Abstract

Mitochondrial processing peptidase (MPP) is a heterodimeric enzyme which belongs to M16B subfamily of metaloendopeptidases. A universal function of this enzyme is in recognition and cleavage of great number of mitochondrial preprotein presequences, which differ in length and amino acid sequence. MPP consists of catalytical β-MPP and probably recognizing α-MPP. The most conservative region in α-MPP is GRL – glycine-rich loop. Its function is supposed in primary interaction with preprotein presequence. It is possible to study conformational change of GRL after binding the substrate by fluorescence experiments.

In this diploma thesis the constructs coding the α-MPP with the single reporter tryptophan residue in the position 289 or 299 were prepared using site-directed mutagenesis. These forms of α-MPP were produced in *E. coli* BL21(DE3)+pGroESL. Activities of MPP dimer containing α-MPP with the single tryptophan residue in the reporter position were compared with MPP from wild type of *S. cerevisiae*. Used substrate was yeast malate dehydrogenase precursor with fused presequence (pMDH) from three organisms (yeast, mouse and melon). These presequences differ in their length. Activities of MPP dimer containing α-MPP with the single reporter tryptophan residue in the position 289 were about 70 % while activities of MPP dimer containing α-MPP with the single reporter tryptophan residue in the position 299 were about 90 %.

Studying of dynamics of reporter tryptophan residue in GRL in the position 289 or 299 was performed by time-resolved fluorescence spectroscopy. The measurement results show that yeast pMDH binds into α-MPP. The mobility of this very flexible loop is reduced after binding the substrate. Results confirmed similar conformational change in the proximity of both reporter positions.