551b Comb-Shaped Polyethylene Glycol-Modified Subtilisin Soluble and Highly Active in Ionic Liquids

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Subtilisin Carlsberg conjugated with comb-shaped polyethylene glycol was solubilized in common ionic liquids without adding water, and exhibited higher transesterification activity in ionic liquids than in organic solvents commonly used for enzymatic biotransformation.

Many researchers are becoming increasingly interested in the application of ILs as reaction media for biotransformation. Recent works showed that enzymes exhibited their catalytic activities in pure ILs or IL/aqueous biphasic systems, providing many advantages such as high conversion rates, high enantioselectivity, and increased stability of enzymes. However, one of the most significant limitations in enzymatic reactions in ILs is the relatively low activity of enzymes suspended in ILs. Some ILs are known to dissolve enzymes with or without a small amount of water, however, dissolved enzymes show little catalytic activity presumably due to their conformational change in ILs. To overcome this limitation, several studies have been conducted to enhance enzymatic activity in ILs, involving the addition of a small amount of water to ILs, and immobilization of enzyme with solid supports.

Polyethylene glycol (PEG) shows high solubility in ILs, and was found to provide high stability and dispersibility of enzymes in ILs. We previously showed the effective activation of lipases in ILs by physical complexation of lipases with PEG 20,000. Although some researchers attempted to improve solubility and activity of enzymes in ILs by covalent modification with PEG, chemical modification of enzymes with linear PEG did not offer sufficient solubility and catalytic activity in ILs.

Here, we report the use of comb-shaped PEG as an enzyme modifier to solubilize an enzyme in ILs. Comb-shaped PEG, PM13 is a copolymer derivative of PEG and maleic anhydride with an approximate molecular weight of 13,000 and has multivalent reactive sites, acid anhydrides, which react preferentially with amino groups in a protein molecule. The potential utility of PM13 was validated in enzymatic catalysis in organic solvents. In the present study, we have demonstrated perfect solubilization and marked enhancement of catalytic activity of an enzyme in ILs by modification with PM13. To our knowledge, this great superiority of enzymatic catalysis in pure ILs compared with organic solvents has not yet been reported.

In summary, we showed that comb-shaped PEG, PM13, is an excellent modifier to solubilize enzymes in ILs. This approach offers high enzymatic activity in pure ILs without immobilization of enzyme or addition of small amounts of water. We believe that this paper will stimulate researchers to develop further applications in the field of enzymology for the use of ILs.