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Crystallization and preliminary X-ray diffraction analysis of glutamate dehydrogenase from an aerobic hyperthermophilic archaeon, *Aeropyrum pernix* K1

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Glutamate dehydrogenase from an aerobic hyperthermophilic archaeon, *Aeropyrum pernix* K1, was crystallized by the hanging-drop vapour-diffusion method using polyethylene glycol (PEG) 400 as the precipitant. The crystals belong to the hexagonal space group $P6_3$, with unit-cell parameters $a = b = 98.9$, $c = 394.8$ Å, $\alpha = \beta = 90$, $\gamma = 120^\circ$. The asymmetric unit contained one hexamer of the enzyme, giving a crystal volume per enzyme mass (V_M) of 1.98 Å^3 Da^{-1} and a solvent content of 37.3%. The X-ray diffraction data were collected to a resolution of 3.0 Å at the BL6B beamline in the Photon Factory with an overall R_{sym} of 13.8% and a completeness of 87.1%.

1. Introduction

More than 60 strains of physiologically different hyperthermophiles have been isolated from high-temperature environments in the last two decades (Hutchins *et al.*, 2001). Most of the hyperthermophiles growing at a temperature around the boiling point of water are known to be anaerobic organisms and obtain energy by fermentation or with a non-oxygenic respiratory system. *A. pernix* K1 isolated from a coastal solfataric vent is a unique hyperthermophilic archaeon that grows heterotrophically at the optimum temperature of 363–368 K under absolutely aerobic conditions (Sako *et al.*, 1996). This is the only strictly aerobic organism growing optimally at a temperature above 363 K. Thus, the energy metabolism, synthesis and metabolism of the cell components, and properties of the enzymes of *A. pernix* K1 are expected to be different from many other anaerobic hyperthermophiles such as *Pyrococcus furiosus* and *Thermococcus litoralis*.

We have already investigated the structure and function of glutamate dehydrogenases (GluDHs) from marine and continental hyperthermophilic archaea (Ohshima & Nishida, 1993, 1994; Kujo & Ohshima, 1998; Kujo *et al.*, 1999; Bhuiya *et al.*, 2000). Recently, we purified an NADP-dependent GluDH from *A. pernix* K1 and cloned the gene encoding the enzyme (Bhuiya *et al.*, 2000). We compared the primary structure of the enzyme with those of the other GluDHs from hyperthermophilic archaea and performed a phylogenetic analysis. As a result, it was revealed that the GluDH from *A. pernix* K1 was clustered with those from aerobic thermophiles (*Sulfolobus solfataricus*, *S. shibatae*) and the anaerobic hyperthermophile *Pyrobaculum islandicum* and was separated from another cluster of the enzymes from *Thermococcales* such as *Pyrococcus* and *Thermococcus* (Bhuiya *et al.*, 2000). The two clusters clearly reflected the difference between Crenarchaeota (the former cluster) and Euryarchaeota (the latter), the two kingdoms of Archaea. The presence of the citric acid cycle has been demonstrated in *Sulfolobus* (Danson, 1988), *Pb. islandicum* (Selig & Schönheit, 1994) and also in *A. pernix* K1 (Kawarabayasi *et al.*, 1999). However, it has not yet been reported in cells of the members of the *Thermococcales* and the principal function of GluDHs from the *Thermococcales* has been suggested to be L-glutamate biosynthesis coupled with L-alanine production (Ohshima & Nishida, 1993; Kengen & Stams, 1994; Kobayashi *et al.*, 1995). Thus, we have predicted that the physiological role of GluDHs from *Sulfolobus*, *Pb. islandicum* and *A. pernix* K1 is distinct from that of the *Thermococcales* enzymes and may be linked to the citric acid cycle *via* 2-oxoglutarate in the cells of these organisms (Bhuiya *et al.*, 2000).

Recently, we demonstrated that the temperature dependence of the kinetic parameters for the GluDH from *A. pernix* K1 is remarkably different from that for the enzymes from *Thermococcales* (Bhuiya *et al.*, 2002). The K_m values for L-glutamate of the GluDHs from *P. furiosus* and isolate AN1 determined at around 323 K are low compared with those determined at higher temperatures (Robb *et al.*, 1992; Hudson *et al.*, 1993). For *A. pernix* K1 GluDH, K_m decreased and the catalytic efficiency markedly increased with an increase in temperature from 323–363 K (Bhuiya *et al.*, 2002). This suggests that the conformational state that is responsible for the kinetic properties of the enzyme is different from that of the *Thermococcales* enzyme. The three-dimensional structures of the GluDHs from *Thermococcales* in Euryarchaeota have

already been determined, *i.e.* GluDHs from *P. furiosus* (Yip *et al.*, 1995), *T. litoralis* (Britton *et al.*, 1999) and *T. profundus* (Nakasako *et al.*, 2001). However, they have not been determined for the enzymes from either the species in the Crenarchaeota or the aerobic hyperthermophiles. The *A. pernix* K1 GluDH showed sequence identities of 51.1, 49.4 and 48.6% to GluDHs from *T. profundus*, *T. litoralis* and *P. furiosus*, respectively (Bhuiya *et al.*, 2000). Comparison of the structure of the *A. pernix* K1 GluDH with those of the *Thermococcales* enzymes may help to elucidate the relationship between the physiological function and the differences in the conformational state of GluDHs from hyperthermophilic archaea. Therefore, we decided to determine the three-dimensional structure of *A. pernix* K1 GluDH. We now describe the crystallization and preliminary X-ray diffraction analysis of GluDH from the aerobic hyperthermophilic archaeon *A. pernix* K1.

2. Materials and methods

2.1. Crystallization

GluDH was purified from the crude extract of *A. pernix* K1 (JCM 9820) as described previously (Bhuiya *et al.*, 2000). The purified enzyme was dialyzed against 10 m*M* MOPS–NaOH pH 7.0 and concentrated to 10 mg ml^{-1} for the crystallization trials. The initial screening for crystallization of the GluDH was carried out using Crystal Screens (Hampton Research, USA) by the hanging-drop vapour-diffusion method at room temperature. Crystals were grown in hanging drops consisting of 2 µl of enzyme solution with an equal volume of a reservoir solution consisting of 20%(*w*/*v*) PEG 400, 200 m*M* $CaCl_2$ and 100 m*M* HEPES–KOH pH 7. Crystals of diffraction quality appeared within 24 h and reached maximum dimensions of 0.3 × 0.3 × 0.15 mm within 2–3 d (Fig. 1).

2.2. X-ray measurements and data processing

The crystals were mounted in a capillary and diffraction data were collected using beamline BL6B (λ = 1.00 Å) at the Photon Factory (Tsukuba, Japan) using a Weissenberg camera. The crystal-to-detector distance, oscillation angle per image and coupling constant were set to 57.3 cm, 3° and 1.0, respectively. The crystals diffracted to a resolution limit of 3.0 Å. The data were processed with *DENZO* and *SCALEPACK* from the *HKL* program suite (Otwinowski & Minor, 1997). The space group was determined to be $P6_3$ or $P6_322$ based on systematic absences. $P6_3$ was the most feasible space group judged by the statistics from *SCALEPACK*. A summary of the data statistics is shown in Table 1. A self-rotation function showed a sixfold axis of crystallographic symmetry; however, it did not show twofold or threefold non-crystallographic symmetry. Assuming six subunits of GluDH, each of molecular mass 46 kDa, in the asymmetric unit, the crystal volume per enzyme mass (V_M) and the solvent content were calculated to be 1.98 Å^3 Da^{-1} and 37.3%, respectively. These values are within the frequently observed ranges for protein crystals (Matthews, 1968).

One hexamer occupied an asymmetric unit for GluDH from *T. profundus* (Nakasako *et al.*, 2001) and *T. litoralis* (Britton *et al.*, 1999) and one trimer for *P. furiosus* (Yip *et al.*, 1995). The crystal structure analysis is under way by the molecular-replacement method using the structures of GluDH from *T. profundus* (Nakasako *et al.*, 2001), *T. litoralis* (Britton *et al.*, 1999) and *P. furiosus* (Yip *et al.*, 1995). We have not yet obtained a clear solution, but are now attempting the use of different models and programs. Furthermore, we are also trying to solve the phases using the heavy-atom isomorphous replacement method.

Table 1
Data collection and processing statistics of *A. pernix* K1 GluDH.

Values in parentheses are for the highest resolution shell.

Beam source	KEK-PF BL6B
Detector	Weissenberg camera
Distance (cm)	57.3
Space group	$P6_3$
Unit-cell parameters (Å, °)	$a = b = 98.9$, $c = 394.8$, $\alpha = \beta = 90$, $\gamma = 120$
Max. resolution (Å)	3.0
Unique reflections	37949
Redundancy	5.2
Resolution range (Å)	50–3.0 (3.16–3.0)
Completeness (%)	87.1 (87.9)
$R_{sym}(I)$†	0.138 (0.297)
$I/\sigma(I)$	4.0

† $R_{sym}(I) = \sum_j \sum_i |I_{ij} - \langle I_j \rangle| / \sum_i \sum_j I_{ij}$.

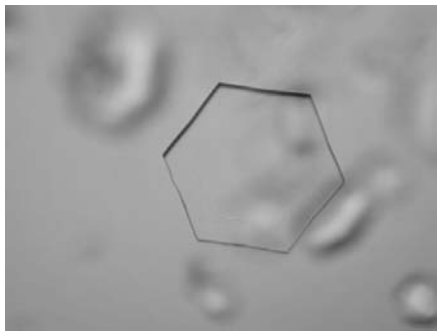

Figure 1
Hexagonal crystal of *A. pernix* K1 GluDH. The dimensions of the largest crystal are 0.3 × 0.3 × 0.15 mm.

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