Immobilization of *sn*-2 rRegioselective Lipase on Modified Epoxy Support

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Despite potential and demand for *sn*-2 regioselective lipases in the industry, such lipases are not cost-effective for commercial use. The financial limitations of this enzyme application are mostly caused by the instability of the enzyme under reaction condition. Furthermore, *sn*-2 regioselective lipases have been reported to be less selective than *sn*-1,3 regioselective lipases. To address these problems, in this study, *sn*-2 regioselective lipase from *Cordyceps militaris* was immobilized on modified epoxy support. The epoxy functional group covalently bonds with various side chains of amino acids, mainly ε-amino group of lysine. As covalent bonding between epoxy group and amino group is slow enough at neutral pH, adequate modification of the support can promote orientation-directed adsorption before covalent bonding occurs. Based on Alphafold2 and adaptive Poisson-Boltzmann solver, structure of the lipase was predicted to possess a surface with positive, negative charges along with external histidine of which side chain is oriented outward. Considering the characteristics of the epoxy support and the lipase structure, the epoxy support was modified with ethylene diamine, iminodiacetic acid, and iminodiacetic–copper chelate. Each modification was designed to induce the orientation of enzyme-support interaction towards the negatively charged surface, positively charged surface, and surface with the external histidine group. The degree of support modification was determined by the titration of amino group in ethylene diamine or ICP-MS method for copper detection. Then, the lipase was immobilized through adsorption and multipoint covalent bonding. The yield of the immobilization was accessed by comparing the initial enzyme activity with the remaining enzyme activity in the reaction solution. Subsequently, the stereoselectivity of each enzyme-support complex was evaluated on triolein using a HPLC-ELSD system. Results suggest that the regioselectivity of the immobilized lipase was influenced by the different orientations and enzyme-support interaction sites.