Substrate specificity of the MBP-SelU fusion protein (Escherichia coli tRNA 2-selenouridine synthase)

P-01.2-25

P. Szczupak[I], M. Sierant[I], B. Nawrot[I]

[I]Centre of Molecular and Macromolecular Studies Polish Academy of Sciences, Lodz, Poland

Transfer RNAs (tRNAs) constitute a unique group of RNA oligonucleotides, which is characterized by the highest content of modified nucleosides. The 5-substituted nucleosides, like 2-thiouridines (R5S2U), S-geranyl-2-thiouridines (R5geS2U), and 2-selenouridines (R5Se2U), found in bacterial tRNAs specific for Lys, Glu, and Gln at the first position of their anticodons (a position 34, the wobble position), play a pivotal role in the decoding of genetic information. Bacterial tRNA 2-selenouridine synthase (SelU, MnmH) exerts a two-step post-transcriptional transformation of (c)mnm5S2U-tRNA to (c)mnm5Se2U-tRNA via a (c)mnm5geS2U-tRNA intermediate.[1] In the current studies we used an MBP-SelU fusion protein, with a maltose binding protein (MBP) tag attached to the N-terminus of the wild type SelU synthase. MBP-SelU (84.4 kDa) shows high activity in the S-geranylation (> 90%) and selenation (~100%) of model 17-mer oligonucleotides mimicking the anticodon-stem-loop (ASL) of $tRNA^{Lys}$. These models contained at the position corresponding to the wobble position in tRNA anticodon either an S2U or geS2U unit, respectively. The MBP-SelU activity and the minimum length of the RNA oligonucleotide substrate, as well as the influence of the position of modified nucleoside and flanking sequences on the enzyme/substrate recognition were determined. We confirmed that the MBP-SelU protein binds the bacterial $tRNA^{Lys}$, $tRNA^{Glu}$ and $tRNA^{Gln}$. Using Microscale Thermophoresis (MST) technique the constants for binding of Cy3-labeled 17-mer oligonucleotides (containing U, S2U, geS2U or Se2U) to MBP-SelU protein were determined. The geS2U-RNA exhibits the highest affinity for MBP-SelU (Kd=3.946 ±0.41 μM) in comparison with S2U-RNA (Kd= 18.54 ±3.01 μM), Se2-RNA (Kd=27.33 ±4.33 μM).

Acknowledgements: This research was financially supported by The National Science Centre in Poland, Grant number [UMO-2018/29/B/ST5/02509] for years 2019–2022.

[1] Sierant M et al. (2018) FEBS Lett. 592, 2248-2258