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Choline Chloride- and Betaine-Based Deep Eutectic Solvents Increase Lipase Activity and Thermal Stability

Miša Mojca Cajnko, Mejrema Nuhanović, Uroš Novak, Blaž Likozar
National Institute of Chemistry, Hajdrihova 19, Ljubljana, Slovenia

Deep eutectic solvents (DES) are a class of green solvents that have immense potential in several fields like organic and catalysis, material chemistry and electrochemistry, as well as in extraction processes [1]. DES have many advantages as a reaction media in biocatalytic reactions such as esterification, polymerization and hydrolysis, due to their low toxicity, biodegradability, easy preparation and low cost [2]. These solvents have also been shown to have a high solubilizing capacity for various substrates with poor or no water solubility [3] and have been used as stabilizing reaction media in biocatalytic reactions with enzymes like laccases and lipases [4]–[6].

The objective of this preliminary research was to determine the effects of DES on the activity of lipase which could potentially be used for modification of polymers with very poor water solubility. We prepared 15 different DES, 13 with choline chloride (ChCl) as H-bond acceptor (HBA) and sugar and non-sugar H-bond donor (HBD) and 2 with betaine (Bet) as HBA and non-sugar HBDs, and tested their effects on the activity of immobilized lipase B from *Candida antarctica* (Novozym 435). Lipase was first subjected to different temperatures in order to determine its stability. Two milligrams of enzyme was incubated in Tris/HCl buffer at 30 - 90°C for 30 minutes, the buffer then was removed and a substrate solution was added. The experiments with DES were performed in a similar manner. However, the lipase substrate used in our experiments was sensitive to both temperature and pH and subsequently, enzyme activity could not be measured directly in DES. Therefore, the enzyme was first incubated in the selected DES for 30 minutes at 30°C, the DES was then diluted with distilled water and removed and the enzyme washed again 2 times with distilled water. Substrate solution was then added and enzyme activity measured. The same experiment was performed at 80°C. The results showed a decrease in lipase activity with increase in temperature, where only minimal activity at 80°C and none at 90°C. However, when the enzyme was incubated in DES at 30°C, its activity was increased over 1.5-times in all DES and over 2-times in some ChCl-based DES compared to Tris/HCl buffer. Most DES also showed a significant thermostabilizing effect where lipase activity after incubation at 80°C was 5 to 7-times higher compared to Tris/HCl buffer for DES with non-sugar HBDs and even higher for DES with sugar HBDs. Our results show strong thermostabilizing effects of selected DES mixtures which could allow biocatalytic reactions at higher temperatures without significant loss of enzyme function. Furthermore, these DES show activating effects even after they themselves have been removed, opening potential new possibilities for increasing enzyme activities and subsequent product yields, including for substrates that are sensitive to DES (their components or pH).

Key words: lipase; deep eutectic solvents; activation; thermostability

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