CRYSTALLIZATION OF *E. COLI* ASPARTOKINASE I – HOMOSERINE DEHYDROGENASE I

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1. Introduction

E. coli aspartokinase I –homoserine dehydrogenase I* is a bifunctional and regulatory protein involved in the biosynthesis of L-threonine from aspartic acid [1]. It is composed of four identical polypeptide chains of a mol. wt. of about 86 000 [2], each chain carrying a kinase active site on its amino-terminal half and a dehydrogenase active site on its carboxy-terminal half [3]. A model has been proposed for the arrangement of the chains in the tetrameric molecule [4].

We wish to report the crystallization of the protein in the presence of two of its ligands, L-threonine, which is an allosteric inhibitor of both catalytic functions, and NADPH, a substrate of the dehydrogenase activity.

2. Methods and results

A sample of purified enzyme [5] was kindly given to us by P. Cossard and L. Sibilli of this laboratory, as the ammonium sulfate precipitate. About 10 mg of protein were centrifuged, resuspended in 0.5 ml of 1.0 M ammonium sulfate, 1 mM L-threonine, 1 mM dithiothreitol (pH adjusted to 5.7), and dialyzed overnight at 4°C against the same buffer. The sample was then transferred to dialysis cells (25 μl) which were sealed with dialysis tubing and placed in glass vials containing 2 ml of 1.2 M ammonium sulfate, L-threonine and dithiothreitol as above, plus 0.5 mM NADPH (final pH 5.7). Alternatively, 100–200 μl of protein solution were placed in 2 ml plastic dishes, NADPH and 2 M ammonium sulfate were added to give the same final concentrations (0.5 mM and 1.2 M), and the dishes were sealed with microscope cover-slides and immersion oil. In either case crystals were found to grow within three to five days at 4°C. No crystal was obtained when threonine or NADPH was ommitted, but 2 mM NADP appeared to substitute for NADPH. The crystals did not grow at 20°C, and crystals grown at 4°C redissolved when brought to room temperature in their mother liquor, unless the ammonium sulfate concentration was raised.

Two crystal habits were observed under these conditions: rectangular prisms with square or octagonal section, and pyramids or dipyramids with square section. The latter reached 0.8 mm in length and 0.4 mm across (fig. 1), and could be used for diffraction studies. The two types of crystals may or may not be isomorphous; they were sometimes found to grow in the same dish.

Crystals of the pyramidal habit were transferred to 1.4 M ammonium sulfate, dithiothreitol, threonine and NADPH as above, and mounted in quartz capillaries at 20°C. X-ray diffraction patterns were recorded with a Nonius precession camera, using Ni filtered Cu Kα radiation from an Elliott rotating anode generator. Diffraction could only be observed at low resolution (about 8 Å), mainly because of rapid disordering of the crystals in the X-ray beam. Weak patterns were obtained with the beam parallel to the long axis of the crystals: they were indicative of tetragonal symmetry, which was confirmed when the more intense hO1 (fig.

* Aspartokinase: EC 2.7.2.4; homoserine dehydrogenase: EC 1.1.1.3.

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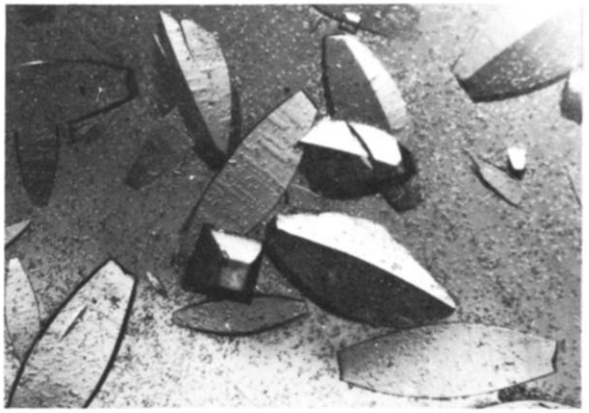

Fig. 1. Crystalline aspartokinase I–homoserine dehydrogenase I. Pyramidal crystals grown in plastic dishes at 4°C.

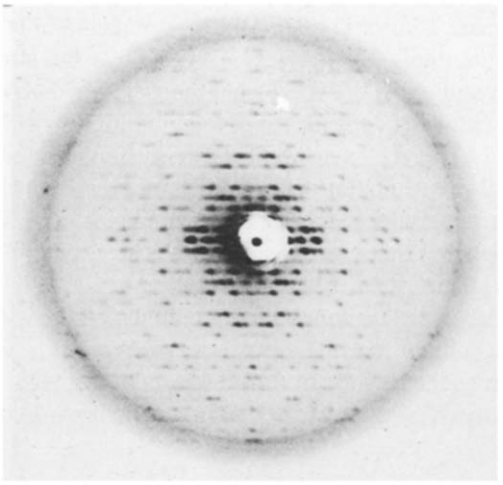

Fig. 2. A 5° precession photograph of the h01 zone. Precession around b*; c* along the spindle. The photograph was taken using a 0.3 mm collimator, and a 7 hr exposure at 40 kV and 50 mA. The crystal to film distance was 100 mm.

2) and Ok1 zones were found to be identical, at least to 12 Å resolution. Symmetry and systematic absences in these zones are compatible with the space group being I422 or $I4_122$. Packing considerations favour $I4_122$.

3. Discussion

The asymmetric unit (1/16 of the body-centered unit cell in either space group) has a volume of 1.0×10^6 Å^3 (table 1), and must therefore contain one tetrameric molecule with a molecular weight of nearly 350 000, occupying 47% of the total volume (from $v = 0.737$ cm^3/g [5]), a value within the usual range [6]. Thus, although the aspartokinase I–homoserine dehydrogenase I molecule is likely to have at least approximate 222 point symmetry [7], this symmetry is not expressed in the crystalline arrangement. Chemical studies of the dehydrogenase active sites do indeed suggest that the four substrate binding sites may not be equivalent [8], preventing therefore the 222 symmetry of the molecule from being exact in the presence of the nicotinamide coenzymes. We may however hope to find crystal forms with smaller asymmetric units in the presence of other ligands of the protein.

Table 1
Unit cell parameters

Space group:	I422 or $I4_122$
Multiplicity:	$Z = 16$
Parameters*:	$a = b = 180.5$ Å, $c = 493$ Å
Volume of asymmetric unit:	1.004×10^6 Å^3

* from precession photographs of the h01 and 0k1 zones.

Acknowledgements

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References

[1] Cohen, G. N. (1969) Curr. Top. Cell. Reg. 1, 183.
[2] Falcoz-Kelly, F., Janin, J., Saari, J. C., Véron, M., Truffa-Bachi, P. and Cohen, G. N. (1972) Eur. J. Biochem. 28, 507.
[3] Véron, M., Falcoz-Kelly, F. and Cohen, G. N. (1972) Eur. J. Biochem., 28, 520.
[4] Véron, M., Saari, J. C., Villar-Palasi, C. and Cohen, G. N. (1973) Eur. J. Biochem., 38, 325.
[5] Truffa-Bachi, P., van Rapenbusch, R., Janin, J., Gros, C. and Cohen, G. N. (1968) Eur. J. Biochem., 5, 73.
[6] Matthews, B. W. (1968) J. Mol. Biol., 33, 491.
[7] Mackall, J. C. and Neet, K. E. (1974) Eur. J. Biochem., 42, 275.
[8] Hirth, C. G., Véron, M., Villar-Palasi, C., Hurion, N. and Cohen, G. N. (1974) Eur. J. Biochem., in press.