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Crystal Structure of *Sus scrofa* Quinolinate Phosphoribosyltransferase in Complex with Nicotinate Mononucleotide

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1. Introduction

Quinolinate phosphoribosyltransferase (QAPRTase) is an essential enzyme in the first step of NAD^+ biosynthesis, catalyzing the transfer of the phosphoribosyl moiety from PRPP to QUIN to generate NAMN. Here, we report the 2.1 Å resolution crystal structure of *Sus scrofa* QAPRTase (*Ss*-QAPRTase) in complex with NAMN [1]. Our results represent the first crystal structure of a mammalian hexameric QAPRTase with its reaction product and may provide structural information useful for understanding the mode of binding of NAMN with eukaryotic QAPRTases and for designing drugs specifically targeting the QAPRTases of pathogenic bacteria rather than those of mammals.

2 Experiment

The co-crystals of the *Ss*-QAPRTase with NAMN were grown in reservoir solution (100 mM Tris–HCl, pH 8.0, 16–24% (*w/v*) PEG 8000, 150–200 mM ammonium acetate, and 5 mM NAMN) at room temperature (294±1 K) using the hanging-drop vapor diffusion method. The X-ray diffraction data were collected on the 18B beamline at the Photon Factory (Tsukuba, Japan). The data set was processed and scaled with HKL2000 [2]. The *Ss* QAPRTase –NAMN cocrystal diffracted to 2.1 Å and belongs to the *P*321 space group with cell dimensions $a = b = 119.1$, $c = 93.7$ Å , $c = 120.0°$. The structure was solved via molecular replacement with PHASER [3] using the dimeric structure of human QAPRTase (PDB ID: 2JBM) as the search model.

3 Results and Discussion

Overall Structure and NAMN Binding Site

The monomer of *Ss*-QAPRTase comprises ten β-strands and twelve a helices arranged into two structural domains, the N-terminal open-face β-sandwich domain (N-lobe) and the C-terminal α/β-barrel domain (C-lobe) (Figure 1). The secondary structure elements of the N-lobe consist of β1, β2, β3, β10, and α1– α5. The top layer of the sandwich is a four-stranded antiparallel β sheet consisting of β-strands β1, β2, β3, and the end of the C-terminal β10 strand. Helices (α3–α5) form the second layer of the sandwich. The N-terminal domain is a triple-layered sandwich, as the N-terminal α4– α5 helices stacks on the top of helix α2. The N-terminal domain ends with the longest a helix, α5, which also marks the start of the α/β-barrel. The C-terminal domain is an α/β-barrel structure consisting of six β strands and seven α helices.

The NAMN binding sites are located at the interfaces between the N-lobe of one subunit and the C-lobe of the other subunit in a dimer and are composed of residues from both subunits. The 3-carboxyl group of nicotinate moiety and the phosphate group occupy the basic pockets, whereas the hydroxyl groups of ribose ring make hydrogen bonds with the cavity consisting of Glu201 and Asp222 (Figure 2). The nicotinate ring of NAMN is located between the β4 and β5 strands, and the ribose phosphate groups extend across the barrel toward β strands β8 and β9 (Figure 1). The ribose hydroxyl group oxygen atoms of NAMN are within hydrogen-bonding distance of Glu201 and Asp222. The phosphate group of NAMN makes hydrogen bonds with the main chain nitrogens of Gly249, Gly250, and Gly270 and the side chain nitrogens of Lys139, Asn223, and Gln274. In addition, Arg138, His160, Arg161, and Lys171 form a basic pocket and contribute to the hydrogen interaction with the 3-carboxyl group of the nicotinate moiety of NAMN.

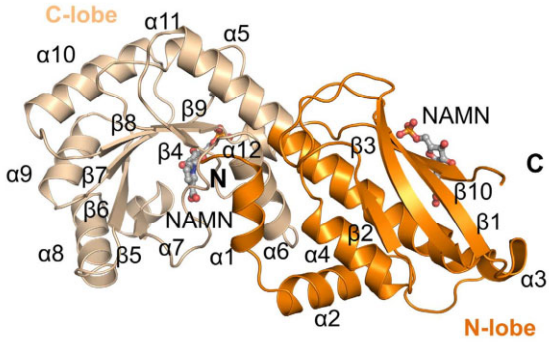

Figure. 1 Overall structure of monomer *Ss*-QAPRTase-NAMN complex. NAMN is shown as gray sticks.

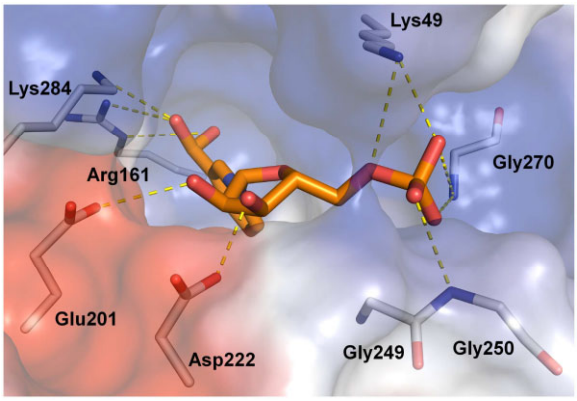

Figure. 2 Surface representation of the *Ss*-QAPRTase active site. The surface of the protein is colored based on the electrostatic potential. NAMN (orange) and residues in the active site are shown in sticks. The hydrogen bonds between a ligand and the active site residues are shown as dashed yellow lines.

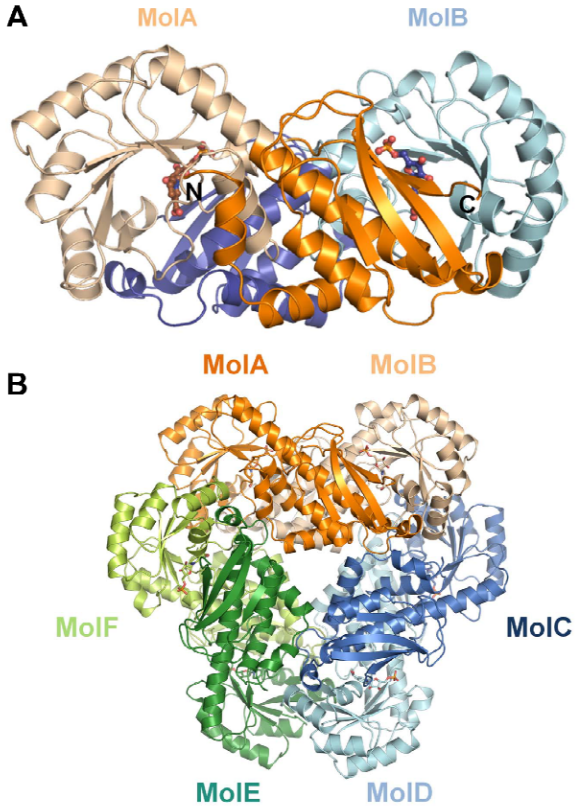

Figure. 3 Dimer and hexamer organization of *Ss*-QAPRTase. (A) *Ss*-QAPRTase dimer structure. The other subunit is displayed in blue. (B) Hexamer structure. Dimer subunits were colored in orange, blue, and green. The lighter color indicates the other subunit of each dimer.

Dimer and Hexamer Organization

Ss-QAPRTase forms a dimer via interaction between the N-lobe of one subunit and the C-lobe of the adjacent subunit (Figure 3A). The dimeric interface of *Ss*-QAPRTase buries approximately 3200 Å^2 of the protein surface, which represents approximately 23.6% of the total accessible surface area of each subunit. The root mean square deviations (RMSDs) between corresponding C_α atoms of two subunits in the asymmetric unit is 0.44 Å. Dimerization is thought to be important in increasing substrate specificity and proper enzymatic function, as has been shown in all prior QAPRTase structures.

Ss-QAPRTase forms a hexamer organized as a trimer of dimers (Figure 3B). The three dimers of porcine QAPRTase form a hexamer with a triangular structure. The hexamer has approximate dimensions of 110×110×60 Å. The surface area of *Ss*-QAPRTase that is buried by the hexamer formation is approximately 2900 Å^2 per dimer, which represents approximately 14% of the total surface area. Ionic and van der Waals interactions are the predominant contributors to the stabilization of the dimer and hexamer structure, respectively, rather than other non-covalent bonds.

4. Acknowledgement

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References

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