

Crystal Structure of *Escherichia coli* Polynucleotide Phosphorylase

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In eukaryotes and prokaryotes, RNA degradation is an important biological function and executed by some of specific ribonucleases. In eukaryotes, exosome is the most important complex for RNA degradation. In contrast, the bacterial trimeric polynucleotide phosphorylase (PNPase) is homologous to exosome and has similar domains organization with eukaryotic exosome. It's also play an important role in RNA degradation. Both of them are phosphorolytic 3' to 5' riboexonuclease that use inorganic phosphate as a nucleophil to attack RNA and release ribonucleotide 5' diphosphate products.

E. coli PNPase functions in mRNA degradation and tRNA 3' end processing in cell. Although PNPase has two RNase PH domains, only the second core domain has exoribonuclease activity. In the other hand, PNPase could form trimer. The trimer structure of PNPase show the high similarity to archeal exosome and human exosome which supports that a common mechanism for RNA degradation in all kingdoms of life.

Here we show the crystal structure of *E. coli* full-length PNPase and C terminal KH/S1 domain truncated PNPase which X-ray diffraction data were collected using synchrotron radiations at NSRRC 13C1 beam lines.

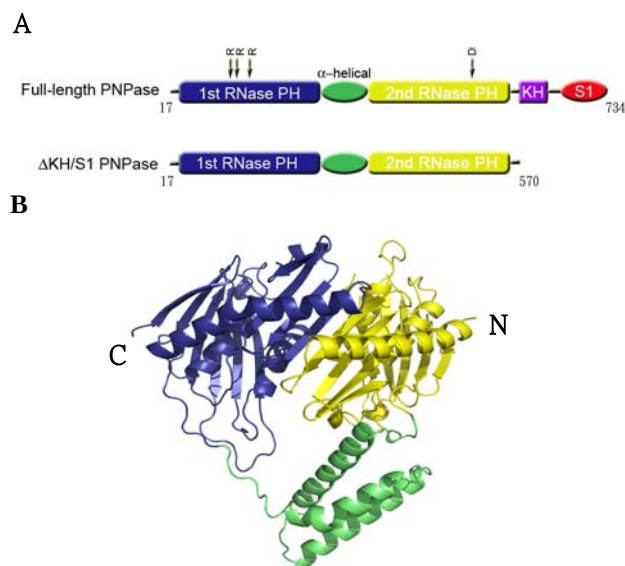


Figure 1. A. domain structure of full-length and C terminal truncated PNPase (Δ KH/S1 PNPase). B. Crystal structure of full-length PNPase. PNPase has three major domains, first and second core domains are both homologous to RNase PH and shown in yellow and blue respectively. A small α -helix domain connects these two core domains, which is shown in green. The third major domain, S1/KH domain was disordered in our structure.

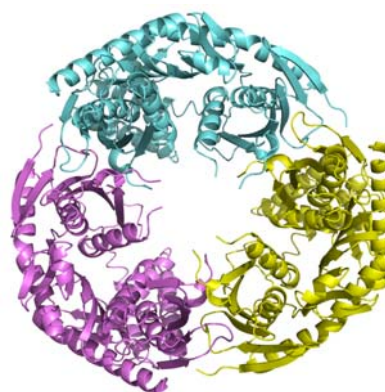


Figure 2. Schematic drawing of *E. coli* PNPase trimer. Three subunits are colored in yellow, purple and light blue. Six RNase PH domains reveal a ring-like structure. The ring has a diameter of 95 Å and displays a central channel with 30 Å length.

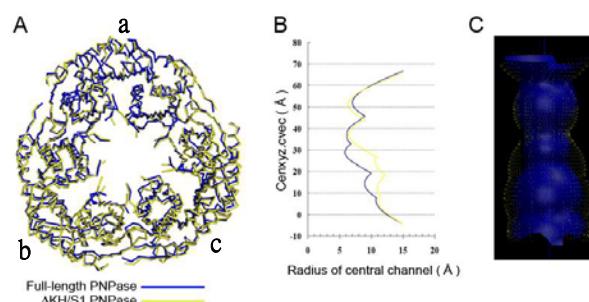


Figure 3. A. Structure superposition of WT (blue) and Δ S1/KH PNPase (yellow). Superposition of one of the monomers (molecule a) between WT and Δ S1/KH PNPase gave an average RMSD of 1.1 Å for 417 C α atoms. However, The RMSD are 1.6 Å and 1.7 Å for the molecule b and c, respectively. This result shows that Δ S1/KH PNPase is expanded and bigger than WT one. B. Comparison of the radius of central channel between WT and Δ S1/KH PNPase. Deletion mutant (Δ S1/KH PNPase) has a larger channel. C. Comparison of the central channel size of full-length and truncated PNPase. The channel size of truncated PNPase is larger than full-length PNPase.