Biochemical Engineering Journal 109 (2016) 35–42

Contents lists available at ScienceDirect

Biochemical Engineering Journal

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

Regular article

Effects of ionic liquids on the hydrolysis of casein by lumbrokinase

Yunchang Fan [a], Xing Dong [a], Yingying Zhong [b], Jing Li [c], Juan Miao [a,*], Shaofeng Hua [a], Yan Li [a], Boyu Cheng [a], Wenqiong Chen [a]

[a] *College of Physics and Chemistry, Henan Polytechnic University, Jiaozuo 454003, China*
[b] *Technology Center of Ningbo Entry—Exit Inspection and Quarantine Bureau, Ningbo 315012, China*
[c] *Yunnan Academy of Tobacco Science, Kunming, China*

ARTICLE INFO

Article history:
Received 11 September 2015
Received in revised form
12 December 2015
Accepted 30 December 2015
Available online 6 January 2016

Keywords:
Lumbrokinase
Ionic liquids (ILs)
Affinity
Protein
Enzyme activity
Proteolysis

ABSTRACT

In this work, the hydrolysis of casein by lumbrokinase in the presence of ionic liquids (ILs) was studied. The experimental results indicated that the effects of ILs on the casein hydrolysis depended on their concentrations; at low IL concentration, the addition of ILs could remarkably increase the lumbrokinase activity due to enhancing the hydrophilicity of casein by forming the IL-casein complex. However, at higher IL concentration, significant decrease in lumbrokinase activity was observed because the presence of ILs disturbed the hydrogen bond network in the active site of lumbrokinase. At the IL concentrations beyond their critical micelle concentrations, the incorporation of substrate into the micellar pseudo-phase also contributed to the decrease in lumbrokinase activity. When a more hydrophilic substrate was adopted, the effects of ILs on the lumbrokinase activity were negative in all cases. Fluorescence analysis indicated that hydrogen bonding was the main driving force ruling the interaction of ILs and lumbrokinase.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Lumbrokinase is an enzyme isolated from earthworm with strong proteolytic and fibrinolytic activity and molecular weights of 24.6–33.0 kDa; it has high thermal stability, broad pH optima (pH 4–12) and high tolerance to organic solvents [1,2]. Lumbrokinase can dissolve blood fibrin clots and is used clinically as a thrombolytic agent [1,2]. In addition, lumbrokinase is a trypsin-like serine protease and can hydrolyze casein that is the most abundant milk protein [3]. Casein hydrolysates are widely used in microbial culture, food, drug and feed industries [4–6]. Furthermore, lumbrokinase has also found a lot of applications in nutraceutical products, food and cosmetics industries [7].

Studies on enhancing enzyme activity have always exhibited great significance from basic research to industrial applications. Ionic liquids (ILs) have emerged as better alternatives to conventional solvents. They are simply salts and entirely composed of ions that are liquid in a wide temperature range (their melting points are usually close to room temperature) [8,9]. The applications of ILs in enzymatic reactions have been reported in literature [10–19]. It was found that the enzyme activity in IL media or IL-based aqueous solutions highly depended on the substrates and the physicochemical properties of ILs. Yang and coworkers reported that both hydrolytic and transesterification activities of lipase (corn oil as substrate) were significantly enhanced in the IL, 1-butyl-3-methylimidazolium hexafluorophosphate ($[C_4mim]PF_6$) as compared to the organic solvent, hexane [13]. However, remarkable decrease in the hydrolytic activity of lipase (*p*-nitrophenyl laurate as substrate) in hydrophilic IL-based aqueous solutions was observed; the degree of inhibition on lipase activity related to the chemical structures of ILs; long alkyl chain in the IL cation resulted in strong activity inhibition [14]. Debnath et al. reported that in the cationic reverse micelles of cetyltrimethylammonium bromide (solvents: isooctane + *n*-hexanol), the hydrolytic activity of trypsin using *N*-α-benzyloxycarbonyl-*L*-lysine *p*-nitrophenyl ester hydrochloride as the substrate was enhanced in the presence of hydrophilic ILs by improving the nucleophilicity of water in the vicinity of enzyme through hydrogen bonding [15]. Despite of the progress made in this field, the molecular interaction mechanisms of ILs and enzymes, which are great of importance for the applications of ILs in biotechnology, are not well understood.

In this work, by means of spectroscopy, a series of imidazolium ILs, $[C_nmim]X$ (n = 2, 4, 6; X = Br^-, Cl^-, $CF_3SO_3^-$), was selected to investigate their effects on the lumbrokinase activity. This selection enables us to explore how the nature of ILs, achieved by changing the alkyl chain length in the cation and the types of anions,

* Corresponding author. Fax: +863913987815.
E-mail address: juanmiao2013@163.com (J. Miao).

http://dx.doi.org/10.1016/j.bej.2015.12.020
1369-703X/© 2015 Elsevier B.V. All rights reserved.

influences the lumbrokinase activity. The aim of this work was to understand the molecular interaction mechanisms of ILs and lumbrokinase and to obtain important information that may be useful for the practical applications.

2. Experimental

2.1. Materials

Lumbrokinase (>12000 units mg^{-1}), casein and diethylsafraninazodimethylaniline chloride (Janus green B, dye content 65%) was purchased from Aladdin Reagent Co. (Shanghai, China). Nα-Benzoyl-DL-arginine *p*-nitroanilide hydrochloride (98%, BAPA) was purchased from Sigma–Aldrich (St. Louis, USA). 1-Butyl-3-methylimidazolium chloride (99%, $[C_4mim]Cl$), 1-butyl-3-methylimidazolium bromide (99%, $[C_4mim]Br$), 1-butyl-3-methylimidazolium trifluoromethanesulfonate (99%, $[C_4mim]CF_3SO_3$), 1-ethyl-3-methylimidazolium bromide (99%, $[C_2mim]Br$), and 1-hexyl-3-methylimidazolium bromide (99%, $[C_6mim]Br$) were obtained from Lanzhou Institute of Chemical Physics of the Chinese Academy of Sciences (Lanzhou, China). All the other chemicals are analytical grade unless stated otherwise. Ultrapure water (18.2 MΩ cm) produced by an Aquapro purification system (Aquapro International Co., Ltd., Dover, DE, USA) was used throughout the experiments.

The stock solutions of lumbrokinase (5.0 g L^{-1}), Janus green B (10^{-3} mol L^{-1}), casein (10.5 g L^{-1}) and ILs (0.50–5.0 mol L^{-1}) were prepared by Tris–HCl buffer (50 mmol L^{-1}, pH 8.0); 10% (wt%) of acetocaustin was prepared by water. All the stock solutions were stored in the dark at $0-4$ °C. The solution of BAPA (0.40 g L^{-1}) was prepared as follows: 40 mg of BAPA was dissolved in 1.0 mL of dimethyl sulfoxide and then diluted to 100 mL by Tris–HCl buffer (50 mmol L^{-1}, pH 8.0) prewarmed to 37 °C; this solution was prepared daily and kept at 37 °C while in use.

2.2. Measurements of lumbrokinase activity

Lumbrokinase activity, with casein as substrate, was measured via the method reported in the literature with minor modification [1,20]. Briefly, 0.2 mL of lumbrokinase solution (5.0 g L^{-1}), 6.8 mL of Tris–HCl buffer (50 mmol L^{-1}, pH 8.0), and 3.0 mL of casein solution (10.5 g L^{-1}) were added into a 10-mL glass-stoppered tube. Before addition, all the solutions were incubated at 37 °C for 20 min at least. After incubation at 37 °C for 10 min, the enzymatic reaction was inactivated by adding 2.0 mL of acetocaustin (10%); the absorbance of the resulting solution was measured by an Evolution 201 UV-Visible Spectrophotometer (Thermo Fisher Scientific, Tewksbury, USA) at 280 nm, wherein the lumbrokinase activity was defined as the absorbance variation for a 10-min duration (ΔA_0 min^{-1}).

The lumbrokinase activity, with BAPA as substrate, was measured in a similar way: 0.2 mL of lumbrokinase solution (5.0 g L^{-1}), 4.9 mL of Tris–HCl buffer (50 mmol L^{-1}, pH 8.0), and 5.0 mL of BAPA solution (0.40 g L^{-1}) were added into a 10-mL glass-stoppered tube. Before addition, all the solutions were incubated at 37 °C for 20 min at least. After incubation at 37 °C for 10 min, absorbance of the resulting solution was measured by the spectrophotometer at 400 nm, wherein the lumbrokinase activity was also defined as the absorbance variation for a 10-min duration (ΔA_0 min^{-1}).

To study the effects of ILs, a specified amount of an IL was added into the above enzymatic reaction system, the lumbrokinase activity in the presence of an IL was registered as ΔA min^{-1}; the relative activity of the enzyme with and without the presence of an IL was thus expressed as $\Delta A/\Delta A_0$.

2.3. Fluorescence measurements

Fluorescence spectroscopic analysis was conducted on a Cary Eclipse fluorescence spectrophotometer (Agilent, Santa Clara, USA) equipped with 1.0 cm quartz cells and a thermostatic bath. Typically, 1.25 mL of lumbrokinase solution (5.0 g L^{-1}), and a known concentration of an IL were added to a 10.0 mL standard flask and diluted by Tris–HCl buffer solution (50 mmol L^{-1}, pH 8.0) to the volume. Fluorescence emission spectra of lumbrokinase were measured in the range of 285 to 500 nm (λ_{em}) with excitation wavelength (λ_{ex}) at 280 nm. To measure the fluorescence emission spectra of casein, an aqueous solution (pH 8.0) containing 0.21 g L^{-1} of casein and a known concentration of an IL was scanned by the fluorescence spectrophotometer at λ_{ex} = 280 nm and λ_{em} = 290–500 nm. The slit widths for both excitation and emission were 5 nm.

2.4. Measurements of the octanol—water partition coefficients (P_{ow}) of ILs

The P_{ow} values of the five ILs were measured per the methods already reported [21,22]. Typically, the solutions of ILs (0.10 mol L^{-1} for each) were prepared by Tris–HCl buffer (50 mmol L^{-1}, pH 8.0) saturated by octanol; 10.0 mL of the solution of a specific IL and 10.0 mL of octanol saturated with Tris–HCl buffer (50 mmol L^{-1}, pH 8.0) were mixed under stirring for 30 min at 298 K. After phase separation by centrifugation, both the octanol and water

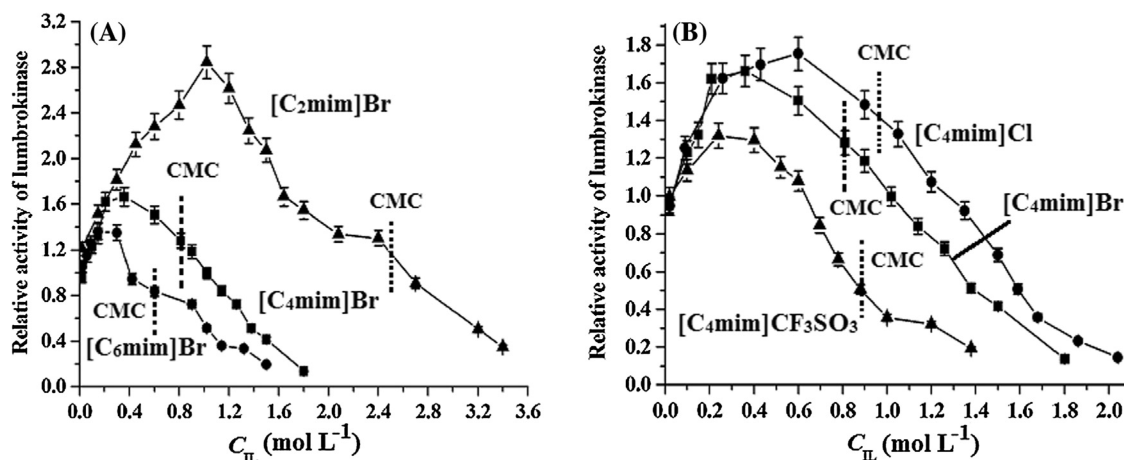

Fig. 1. Effects of ILs on the hydrolysis of casein by lumbrokinase. The vertical dashed lines indicated the IL CMC values.

Download English Version:

https://daneshyari.com/en/article/6483960

Download Persian Version:

https://daneshyari.com/article/6483960

Daneshyari.com