Acta Cryst. (1995). D51, 245–247

Crystallization and preliminary X-ray diffraction studies of *Escherichia coli* glyceraldehyde-3-phosphate dehydrogenase.

By LAURENCE OLIVIER, GEORGES BUISSON and ERIC FANCHON, *Laboratoire de Cristallographie Macromoléculaire, Institut de Biologie Structurale (CEA-CNRS), Avenue des Martyrs* 41, 38027 *Grenoble CEDEX* 1, *France*, CATHERINE CORBIER and GUY BRANLANT, *Laboratoire d'Enzymologie et de Génie Génétique, Université de Nancy* I (*URA-CNRS*) 457, *BP* 239, 54506 *Vanduvre-lès-Nancy CEDEX, France* and OTTO DIDEBERG,* *Laboratoire de Cristallographie Macromoléculaire, Institut de Biologie Structurale (CEA-CNRS), Avenue des Martyrs* 41, 38027 *Grenoble Cedex* 1, *France*

(*Received* 20 *April* 1994; *accepted* 14 *October* 1994)

Abstract

Phosphorylating glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a key enzyme in glycolysis. Single crystals of NAD-dependent GAPDH from *Escherichia coli* have been obtained by vapour diffusion at room temperature using trisodium citrate as precipitant. In almost the same crystallization conditions, two kinds of crystals were found to be suitable for X-ray diffraction. The crystals with only one half of a tetramer in the asymmetric unit were chosen for high-resolution analysis. They belonged to space group $C222_1$, with cell dimensions a = 79.1, b = 189.6 and c = 122.2 Å. These crystals diffracted to 1.8 Å resolution.

Introduction

Phosphorylating glyceraldehyde-3-phosphate dehydrogenase (GAPDH) catalyzes the reversible oxidation of D-glyceraldehyde-3-phosphate into 1,3-diphosphoglycerate. Two types are known. The first (E.C. 1.2.1.12) is a key enzyme of the glycolytic pathway which is either strictly NAD-dependent as described for eubacteria and eukaryotes (Harris & Waters, 1976) or dual coenzyme NAD(P)-dependent as observed in archaebacteria (Fabry, Lehmacher, Bode & Hensel, 1988). The second (E.C. 1.2.1.13), closely related to the glycolytic type, is involved in the Calvin cycle and exhibits a dual-coenzyme specificity (Ferri, Comerio, Iadarola, Zapponi & Speranza, 1978). Apart from its essential role in the glycolytic pathway, various other cellular roles have been recently attributed to the strictly NAD-dependent GAPDH (Forthergill-Gilmore & Michels, 1992).

Glycolytic GAPDH's have four identical subunits with M_r about 36 000 per subunit. Currently, more than 60 amino-acid sequences of GAPDH's of eubacteria and eukaryotic origin are listed in the Swiss Protein Database. These all have high sequence similarity with, for example, 65% identity between *E. coli* and human GAPDH's.

X-ray structures of the enzymes isolated from lobster muscle (Rossmann, Ford, Watson & Banaszak, 1972; Moras *et al.*, 1975; Murthy, Garavito, Johnson & Rossmann, 1980), from human muscle (Watson, Duée & Mercer, 1972), from *Bacillus stearothermophilus* (Biesecker, Harris, Thierry, Walker & Wonacott, 1977; Skarzynski, Moody & Wonacott, 1987), from *Trypanosoma brucei* (Vellieux *et al.*, 1993) and from *Palinurus versicolor* (Lin *et al.*, 1993) have been determined. In all these structures, the tetramer has a 222 symmetry, the twofold axes are labeled *P*, *Q* and *R* (Buehner, Ford, Moras, Olsen & Rossmann, 1974). Despite the knowledge, at atomic resolution, of some enzyme structures of this family more information for the *E. coli* protein is needed at the molecular level.

We report here the crystallization of the *E. coli* NAD-dependent GAPDH derived from the *gap* A gene (Branlant & Branlant, 1985; Alefounder & Perham, 1989). Knowledge of the three-dimensional structure will allow us to study, using site-directed mutagenesis, structure–function relationships. A series of mutants affecting amino acids directly related to catalysis or involved in substrate or coenzyme binding have been constructed and characterized (Soukri *et al.*, 1989; Corbier *et al.*, 1990; Corbier, Della Seta & Branlant, 1992, Corbier, Michels, Wonacott & Branlant, 1994). Futhermore, a comparison of the *E. coli* GAPDH structure with the well refined structure of the *B. stearothermophilus* GAPDH will help to delineate more precisely the conserved and variable parts of the structure.

Experimental

Purification and crystallization

The wild-type enzyme was genetically overexpressed in *E. coli* and purified by the method described by Mougin *et al.* (1988) except that purification was carried out by FPLC using a Q-sepharose resin. The enzyme was homogeneous when examined by polyacrylamide gel electrophoresis under denaturating conditions. Protein solutions (10 mg ml^{-1}) were prepared in 10 m*M* Tris–HCl (pH = 7.5) containing 1 m*M* dithiothreitol (DTT) and 0.1 m*M* azide.

The experimental conditions to produce large crystals suitable for X-ray analysis were as follows: 3 µl of GAPDH solution were mixed in a droplet with an equal volume of a reservoir solution containing for the first form: 1.45 *M* trisodium citrate, 1 m*M* EDTA, 1 m*M* DTT, 0.1 m*M* azide, 0.3 m*M* NAD, 100 m*M* Tris–HCl (pH = 7.5), and for the second form: 1.35 *M* trisodium citrate, 1 m*M* EDTA, 1 m*M* DTT, 0.1 m*M* azide, 0.3 m*M* NAD and 100 m*M* HEPES (pH = 7.5). After one month, both types of crystals grew to a size of 1.0 × 0.7 × 0.5 mm.

X-ray diffraction pattern

Crystals were mounted and sealed in capillary tubes. Unit-cell parameters and space groups were determined using a precession camera. Crystal I belongs to the orthorhombic space group $P2_12_12$ with a = 133.7, b = 154.1 and c = 156.1 Å. Comparing this unit-cell volume to that of holoenzyme crystals from *B. stearothermophilus* (Skarzynsky *et al.*, 1987) confirms that the *E. coli* unit cell contains 32 monomers of 35.5 kDa. Since the $P2_12_12$ space group has four equivalent positions, this

* Author to whom all correspondence should be sent.

crystal form contains two complete tetramers per asymmetric unit. The solvent content is 41%. The packing density is V_m = 2.8 Å^3 Da^{-1}, in good agreement with V_m values known for other proteins (Matthews, 1968). The space group of crystal II is also orthorhombic, $C222_1$, with a = 79.1, b = 189.6 and c = 122.2 Å. With 16 monomers per unit cell and eight equivalent positions, each tetramer must sit on a crystallographic twofold axis; therefore the asymmetric unit contains one dimer and the molecule has at least one exact twofold axis of symmetry. The V_m value is 3.2 Å^3 Da^{-1} and the solvent fraction is 48%.

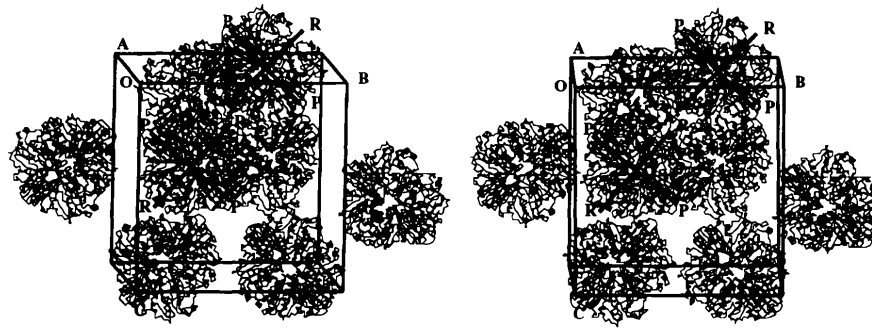
X-ray diffraction data were collected for both crystals on the FAST Enraf–Nonius area detector using a rotating-anode generator. For crystal I, space group $P2_12_12$, a total of 38 329 diffraction intensities were collected to 4.0 Å, giving 21 181 unique reflections after the program *PROCOR* of *MADNES* (Messerschmidt & Pflugrath, 1987; Kabsch, 1988). This represents nearly 82% of the complete data with an agreement factor R_{sym} = 5% on equivalent intensities. For crystal II, space group $C222_1$, a total of 60 338 diffraction intensities was processed. They were merged into 18 294 unique reflections, the R_{sym} value was 3% and the data set was 86% complete to 2.9 Å.

Results and discussion

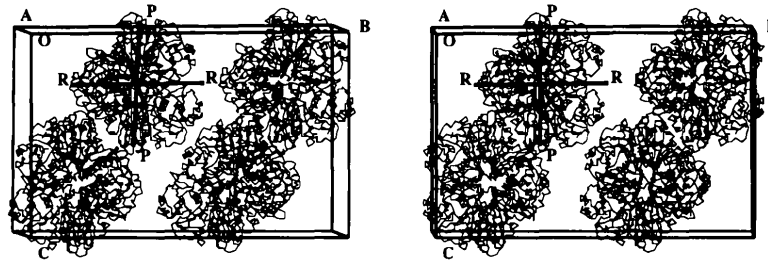
The three-dimensional structure of *B. stearothermophilus* GAPDH (Skarzynsky *et al.*, 1987), from which solvent and NAD molecules were eliminated, was used as a model for molecular-replacement methods.

Classical programs failed to find a solution for crystal I because of the presence of eight monomers per asymmetric unit. Eventually, a discernible molecular-replacement solution for both rotation and translation functions was found with the help of the program *AMoRe* written by Navaza (1994). A cross-rotation-function map was calculated using the program *ROTTING* (Navaza, 1987, 1994). Data were included between 15.0 and 4.0 Å with a radius of integration of 53.3 Å. Only three peaks above half the maximum value of the function were obtained. The orientation corresponding to the first peak of the *ROTTING* map was used in the calculation of the translation function. The map was poorly contrasted. The first tetramer was positioned according to the first peak and a translation function was calculated for each of the three peaks of the cross-rotation function. In this way, the two orientations could be identified by analysis of correlation coefficients between observed and calculated structure factors. The correlation coefficient was 42.2% for the correct solution but as low as 30.5% for incorrect ones. The parameters were optimized by the program *FITTING* (Castellano, Oliva & Navaza, 1992); the *R* factor dropped from 49.7 to 46.5% and the correlation coefficient increased from 42.2 to 49.5% for 21 181 reflections between 15.0 and 4.0 Å. Comparison of the rotation matrices for the two tetramers indicated a very similar orientation for both tetramers. The angles between equivalent axis are 11, 10 and 5° for *P*, *Q* and *R*, respectively. This observation might explain why we failed to find the molecular-replacement solution with other programs.

The structure of crystal II was solved by molecular-replacement techniques, as implemented in the program package *MERLOT* (Fitzgerald, 1988). Each of the possible dimers (*OP*, *OQ*, *OR*) was used as the search model. Only the *OP* dimer gave significant signals, indicating that the asymmetric unit of the *E. coli* GAPDH crystals contained the *OP* dimer. The orientation of the *OP* dimer in the asymmetric unit was found by performing Crowther's fast-rotation function (Crowther, 1972) on a search grid of 2.5° in α and 5° in β



(*a*)



(*b*)

Fig. 1. The packing of the *E. coli* GAPDH molecules in the two crystal forms as viewed down the *a* axis: (*a*) crystal form I, (*b*) crystal form II.

and γ (where α, β and γ are Euler angles) and using the data between 8.0 and 4.2 Å. The search yielded a first peak with a maximum at 4.63 r.m.s. units above background. The next peak appeared at 89% of the maximum value. The position of the dimer was found by using the translation function of Crowther & Blow (1967). All three sections yielded a consistent solution at fractional coordinates $[u, v, w] = 0, 0.67, 0.5$. The molecular twofold axis *R* coincides with the crystallographic twofold axis *b*. Finally, the rotational and translational parameters were optimized by a rigid-body refinement of the program *X-PLOR* (Brünger, 1992). The *R* value was 43.7% for 1361 reflections between 8.0 and 6.0 Å. The structure refinement is now underway. The packing of GAPDH molecules in both crystal forms is shown in Fig. 1.

In summary, we have obtained well ordered crystals of glyceraldehyde-3-phosphate dehydrogenase from *E. coli*. These crystals offer the opportunity for high-resolution structural analysis of the wild-type and mutated enzymes.

We thank the IMABIO programme for financial support. We are grateful to Dr P. Gouet for much help and advice.

References

ALEFOUNDER, P. R. & PERHAM, R. N. (1989). *Mol. Microbiol.* **3**, 723–732.

BIESECKER, G., HARRIS, J. I., THIERRY, J. C., WALKER, J. E. & WONACOTT, A. J. (1977). *Nature (London)*, **266**, 328–333.

BRANLANT, G. & BRANLANT, C. (1985). *Eur. J. Biochem.* **150**, 61–66.

BRÜNGER, A. T. (1992). *X-PLOR Manual, Version* 3.0, Yale Univ., New Haven, CT, USA.

BUEHNER, M., FORD, G. C., MORAS, D., OLSEN, K. W. & ROSSMANN, M. G. (1974). *J. Mol. Biol.* **82**, 563–585.

CASTELLANO, E. E., OLIVA, G. & NAVAZA, J. (1992). *J. Appl. Cryst.* **25**, 281–284.

CORBIER, C., DELLA SETA, F. & BRANLANT, G. (1992). *Biochemistry*, **31**, 12532–12535.

CORBIER, C., MICHELS, S., WONACOTT, A. & BRANLANT, G. (1994). *Biochemistry*, **33**, 3260–3265.

CORBIER, C., MOUGIN, A., MELY, Y., ADOLPH, H. W., ZEPPEZAUER, M., GERARD, D., WONACOTT, A. & BRANLANT, G. (1990). *Biochimie*, **72**, 545–554.

CROWTHER, R. A. (1972). In *The Molecular Replacement Method*, edited by M. G. ROSSMANN. New York: Gordon and Breach.

CROWTHER, R. A. & BLOW, D. M. (1967). *Acta Cryst.* **23**, 544–548.

FABRY, S., LEHMACHER, A., BODE, W. & HENSEL, R. (1988). *FEBS Lett.* **237**, 213–217.

FERRI, G., COMERIO, G., IADAROLA, P., ZAPPONI, M. C. & SPERANZA, M.L. (1978). *Biochem. Biophys. Acta*, **522**, 19–31.

FITZGERALD, P. M. D. (1988). *J. Appl. Cryst.* **21**, 273–278.

FORTHERGILL-GILMORE, L. A. & MICHELS, P. A. M. (1992). *Prog. Biophys. Mol. Biol.* **59**, 105–123

HARRIS, J. I. & WATERS, M. (1976). In *The Enzymes*, edited by P. D. BOYER, Vol. 13, 3rd ed., pp. 1–49. New York: Academic Press.

KABSCH, W. (1988). *J. Appl. Cryst.* **21**, 916–924.

LIN, Z.-J., LI, J., ZHANG, F.-M., SONG, S.-Y., YANG, J., LIANG, S.-J. & TSOU, C.-L. (1993). *Arch. Biochem. Biophys.* **302**, 161–166.

MATTHEWS, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.

MESSERSCHMIDT, A. & PFLUGRATH, J. W. (1987). *J. Appl. Cryst.* **20**, 306–315.

MORAS, D., OLSEN, K. W., SABESAN, M. N., BUEHNER, M., FORD, G. C. & ROSSMANN, M. G. (1975). *J. Biol. Chem.* **250**, 9137–9162.

MOUGIN, A., CORBIER, C., SOUKRI, A., WONACOTT, A., BRANLANT, C. & BRANLANT, G. (1988). *Protein Eng.* **2**, 45–48.

MURTHY, M. R. N., GARAVITO, R. M., JOHNSON, J. E. & ROSSMANN, M. G. (1980). *J. Mol. Biol.* **138**, 859–872.

NAVAZA, J. (1987). *Acta Cryst.* A**43**, 645–653.

NAVAZA, J. (1994). *Acta Cryst.* A**50**, 157–163.

ROSSMANN, M. G., FORD, G. C., WATSON, H. C. & BANASZAK, L. J. (1972). *J. Mol. Biol.* **64**, 237–249.

SKARZYNSKI, T., MOODY, P. C. E. & WONACOTT, A. J. (1987). *J. Mol. Biol.* **193**, 171–187.

SOUKRI, A., MOUGIN, A., CORBIER, C., WONACOTT, A., BRANLANT, C. & BRANLANT, G. (1989). *Biochemistry*, **28**, 2586–2592.

VELLIEUX, F. M. D., HAJDU, J., VERLINDE, C. L. M. J., GROENDIJK, H., READ, R. J., GREENHOUGH, T. J., CAMPBELL, J. W., KALK, K. H., LITTLECHILD, J. A., WATSON, H. C. & HOL, W. G. J. (1993). *Proc. Natl Acad. Sci. USA*, **90**, 2355–2359.

WATSON, H. C., DUÉE, E. & MERCER, W. D. (1972). *Nature (London)*, **240**, 130–139.