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Crystallization of the glutamate dehydrogenase from the hyperthermophilic archaeon *Thermococcus litoralis*

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Abstract

The NADP$^+$-dependent glutamate dehydrogenase from *Thermococcus litoralis* has been crystallized by the hanging-drop method of vapour diffusion using an ammonium sulfate and PEG mixture as the precipitant. The crystals belong to the monoclinic system and are in space group $C2$ with unit-cell dimensions $a = 142.7$, $b = 202.0$, $c = 125.8$ Å with $\beta = 113.1°$ with a hexamer in the asymmetric unit. *T. Litoralis*, a hyperthermophilic organism, belongs to the family of Archaea and has a maximum growth temperature of about 370 K. The glutamate dehydrogenase isolated from this organism has a half-life of 2 h at 373 K and a comparison of this structure with that of other GluDH's from hyperthermophilic organisms and from mesophiles will contribute to an understanding of the molecular mechanisms which underlie thermostability.

1. Introduction

Thermococcus litoralis is a hyperthermophilic marine organism with a maximal growth temperature of 370 K and which is involved in the fermentation of peptides rather than carbohydrates (Neuner, Jannasch, Belkin & Stetter, 1990). Like most of the hyperthermophiles isolated to date, *T. litoralis* is a member of the Archaea, and is able to grow only in the presence of elemental sulfur. The organism was originally isolated from shallow submarine solfataras at Lucrino, near Naples, Italy. It is able to grow optimally at 361 K, although its metabolic responses are similar to *Pyrococcus furiosus*, a hyperthermophile growing optimally at 373 K. Little is known about the primary metabolic pathways of these organisms, and it is significant that, like several of the hyperthermophiles examined so far, *T. litoralis* contains extremely high levels of the NADP$^+$-dependent GluDH corresponding to about 5% of the soluble cell protein (Ma, Robb & Adams, 1994). The enzyme has fivefold higher affinity for NADP$^+$ compared with NADPH, and is unable to utilize NAD$^+$ or NADH as cofactors, suggesting that it is a key catabolic enzyme for the utilization of glutamate arising from the degradation of peptides. Like all of the GluDH's characterized from Archaea so far, the enzyme is a hexamer with subunits of $M_r = 45\,000$. The enzyme has a half-life of 2 h at 373 K, and is approximately five times less stable than the homologous enzyme from the hyperthermophile *P. furiosus* (Klump *et al.*, 1992). The comparison of the amino-acid sequences of *P. furiosus* and *Thermococcus litoralis* GluDH's has shown that they have a sequence identity of 87%. One feature of the few residues that are different is the repeated replacement of Val in the *T. litoralis* enzyme by Ile in the *P. furiosus* enzyme which leads to the formation of a tight cluster of five isoleucines at the core of the N-terminal domain of the latter, which may have an effect on their relative thermostabilities, (Britton *et al.*, 1995). Recently the solution of the three-dimensional structure of the *P. furiosus* GluDH (Yip *et al.*, 1995) and the comparison of this structure with that from the mesophile *C. symbiosum* has highlighted a possible role for extended networks of ion pairs in the adaptation of enzymes to extreme temperatures. The structure determination of the *T. litoralis* GluDH will permit a detailed comparison of these molecules to be made in an attempt to correlate their differences in thermostability with the extent of ion-pair formation and packing changes. This should provide deeper insights into the molecular basis of thermostability.

2. Experimental

T. litoralis GluDH was purified as described previously (Ma *et al.*, 1994) and stored at 277 K at a concentration of about 45 mg ml^{-1} in a buffer containing 50 m*M* Tris–HCl pH 7.8, 2 m*M* DTT and 10% glycerol. For the crystallization trials, an aliquot of the protein was dialysed against a buffer containing 50 m*M* HEPES, pH 8. Trials were then conducted using the hanging-drop method of vapour diffusion mixing samples (4 µl) of the protein solution at a concentration of ~35 mg ml^{-1} with a range of precipitants of equal volume and storing the trials at 290 K. Using a mixture of different ammonium sulfate solutions (1.6–1.7 *M*) in 0.1 *M* HEPES at pH 7 or 8 containing 1.5% of PEG 8000, crystals, with rhomboidal morphology, appeared after 2–3 weeks and continued to grow over a further 2–3 weeks to reach dimensions of 0.8 × 0.4 × 0.4 mm. These crystals were extremely sensitive to any changes in the mother liquor, and hence could not be stabilized. For crystallographic analysis they were mounted directly from the drop.

3. Results and discussion

Preliminary X-ray images were recorded on station PX9.5 at the CCLRC Daresbury Laboratory SRS and indicated that the crystals diffract to beyond 3 Å (Fig. 1). Inspection of these oscillation images combined with the use of the cell reduction package *REFIX* (Kabsch, 1988) led us to deduce that the crystals had a monoclinic lattice with cell dimensions of $a = 142.7$, $b = 202.0$ and $c = 125.8$ Å with $\beta = 113.1°$. Analysis of the diffraction pattern showed that reflections with $h + k = 2n + 1$ were systematically absent identifying the space group to be $C2$. Gel-filtration studies of the *T. litoralis* have established that this enzyme is a hexamer (Ma *et al.*, 1994). Given that the cell volume is 3.33×10^6 Å^3 and the molecular weight of a GluDH subunit is (M_r) 45 000, the V_m value is 3.083 Å Da^{-1} assuming that there is a hexamer in the asymmetric unit. This value falls in the range given by

Matthews (1977) whereas the value for a trimer (6.173 Å Da^{-1}) is outside the observed range.

Approximately 90° of data were collected from five crystals at stations PX9.5 and PX7.2 at the CCLRC Daresbury Laboratory SRS to 2.70 Å resolution using the rotation method of data collection and with rotations of either 1.5 or 1.0° per frame. Observations were recorded on a small (PX7.2) and large (PX9.5) MAR Research image plate at X-ray wavelengths of 1.488 and 1.209 Å, respectively, to give 165 993 measurements of which 73 082 were independent. The data set was processed using the *MOSFLM* (Leslie, 1992) and *CCP*4 (Collaborative Computational Project, Number 4, 1994) software packages and gave a merging *R* factor of 7.7% with 83% completeness from 20 to 2.8 Å resolution.

A self-rotation function was calculated with the program *POLARRFN* (*CCP*4; Collaborative Computational Project, Number 4, 1994) using data from 10 and 5 Å resolution with a radius of integration of 30 Å. A peak with 44% of the height of the origin on section $\kappa = 120°$ indicated the presence of a non-crystallographic threefold at $\omega = 85$, $\varphi = 95°$ (Fig. 2). In addition, a series of peaks occur on the $\kappa = 180°$ section close to $\varphi = 0°$ which potentially correspond to non-crystallographic twofolds perpendicular to the threefold axis. These results are consistent with a hexamer with 32 symmetry being aligned in the cell with its molecular threefold axis lying very close to the *b* axis. Subsequently a cross-rotation function was calculated using using a hexameric model of GluDH from *P. furiosus* as a search model (Yip *et al.*, 1995) with a 25 Å radius of integration and this gave a clear solution consistent with the self-rotation function. The search model was rotated and a translation function was calculated using the program *TFFC* (*CCP*4; Collaborative Computational Project, Number 4, 1994) and again a distinct solution with a peak height of 15.5σ was obtained (Fig. 3). Analysis of the packing indicated that there were no poor steric interactions and refinement of this structure is underway. The comparative analysis of the *T. litoralis* GluDH structure with structures of the mesophilic (*C. symbiosum*) and the hyperthermophilic (*P. furiosus*) GluDH's will provide further information about the molecular basis of the adaptation of enzymes to life at extreme temperatures.

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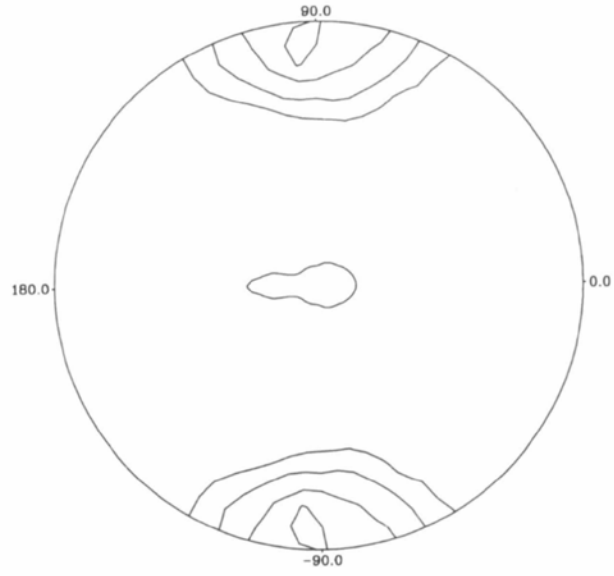

Fig. 2. A stereographic projection of the $\kappa = 120°$ section of the self-rotation function of the *T. litoralis* GluDH native data. The two non-crystallographic threefold axes related by the crystallographic twofold can be seen close to the perimeter of the plot ($\omega = 85°$).

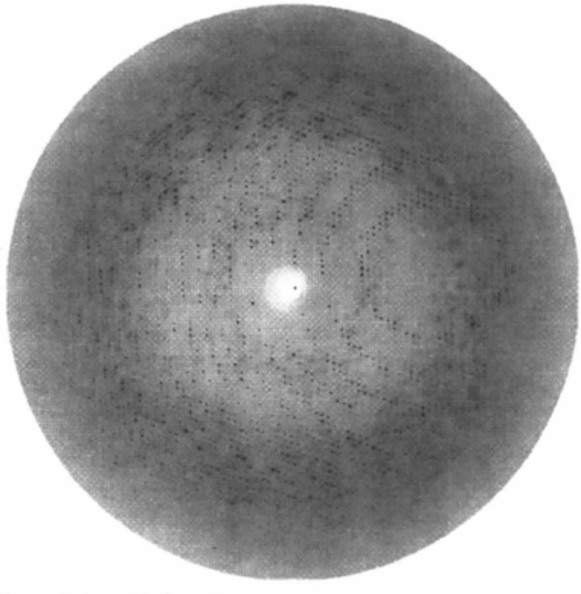

Fig. 1. A 1° oscillation photograph from a crystal of GluDH from *T. litoralis*. This image was taken on a MAR Research image plate on station PX9.5 at the SRS at CCLRC Daresbury Laboratory and is at a resolution of 2.7 Å to the edge of the plate.

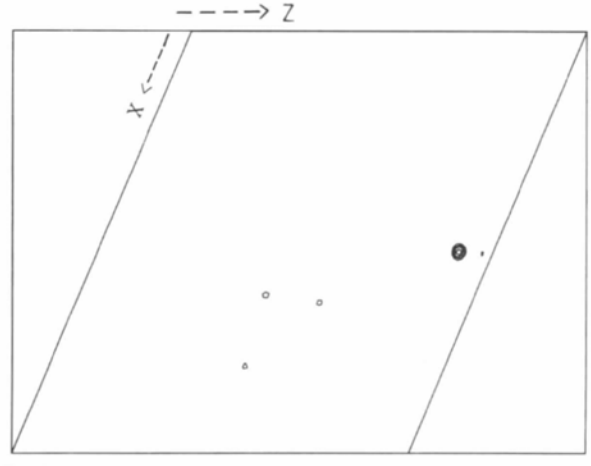

Fig. 3. A section through the translation-function solution for GluDH from *T. litoralis* in space group *C*2 calculated using *TFFC* (Collaborative Computational Project, Number 4, 1994) and using a hexamer of GluDH from P. furiosus as the search model. This section is at $Y = 0$ and covers the range from $X = 0$ to $X = \frac{1}{2}$ and $Z = 0$ to $Z = \frac{1}{2}$ and is contoured from 0.2 arbitrary units at intervals of 0.2.

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