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Purine nucleoside phosphorylase activity decline is linked to the decay of the trimeric form of the enzyme

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ABSTRACT

Homotrimeric mammalian purine nucleoside phosphorylase (PNP) plays a key role in the nucleoside and nucleotide metabolic salvage pathway. Each monomer in the active PNP trimer is composed of a central β-sheet flanked by several α-helices. We investigated the stability of calf PNP using analytical ultracentrifugation, differential scanning calorimetry, circular dichroism, and UV absorption spectroscopy. The results demonstrate that the activity decline (due to protein aging after isolation from cells) of wild type PNP and its two mutants with point mutations in the region of monomer–monomer interface, is accompanied by a decrease of the population of the trimeric enzyme and an increase of the population of its aggregated forms. The data do not indicate a significant population of either folded or unfolded PNP monomers. The enzyme with specific activity lower than the maximal shows a decrease of the helical structure, which can make it prone to aggregation. The presence of phosphate stabilizes the enzyme but leads to a more pronounced aggregation above the melting temperature. These results suggest that the biological role of packing of the PNP monomers into a trimeric structure is to provide the stability of the enzyme since the monomers are not stable in solution.

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Introduction

Purine nucleoside phosphorylases (PNPs)[1] are homooligomeric proteins that belong to one of the two classes: (1) low molecular mass trimeric PNPs from mammals and some microorganisms (for example *Cellulomonas* species) and (2) high molecular mass hexameric PNPs found in mainly bacteria [1–3].

Mammalian purine nucleoside phosphorylases (PNP, E.C. 2.4.2.1) form a noncovalent complex consisting of three identical subunits (Fig. 1A). The amino-acid sequences of these PNPs are highly conserved, including at the trimer forming interface (Fig. 1B). The molecular mass of each subunit is c.a. 31.7 kDa. PNP plays a key role in the metabolism of purines, purine nucleosides and nucleotides, catalyzing the reversible phosphorolytic cleavage of the glycosidic bond of 6-oxo-purine ribo- and 2′-deoxyribonucleosides. Defects in the PNP activity in humans lead to selective immunodeficiency caused by the incorrect T-cell proliferation [1,4]. Because of potential applications of PNP inhibitors as selective immunosuppressive drugs and anticancer agents, the structure and mechanism of PNP have been a subject of intensive biomedical and pharmacological studies [5–7].

A close contact between identical monomers in the active PNP trimer, the localization of the active site at the interfaces of two monomers, and the contribution of Phe159 from the neighboring subunit to the active-site pocket [8,9] suggest a communication between subunits of the active trimer. However, our recent experiments [10] argue against the importance of the inter-monomer interactions in the PNP catalysis. We have shown that the postulated [11] total inhibition of the PNP trimer by binding of only one molecule of a very potent transition-state analogue inhibitor – immucillin H (ImmH) does not occur. This means that the "one-third-of-the-sites" inhibition model is not applicable to mammalian PNP. Thus, the simplest model of inhibition, which assumes an independent active site in each monomer, is sufficient to describe the PNP–ligand interaction not only for ImmH, but also for another transition-state analogue and ground-state analogue inhibitors.

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[1] *Abbreviations used:* PNP, purine nucleoside phosphorylase; Ino, inosine; GdnHCl, guanidinium hydrochloride; DSC, differential scanning calorimetry; CD, circular dichroism; AUC, analytical ultracentrifugation; $s_{20,w}$, standard sedimentation coefficient at 20 °C in water.

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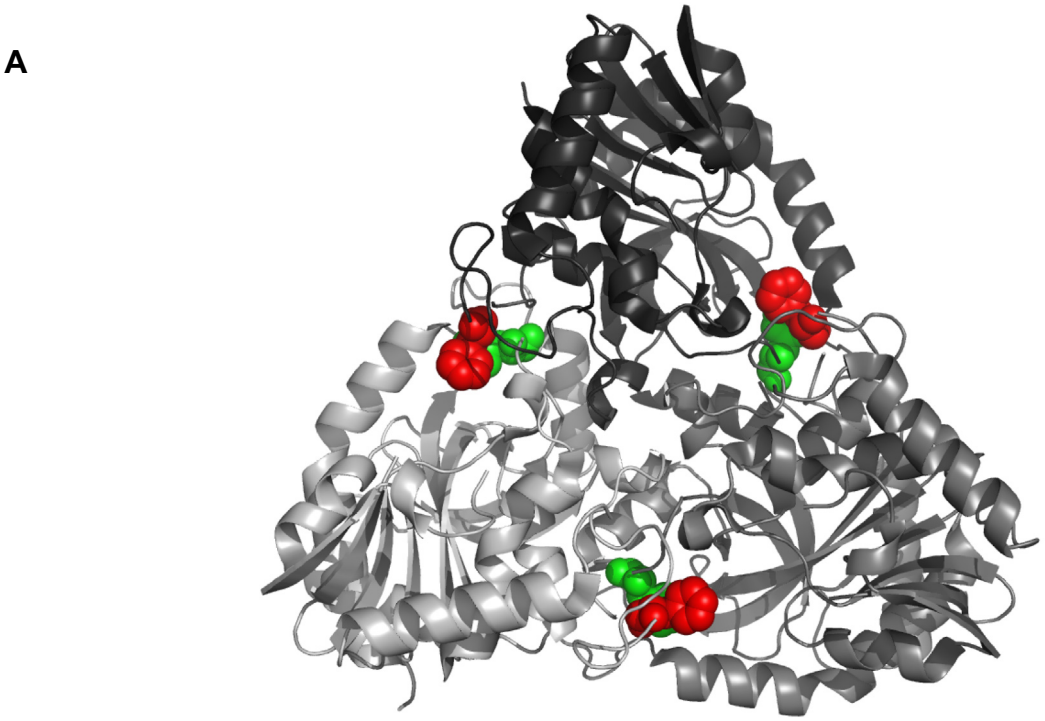

B

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1    MENGYTYEDY  KNTAEWLLSH  TKHRPQVAII  CGSGLGGLTD  KLTQAQIFDY  GEIPNFPRST
1    MANGYTYEDY  QDTAKWLLSH  TEQRPQVAVI  CGSGLGGLVN  KLTQAQTFDY  SEIPNFPEST
1    MENEFTYEDY  ETTAKWLLQH  TEYRPQVAVI  CGSGLGGLTA  HLKEAQIFDY  NEIPNFPQST
1    MENEFTYEDY  QRTAEWLRSH  TKHRPQVAVI  CGSGLGGLTA  KLTQPQAFDY  NEIPNFPQST

61   VPGHAGRLVF  GFLNGRACVM  MQGRFHMYEG  YPLWKVTFPV  RVFHLLGVDT  LVVTNAAGGL
61   VPGHAGRLVF  GILNGRACVM  MQGRFHMYEG  YPFWKVTFPV  RVFRLLGVET  LVVTNAAGGL
61   VQGHAGRLVF  GLLNGRCCVM  MQGRFHMYEG  YSLSKVTFPV  RVFHLLGVET  LVVTNAAGGL
61   VQGHAGRLVF  GFLNGRSCVM  MQGRFHMYEG  YSLSKVTFPV  RVFHLLGVDT  LVVTNAAGGL

121  NPKFEVGDIM  LIRDHINLPG  FSGQNPLRGP  NDERFGDRFP  AMSDAYDRTM  RQRALSTWKQ
121  NPNFEVGDIM  LIRDHINLPG  FSGENPLRGP  NEERFGVRFP  AMSDAYDRDM  RQKAHSTWKQ
121  NPNFEVGDIM  LIRDHINLPG  FCGQNPLRGP  NDERFGVRFP  AMSDAYDRDM  RQKAFTAWKQ
121  NPKFEVGDIM  LIRDHINLPG  FCGQNPLRGP  NDERFGVRFP  AMSDAYDRDM  RQKAFNAWKQ

181  MGEQRELQEG  TYVMVAGPSF  ETVAECRVLQ  KLGADAVGMS  TVPEVIVARH  CGLRVFGFSL
181  MGEQRELQEG  TYVMLGGPNF  ETVAECRLLR  NLGADAVGMS  TVPEVIVARH  CGLRVFGFSL
181  MGEQRKLQEG  TYVMLAGPNF  ETVAESRLLK  MLGADAVGMS  TVPEVIVARH  CGLRVFGFSL
181  MGEQRELQEG  TYIMSAGPTF  ETVAESCLLR  MLGADAVGMS  TVPEVIVARH  CGLRVFGFSL

241  ITNKVIMDYE  SLEKANHEEV  LAAGKQAAQK  LEQFVSILMA  SIPLPDKAS   PNP HUMAN
241  ITNKVIMDYE  SQGKANHEEV  LEAGKQAAQK  LEQFVSLLMA  SIPVSGHTG   PNP BOVINE
241  ITNKVVMDYE  NLEKANHMEV  LDAGKAAAQT  LERFVSILME  SIPLPDRGS   PNP MOUSE
241  ITNKVVMDYN  NLEKASHQEV  LEAGKAAAQK  LEQFVSILME  SIPPRERAN   PNP RAT
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Fig. 1. (A) The structure of purine nucleoside phosphorylase from calf spleen (PDB structure 3FUC [17]). Three identical subunits are shown in different shades of gray. The two sites of mutation – Phe159 (red) and Phe200 (green) are marked in each subunit. (B) Alignment of the PNP sequences from human, bovine, mouse and rat from UniProt database [20]. The conserved residues at the trimer-forming interface are highlighted in yellow, the other conserved residues are shown in gray, and the non-conserved residues at the trimer-forming interface are marked in blue. Phe159 and Phe200 at the trimer-forming interface are shown in red and green, respectively. The residues at the trimer-forming interface were identified by Hot Point [28].

On the other hand, the process of dissociation of PNP has been previously investigated [12–17] with the conclusion that wild type PNPs do not dissociate into active monomers. However, the question of the monomeric form as a possible intermediate state in protein assembly and aging is still open.

To better understand the reasons why the mammalian PNP is homotrimeric, we have compared the biochemical properties of the wild type calf PNP and its two mutants with Phe159 or Phe200 substituted by tryptophan (Fig. 1). In the wild type PNP, both Phe159 and Phe200 are located at the subunit interface and

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