).

A comparative study based on the changes in the lipase activity, thermal stability, structure and associated binding energetics was conducted to find the influence of spacer length on the surfactant–rhL interaction.

2. Materials and methods

2.1. Materials

p-Nitrophenyl acetate was procured from Sigma Aldrich (St. Louis, MO, USA). Novel ionic liquid-type cationic gemini imidazolium surfactants of varying spacer lengths, [C_{16}-3-C_{16}im]Br_2 and [C_{16}-12-C_{16}im]Br_2, were provided by Prof. S. Bhattacharya, Department of Organic Chemistry, Indian Institute of Science, Bangalore (India). All other reagents were of analytical grade.

2.2. Lipase

Lipase of *R. oryzae* NRRL 3562 was produced and further purified by following the procedure of Kumari et al. [27] and Adak and Banerjee [28], respectively.

2.3. Effect of gemini surfactants on the enzyme activity

The enzyme (10 μM) activity was determined in the presence of varying concentration of gemini surfactants (0–100 μM) following the standard method developed by De Caro et al. [29]. The assay was carried out at 30 °C and pH 7 as according to previous study [28], the enzyme showed maximum stability under these conditions. Both the enzyme and the surfactants were dissolved in 10 mM phosphate buffer (pH 7) and assays were conducted in triplicate against suitable blanks containing corresponding concentration of surfactant and substrate except enzyme. One unit (IU) of lipase activity equals to the amount of enzyme that releases 1 μmole of *p*-nitrophenol per minute under the assay conditions.

2.4. Study of gemini surfactants induced structural changes in the enzyme

2.4.1. UV–vis spectroscopy

UV–vis spectra of lipase (10 μM) in the presence of varying concentration of gemini surfactants (0–80 μM) was recorded employing UV-Vis spectrophotometer (Agilent). Quartz cuvettes of 1 cm path length were used for scanning the samples in the range of 250–300 nm against suitable controls (with corresponding amount of surfactant).

2.4.2. Circular dichroism (CD) spectroscopy

Far-UV CD spectra of the enzyme (10 μM) with gemini surfactants at 0–80 μM concentrations were recorded by JASCO J-810 CD Spectropolarimeter. The range of 200–240 nm was scanned with 0.1 nm step resolution and 50 nm s^{-1} scan speed. All the spectra were recorded at 30 °C in N_2 atmosphere using 0.1 cm path length quartz cell. Final spectra, representing average of three scans, were corrected by subtracting the base line recorded for every medium. Prediction of secondary structure was carried out with the standard spectral analysis software provided by the manufacturer, based on the reference CD spectra of distinct proteins given by Yang et al. [30].

2.5. Binding thermodynamics study by isothermal titration calorimetry (ITC)

ITC (VP cal 2000) studies were undertaken to evaluate the interactions involved in binding between the gemini surfactants and enzyme. Both enzyme and surfactants (in 10 mM phosphate buffer, pH 7) were degassed extensively prior to initiating the experiments. Enzyme (10 μM) filled in the sample cell was titrated by [C_{16}-3-C_{16}im]Br_2 and [C_{16}-12-C_{16}im]Br_2 (500 μM) taken in the injector. Total 25 injections of 10 μL of surfactant were carried out at 120 s interval and 307 rpm. Heat evolved during each injection was measured by the instrument. The data were analyzed by Origin 8 software supplied along with the instrument after subtracting heat of dilution of the surfactants, obtained separately by injecting into the buffer.

2.6. Thermal stability study

2.6.1. Enzyme activity assay at varying temperatures

To determine the effect of the surfactants on the thermal stability of the enzyme, rhL (10 μM) was incubated with the gemini surfactants (20 μM) for 1 h at 60 and 70 °C. These two temperatures were chosen as lipase showed good stability till 50 °C in an earlier study [28]. After incubation the enzyme activity was measured in triplicate employing *p*NPA as substrate against suitable controls. Residual activity (%) was calculated based on the activity of lipase without any addition at t_0 time of incubation.

2.6.2. Differential scanning calorimetry (DSC)

DSC measurements of lipase with and without gemini surfactants were performed using Pyris Diamond Differential Scanning Calorimeter (Perkin Elmer). Lipase (10 μM) was incubated for one hour at pH 7, 30 °C in the presence of gemini surfactants (20 μM). An