Making *Bacillus subtilis* Lipase A Amenable for Structural Biology: Protein Preparation and Solubilization

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Lipase A from Bacillus subtilis is one of the best known esterases and a good target for examination of concepts in biocatalysis. Recently, thermostable mutants of the enzyme have been obtained in our laboratory using B-FIT driven iterative saturation mutagenesis [1,2]. The evolved mutants are stable at temperatures higher than 90 ^{o}C, whereas the wild-type enzyme is stable at temperatures up to 50 ^{o}C. The availability of the thermostable mutants opens up the opportunity of studying biophysical factors responsible for elevated thermostability of proteins using both nuclear magnetic resonance (NMR) spectroscopy and X-ray crystallography [3]. Here, optimization of protein sample preparation is reported.

Structural biologists require homogenous protein samples for studies. The expression of the lipase was optimized and various systems have been tested. Finally, the heterologous insoluble intracellular expression from *Escherichia coli* turned out to be a very efficient method of protein expression. The purification protocol consists of solubilization of inclusion bodies in chaotrope, subsequent re-folding followed by hydrophobic interaction and cation exchange chromatographies. The procedure yielded homogenous *B. subtilis* lipase A. This protocol allows efficient isotope labeling required by protein NMR when minimal medium is used.

Lipase A from *B. subtilis* is a hydrophobic protein, therefore the solubility of the protein is limited. Whereas high protein concentration is not required to grow high-quality crystals for X-ray crystallography [3-6], the concentration issue is critical for protein NMR. Solubility studies using detergents, chaotropic reagents and organic solvents as protein solubilizers are reported here.

References:

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