Summary of doctoral dissertation of Daria Krefft, Msc

TthHB27I is REase-MTase naturally occurring in thermophilic bacteria *Thermus thermophilus* HB27 (*T. thermophilus* HB27). It belongs to the Thermus family of thermostable enzymes discovered in 2003 (*Skowron et al., 2003*), which includes REases-MTases Type II, showing similarities at the amino acid sequence level and location of the domains responsible for both REase and MTase activities within the same polypeptide. The enzymes belonging to this family have features characteristic to 3 sub-Types: IIC, IIG and IIS, and additionally show similarities to Type I and Type III REases.

As part of this work, the isolation protocol of active TthHB27I REase-MTase from strain *T. thermophilus* HB27 was developed. Due to the very low content of the enzyme in native host cells, the overproduction of the desired protein was optimized by cloning and expression of two versions of the *tthHB27IRM* gene in *E. coli* cells. In the first case, the overproduction of the biologically active enzyme was conducted by coexpression of the native wt-*tthHB27IRM* gene together with the genes coding for chaperone proteins. The next approach was to use the syn-*tthHB27IRM* gene optimized for expression in *E. coli* cells, obtained by chemical synthesis. Gene optimization was done by adjusting the codons used to that optimal for *E. coli* and reducing possible secondary mRNA structures that could affect the course of transcription and, consequently, protein translation. To isolate the recombinant enzyme the purification protocol developed for the native enzyme was used with small modifications was used.

The experiments carried out on the purified enzyme preparation provided a wealth of information on the biochemical and physicochemical properties of TthHB27I REase-MTase, which until now was only known to be the Tth111II isoschizomer. The sequence recognized by the enzyme was confirmed and the precise DNA strands cleavage positions were determined. The reaction conditions necessary to obtain REase and MTase activity were established and the effect of SAM cofactor and its analogues on REase activity was evaluated. In addition, the thermodynamic stability of the protein was examined using three techniques: CD, DSC and DSF.