Poly(A)-specific ribonuclease: mechanism of catalysis and substrate specificity

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Gene expression is complicated process that follows the formation of a gene product form its transcription (copying the information from DNA to RNA) to synthesis of functional RNA or protein. The process is the basis of the life of a cell and it requires a proper regulation. One of the regulatory mechanisms is mRNA turnover. By definition this is the time an mRNA is 'living' in the cell, or the period from its synthesis to its degradation. There are several degradation pathways, but most of the mRNAs are degraded via deadenylation-dependent decay. The first step of this pathway is deadenylation or removal of mRNA poly(A) tail which is performed by enzymes called deadenylases. Poly(A) – specific ribonuclease (PARN) is one of the deadenylases involved in mRNA turnover. The enzyme is a processive and highly specific 3' – 5' exoribonuclease, e.g. degrades only the mRNA tail starting form the last nucleotide. PARN uses two magnesium ions for the catalysis, coordinated by four conserved amino acids. The catalytic cycle is composed of two sequential steps – translocation and hydrolysis, which are repeated until the substrate is completely degraded. This project has examined the importance of divalent metal ions, such as magnesium, manganese and zinc, for the activity and specificity of PARN. It also studied the role of a fifth conserved amino acid – histidine in the catalysis.

Our results showed that the substrate specificity of PARN was altered when Mg^{2+} was replaced by Mn^{2+} or Zn^{2+}. This suggests that at least one of the metal ions is involved in the recognition of the substrate. The substitution of the divalent metal ions and His also led to changes in the catalytic cycle. The metal ion substitutions generally enhanced the reaction, and eased the substrate translocation. The replacement of His residue also affected this step of the cycle. The mutants that we tested showed lower reaction rate. Furthermore, the titrations with some of the mutant enzymes indicated that the reaction might be reversible. Based on the results it can be stated that the divalent metal ions have diverse roles for the enzyme activity. Moreover, their limited coordination states suggest the presence of a third metal ion in the active site. Additionally, the experiments with the mutant enzymes revealed that His is not essential for the enzyme activity. However, the amino acid plays an important role in the catalytic cycle, and its substitution leads to decreased activity and problematic translocation. The possibility that PARN can perform the reverse reaction – ligation, will be exploited further.

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