Structural insights into the dimeric human PNPase revealing why the disease-linked mutants exhibit lower RNA import and degradation activities

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ABSTRACT

Polynucleotide phosphorylase (PNPase) is an evolutionary conserved 3'-to-5' exoribonuclease that functions differently among species, ranging from RNA turnover in bacteria to RNA transport in mammals. In human, PNPase is essential for importing a subgroup of noncoding RNAs, including 5S rRNA, MRP RNA and RNase P RNA, into the mitochondria. Mutations in PNPase are thus linked to impair in the RNA import pathway and severe mitochondrial dysfunction and diseases. PNPase is primarily assembled into a trimeric conformation with a central channel for binding of a single-stranded RNA and leading its 3' end into the active site for degradation. In this study we show that the trimeric assembly of PNPase is affected by the disease-linked mutations, including Q378R and E475G. PNPase is oligomerized into a dimeric conformation after introducing the disease-linked mutations, and these PNPase mutants have lower RNA-binding and degrading activities as compared to the wild-type PNPase. Moreover, we found that S1 domain of PNPase is responsible for the interaction with the stem-loop motif of imported RNAs. We further determined the crystal structure of the dimeric form of the S1-truncated PNPase at a resolution of 2.8 Å. Combining with small angle X-ray scattering (SAXS), we showed that both of the RNA-binding KH and S1 domains are not fully accessible in the dimeric structure, explaining why these dimeric PNPase mutants interact with RNA poorly. Taken together these results show that mutations at the interface of the trimeric PNPase tend to produce a dimeric protein with obstructed RNA-binding surfaces, thus impairing both of its RNA import and degradation activities and leading to mitochondria disorders.