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Crystal structure of a menaquinone biosynthetic enzyme, *Tth*MqnD, complexed with its product, 1,4-dihydroxy-6-naphthoate

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Introduction

Recently, an alternative menaquinone (vitamin K_2) biosynthetic pathway was found in some organisms [1]. Since human and their commensal intestinal bacteria, including lactobacilli, lack the alternative manaquinone biosynthetic pathway, this enzyme in pathogenic species, such as *Helicobacter pylori* and *Campylobacter jejuni*, is an attractive target for the development of chemotherapeutics. Here we report the structure of MqnD from *Thermus thermophilus* HB8 (*Tth*MqnD: TTHA1568), an enzyme in the alternative menaquinone biosynthetic pathway, co-crystallized with its product, 1, 4-dihydroxy-6-naphtoate.

Materials and methods

The *Tth*MqnD was crystallized at 20ºC using the hanging drop vapor diffusion method. 1 μl of *Tth*MqnD was mixed with the same volume of well solution (0.1 M Tris-HCl (pH 7.8), 0.75 M Potassium sodium tartrate, 1% PEG 5000 MME). The co-crystal with the product (1, 4-dihydroxy-6-naphthoate) was obtained by soaking experiments. X-ray diffraction data collections were performed at Photon Factory BL-5A, 17A and NE3A at 100 K with Paratone-N and paraffin oil as a cryoprotectant. The structure was solved by molecular replacement using MOLREP with the *Tth*MqnD structure with tartrate (PDB: 3A3U) [2]. The crystal structure was refined to 1.55 Å resolution using REFMAC5.

Results and discussion

The structure comprises two domains with α/β structures, a large domain and a small domain. There is no difference in the overall structures between the crystals with the product and tartrate. The product compound is bound to the pocket between the two domains. Several residues in the pocket are highly conserved in the MqnD orthologs. The complex structure suggests that these residues are important for substrate binding and/or catalysis. Amide hydrogens of Thr107 and Ala108, and hydroxyl group of Ser57 form hydrogen bonds to the carboxy group of the product (Fig. 1A). Leu176 and Tyr234 have hydrophobic interactions with naphthalene ring of the product. In addition, residues Asp38 and His145 form a hydrogen-bonding network via water molecule (Fig. 1B), suggesting that His145 can function as the catalytic base. Asn13 is located very close to the product, implying that amide carbonyl oxygen of Asn13 may stabilize carbocation of putative reaction intermediate. From these points, we propose putative reaction mechanism of MqnD. This high-resolution structure should contribute toward the development of the inhibitors.

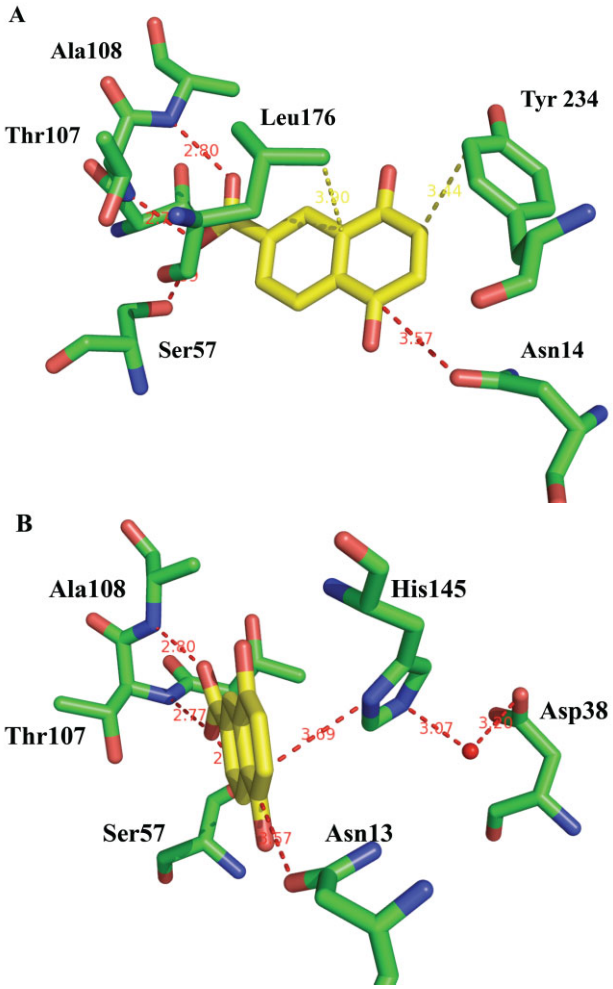

Fig. 1. Closed-up view of the putative active site of the *Tth*MqnD.

References

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