ABSTRACT

Many man-made applications like ELISA, liposomal drug delivery, vaccine, biosensor, biosorbents and biocatalyst require proteins to perform their functions in the solid-liquid interface while anchored to solid surfaces. Anchoring hydrophilic proteins to hydrophobic surfaces is a major challenge even for biological systems. Bacteria have overcome this challenge by evolving lipoproteins to perform a variety of functions at membrane-aqueous interface; while one end of hydrophilic protein is anchored to its membrane by a lipid moiety, N-acyl-S-diacylglyceryl cysteine, the rest of the protein is in the aqueous phase functioning normally. However, bacterial lipid modification mechanism remains unexploited for man-made applications. Based on the current understanding of this ubiquitous and unique bacterial lipid modification, it is possible to use E.coli, the popular recombinant protein-expression host, for converting a protein to a lipoprotein with the characteristic hydrophobic anchor at the N-terminal end. Two different lipid modification strategies, suitable for proteins with or without signal sequences, were designed using periplasmic enzyme Shigella apyrase as a model. In the first one, its native signal sequence was engineered at the C-terminal end to incorporate Lipobox (LAGC), an essential structural determinant for lipid modification. In the second strategy, the signal sequence containing lipobox and +2 Ser of an abundantly expressed Braun’s lipoprotein (Lpp) was fused to
mature sequence of apyrase. The engineered apyrase, when expressed using BL21-DE3 and GJ1158, *E. coli* hosts for T7-expression, the originally soluble periplasmic enzyme, became bound to inner membrane. Actual lipid modification was proved by mobility shift in Tricine SDS-PAGE and mass spec analysis. Substrate specificity and specific activity measurements indicated functional integrity after lipid modification. Owing to a variety of applications possible for lipid modification two different generic lipoprotein-expression vectors with the convenience of multiple cloning site were constructed based on the above two strategies. The expression and lipid modification of apyrase by these vectors were found to be comparable with their parent plasmids, but lower compared to native apyrase. In spite of currently proposed outer membrane targeting signal included in the strategy based on fusing of Lpp signal sequence, apyrase was found only in the inner membrane. Since surface display of proteins has powerful applications like whole-cell-vaccine, combinatorial library screening, biosorbents and biocatalysts outer membrane targeting signals of lipoproteins were further analyzed by bioinformatics studies. Apart from sequence determinants adjacent to lipid-modifiable Cys, an amphipathic β-strand, normally seen in outer membrane proteins, seems to be an important signal in outer membrane targeting. In majority of outer membrane lipoproteins lacking amphipathic β-strand we found Gln at +2 position (next to Cys) in the mature sequence. This finding was tested experimentally by inserting +2Q in lipid-modified apyrase. In conclusion, convenient protein-engineering strategies for converting non-
lipoprotein to lipoprotein with minimal fusion have been devised and tested successfully. Based on these strategies, two different generic lipoprotein-expression vectors were constructed.