

MOLECULAR CHARACTERIZATION OF THE SERYL-tRNA SYNTHETASE
(SerARS) OF *TRYPANOSOMA BRUCEI*.

Evangelista, J.P.¹, Thiemann, O.H.¹

¹Centro de Biotecnologia Molecular Estrutural - CBME, Instituto de Física de São Carlos, Universidade de São Paulo, São Carlos - SP, Brazil.

The study of the translation process attracts the interest of several groups due to its central role in the general metabolism of the cell. In particular, interest has been growing on the study of the amino acid selenocysteine due to its unique synthesis pathway and cellular importance. This pathway is initiated by the Seryl-tRNA synthetase coupling Serine to a UGA codon decoding tRNA encoded by the SelC gene. We have cloned the Seryl-tRNA Synthetase (SerARS) from *Trypanosoma brucei* to investigate the functional and structural elements that allow this enzyme to bind all Ser-tRNA as well as to SelC. The *Trypanosoma brucei* Seryl-tRNA Synthetase (SerARS) was expressed in *Escherichia coli* BL21 (DE3) strain and induced with 0.1mM IPTG. The recombinant SerARS enzyme was purified, after ammonium sulfate and hydrophobic chromatography (phenyl-sepharose) fractionation and metal-chelate affinity chromatography (Ni-NTA column), eluting with 250mM of imidazole. The fractions were analyzed in 15% SDS-PAGE confirming the monomeric size of 54KDa. Dynamic Light Scattering (DLS) experiments determined the hydrodynamic radius of 4,32nm corresponding to a molecular mass of 108 KDa, consistent with a dimeric state. This result was confirmed by native gel experiments. All four *T. brucei* Ser-tRNA isoacceptors and SelC tRNA are available by in-vitro transcription. Binding experiments to identify the prevalent interactions of the tRNAs and Ser-RS are under way on an VP-ITC Microcalorimeter (MicroCal).

Supported by FAPESP

Keywords: selenocysteine, aminoacyl-tRNA synthetase, Seryl-tRNA synthetase.