Selenocysteine synthase (SELA) and $tRNA^{sec}_{uca}$ Interaction: Structural and Functional Investigations

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The amino acid selenocysteine represents the main biological form of the element selenium, and its co-translational incorporation into selenoproteins occurs at a UGA termination codon in phase with the remaining message. The selenocysteine synthesis is a result of a complex molecular machinery. In *Escherichia coli* the main proteins involved in this pathway are: Selenocysteine synthase (SELA), Elongation factor of selenocysteine (SELB or EFSec), Selenophosphate syinthetase (SELD) and a specific $tRNA^{sec}$ (SELC). After the aminoacylation of $tRNA^{sec}$, serine is converted into selenocysteine by a reaction catalyzed by the SELA protein, a pyridoxal-5´-phosphate containing enzyme. This cofactor interacts with seryl-$tRNA^{sec}$, resulting in the synthesis of an intermediate molecule (aminoacrylyl-$tRNA^{sec}$). In order to better elucidate the stability and dynamics of the SELA homodecamer formation, thermal denaturation assays through the technique of circular dicroism were carried out to enable a better understanding about its kinetic of unfolding as a result of temperature changes. As this protein establishes a specific interaction with $tRNA^{sec}$, affinity assays are being carried out using the technique of spectroscopy of fluorescence for the determination of the SELA oligomerization constant and the SELA - $tRNA^{sec}$ binding constant. Later, binding assays will be performed between SELA protein and mutant tRNAs, which will have total substitutions in each arm, with the purpose of finding the most important regions involved in this interaction. This analysis may contribute to a more detailed structural characterization of these elements of the selenoproteins pathway, as well as allowing the possibility of better understanding of their interactions with other components of this pathway.

Keywords: Selenocysteine synthase, $tRNA^{sec}$, spectroscopy of fluorescence

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