ABSTRACT

Lipases have emerged as one of the most important biocatalysts in the field of biosynthesis. They posses unique ability to carry out diverse range of reactions, remain active even in water restricted environment and act at interfaces. In many lipase mediated biotransformations, industrial applications and biotechnological studies surfactants have played essential role. They have been found to impart stability, modify specificity and even enhance the activity of lipases. Development of novel surfactants opens an unexplored area of research involving their application in lipase modification as well as biosynthesis.

First, in the present study an attempt was made to purify lipase from *Rhizopus oryzae* NRRL 3562 in minimum steps attaining both high purification fold and yield. Only two simple steps of purification involving ammonium sulphate precipitation followed by gel filtration chromatography led to a yield of 51.39% and fold increase of 37. Lipase with specific activity of 450 IU/mg gave a single band on both native and SDS PAGE, showing purification to homogeneity. Further characterization studies of the purified lipase were conducted. It was found to be a low molecular weight lipase, of 14.45 kDa and had optimum temperature and pH of 30-40 $^{\circ}$C and 9 respectively. The purified lipase showed specificity towards long chain ($C_{16\text{-}18}$) *p*-Nitrophenyl esters and low K_m of 7.76 µM. Stability at elevated temperatures, wide pH range and in presence of solvents makes this small lipase a potent candidate for transesterification reactions.

Further work was carried out to gain an insight into the effects of new ionic liquid-type imidazolium cationic surfactants on the structure and function of the lipases. Changes in the activity, stability and structure of purified *R. oryzae* lipase in the presence of novel surfactants, (C_{16}mim)Br, [C_{16}-3-C_{16}im]Br_2 and [C_{16}-12-C_{16}im]Br_2 were probed. Both the activity and stability were found to be enhanced in presence of the surfactant at low concentration followed by inhibition at high concentration. Surfactant with shorter spacer length, [C_{16}-3-C_{16}im]Br_2 was found to be better in improving the hydrolytic activity (97%) and thermal stability of the lipase. Investigations by ultraviolet-visible spectroscopy and circular dichroism revealed structural changes leading to rise in β-sheet content and lowering of α-helix at low surfactant concentrations. Deactivation at high concentration correlated with greater

structural changes depicted by spectroscopic studies. Isothermal titration calorimetric studies showed the binding to be spontaneous in nature involving non-covalent interactions. High negative value of entropy signifies exposure of hydrophobic domains and increase in structural rigidity, which correlates with active site being more accessible and rigid in presence of the surfactant. The forces involved in binding were found to differ for the gemini surfactants proving that the spacer length is an important factor which governs the interaction. Application of these surfactants hold greater potential in the field of lipase based biotransformations, enzyme structural modifications and studies.

Lastly, application of the combination of novel ionic liquid-type imidazolium cationic surfactants with lipase for biosynthesis was explored. For this modification of starch by esterification was targeted. Lipases, as catalysts have emerged as a promising alternative to chemical processes for starch esterification. Although ionic liquids and microwave assisted heating are emerging as green technology yet their use along with lipases for starch modification has not been probed. The present study deals with esterification of corn starch employing *R. oryzae* lipase, microwave irradiation and novel imidazolium surfactants. At 80% irradiation, 1:3 starch/oleic acid molar ratio, 150 IU enzyme, and 50 µmol of $[C_{16}\text{-}3\text{-}C_{16}im]Br_2$ maximum degree of substitution (DS= 2.75) was attained. The modified starch showed better hydrophobicity and thermoplasticity with corresponding structural changes depicted by FTIR, XRD and SEM. These properties advocate the usefulness of the modified starch in food and biopolymer sectors.

Keywords: *Rhizopus oryzae*, Lipase, Purification, Characterization, Novel imidazolium surfactants, Interactions, Starch esterification