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Structure of apo-glyceraldehyde-3-phosphate dehydrogenase from *Synechococcus* PCC7942

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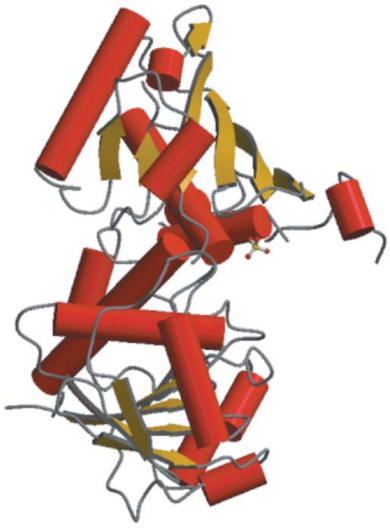

The crystal structure of NADP-dependent apo-glyceraldehyde-3-phosphate dehydrogenase (apo-GAPDH) from *Synechococcus* PCC 7942 is reported. The crystal structure was solved by molecular replacement and refined to an R of 21.7% and R_{free} of 27.5% at 2.9 Å resolution. The structural features of apo-GAPDH are as follows. The S-loop has an extremely flexible conformation and the sulfate ion is only taken into the classical P_i site. A structural comparison with holo-GAPDHs indicated that the S-loop fixation is essential in the discrimination of NADP and NAD molecules.

1. Introduction

NADP-dependent glyceraldehyde-3-phosphate dehydrogenase (NADP-dependent GAPDH) catalyzes the second reaction of the photosynthetic carbon reduction cycle of higher plants and algae. The enzyme utilizes both NADP(H) and NAD(H) as coenzymes, with a greater specificity for NADP(H). In higher plants, photosynthetic NADP-dependent GAPDH exists in the form of either a homotetramer A_4-GAPDH consisting of a subunit A or a heterotetramer A_2B_2-GAPDH consisting of two subunits, A and B, in stoichiometric amounts (Cerff, 1978; Shih *et al.*, 1991; Scagliarini *et al.*, 1998). The activity of A_2B_2-GAPDH is regulated *in vivo* by a system consisting of ferredoxin, thioredoxin f and ferredoxin/thioredoxin reductase (Buchanan, 1980; Cséke & Buchanan, 1986), whereas the activity of A_4-GAPDH is not regulated by this system (Scagliarini *et al.*, 1998). The A_2B_2 tetramer is regulated by the organizing oligomer $(A_2B_2)_4$, which has little GAPDH activity (Pupillo & Guiliani Piccari, 1975; Trost *et al.*, 1993; Baalmann *et al.*, 1995; Scagliarini *et al.*, 1998). NADP(H) stabilizes the active form of A_2B_2, whereas NAD(H) induces oligomerization. The activity of A_2B_2-GAPDH is also regulated by the reversible dissociation of the supramolecular complex PRK–CP12–GAPDH, involving phosphoribulokinase (PRK), the regulatory peptide CP12 and GAPDH, mediated by NADP(H) (Wedel *et al.*, 1997; Graciet *et al.*, 2003).

Coenzyme specificity is an important property of NADP-dependent GAPDH that is linked to its regulation. To clarify the structural features responsible for the recognition of NADP, crystal structures of NADP-dependent GAPDH from *Methanothermus fervidus* in complex with NADP (Charron *et al.*, 2000) and the non-regulatory A_4 isoforms from spinach chloroplast in complex with NADP (Fermani *et al.*, 2001) and with NAD (Falini *et al.*, 2003) and mutants of the A_4 isoform in complex with NADP (Sparla *et al.*, 2004) have been determined. Recently, we have determined the crystal structure of NADP-dependent GAPDH from the cyanobacterium *Synechococcus* PCC 7942 (*S.* 7942) at 2.5 Å resolution (Kitatani *et al.*, 2006). There is as yet no report concerning NADP-dependent apo-GAPDH, although comparison between apo-GAPDH and NADP-dependent GAPDH complexed with a coenzyme (holo-GAPDH) is expected to be highly informative.

Here, we report the first crystal structure of the NADP-dependent apo-GAPDH from *S.* 7942 at 2.9 Å resolution. A crystal suitable for X-ray diffraction analysis was obtained in microgravity. The activity of the enzyme from *S.* 7942 is not regulated by the ferredoxin/thioredoxin system, but it is regulated by the reversible dissociation

of the supramolecular complex PRK–CP12–GAPDH, mediated by NADP(H) (Tamoi *et al.*, 1996, 1998, 2005). A detailed study of the three-dimensional structure of apo-GAPDH by comparing it with those of the holo-GAPDHs should provide very important information regarding the recognition mechanism for the two coenzymes NADP and NAD.

2. Materials and methods

The NADP-dependent apo-GAPDH from *S.* 7942 was crystallized by the hanging-drop vapour-diffusion technique using ammonium sulfate as a precipitant (Nakamura *et al.*, 2001). However, the crystals showed high mosaicity and diffracted weakly to a maximum resolution of 3.5 Å. The crystal quality was improved using the gel-tube method (Tanaka *et al.*, 2004) in microgravity (Kinoshita *et al.*, 2005). A glass tube filled with protein solution concentrated to 7.5 mg ml^{-1} in 50 m*M* HEPES buffer pH 7.5 containing 30 m*M* NaCl was placed into the 1% agarose gel in the bottom of the outer box. The precipitant solution, which was 15 times the volume of the protein solution, was poured on top of the agarose layer. The precipitant solution contained 60% saturated ammonium sulfate, 0.1 *M* citrate buffer pH 4.8 and 0.2 *M* potassium sodium tartrate. The Granada Crystallization Facility (GCF), developed by the European Space Agency and the University of Granada, was purchased from Hampton Research and was used as the outer box for the gel-tube crystallization. Five tubes of 60 mm in length and 0.5 mm in diameter were placed into a GCF. The Russian Service Module developed by the Russian Federal Space Agency was used for the space experiment. The crystallization was carried out for eight weeks. The crystal grew to maximum dimensions of 0.3 × 0.07 × 0.3 mm (Fig. 1).

Diffraction data from the crystals were collected on an ADSC CCD detector using synchrotron radiation of wavelength 1.00 Å at the BL12B2 station of SPring-8, Japan. The crystal-to-detector distance was 220 mm and the oscillation range was 1° per image. For data collection at 100 K, the crystals were loop-mounted in a cryoprotectant solution containing 60% saturated ammonium sulfate, 0.1 *M* citrate buffer pH 4.8, 0.2 *M* potassium sodium tartrate and 20%(*v*/*v*) glycerol. The crystal diffracted to 2.9 Å resolution. The crystal belonged to monoclinic space group *C*2, with unit-cell parameters a = 151.4, b = 79.8, c = 207.2 Å, β = 102.1°. Data integration and scaling were performed with the *HKL*-2000 suite of programs (Otwinowski & Minor, 1997). The statistics for the intensity data are shown in Table 1.

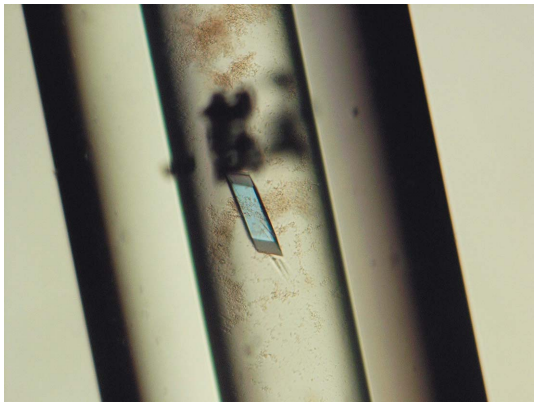

Figure 1
Crystal of apo-GAPDH from *S.* 7942.

Table 1
Summary of data-collection and refinement statistics.

Values in parentheses are for the highest resolution shell.

Data-collection statistics	
X-ray source	SPring-8, BL12B2
Wavelength (Å)	1.00
Temperature (K)	100
Resolution (Å)	2.9 (3.0–2.9)
Space group	*C*2
Unit-cell parameters (Å, °)	a = 151.4, b = 79.8, c = 207.2, β = 102.1
$R_{\rm merge}$† (%)	7.5 (34.0)
$I/\sigma(I)$	4.5 (2.0)
No. of observed reflections	844582
No. of unique reflections	53808
Completeness (%)	98.9 (100)
Mosaicity (°)	0.87
Model details	
Protein residues	1932
Sulfate ions	6
Water molecules	317
Refinement statistics	
Resolution (Å)	50–2.9
R factor‡ (%)	21.7 (31.8)
$R_{\rm free}$§ (%)	27.5 (40.7)
R.m.s.d. in bond distances (Å)	0.010
R.m.s.d. in bond angles (°)	1.4
Average B value	
Overall (Å^2)	60.7
Sulfate ions (Å^2)	51.4
Water molecules (Å^2)	39.4
Ramachandran plot	
Most favoured region (%)	82.9
Additionally allowed regions (%)	15.6
Generously allowed regions (%)	1.3
Disallowed regions (%)	0.2

† $R_{\rm merge} = \sum_{hkl} |I - \langle I\rangle| / \sum_{hkl} \langle I\rangle$. ‡ R factor $= \sum ||F_{\rm obs}| - |F_{\rm calc}|| / \sum |F_{\rm obs}|$. § $R_{\rm free}$ was calculated using 10% of the data.

The structure of apo-GAPDH from *S.* 7942 was solved by the molecular-replacement method with the program *EPMR* (Kissinger *et al.*, 1999) using the structure of holo-GAPDH from *S.* 7942 as a search model (PDB code 2d2i). The cofactor, the sulfate ions and the water molecules were not included in the model. The refinement procedures were carried out with the program *CNS* (Brünger *et al.*, 1998) applying noncrystallographic symmetry (NCS) restraints. The model was examined and rebuilt manually with the graphics program *O* (Jones *et al.*, 1991). After several cycles of energy minimization and *B*-factor refinement, the $F_{\rm o} - F_{\rm c}$ difference map showed an electron density of more than 4σ that was assigned to a sulfate ion, but did not show any densities corresponding to the NADP molecule at the coenzyme-binding site. Water molecules were added to the model at locations with $F_{\rm o} - F_{\rm c}$ densities higher than 2.5σ and hydrogen-bonding stereochemistry using the water-pick function of *CNS*. The final R factor and $R_{\rm free}$ were 21.7 and 27.5%, respectively. The stereochemistry of the final model was analyzed with the program *PROCHECK* (Laskowski *et al.*, 1993). A Ramachandran plot shows 82.9% of the residues in most favoured regions and the remaining residues in additionally allowed regions. Only Ala127 of monomers *O*, *P*, *Q* and *R* are in disallowed regions. Ala127 projects into the solvent region and the *B* factors of Ala127 and adjacent residues are higher than surrounding residues. This region including Ala127 might have a flexible conformation. The final refinement statistics are summarized in Table 1.

3. Results and discussion

The final model comprises six monomers of NADP-dependent apo-GAPDH from *S.* 7942, six sulfate ions and 317 water molecules in an

asymmetric unit. The six monomers, *O*, *P*, *Q*, *R*, *O*′ and *P*′, were named according to *Bacillus stearothermophilus* GADPH (Biesecker *et al.*, 1977). Monomers *O*, *P*, *Q* and *R* form a tetramer *OPQR*. Monomers *O*′ and *P*′ are close to the cell origin and the crystallographic equivalents *Q*′ and *R*′ are created by twofold symmetry operation to form the tetramer *O*′*P*′*Q*′*R*′. The crystal packing of apo-GAPDH is the same as in the holo-GAPDH from *S.* 7942 in complex with NADP (Kitatani *et al.*, 2006). Although the two tetramers differ crystallographically, the difference in their quaternary structures cannot be regarded as significant.

The whole structure of the monomer is shown in Fig. 2. In all six monomer structures, the 42 amino-acid residues from the C-termini were disordered and did not show clear electron densities for assignment, possibly because they are located on the surface of the tetramer. The six monomers present very similar conformations. The monomer structure consists of a cofactor-binding domain and a catalytic domain. The cofactor-binding domain has a Rossmann fold (Rossmann *et al.*, 1975) in which six β-strands form a parallel β-sheet and six α-helices lie on both sides of the sheet. The catalytic domain is made up of an eight-stranded antiparallel β-sheet surrounded by seven α-helices.

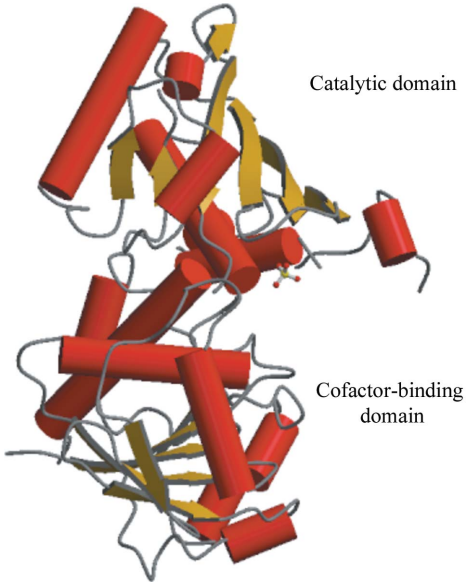

Figure 2
Overall view of the monomer of apo-GAPDH from *S.* 7942. The sulfate ion is shown as a ball-and-stick model. This figure was produced using *MOLSCRIPT* (Kraulis, 1991) and *RASTER3D* (Merritt & Murphy, 1994).

The overall monomer conformation of apo-GAPDH is essentially similar to that of holo-GAPDH as shown in Fig. 3. However, interestingly, 16 amino-acid residues from 185 to 200 in the S-loop region did not show clear electron density for assignment, indicating that the region has an extremely flexible conformation in apo-GAPDH. On the other hand, in holo-GAPDH, the S-loop composed of 26 amino-acid residues from 183 to 208 was fixed in one conformation and the hydroxyl group of Ser194 belonging to the S-loop formed a hydrogen bond with one of the 2′-phosphate O atoms of NADP located in a coenzyme-binding site of a symmetry related monomer. The S-loop fixation and the hydrogen-bond formation were also found in the crystal structure of A_4-GAPDH from spinach chloroplasts in complex with NADP (Fermani *et al.*, 2001). However, the hydrogen bond cannot be considered to play a predominant role in the S-loop fixation, because the fixation also occurred in the complex of A_4-GAPDH with NAD (Falini *et al.*, 2003) and the mutant of A_4-GAPDH with NADP, in which the Ser of the S-loop was replaced by Ala (Sparla *et al.*, 2004).

Another interesting structural feature was found in relation to the conformational change of the S-loop. GAPDHs have two anion-binding sites, P_s (substrate phosphate ion site) and the new or classical P_i (inorganic phosphate ion site), around the catalytic Cys residue (Moras *et al.*, 1975). The inorganic phosphate ion site is divided into two distinct sites called the 'new P_i' site and the 'classical P_i' site that are conserved in almost all GAPDH structures (Kim & Hol, 1998; Yun *et al.*, 2000). Sulfate ions are taken into both the P_s and classical P_i sites in the crystal structures of holo-GAPDHs. However, the apo-GAPDH only possesses a sulfate ion in the classical P_i site, despite the fact that a concentrated solution of ammonium sulfate was used in crystallization. The sulfate ion located in the P_s site was hydrogen bonded to the nicotinamide ribose O2′ atom of NADP, the O^{γ} atom of Thr185 and the $N^{\eta 1}$ atom of Arg200 in holo-GAPDH.

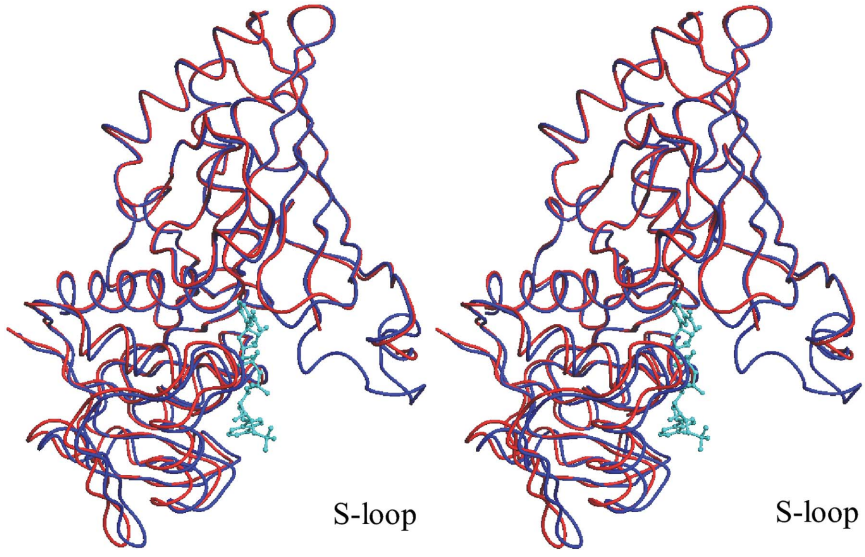

Figure 3
Stereoview of the superimposition of the monomers of apo-GAPDH (red) and holo-GAPDH (blue) from *S.* 7942 in a C^{α} trace. The NADP molecule is coloured cyan. This figure was produced using *DINO* (http://www.dino3d.org).

This means that the sulfate ion cannot bind to the P_s site in the absence of the coenzyme and the hydrogen bonds between the sulfate ion and the protein play an important role in the S-loop fixation. *In vivo*, the substrate and coenzyme probably bind simultaneously in the active site and coenzyme-binding site of GAPDH, respectively, and the conformation of the S-loop will be fixed to cover the active and coenzyme-binding sites by forming hydrogen bonds between the phosphate groups of the substrate and protein. The S-loop fixation makes possible the formation of the hydrogen bond between the 2′-phosphate O atom of NADP located in a coenzyme-binding site of a symmetry-related monomer and Ser194 belonging to the S-loop. Since the two coenzymes only differ structurally in the phosphate group esterified at the 2′ position of adenosine ribose, the S-loop fixation is considered essential in the discrimination of NADP and NAD molecules.

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References

Baalmann, E., Backhausen, J. E., Rak, C., Vetter, S. & Scheibe, R. (1995). *Arch. Biochem. Biophys.* **324**, 201–208.

Biesecker, G., Harris, J. I., Thierry, J. C., Walker, J. E. & Wonacott, A. J. (1977). *Nature (London)*, **266**, 328–333.

Brünger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, J.-S., Kuszewski, J., Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T. & Warren, G. L. (1998). *Acta Cryst.* D**54**, 905–921.

Buchanan, B. B. (1980). *Annu. Rev. Plant. Physiol.* **31**, 341–374.

Cerff, R. (1978). *Eur. J. Biochem.* **82**, 45–53.

Charron, C., Talfournier, F., Isupov, M. N., Littlechild, J. A., Branlant, G., Vitoux, B. & Aubry, A. (2000). *J. Mol. Biol.* **297**, 481–500.

Cséke, C. & Buchanan, B. B. (1986). *Biochim. Biophys. Acta*, **853**, 43–63.

Falini, G., Fermani, S., Ripamonti, A., Sabatino, P., Sparla, F., Pupillo, P. & Trost, P. (2003). *Biochemistry*, **42**, 4631–4639.

Fermani, S., Ripamonti, A., Sabatino, P., Zanotti, G., Scagliarini, S., Sparla, F., Trost, P. & Pupillo, P. (2001). *J. Mol. Biol.* **314**, 527–542.

Graciet, E., Gans, P., Wedel, N., Lebreton, S., Camadro, J. M. & Gontero, B. (2003). *Biochemistry*, **42**, 8163–8170.

Jones, T. A., Zou, J.-Y., Cowan, S. W. & Kjeldgaard, M. (1991). *Acta Cryst.* A**47**, 110–119.

Kim, H. & Hol, W. G. (1998). *J. Mol. Biol.* **278**, 5–11.

Kinoshita, T., Maruki, R., Warizawa, M., Nakajima, H. & Nishimura, S. (2005). *Acta Cryst.* F**61**, 346–349.

Kissinger, C. R., Gehlhaar, D. K. & Fogel, D. B. (1999). *Acta Cryst.* D**55**, 484–491.

Kitatani, T., Nakamura, Y., Wada, K., Kinoshita, T., Tamoi, M., Shigeoka, S. & Tada, T. (2006). *Acta Cryst.* F**62**, 315–319.

Kraulis, P. J. (1991). *J. Appl. Cryst.* **24**, 946–950.

Laskowski, R. A., MacArthur, M. W., Moss, D. S. & Thornton, J. M. (1993). *J. Appl. Cryst.* **26**, 283–291.

Merritt, E. A. & Murphy, M. E. P. (1994). *Acta Cryst.* D**50**, 869–873.

Moras, D., Olsen, K. W., Sabesan, M. N., Buehner, M., Ford, G. C. & Rossmann, M. G. (1975). *J. Biol. Chem.* **250**, 9137–9162.

Nakamura, Y., Tada, T., Wada, K., Kinoshita, T., Tamoi, M., Shigeoka, S. & Nishimura, K. (2001). *Acta Cryst.* D**57**, 879–881.

Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.

Pupillo, P. & Giuliani Piccari, G. (1975). *Eur. J. Biochem.* **51**, 475–482.

Rossmann, M. G., Liljas, A., Brändén, C. I. & Banaszak, L. J. (1975). *The Enzymes*, edited by P. D. Boyer, Vol. 11, pp. 61–102. New York: Academic Press.

Scagliarini, S., Trost, P. & Pupillo, P. (1998). *J. Exp. Bot.* **49**, 1307–1315.

Shih, M. C., Heinrich, P. & Goodman, H. M. (1991). *Gene*, **104**, 133–138.

Sparla, F., Fermani, S., Falini, G., Zaffagnini, M., Ripamonti, A., Sabatino, P., Pupillo, P. & Trost, P. (2004). *J. Mol. Biol.* **340**, 1025–1037.

Tamoi, M., Ishikawa, T., Takeda, T. & Shigeoka, S. (1996). *Biochem. J.* **316**, 685–690.

Tamoi, M., Miyazaki, T., Fukamizo, T. & Shigeoka, S. (2005). *Plant J.* **42**, 504–513.

Tamoi, M., Murakami, A., Takeda, T. & Shigeoka, S. (1998). *Biosci. Biotechnol. Biochem.* **62**, 374–376.

Tanaka, H., Inaka, K., Sugiyama, S., Takahashi, S., Sano, S., Sato, M. & Yoshitomi, S. (2004). *J. Synchrotron Rad.* **11**, 45–48.

Trost, P., Scagliarini, S., Valenti, V. & Pupillo, P. (1993). *Planta*, **190**, 320–326.

Wedel, N., Soll, J. & Paap, B. K. (1997). *Proc. Natl Acad. Sci. USA*, **94**, 10479–10484.

Yun, M., Park, C. G., Kim, J. Y. & Park, H. W. (2000). *Biochemistry*, **39**, 10702–10710.