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Crystal structure of thermostable NAD(P)H-dependent carbonyl reductase from the hyperthermophilic archaeon *Aeropyrum pernix* K1

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1 Introduction

A gene encoding NAD(P)H-dependent carbonyl reductase (CR) from the hyperthermophilic archaeon *Aeropyrum pernix* K1 was overexpressed in *Escherichia coli*. The expressed enzyme was the most thermostable CR found to date. *A. pernix* CR catalyzed the reduction of various carbonyl compounds including ethyl 4-chloro-3-oxobutanoate and 9,10-phenanthrenequinone, similar to the thyroidectomised (Tx) CR from chicken fatty liver [1, 2]. However, *A. pernix* CR exhibited significantly higher K_m values against several substrates than Tx chicken fatty liver CR. The three-dimensional structure of *A. pernix* CR was determined at a resolution of 2.09 Å. The overall fold of *A. pernix* CR showed moderate similarity to that of Tx chicken fatty liver CR; however, *A. pernix* CR had no active-site lid unlike Tx chicken fatty liver CR. Consequently, the active-site cavity in the *A. pernix* CR was much more solvent-accessible than that in Tx chicken fatty liver CR. This structural feature may be responsible for the enzyme's lower affinity for several substrates and NADPH. The factors contributing to the much higher thermostability of *A. pernix* CR were also analyzed by comparing its structure with that of Tx chicken fatty liver CR. This is the first report regarding the characteristics and three-dimensional structure of hyperthermophilic archaeal CR.

2 Experiment

Single-wavelength (1.0 Å) data for *A. pernix* CR was collected on the beamline AR-NE3A at the Photon Factory. The data were processed using HKL2000 and the CCP4 program suite.

3 Results and Discussion

The structure of the NADPH-bound *A. pernix* CR was determined using molecular replacement (MR) method and was refined at a resolution of 2.09 Å to a crystallographic *R*-factor of 22.9% and a free *R*-factor of 24.2% [3]. The asymmetric unit consisted of one dimer with a solvent content of 64.5%, which corresponds to a Matthew's coefficient of 3.47 $Å^3Da^{-1}$. The final structure showed good geometry with no Ramachandran outliers, and consisted of 477 amino acid residues, two NADPH molecules and 244 water molecules (Fig. 1, 2). The atomic coordinates and structure factor (code 5B1Y) have been deposited to the Protein Data Bank (PDB).

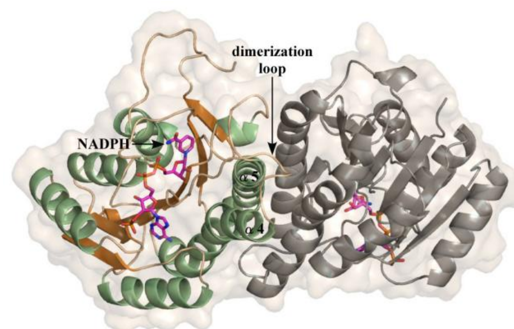

Fig.1 The dimeric structure of *A. pernix* CR. The α-helices and β-strands are shown in green and orange, respectively. The adjacent subunit is shown in gray. The dimerization loop is involved between the two subunits. NADPH molecule (magenta) is shown as a stick model. Oxygen and nitrogen atoms are shown in red and blue, respectively.

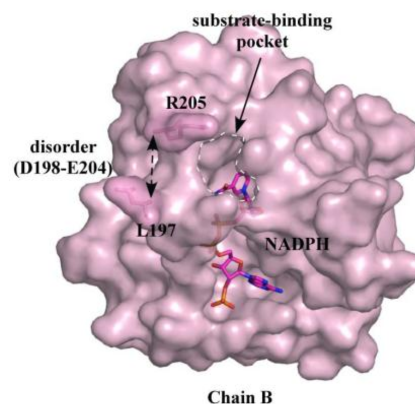

Fig.2 The substrate binding cavity structure of *A. pernix* CR. The structure of *A. pernix* CR is shown in pink. NADPH (magenta) and the disorder region (L197 and R205) are shown as stick models. The substrate-binding pocket structure of *A. pernix* CR is indicated. Atoms are colored as described for Fig. 1.

References

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[3] Y. Fukuda *et al*., *Enzyme Microb. Technol.* (2016) **91**, 17-25.

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