Mechanistic and structural study of an enzyme that substitutes sulfur for selenium in tRNAs

Besides its presence within the rare amino acid selenocysteine in enzymes, selenium is found in modified nucleosides in certain tRNAs (1). The most abundant selenonucleoside, present at the wobble position 34 of tRNAs belonging to the three domains of life, is thought to play a role in the fine tuning of codon-anticodon interactions and thus in translation fidelity. During the last two decades, considerable progress has been made in identifying genes involved in tRNA modification; however the enzymology of the encoded proteins remains to be studied. The present project specifically aims at characterizing biochemically and structurally 2-seleno-uridine-tRNA synthase that catalyzes the replacement of the sulfur atom in 5-methylaminomethyl 2-thiouridine (mnm’sU) at position 34 of tRNAs with a Se atom to form 5-methylaminomethyl 2-selenouridine (mnm’se3U) using selenophosphate as a selenium-donor. The same enzyme has also been shown to be responsible for the formation of the newly discovered S-geranyl-2-thiouridine, a modification induced by cellular stress (2), and it was subsequently proposed that S-geranyl-2-thiouridine serves an intermediate product in the transformation of 2-thiouridine to 2-selenouridine (3).

The E. coli enzyme MnmH encoded by the ybbB gene is composed of an N-terminal catalytic rhodanese domain and a C-terminal domain containing a P-loop motif (4). Rhodaneses are sulfurtransferases, which catalyze the transfer of sulfane sulfur from thiosulfate to cyanide in vitro, via a persulfide attached to an invariant catalytic Cys residue. In archaea such as the Methanococcales, a bipartite ortholog of MnmH is present with two proteins acting in trans (5).

The goal is to investigate the molecular mechanisms by which the sulfur atom in 2-thiouridine is replaced with Se, and MnmH specifically recognizes its tRNA substrates. The genes encoding the E. coli and Methanocaldococcus jannaschii enzymes have been cloned and the different steps of the project are:

(i) purification of the enzymes
(ii) Characterization of the selenium-transferase and ATPase activities
(iii) Understanding the role the P-loop motif and identifying key residues, in particular cysteines involved in S/Se transfer using site-directed mutagenesis
(iv) Decipher the catalytic mechanisms by trapping Se-containing intermediates and using mass spectrometry for analysis
(v) Determination of the three-dimensional structure of the enzymes alone, and in complex with their substrates, in particular tRNA, using X-ray crystallography

References


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