Analysis of selenophosphate synthetase association with selenocysteine synthase-tRNA^{sec} binary complex

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INTRODUCTION: Investigation of the genetic code translation to proteins is of interest due to its central role in the cellular metabolism, in particular, the investigation of the 21st amino acid selenocysteine, the major biological form of selenium. Its incorporation into selenoproteins occurs via a cotranslational process in response to a UGA in-frame stop codon and requires a complex enzymatic machinery that comprises Selenocysteine Synthase (SelA), specific elongation factor (SelB), Selenophosphate Synthetase (SPS), specific tRNAsec (SelC), SElenoCysteine Insertion Sequence (SECIS) and Seryl-tRNA Synthetase (SerRS). Since selenium compounds are highly toxic in cellular environment, selenium association with proteins complexes throughout its metabolism is suggested to be essential for cell survival. However, macromolecular interactions between the different proteins have not been characterized yet. OBJECTIVES: In this study, we investigate selenophosphate synthetase association with selenocysteine synthase-tRNAsec binary complex. MATERIALS AND METHODS: We produced recombinant *Escherichia coli* SPS and SelA in BL21(DE3) and WL81640(DE3) *E.coli* strains, respectively. The tRNAsec was obtained by in vitro transcription. Fluorescein labelled tRNAsec interaction with SelA was monitored by Fluorescence Anisotropy Spectroscopy, which was also applied to investigate SPS interaction with SelA-tRNAsec binary complex. RESULTS AND DISCUSSION: We show that SPS cooperatively interacts with decameric SelA-tRNAsec complex with an apparent dissociation constant of 610 nM. The resulting 1.3 MDa ternary complex dimensions are 4 nm in height and 27 nm in diameter as observed by both atomic force and cryo-electron microscopies. Binding interfaces were determined for SPS and SelA by hydrogen/deuterium exchange mass spectrometry and we observed that SPS, not SelA, undergoes a conformational change upon the ternary complex formation. SPS glycine-rich N-terminal region showed to be crucial for SPS-SelA-tRNAsec interaction and in vivo studies revealed that it is essential for selenoprotein biosynthesis. CONCLUSION: Together, our results give new insights into the selenium delivery system in the selenocysteine incorporation pathway necessary for keeping cell under nontoxic levels of selenium.

Keywords: Selenocysteine, Selenophosphate Synthetase, Selenocysteine Synthase.

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