Cloning, Expression, Purification and Structural Analysis of *Escherichia coli* Selenophosphate Synthetase

Silva, I. R.[1]; Faim, L. M.[1]; Sculaccio, S. A.[1]; Thiemann, O. H.[1]

[1]Instituto de Física de São Carlos, Universidade de São Paulo, São Paulo, Brazil

The main biological form of selenium, in several organisms, is the amino acid selenocyteine (Sec, U), which is incorporated in the emerging polypeptide at specific UGA stop codons. In *Escherichia coli*, this incorporation requires the genes that codify for selecysteine synthase (SELA), seryl-tRNA synthetase (SerRS), a specific $tRNA^{Sec}$ (SELC), selenophosphate synthetase (SELD) and a selenocysteine-specific translation elongation factor (SELB). Selenophosphate synthetase (SELD, EC 2.7.9.3), the product of the selD gene, produces the biologically active selenium donor compound, monoselenophosphate, from ATP and selenide. The selD gene in *E. coli* is 1041 base pairs long encoding a protein of 347 amino acids with molecular mass of 37 kDa. The open reading frame for selD was cloned into the vector pet28a(+) (Novagen) and the recombinant SELD was overexpressed in *E. coli* at 37ºC for 3 hours by IPTG induction. The SELD protein was purified by metal-chelate affinity chromatography. The eluted fraction with recombinant protein was concentrated up to ~7.0 mg/ml by ultra filtration and the product was submitted to Thrombin Protease cleavage of the N-terminal 6-Histidine tail. In order to purify the product of the protease cleavage reaction and to determine its molecular mass and oligomeric state, size exclusion chromatography was employed. The purified SELD was used for Dynamic Light Scattering (DLS) and native gel analysis. The result indicates a protein with molecular mass consistent with dimeric protein. Small Angle X-ray Scattering (SAXS) are been employed to determine the structural organization of the dimer and the results are being analyzed.

Keywords: *Escherichia coli*, selD, Selenocysteine, Selenophosphate synthetase

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