484g Enhance and Modulate Substrate Permeability for Whole-Cell Biocatalysis through Cellular Membrane Engineering

Ye Ni, Xuan Guo, John Reye, and Rachel Ruizhen Chen

Obtaining rapid substrate permeation through outer membranes is a common and formidable obstacle in whole-cell biocatalysis. In fact, the rate of entry of both hydrophobic and hydrophilic molecules is often the rate-limiting step of a whole-cell biocatalysis reaction. To address this issue, we apply molecular engineering tools to modify membrane compositions and mass transfer barrier properties of cell envelopes. E. coli mutants carrying outer membrane mutations in lipopolysaccharide (LPS) synthesis and lipoprotein (in particular, a mutant deficient in the enzyme catalyzing the first step of lipid A synthesis and a mutant with a dysfunctional Braun's lipoprotein) were used. Several substrate molecules spanning a wide spectrum of size and hydrophobicity (nitrocefin, a hydrophobic tetrapeptide, toluene, UDP-Glucose) were used to probe the mutation effect. Drastic reaction acceleration (up to 20 fold) was observed. While beneficial effects of both mutations are observed with all substrates tested, the gains to be realized by mutating the outer membrane are dependent on the nature of the substrate. For example, while lipoprotein mutation rendered a barrier-less condition (total elimination of outer membrane permeability barrier) for a tetrapeptide molecule, the same mutation increased the permeability for nitrocefin by only 20%. Size and hydrophobicity of the molecules appear to be particularly important in determining the beneficial effect of the mutations. Surprisingly, for the lipoprotein mutation, the significant acceleration comes with little adverse effects on the cell's ability for growth and recombinant protein expression. The molecular engineering approach was compared to several common empirical permeabilizing methods such as the freeze-thaw and the EDTA treatment. The molecular engineering approach was shown to give better permeabilization and less unintended damage to the cell. Besides increasing permeability, we are also developing methods for tuning permeability for temporal and spatial control of biocatalysis. Applications envisioned include relocation of inhibitive products to a desired cellular compartment. One approach investigated involves using peptides that are capable of modifying cellular membrane permeability. A 23-residue peptide was expressed as a fusion with a maltose-binding protein. The presence of the peptide increased the permeability of substrates for both *ƒ*"-lactamase and *ƒ*"-galactosidase, increasing the whole-cell catalyzed reactions by several fold. Molecular engineering is highly effective in addressing permeability issues in whole-cell biocatalysis. The ability to modulate cellular permeability opens up new possibilities to engineer whole-cell biocatalysts and exciting opportunities to make whole-cell biocatalysis a more competitive and accessible technology.