Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 159 (2016) 128–133

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

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

Spectroscopic studies on the inhibitory effects of ionic liquids on lipase activity

Yunchang Fan [a], Xing Dong [a], Xiaojing Li [a], Yingying Zhong [b], Jichuan Kong [a], Shaofeng Hua [a], Juan Miao [a,*], Yan Li [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

ARTICLE INFO

Article history:
Received 28 September 2015
Received in revised form 15 January 2016
Accepted 23 January 2016
Available online 25 January 2016

Keywords:
Ionic liquids (ILs)
Fluorescence spectroscopy
Lipase
Hydrophobicity
Hydrogen bonding

ABSTRACT

The effects of ionic liquids (ILs) on the lipase activity were studied by UV–Vis spectroscopy and the IL-lipase interaction mechanism at the molecular level was investigated by fluorescence technique. Experimental results indicated that the lipase activity was inhibited by ILs and the degree of inhibition highly depended on the chemical structures of ILs. The inhibitory ability of the Cl^-- and Br^--based ILs increased with increasing the alkyl chain length in the IL cation. Thermodynamic parameters, enthalpy change (ΔH) and entropy change (ΔS) were obtained by analyzing the fluorescence behavior of lipase with the addition of ILs. Both ΔH and ΔS were positive suggesting hydrophobicity was the major driven force for the Cl^-- and Br^--based ILs. For the BF_4^--, $CF_3SO_3^-$-, ClO_4^-- and $N(CN)_2^-$-based ILs, hydrogen bonding was the main driven force. For a more comprehensive understanding of the effects of ILs on lipase activity, the roles of hydrophobicity and hydrogen bonding must be considered simultaneously. A regression-based equation was developed to describe the relationship of the inhibitory ability of ILs and their hydrophobicity and hydrogen bonding ability.

1. Introduction

In the last decade, ionic liquids (or called room temperature molten salts) have received increasing attention as environmentally benign solvents and functional materials due to their non-detectable vapor pressure, high thermal and chemical stability, and tunable properties such as polarity, viscosity, solubility and hydrophobicity through the appropriate adjustment of the combination of the cation and anion [1,2]. Many reviews have highlighted the advantages of the use of ILs as media for dissolving cellulose [3], capturing CO_2 [4], organic synthesis [5] and biocatalysis [6]. Despite the nonvolatility, ILs do have remarkable solubility in water, which may cause the release of them into the environment. Besides, the wide use of these solvents may inevitably result in accidental discharge and contamination. It is the properties making ILs be interested such as high thermal and chemical stability and nonvolatility that lead us to consider the potential problems with their toxicity and accumulation in the environment. Several excellent reviews [7–9] have summarized the toxicity of ILs to organisms and shown that their toxicity depends on the chemical structures of cation and anion with the former having a more dramatic effect. Enzymes as biological catalysts are responsible for supporting many chemical reactions in all living things and their activity assays are useful tools to estimate the environmental risk and toxicity of pollutants [10]. Lipases play an essential role in the digestion and transformation of fats in most living organisms. Once pollutants enter the human or animal bodies, they will interact with lipases, affect their activities and cause pathological changes [11–13]. Therefore, lipases have been widely used as biomarkers for evaluation of the toxic effects of pollutants [11–13]. At present, the effects of ILs on the lipase activity have been widely reported [14–17]. For example, Ventura and coworkers [14] studied the effects of hydrophilic ILs on the activity of lipase. It was found that the increase in the IL concentration caused a decrease in the lipase activity, which was ascribed to the effects of ILs on the thermodynamic water activity. The increase in the alkyl chain length of the IL cation also reduced the enzymatic activity by obstruction of its non-polar active site. The effects of IL anions on the lipase activity might relate to their hydrogen-bonding ability. Klähn et al. [15] studied the influence of lipase–ion interaction on the structure of the lipase–IL interface with molecular dynamics (MD) simulations and an aggregation of long alkyl chain of the IL cation close to the active site entrance of lipase was observed. This aggregation substantially impeded substrate binding to enzyme, which would compromise the lipase activity. Despite the progress made in this field, there are still some questions: I) what are the driven forces ruling the interaction of an IL and lipase? II) For the different types of anions, do they have the same driven forces?

In order to explore these questions, a series of N-methylimidazolium- and pyridinium-based ILs with different alkyl chain length and anions

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

http://dx.doi.org/10.1016/j.saa.2016.01.047
1386-1425/

were selected to study their effects on the lipase activity. This selection enables us to investigate how the nature of the IL cation and anion affect the lipase activity. The aim of this work was therefore to explore the driven forces underlying the interaction of an IL and lipase with the aid of spectroscopic methods.

2. Experimental

2.1. Materials

Lipase from Candida (>100 units mg^{-1}) was purchased from Aladdin Reagent Co. (Shanghai, China). 4-Nitrophenyl acetate (NPA) was purchased from Sigma-Aldrich Co. (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-butyl-3-methylimidazolium tetrafluoroborate (99%, [C_4mim]BF_4), 1-butyl-3-methylimidazolium perchlorate (99%, [C_4mim]ClO_4), 1-butyl-3-methylimidazolium dicyanamide (99%, [C_4mim]$N(CN)_2$), 1-benzyl-3-methylimidazolium chloride (99%, [Bzmim]Cl), 1-benzyl-3-methylimidazolium bromide (99%, [Bzmim]Br), 1-heptyl-3-methylimidazolium chloride (99%, [C_7mim]Cl), 1-heptyl-3-methylimidazolium bromide (99%, [C_7mim]Br), 1-octyl-3-methylimidazolium chloride (99%, [C_8mim]Cl), 1-octyl-3-methylimidazolium bromide (99%, [C_8mim]Br), N-butylpyridinium bromide (99%, [C_4Py]Br), and N-octylpyridinium bromide (99%, [C_8Py]Br) were obtained from Lanzhou Institute of Chemical Physics of the Chinese Academy of Sciences (Lanzhou, China). All the other chemicals were analytical grade unless stated otherwise. Ultrapure water (18.2 MΩ·cm) produced by an Aquapro purification system (Aquapro International Co., Ltd., Dover, USA) was used throughout the experiments.

Phosphate buffer (containing 0.05% of triton X-100, 5.0×10^{-2} mol L^{-1}, pH 7.0) was used to prepare the stock solutions of lipase (2.5 g L^{-1}), NPA (2.5×10^{-2} mol L^{-1}) and ILs (0.050 to 4.0 mol L^{-1}) and to control the pH of the aqueous phase. All the stock solutions were stored in the dark at 0–4 °C.

2.2. Measurements of Lipase Activity

Lipase activity, with NPA as substrate, was measured via the method reported in the literature with minor modification [14,18]. Briefly, 1.5 mL of lipase solution (2.5 g L^{-1}), 8.0 mL of phosphate buffer, and 0.5 mL of NPA solution (2.5×10^{-2} mol 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 30 min, the absorbance of the resulting solution was measured by an Evolution 201 UV–Visible Spectrophotometer (Thermo Fisher Scientific, Tewksbury, USA) at 400 nm, wherein the lipase activity was defined as the absorbance variation for a 30-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 lipase 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 $\Delta A/\Delta A_0$. The half maximal inhibitory concentration (IC_{50}), i.e., the concentration of an IL causing a decrease of 50% on lipase activity was used to quantitatively evaluate the inhibition effectiveness of an IL on the lipase activity.

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, 5.0 mL of lipase solution (2.5 g L^{-1}), and a known concentration of an IL were added into a 10.0 mL standard flask and diluted by phosphate buffer solution to the volume. Fluorescence emission spectra of lipase were measured in the range of 310 to 500 nm with excitation wavelength at 290 nm. The slit widths for both excitation and emission were 10 nm.

2.4. Measurements of the Octanol–Water Partition Coefficients (P_{ow}) of ILs

The P_{ow} values of the fourteen ILs were measured per the methods already reported [19,20]. Typically, the solutions of ILs (0.10 mol L^{-1} for each) were prepared by phosphate buffer saturated by octanol; 10.0 mL of the solution of a specific IL and 10.0 mL of octanol saturated with phosphate buffer were mixed under stirring for 30 min at 298 K. After phase separation by centrifugation, both the octanol and water phases were injected into the Agilent 1200 high performance liquid chromatograph (HPLC) (Agilent, Santa Clara, USA) equipped with a variable wavelength detector (VWD) and an autosampler. An Amethyst C18-H column (4.6 mm × 150 mm, 5 μm, Sepax Technologies Inc., Newark, USA) was used to separate the ILs, and the column temperature was set at 30 °C. The injection volume was 1.0 μL and detection wavelength was 220 nm. The mobile phase was the mixture of methanol and 2.0×10^{-3} mol L^{-1} of aqueous sodium 1-heptanesulfonate solution: 70% (*v*/v) of methanol was adopted for the analysis of $[C_8mim]^+$-based ILs; for the analysis of $[C_7mim]^+$-, $[Bzmim]^+$- and $[C_8Py]^+$-based ILs, 60% (*v*/v) of methanol was adopted, and 40% (*v*/v) of methanol was used to separate all the other ILs. The flow rate of mobile phase was set at 0.8 mL min^{-1}. The P_{ow} value was calculated by the equation:

$$P_{ow} = C_{octanol}/C_{water} \quad (1)$$

where $C_{octanol}$ and C_{water} are the concentrations of a specific IL in the octanol and aqueous phases, respectively.

2.5. Measurements of Kamlet–Taft Polarity Parameters

The Kamlet–Taft polarity parameters, α (hydrogen bond donating ability), β (hydrogen bond accepting ability) and π^* (dipolarity/polarisability) of the ILs studied in this work were determined using the three dyes, Reichardt's dye (RD), N,N-diethyl-4-nitroaniline (DENA) and 4-nitroaniline (NA). The concentrations of DENA and NA in the ILs were both 5.0×10^{-5} mol L^{-1} and the concentrations of RD in ILs ranged from 2.18×10^{-4} to 8.7×10^{-3} mol L^{-1}. The maximum absorption wavelengths (λ_{max}) of the three dyes were measured by using the Evolution 201 UV–Visible Spectrophotometer. The Kamlet–Taft polarity parameters were then calculated using the equations [21]:

$$\nu_{max}(cm^{-1}) = 10^4/\lambda(nm) \quad (2)$$

$$E_T\,(30) = 28592/\lambda_{max}\,(RD) \quad (3)$$

$$\pi^* = 0.314\,(27.52 - \nu_{max}\,(DENA)) \quad (4)$$

$$\alpha = 0.0649\,E_T\,(30) - 2.03 - (0.72\pi^*) \quad (5)$$

$$\beta = (1.035\,\nu_{max}\,(DENA) + 2.64 - \nu_{max}\,(NA))/2.8. \quad (6)$$

All pH values were tested with a pHS-3B digital pH meter (Shanghai Leici Instrument Factory, Shanghai, China) equipped with a combined glass electrode.

All the above experiments were conducted in triplicate and the data presented in this work are an average of the obtained values.

3. Results and Discussion

3.1. Effects of ILs on the Lipase Activity

To facilitate the evaluation of the effects of chemical structures of ILs on the lipase activity, the ILs were divided into four groups: I) [C_4mim]Cl, [Bzmim]Cl, [C_7mim]Cl and [C_8mim]Cl; II) [C_4mim]Br, [Bzmim]Br, [C_7mim]Br and [C_8mim]Br; III) [C_4Py]Br and [C_8Py]Br; IV) [C_4mim]Cl, [C_4mim]Br, [C_4mim]CF_3SO_3, [C_4mim]BF_4, [C_4mim]$N(CN)_2$ and [C_4mim]ClO_4. The ILs in groups I to III have the same anion but different cations; ILs in group IV have the same cation but different anions.