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Structure Report

Crystal structure of MqnD (TTHA1568), a menaquinone biosynthetic enzyme from *Thermus thermophilus* HB8

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ABSTRACT

In many microorganisms, menaquinone is an essential lipid-soluble electron carrier. Recently, an alternative menaquinone biosynthetic pathway was found in some microorganisms [Hiratsuka, T., Furihata, K., Ishikawa, J., Yamashita, H., Itoh, N., Seto, H., Dairi, T., 2008. An alternative menaquinone biosynthetic pathway operating in microorganisms. Science 321, 1670–1673]. Here, we report the 1.55 Å crystal structure of MqnD (TTHA1568) from *Thermus thermophilus* HB8, an enzyme within the alternative menaquinone biosynthetic pathway. The structure comprises two domains with α/β structures, a large domain and a small domain. L(+)-Tartaric acid was bound to the pocket between the two domains, suggesting that this pocket is a putative active site. The conserved glycine residues at positions 78, 80 and 82 seem to act as hinges, allowing the substrate to access the pocket. Highly conserved residues, such as Asp14, Asp38, Asn43, Ser57, Thr107, Ile144, His145, Glu146, Leu176 and Tyr234, are located at this pocket, suggesting that these residues are involved in substrate binding and/or catalysis, and especially, His145 could function as a catalytic base. Since humans and their commensal intestinal bacteria, including lactobacilli, lack the alternative menaquinone biosynthetic pathway, this enzyme in pathogenic species, such as *Helicobacter pylori* and *Campylobacter jejuni*, is an attractive target for the development of chemotherapeutics. This high-resolution structure may contribute toward the development of its inhibitors.

1. Introduction

The genome sequence of the extremely thermophilic bacterium *Thermus thermophilus* HB8, published by the Structural-Biological Whole Cell Project (www.thermus.org), revealed that over one-third of the 2238 proteins encoded by the genome are function-unknown proteins. TTHA1568 from *T. thermophilus* HB8 is a conserved protein, which consists of 272 amino acid residues with a molecular mass of 30 kDa. TTHA1568 homologs are present within many prokaryotes, including pathogenic species. For example, the TTHA1568 protein shares 44%, 41%, 25% and 23% identities with the conserved hypothetical proteins SCO4326 from *Streptomyces coelicolor* A3(2) (Bentley et al., 2002), AF1704 from *Archaeoglobus fulgidus* DSM 4304 (Klenk et al., 1997), HP0152 from *Helicobacter pylori* 26695 (Tomb et al., 1997), and CJ1674 from *Campylobacter jejuni* NCTC 11168 (Parkhill et al., 2000), respectively (Fig. 1A). TTHA1568 belongs to the DUF191 family of proteins of unknown function in the Pfam database (PF02642) (Finn et al., 2008). The members of the DUF191 protein family are conserved among 82 bacteria and 16 archaea.

Recently, SCO4326 from *S. coelicolor* A3(2), which is an ortholog of TTHA1568, was identified as MqnD, an enzyme within the alternative menaquinone biosynthetic pathway (Hiratsuka et al., 2008). SCO4326 catalyzes the conversion of cyclic de-hypoxanthine futalosine into 1,4-dihydroxy-6-naphthoate (Fig. 1B). It was confirmed that TTHA1568 also catalyzes this reaction (Hiratsuka et al., 2008).

To analyze the structural details of TTHA1568, MqnD from *T. thermophilus* HB8 (*Tth*MqnD), we determined the crystal structure of *Tth*MqnD at 1.55 Å resolution by the multiwavelength anomalous dispersion (MAD) method (Hendrickson, 1991).

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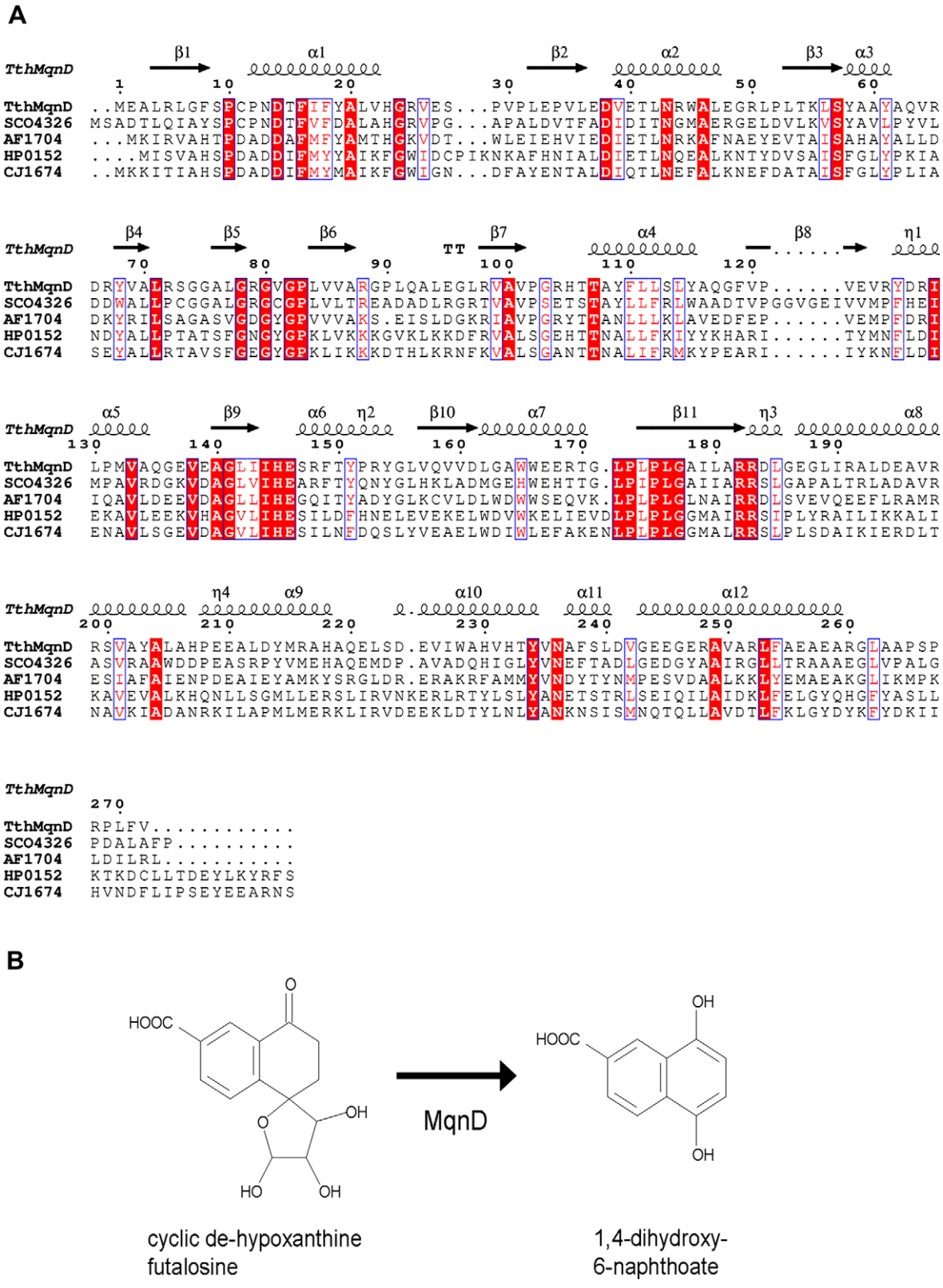

Fig. 1. (A) Sequence alignment of homologs of *Tth*MqnD (TTHA1568). The alignment was generated by ESPript (Gouet et al., 1999) with Clustal W2 (Larkin et al., 2007). The secondary structures of *Tth*MqnD, as determined by DSSP (Kabsch and Sander, 1983), are shown above the sequences (α, α-helix; β, β-strand; η, 3_{10}-helix; TT, β-turn). (B) The enzymatic reaction scheme of MqnD, a menaquinone biosynthetic enzyme (Hiratsuka et al., 2008). All chemical structural formulas were drawn using Symyx Draw (Symyx Technologies).

2. Protein expression, purification and crystallization

The gene encoding full-length *Tth*MqnD (TTHA1568) was cloned into the plasmid vector pET11a (Novagen, Merck). The *Tth*MqnD protein was expressed in *Escherichia coli* BL21(DE3) cultured in LB medium containing 100 μg/ml ampicillin at 37 °C, by induction with 0.5 mM IPTG (at OD_{600} = 0.6–0.8) for 3 h. The *E. coli* lysate was heated at 70 °C for 30 min, and the proteins were