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Crystallization and preliminary X-ray diffraction analysis of NADP-dependent glyceraldehyde-3-phosphate dehydrogenase of *Synechococcus* PCC 7942

Yoshihiro Nakamura,[a]* Toshiji Tada,[a] Kei Wada,[a] Takayoshi Kinoshita,[b] Masahiro Tamoi,[c] Shigeru Shigeoka[c] and Keiichiro Nishimura[a]

[a]Research Institute for Advanced Science and Technology, Osaka Prefecture University, Sakai, Osaka 599-8570, Japan, [b]Exploratory Research Laboratories, Fujisawa Pharmaceutical Co. Ltd, Tsukuba, Ibaraki 300-2698, Japan, and [c]Department of Food and Nutrition, Faculty of Agriculture, Kinki University, Nakamachi, Nara 631-8505, Japan

Correspondence e-mail: ynakam@biochem.osakafu-u.ac.jp

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The NADP-dependent glyceraldehyde-3-phosphate dehydrogenase (GAPDH) of *Synechococcus* PCC 7942 was crystallized in two different forms by the hanging-drop vapour-diffusion method using ammonium sulfate as a precipitant. Form I crystals were hexagonal, space group $P6_5$ or $P6_1$, with unit-cell parameters $a = b = 91.1$, $c = 428.6$ Å, $\gamma = 120^\circ$. Form II crystals were monoclinic, space group $C2$, with unit-cell parameters $a = 152.3$, $b = 80.9$, $c = 213.6$ Å, $\beta = 103.1^\circ$. Native data were collected from a frozen crystal of form I to a resolution of 2.8 Å using synchrotron radiation at SPring-8, whereas form II crystals were easily damaged by radiation at room temperature and increased mosaicity in cryoprotectant solutions. A molecular-replacement solution of the form I crystal was obtained in space group $P6_5$ using the program *AMoRe* and the structure of the NAD-dependent GAPDH from *Bacillus stearothermophilus*.

1. Introduction

Higher plants have two distinct glyceraldehyde-3-phosphate dehydrogenases (GAPDHs): cytosolic NAD-GAPDH (E.C. 1.2.1.12) and chloroplastic NADP-GAPDH (E.C. 1.2.1.13). NAD-GAPDH works in glycolysis/gluconeogenesis only in the presence of a coenzyme, NAD(H). NADP-GAPDH works in the second reaction of the photosynthetic carbon reduction (PCR) cycle after the carbon dioxide fixation reaction to form 3-phosphoglycerate. NADP-GAPDH functions in the presence of either NADP(H) or NAD(H), of which the former is superior to the latter as coenzyme (Wolosiuk & Buchanan, 1976). In chloroplasts of higher plants, NADP-GAPDH exists in the form of either a homotetramer consisting of a subunit GapA or a heterotetramer consisting of two subunits, GapA and GapB (Shih *et al.*, 1991; Scagliarini *et al.*, 1998). The activity of the enzyme is regulated *in vivo* by a system consisting of ferredoxin, thioredoxin *f* and ferredoxin/thioredoxin reductase (Buchanan, 1980; Cséke & Buchanan, 1986). The regulation is caused by Cys residues in the N- and C-termini. This enzyme appears to be activated by light irradiation *in vivo* or by addition of DTT, NADPH or ATP *in vitro* (Wolosiuk & Buchanan, 1976; Wirtz *et al.*, 1982).

Crystal structures have been elucidated for NAD-GAPDHs isolated from various sources, including American and Chinese lobsters (Buehner *et al.*, 1974; Moras *et al.*, 1975; Lin *et al.*, 1993), *Leishmania mexicana* (Kim *et al.*, 1995), *Escherichia coli* (Duée *et al.*, 1996) and *B. stearothermophilus* (Biesecker *et al.*, 1977; Skarzynski *et al.*, 1987). There is only one example of the crystal structure of NADP-GAPDH, from *Methanothermus fervidus* (Charron *et al.*, 2000).

The cyanobacterium *Synechococcus* PCC 7942 (*S.* 7942), however, possesses NADP-GAPDH only in the form of a homotetramer. Since NAD-GAPDH has not been found in *S.* 7942, NADP-GAPDH probably works not only in the PCR cycle but also in glycolysis/gluconeogenesis of the cyanobacterium *in vivo*. This enzyme is not greatly activated by light irradiation *in vivo* (Tamoi *et al.*, 1996, 1998) or by the addition of DTT (Tamoi *et al.*, 1996), NADPH or ATP (Tamoi, unpublished data) *in vitro*. Moreover, the activity is not regulated by a system consisting of ferredoxin, thioredoxin *f* and ferredoxin/thioredoxin reductase (Tamoi *et al.*, 1998). Although these characteristics of this enzyme are quite different from those of the chloroplastic NADP-GAPDH, the homology level of the enzyme from *S.* 7942 with GapA and GapB of tobacco chloroplasts is approximately 65–66% (Tamoi *et al.*, 1996). Differences in their primary structures are mostly at neighbours of the N- and C-termini. In particular, the Cys residues which are found neighbouring the C-terminus and are involved in the light/dark regulation of the chloroplastic enzyme are absent in GAPDH from *S.* 7942 (Tamoi *et al.*, 1996).

The aim of the present study was to clarify the molecular mechanism of these novel properties of NADP-GAPDH from *S.* 7942, which works not only in the PCR cycle but also

in glycolysis/gluconeogenesis *in vivo*, by analyzing its crystal structure. A detailed study of the three-dimensional structure of the enzyme by comparing it with those reported for NAD-GAPDHs and NADP-GAPDH should provide some information about the recognition mechanism for the two coenzymes NADP and NAD. Furthermore, the structural analysis would provide some more information about the activation mechanism brought about by light irradiation and by the addition of NADPH and ATP. Here, we report the crystallization and preliminary X-ray analysis of the NADP-GAPDH originating from *S.* 7942.

2. Materials and methods

2.1. Crystallization

The NADP-GAPDH of *S.* 7942 was overexpressed from *E. coli* BL21(DE3)-pLysS and isolated as reported previously (Tamoi *et al.*, 1996). The purified protein solution was concentrated and ultrafiltrated by use of an Ultrafree (Millipore) filter. The protein solution used for preliminary crystallization was at 5 mg ml^{-1} in 50 m*M* HEPES buffer pH 7.5 containing 30 m*M* NaCl. Crystallization trials were carried out using the hanging-drop vapour-diffusion method. After mixing 1 µl of protein solution with 1 µl of various reservoir solutions, the drops were equilibrated over the respective reservoirs at 293 or 277 K. Initial trials were performed using the commercially available sparse-matrix screening kits Crystal Screen I and II from Hampton Research (Jancarik & Kim, 1991). The initial conditions found to be the best were optimized by varying the concentrations of the protein, precipitants and buffer systems.

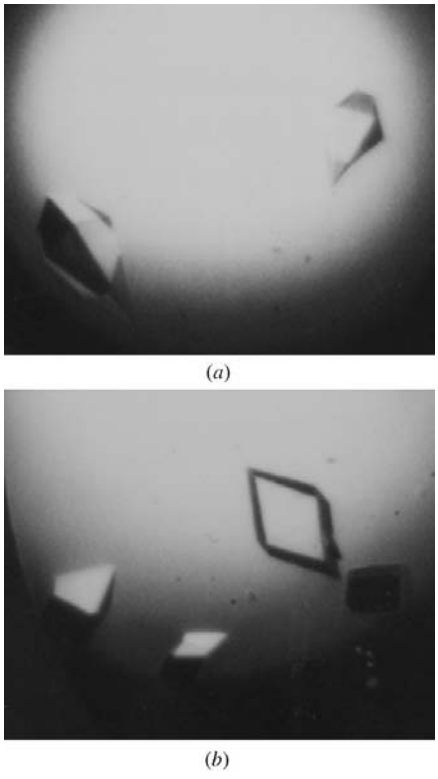

Figure 1
Typical crystals of the NADP-GAPDH of *Synechococcus* PCC 7942. The dimensions of the form I (*a*) and II (*b*) crystals were approximately 0.4 × 0.2 × 0.2 mm and 0.3 × 0.3 × 0.15 mm, respectively.

2.2. Data collection and analysis

Preliminary X-ray diffraction studies were carried out using an R-AXIS IIc image-plate area detector mounted on a Rigaku RU-300 rotating-anode source operating at 40 kV, 100 mA with Cu $K\alpha$ radiation. A complete data set was collected on an R-AXIS IV^{++} image-plate detector using synchrotron radiation of 1.0 Å wavelength at the BL40B2 station of SPring-8, Japan. Data collection was carried out at 100 K with a crystal-to-detector distance of 420 mm, an oscillation range of 1° per image and an exposure time of 90 s per image. The data set was processed using the program *MOSFLM* (Steller *et al.*, 1997) and scaled using the program *SCALA* from the *CCP*4 package (Collaborative Computational Project, Number 4, 1994).

3. Results and discussion

In the initial crystallization trials, small crystals were obtained from several drops containing ammonium sulfate. After optimization of the crystallization conditions, the protein solution was set to 3.5 mg ml^{-1} in 50 m*M* HEPES buffer pH 7.5 containing 30 m*M* NaCl. By mixing 2 µl of the protein solution with 1 µl of the precipitant solutions, two types of crystals, forms I and II, were obtained. Form I was obtained with 0.1 *M* imidazole–HCl buffer pH 7.3 containing 53% saturation of ammonium sulfate, 0.2 *M* potassium sodium tartrate and 10%(*v*/*v*) glycerol at 277 K. Form II was obtained with 0.1 *M* citrate buffer pH 5.2 containing 40% saturation of ammonium sulfate and 0.2 *M* potassium sodium tartrate at 293 K. Under these two conditions, crystals grew to maximal dimensions of 0.4 × 0.2 × 0.2 mm (form I) and 0.3 × 0.3 × 0.15 mm (form II) in two weeks (Fig. 1).

In the initial X-ray analysis, the form I crystal was determined to be hexagonal, space group $P6_5$ or its enantiomorph $P6_1$, with unit-cell parameters $a = b = 91.1$, $c = 428.6$ Å, $\gamma = 120°$. The V_M values were calculated to be 3.2 Å^3 Da^{-1} for one tetramer in the asymmetric unit and 1.6 Å^3 Da^{-1} for two tetramers, giving solvent contents of about 62 and 31%, respectively. The form II crystal was determined to be monoclinic, space group *C*2, with unit-cell parameters $a = 152.3$, $b = 80.9$, $c = 213.6$ Å, $\beta = 103.1°$. Two tetramers were suggested to be in an asymmetric unit from the V_M value of 2.0 Å^3 Da^{-1}, giving a solvent content of about 39%. These values calculated for these two types of crystals are within the range of values tabulated by Matthews (1968).

Crystals of forms I and II mounted in a glass capillary were easily damaged and only diffracted to about 3.5 Å resolution when exposed to Cu $K\alpha$ radiation at room temperature. To avoid damage, the crystals were loop-mounted in cryoprotectant solutions and frozen at 100 K. The growth solution for form I crystals was suitable as a cryoprotectant solution. The crystals diffracted to at least 2.8 Å resolution at SPring-8. A total of 299 698 observed reflections were scaled and reduced to yield a data set containing 41 013 unique reflections with an R_{merge} of 5.5%. The data set was 83.4% complete to 2.8 Å resolution, with data in the 3.0–2.8 Å resolution shell being 71.9% complete. The average $I/\sigma(I)$ value was 6.8 in this shell. A typical X-ray

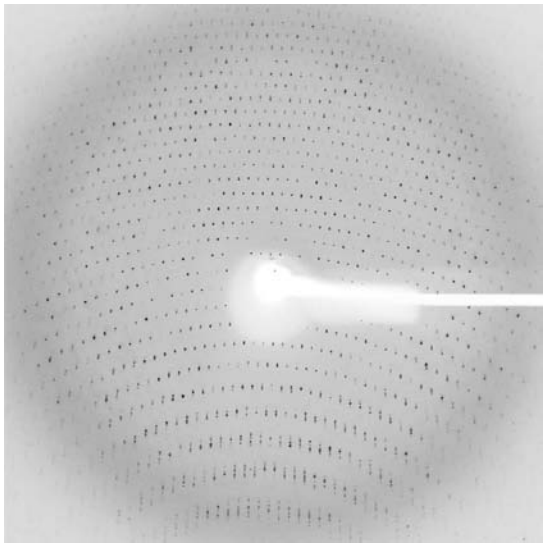

Figure 2
An X-ray diffraction pattern of the form I crystal of NADP-GAPDH.

diffraction pattern of the form I crystal is shown in Fig. 2. Contrarily, data collection from the form II crystal was unsuccessful because of a largely increased mosaicity.

Molecular-replacement calculations were performed using the program *AMoRe* (Navaza, 1994). The structure of NADP-GAPDH from *B. stearothermophilus* (PDB code 1gd1; Skarzynski & Wonacott, 1988) was used as a search model, as the homology was fairly high (59.5%). A clear peak was found with a correlation coefficient of 47.4 and an $R_{\rm factor}$ of 46.1% (15–4 Å) after translation-function calculations in the space group $P6_5$, whereas no significant peak was observed in the space group $P6_1$. Manual modifications of the model structure using the graphic program *O* (Jones *et al.*, 1991) are currently in progress.

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