Patrycja Szczupak „The activity of SelU protein in the synthesis of modified nucleosides in transfer RNAs"

PhD dissertation abstract

Most cellular ribonucleic acids undergo posttranscriptional modifications. To date, about 150 different nucleoside modifications have been identified, of which more than 90 occur in transfer RNAs (tRNAs). One of the most important positions in the tRNA molecule is the 34th position, also known as position 1 of the anticodon or wobble position. The nucleoside at the wobble position of the tRNA interacts with the nucleoside at position 3 of the codon in the mRNA, resulting in the correct reading of the genetic information and the addition of the correct amino acids to the *de novo* synthesized polypeptide chain. More than 40 different modified nucleosides were identified at the wobble position. The bacterial tRNAs specific for lysine, glutamine, and glutamic acid contain 5-substituted 2-thiouridines (R5S2U), 5-substituted 2-selenouridines (R5Se2U) and 5-substituted *S*-geranyl-2-thiouridines (R5geS2U). The enzyme, 2-selenouridine-tRNA synthase (SelU) is responsible for the synthesis of the last two modifications (R5Se2U and R5geS2U). Previously, the SelU enzyme was thought to catalyze two reactions independently, the selenation of 2-thiouridine (S2U→Se2U) in the presence of the selenophosphate anion ($SePO_3^{3-}$) and the geranylation of 2-thiouridine (S2U→geS2U) in the presence of geranyl pyrophosphate (GePP). In 2018, using anticodon-stem-loop (ASL) model oligonucleotides, we demonstrated that the reactions catalyzed by SelU undergo linear. In the first step, S2U-RNA is geranylated and the intermediate geS2U-RNA is selenated to Se2U-RNA (S2U-RNA→geS2U-RNA→Se2U-RNA). Then, the question arose how the SelU enzyme recognizes its substrates and what is the mechanism of R5S2U→R5Se2U conversion in the natural bacterial tRNAs.

The research presented in the dissertation focuses on characterizing the properties of the SelU protein, its substrates, and the reactions catalyzed by the enzyme. The SelU enzyme used in the study was obtained in a fusion with maltose-binding protein (MBP) bound to the N-terminus of SelU. The obtained MBP-SelU fusion protein exhibited much better properties, such as high enzymatic activity and increased stability, compared with the SelU-His_6 variant used in previous studies. It was confirmed that the SelU enzyme is a nucleoprotein that binds geranylated tRNAs. The following types of tRNA were found in the protein-bound fraction: mnm5geS2U-$tRNA^{Lys}$, nm5geS2U-$tRNA^{Lys}$, geS2U-$tRNA^{Lys}$, mnm5geS2U-$tRNA^{Glu}$, nm5geS2U-$tRNA^{Glu}$, and cmnm5geS2U-$tRNA^{Gln}$. Using 17-mer oligoribonucleotides mimicking the anticodon stem loop of $tRNA^{Lys}$ (ASL-RNA) and containing the S2U, geS2U, or Se2U modification, respectively, SelU was shown to exhibit very high substrate specificity in the geranylation reaction. The position of S2U in the RNA chain, the sequences surrounding the S2U modification, and the length of the RNA chain have a crucial influence on the recognition of the RNA substrate by the SelU enzyme. Shifting the S2U modification in the RNA chain from position 34 to other positions in the anticodon-loop (33rd, 35th, or 36th), replacing the nucleoside at position 35th, or shortening the 17-nt oligonucleotide substrate to 7-nt or 3-nt resulted in a significant decrease in the yield of the reaction catalyzed by the SelU enzyme. Moreover, it was shown that a single nucleoside (S2U, geS2U) is not a substrate for SelU. Among the various prenyl compounds present in the cells, only GePP (C_{10}) was confirmed to be a substrate for SelU, whereas other prenyl pyrophosphates such as IPP (C_5), DmaPP (C_5), FPP (C_{15}), and GeGePP (C_{20}) were not recognized as substrates by SelU. The affinity studies of SelU for prenyl pyrophosphates performed by MST technique confirmed

that SelU synthase binds only GePP (K_d ~4.7 μM). The other prenyl pyrophosphates tested did not bind to the SelU protein or their interaction with the protein was very weak (K_d >1 mM). *In silico* analysis confirmed that GePP was the best binding substrate for SelU among all prenyl pyrophosphates tested. Interestingly, all obtained chemically prenylated S2U-RNA derivatives (C_1, C_5, C_{10}, and C_{15}) underwent SelU-catalyzed selenation reaction, although the Se2U-RNA product was formed with different efficiency depending on the type of prenylated substrate (geranyl ≥ farnesyl > dimethylallyl ≫ methyl). The obtained results indicate that SelU exhibits high substrate specificity in the prenylation reaction, while substrate discrimination in the selenation reaction is rather low.

Most importantly, the geranylation and selenation activity of the MBP-SelU protein of full-length natural R5S2U-$tRNA^{Lys}$, $tRNA^{Glu}$ and $tRNA^{Gln}$ (isolated from bacteria with lacking of the SelU gene) was confirmed. The transformation of native bacterial R5S2U-tRNA to R5Se2U-tRNA is a two-step process with the formation of R5geS2U-tRNA as an intermediate. It was shown that the reaction of direct selenation of R5S2U-tRNA to R5Se2U-tRNA does not occur.

The obtained kinetic data and the determined affinity constants of SelU to the tested ASL-RNAs confirm the presumed mechanism of S2U→Se2U conversion in bacterial tRNA. SelU binds R5S2U-tRNA and catalyzes its geranylation to R5geS2U-tRNA. The geranylated intermediate remains bound to the enzyme until the selenophosphate anion ($SePO_3^{3-}$) appears in the reaction environment. Then R5geS2U-tRNA is selenated to R5Se2U-tRNA, which leaves the enzyme and participates in the translation process.