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Review

Key factors affecting the activity and stability of enzymes in ionic liquids and novel applications in biocatalysis

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ABSTRACT

Ionic liquids as eco-friendly solvent media and catalyst play very important roles in many enzymatic reactions such as enzymatic biotransformation and all kinds of biosynthesis due to their unique and tunable physical properties. The changes of the cations or anions types in ionic liquids have important effects on the activity, stability and structures of enzymes. In this critical review, we systematically shed light on the key factors affecting the activity and stability of enzymes in ILs and discussed the relationships among these factors. Moreover, the strategies for improving the enzymes' utility in ionic liquids are exhibited. Finally, we address some novel industrial applications of ionic liquids in biochemical engineering such as the pretreatment of lingocellulosic biomass, transesterification in biodiesel synthesis and non-solvents in biotransformations. This review will thus be helpful for the researchers of various biocatalytic applications.

Contents

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1. Introduction

The history of ionic liquids (ILs) dates back to 1914. The first reported IL was ethylammonium nitrate ([$EtNH_3$][NO_3]), and its m.p. was 13–14 °C [1,2]. Since then, the research of ILs has been increasingly developing and attracting broad interest among researchers. ILs are commonly composed of an organic cation involving imidazolium, pyrrolidinium, pyridinium, ammonium, quanidinium and many other exotic cations with a variety of substituents and an inorganic anion (halides, tetrafluoroborate, hexafluorophosphate and larger anions containing sulfonynol or fluoroalkyl groups). So they belong to salts and are usually viscous liquids with m.p. below 100 °C. Due to the strong interionic interactions, some ILs are still liquids within a broad temperature range below 400 °C [3–5]. The main cations and anions of ILs are shown in Table 1. ILs are classified as green solvents because of their unique properties, e.g., low vapor pressure, outstanding solvation potential, non-flammability, high thermal and excellent chemical stability, as well as their wide liquid range. In addition, the physical and chemical properties of ILs can be finely tuned with the structural change of the cation or anion. ILs as a good solvent can make the solvation of organic and inorganic, polar and non-polar compounds optimizable, and dissolve many compounds being insoluble or sparingly soluble in water and most organic solvents [6]. The dissolving ability of ILs for these compounds, such as cellulose and some compounds having pharmacological activity, commonly depends on the hydrogen bond of anions (presented below) [7]. Due to these outstanding properties of ILs, the traditional organic solvents for enzyme reactions are gradually being replaced by ILs to improve the percent conversion and reduce the environmental pollution. Most enzymatic hydrolyzing reactions often happen in an aqueous environment, but for the hydrophobic substrates, the organic solvents have been used to increase their solubility and to eliminate the hydrolytic reactions of water with the reaction products [8]. However, there are some drawbacks using organic solvents, such as toxicity and flammability and so on. In respect to ILs' non-toxicity and non-volatility, they behave not only in an environmental friendly manner, but also create the possibility to adjust the solubility of the solutes [9]. ILs can be classified into two categories, hydrophobic and hydrophilic ILs; the latter ones can be used as potential solvents for biocatalytic reactions with polar or hydrophilic substrates such as carbohydrates and amino acids, which are insoluble in many common organic solvents, while the former ILs can replace organic solvents for non-polar or hydrophobic substrates [10].

In 1984, Magnuson et al. studied the activity and stability of alkaline phosphatase in [$EtNH_3$][NO_3], with this being the first example of using an enzyme in an IL [11]. However, due to insufficient knowledge of ILs, other researchers did not pay significant attention to this work. Until the year 2000, more and more researches on enzymatic reactions in ILs came forth and some related reports appeared within a few months of each other. Among these, the protease thermolysin was used in IL [bmim][PF_6] containing 5% water for the synthesis of Z-aspartame (*N*-carbobenzoxy-L-aspartame) [10]. Madeira et al. [12] discussed the catalytic activity of free and immobilized *Candida antarctica* lipase B (CAL-B) in two anhydrous ILs [bmim][PF_6] and [bmim][BF_4]. From that time, the enzymes involving lipases, proteases, oxidoreductases, peroxidases and entire cells were examined in ILs as solvents to test their activities and stabilities, and it was found that these enzymes were not denatured and deactivated [13–15]. Recently, many enzymatic reactions, such as transesterification using α-chymotrypsin and perhydrolysis using lipase, have been reported in ILs, and it has been revealed that the physicochemical properties of ILs play important roles in changing the stability, activity and structure of enzymes. Many ILs containing these anions BF_4^-, PF_6^- or Tf_2N^- used as solvents for enzymes show high enantioselectivity, high conversion rates, and less denaturing properties, as well as improved recoverability and recyclability [16]. Therefore, compared with the traditional organic solvents, the advantages of ILs as solvents for enzyme reactions are the following: (1) ILs are tunable solvents designed for particular bioprocesses, but the organic solvents are not; (2) enzymes including CAL-B, *Candida rugosa* lipase (CRL), cytochrome C (Cyt-C), thermolysin, lysozyme, and α-chymotrypsin etc.,commonly show higher operational and thermal stability in ILs, so that bioprocesses can be conducted at high temperatures if required; (3) due to the nonvolatile characteristics, ILs may be used in high-vacuum systems to reduce the pollution from solvent volatilization [12–16].

Many enzymes, however, exhibit the same activity in ILs as that found in traditional organic solvents, and even behave with less magnitude of stabilities and activities than those found in common organic solvents [17]. There is no simple answer as to whether an enzyme is active in a certain IL, because the activity of the enzyme depends on the enzyme-medium-substrate relationship. The factors influencing the catalytic mechanism of enzymes in ILs include anions of ILs, the alkyl chain length in cations, IL polarity, viscosity, ion kosmotropicity (Hofmeister series), hydrophobicity, amphiphilicity and an IL network [18]. But there must be interactions among these factors, and their influences on enzyme stability and activity may be coincidental or opposite.

There are several methods being explored to improve the enzyme's activity and stability, e.g., enzyme immobilization on a solid support, modification of enzyme charge, water-in-IL microemulsions, and designing enzyme compatible ionic solvents [19]. Until now, some new ILs have been designed and synthesized to be more compatible with enzymes. For example, ILs have been synthesized to be water-miscible, partially miscible and immiscible with different polarities through the proper selection of the cations and anions. These tunable properties impact the activity and stability of enzymes. Some reports have shown that the carbohydrates, containing sucrose, lactose, cellulose, β-D-glucose, β-cyclodextrin and sugar alcohols can be dissolved in some ILs rather than common organic solvents [20]. The reason for this is that the anions of these ILs form strong hydrogen bonds with the carbohydrates, making it possible to dissolve and transform them into products via enzymatic reactions [21]. At the same time, some of these ILs may denature enzymes, so there is a strong and urgent need to increase the stability and activity of enzyme in ILs. In this review, we will shed light on the factors that affect the stabilities, activities, and structure of enzymes, and strategies to improve enzymes' utilities in ILs. We hope this work will help researchers understand the mechanisms of enzymatic reactions, and stimulate the flourishing of biocatalytic applications in ILs media.