Acta Cryst. (1998). D**54**, 413–415

Crystallization and preliminary X-ray diffraction studies of homoserine dehydrogenase from *Saccharomyces cerevisiae*

BYRON DELABARRE, SUZANNE L. JACQUES, CATHARINE E. PRATT,† DEREK A. RUTH, GERARD D. WRIGHT AND ALBERT M. BERGHUIS* *at Department of Biochemistry, McMaster University, 1200 Main St. West, Hamilton, Ontario, Canada L8N 3Z5.* *E-mail: berghuis@mcmaster.ca*

(*Received 23 July 1997; accepted 9 September 1997*)

Abstract

Recombinant homoserine dehydrogenase from *Saccharomyces cerevisiae* has been crystallized in three different forms. Crystals of the apo-enzyme belong to the tetragonal space group $P4$ and have unit-cell-dimensions $a = b = 130$ and $c = 240$ Å. The resolution limit for these crystals is 3.9 Å. Crystals of homoserine dehydrogenase grown in the presence of the co-factor NAD^+ have the tetragonal space group $P4_12_12$ or its enantiomorph $P4_32_12$. The unit-cell dimensions for these crystals are $a = b = 80.4$ and $c = 250.2$ Å, and the observed resolution limit is 2.2 Å. Protein crystals grown in the presence of the product L-homoserine and the inert NAD^+ analogue 3-aminopyridine adenine dinucleotide belong to the monoclinic space group $P2_1$ with unit-cell parameters $a = 58.8$, $b = 104.2$, $c = 120.7$ Å, $\beta = 91.9°$. This last crystal form has a diffraction limit of 2.7 Å resolution.

1. Introduction

The frequency and severity of fungal infections have dramatically escalated over the last two decades (Sternberg, 1994). While ten years ago Gram negative bacteria were a major source of infectious diseases, in several medical centres this threat has now been replaced by pathogenic fungi (Graybill, 1995). Individuals with compromised immune systems (*e.g.* AIDS patients, individuals undergoing chemotherapy, organ-transplant recipients and burn victims) are especially susceptible to infections (see for example Bodey, 1993). Several factors have been implicated as a reason for the observed rise in fungal infections. In particular, the increase in the number of immuno-compromised individuals (partly caused by the spread of AIDS) and the development of drug resistance in fungi are both considered to have contributed significantly (Iwata, 1992; Sternberg, 1994; DeMuri & Hostetter, 1995; Graybill, 1995). The latter factor of emerging drug resistance in fungi clearly necessitates the development of new treatment methodologies against fungal infections (Sternberg, 1994). This is underscored by reports of fungal strains which are resistant to all commonly available antifungal agents (Wise *et al.*, 1993).

Exploitation of metabolic differences between species has been a successful strategy in the development of antimicrobial agents. One of the important metabolic differences between fungi and humans is amino-acid biosynthesis. For example, unlike humans, fungi are capable of synthesizing the essential amino acids threonine, isoleucine and methionine through the fungal aspartate pathway (Umbarger, 1978). Therefore, specific inhibitors of this pathway may be useful as fungicides. That this hypothesis holds true is illustrated by the antifungal natural product, (*S*)-2-amino-4-oxo-5-hydroxypentanoic acid, which has been shown to specifically inhibit homoserine dehydrogenase, one of the enzymes in the aspartate pathway (Yamaki *et al.*, 1990, 1992). Fungal homoserine dehydrogenase catalyses a critical common step in the pathway, namely the conversion of aspartate semi-aldehyde to L-homoserine, using the co-factor NAD(P)H. The enzyme from *S. cerevisiae* is 359 amino acids and is active as a dimer (Yamaki *et al.*, 1990). Sequence analysis reveals that the enzyme likely possesses a structural motif known as the Rossmann fold which is common to many NAD(P)H binding proteins (Lesk, 1995).

We have initiated protein crystallographic studies of homoserine dehydrogenase from *S. cerevisiae* in order to investigate the structural requirements for inhibition of this enzyme by compounds such as the natural antifungal product (*S*)-2-amino-4-oxo-5-hydroxypentanoic acid. It is hoped that these studies will provide information that can be used in the development of novel antimicrobial agents for the treatment of fungal infections.

2. Experimental procedures

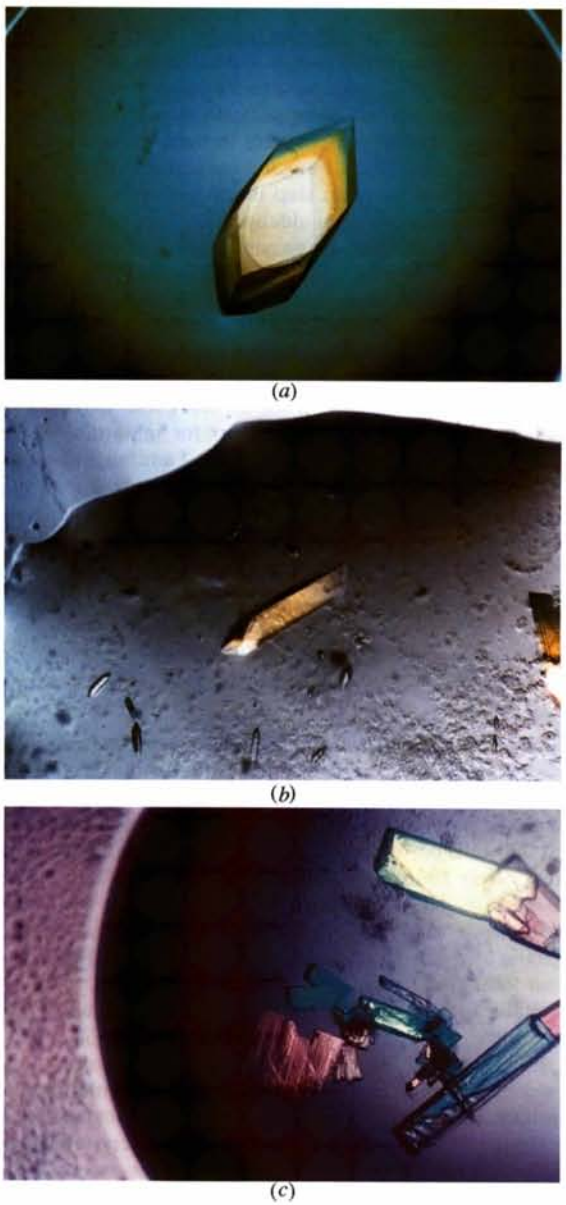
2.1. *Purification and crystallization of homoserine dehydrogenase.*

Homoserine dehydrogenase from *S. cerevisiae* was over-expressed in *Escherichia coli* and purified using procedures described elsewhere (Jacques *et al.*, 1998). Prior to crystallization, the protein was concentrated to an optical density of 9.8 at 280 nm, corresponding to a protein concentration of approximately 10 mg ml^{-1}, in either 10 m*M* HEPES (pH 8.5) or 10 m*M* Tris–HCl buffer (pH 8.5). Homogeneity was tested by Coomassie and silver-stained SDS–PAGE, as well as isoelectric focusing, all of which indicated that the protein was at least 99% pure.

Crystallization experiments were performed using the hanging-drop vapour-diffusion method (McPherson, 1976). The reservoirs contained 1 ml of precipitant solution and the drops contained 5 µl of a 1:1 mixture of protein solution and precipitant. Three different groups of experiments were carried out: crystallization of the apo-enzyme, crystallization of the protein in the presence of fivefold molar excess of the co-factor NAD^+, and crystallization of the protein in the presence of fivefold molar excess of the product L-homoserine and the inert NAD^+ analogue 3-aminopyridine adenine dinucleotide. For the latter co-crystallization experiment, the product of the reaction catalysed by homoserine dehydrogenase, L-homoserine, was used instead of the substrate, aspartate semi-aldehyde, since aspartate semi-aldehyde is an

† Current address: Department of Biochemistry, University of British Columbia, Vancouver, British Columbia, Canada.

unstable compound (Honek, 1997). The NAD^+ analogue used is a competitive inhibitor of HSD with a K_i of 0.22 ± 0.06 m*M* (*cf.* NAD^+ binds to HSD with a K_m of 0.052 ± 0.005 m*M*; Jacques *et al.*, 1997). Initial crystallization conditions were obtained from a sparse-matrix screening of conditions favourable for protein crystal growth (Jancarik & Kim, 1991). Optimal conditions were subsequently determined by varying precipitant and buffer conditions within narrow ranges. Under these conditions, crystals would grow within one to two weeks.



(*a*)

(*b*)

(*c*)

Fig. 1. Three different crystal forms of homoserine dehydrogenase. (*a*) shows a crystal of the apo-enzyme, (*b*) a crystal of the enzyme grown in the presence of NAD^+ and (*c*) a crystal of homoserine dehydrogenase grown in the presence of L-homoserine and an inert analogue of NAD^+. Photographs were taken under polarized light. The scale is approximately 130:1.

Noteworthy in this respect is that the quality of the crystals grown in the presence of NAD^+ was significantly improved by carefully controlling the temperature during crystallization. Without proper temperature control (295 ± 4 K) crystals would only diffract to 3.0 Å resolution. However, by performing the crystallization experiments in a low-temperature incubator (295 ± 0.1 K) the diffraction limit of these crystals could be increased to 2.2 Å resolution.

2.2. *Data collection and processing*

Crystals of the apo-enzyme were mounted in thin-wall glass capillaries with a small amount of mother liquor. Data collection was performed at room temperature. Crystals of homoserine dehydrogenase grown in the presence of product and/or co-factor (analogue) were soaked briefly in a suitable cryo-protectant and then flash frozen in a stream of cold nitrogen. Data collection for these crystals was performed at 95 K.

The X-ray diffraction data was collected with an R-axis IIc imaging system mounted on a rotating copper anode with power settings of 50 kV and 60 mA. The X-ray beam was passed through a nickel filter and a Supper double-mirror focusing system such that the Cu $K\alpha$ radiation was selected. A 0.3 mm collimator was further used to define the beam. Typical oscillation angles used for data collection were 1.0–1.5°. Data processing was carried out with the *DENZO* and *SCALEPACK* data-processing programs (Otwinowski & Minor, 1997). Data manipulations were performed with programs from the *CCP*4 package (Collaborative Computational Project, Number 4, 1994).

3. Results and discussion

As shown in Table 1, crystals of the apo-enzyme diffracted poorly in comparison to those grown in the presence of L-homoserine and/or NAD^+ (analogue). Specifically, crystals grown in the presence of NAD^+ diffracted to 2.2 Å resolution. However, owing to the long cell dimensions along the *c* axis (250.2 Å) and the pixel size of the detector, data collection was complicated and integratable data could only be obtained to a resolution of 2.7 Å. In future, this problem can be circumvented by offsetting the area detector through the use of a 2θ arm.

Analyses of the Matthews coefficients (Matthews, 1968) indicated that one, two or six homoserine dehydrogenase dimers were present in the asymmetric unit, dependent on the crystal form. This implied that non-crystallographic symmetry should be present in our diffraction data. However, no orientations for a non-crystallographic symmetry axis could be identified from the self-rotation function calculated with data collected from the crystals which likely have one dimer in the asymmetric unit, *i.e.* crystals grown in the presence of NAD^+ One possible explanation for this is that the orientation of a symmetry axis relating the two monomers within the putative dimer coincides with a crystallographic symmetry axis. Another possibility is that the molecules are related by a transformation which is mainly translational; however, analysis of the native Patterson maps in the resolution range 40–15 Å was inconclusive. The same observations were made for crystals which likely have two dimers in the asymmetric unit, *i.e.* crystals grown in the presence of L-homoserine and

Table 1. *Crystallization conditions and X-ray diffraction-data statistics for the different crystal forms of homoserine dehydrogenase (HSD)*

Species	HSD	HSD + NAD^+	HSD + L-homoserine + NAD^+ analogue
Crystallization conditions†	2.0 *M* ammonium sulfate 0.1 *M* Tris–HCl (pH 8.5)	2.2 *M* ammonium sulfate 0.1 *M* sodium acetate (pH 4.4)	15–20% PEG 8000 0.1 *M* sodium cacodylate (pH 6.5)
Cryo-protectant	—	2.5 *M* ammonium sulfate 0.1 *M* sodium acetate (pH 4.4) 100% sat. glucose	30% PEG 8000 0.1 *M* sodium cacodylate (pH 6.5)
Space group‡	$P4_1$	$P4_12_12/P4_32_12$	$P2_1$
Unit-cell parameters (Å,°)	$a = b = 130$, $c = 240$	$a = b = 80.4$, $c = 250.2$	$a = 58.8$, $b = 104.2$, $c = 120.7$, $\beta = 91.9$
Molecules per asymmetric unit§	12	2	4
Diffraction limit (Å)	3.9	2.2	2.7
R_{sym}¶ [range (Å)]	—	0.049 [40–2.7]	0.081 [40–2.7]

† The hanging-drop method was used. The conditions of the reservoir solutions are shown. ‡ Based upon symmetry and systematic absences observed in the reduced data. The apo-enzyme space-group assignment was based upon data from a single oscillation frame. § Number of molecules was determined by comparing the Matthews coefficient to typical values for protein crysals. ¶ R_{sym} value is based on intensities. For the apo-enzyme, the complete data set was not collected owing to the limited resolution.

the co-factor analogue. Screening for heavy-atom derivatives is currently in progress.

We thank past and present members of the Berghuis and Wright groups for their assistance. This work was supported by a grant from the Medical Research Council of Canada to AMB (MT 14304) and a strategic grant from the Natural Sciences and Engineering Research Council of Canada to GDW.

References

Bodey, G. P. (1993). *Leuk. Lymphoma*, **11**, Suppl. 2, 127–135.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
DeMuri, G. P. & Hostetter, M. K. (1995). *Pediatr. Clin. North Am.* **4285**, 665–685.
Graybill, J. R. (1995). *Infect. Dis. Clin. North Am.* **9**, 297–311.
Honek, J. F. (1997). Personal communication.
Iwata, K. (1992). *Eur. J. Epidemiol.* **8**, 407–421.
Jacques, S., Pratt, C. E., Kinach, R., Honek, J. F. & Wright, G. D. (1998). *Protein Sci.* Submitted.
Jancarik, J. & Kim, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.
Lesk, A. M. (1995). *Curr. Opin. Struct. Biol.* **5**, 775–883.
McPherson, A. (1976). *Methods Biochem. Anal.* **23**, 249–345.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Sternberg, S. (1994). *Science*, **266**, 1632–1634.
Umbarger, H. (1978). *Annu. Rev. Biochem.* **47**, 532–606.
Wise, K. A., Speed, B. R., Ellis, D. H. & Andrew, J. H. (1993). *Pathology*, **25**, 187–189.
Yamaki, H., Yamaguchi, M., Imamura, H., Suzuki, H., Nishimura, T., Saito, H. & Yamaguchi, H. (1990). *Biochem. Biophys. Res. Commun.* **168**, 837–843.
Yamaki, H., Yamguchi, M., Tsuruo, T. & Yamaguchi, H. (1992). *J. Antibiot. (Tokyo)*, **45**, 750–755.