Proc. KSAM Annual Meeting
June 20-22, 2001, Chonan, Korea

Polycationic Amino acid Fusion System for Simple Purification and Immobilization of Foreign Proteins in Recombinant *Escherichia Coli*

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This research was focused on characterization of polycationic amino acids fusion systems and application of the fusion systems for easy purification and enzyme immobilization of a target protein or an enzyme in recombinant *Escherichia coli.*

Fusion proteins with charged polypeptide tails were constructed for the purpose of simple ion-exchange purification with high purity. Proteome analysis of *E. coli* showed that most intracellular proteins had their isoelectric points below pH 7.0, directing the choice of cationic amino acids as fusion partners. A series of positively charged lysine tails and arginine tails were fused to the C-terminal of a model protein, cyclodextrin glycosyltransferase (CGTase) derived from *Bacillus macerans* and expressed in *Escherichia coli.* The electric charge provided by a series of tails allowed the selective recovery of CGTase from recombinant *E. coli* cell extracts by providing a binding ability to the CGTase which did not have a binding ability by itself. The purification factors were directly affected by the binding strength of tails to the cation exchanger reflected by amino acid species and the length of tails. Among the fusion CGTases, CGTK10ase containing 10 lysine residues as a fusion partner had the strongest binding ability and could be purified to its homogeneity by simple ion-exchange chromatography. In addition to the purification of foreign proteins, the versatility of an ionic amino acid fusion system had many applications such as enzyme immobilization method and efficient solid-state refolding methods.

A variety of enzyme immobilization methods have been used while chemical bonding, whether by cross-linking, copolymerization, or covalent linkage, being the most common. The chemical bonds give very stable enzyme preparations without enzyme leakage. Nonetheless, the method is very expensive and complicated. And used chemicals may not be compatible with some applications because of harsh conditions employed for enzyme immobilization. Adsorption is the simple and less expensive, with minimal chemical requirements and less likelihood of enzyme denaturation. However, the weak nature of the binding forces can cause leakage of the enzyme with changes in pH, ionic strength, and/or temperature.

The concept of charged amino acid fusion can be applied to non-covalent enzyme immobilization as well as to purification. The fusion protein could be directly adsorbed from crude cell extracts on polyanionic matrices in a specific, oriented fashion. Upon non-covalent immobilization by polyionic interactions, the stability of the fusion protein was not affected by pH-, urea-, or thermal-denaturation. The operational stability of the coupled enzyme under conditions of continuous substrate conversion increased significantly compared to the soluble form. CGTase fused with a polycationic tail could be successfully immobilized on a cation exchanger. No loss of enzyme activity during an immobilization process was observed because harsh conditions typically required for covalent immobilization could be avoided. Immobilization of CGTase using a fusion cationic tail did not alter the enzymatic characteristics

significantly. Immobilized CGTK10ase was successfully employed to produce a-CD in a packed bed enzyme reactor.

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