Acta Crystallographica Section F
Structural Biology and Crystallization Communications
ISSN 1744-3091

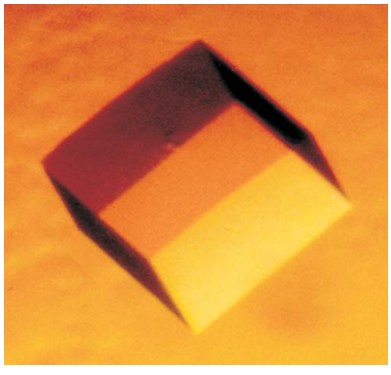
Expression, purification, crystallization and preliminary X-ray analysis of a nucleoside kinase from the hyperthermophile *Methanocaldococcus jannaschii*

Linda Arnfors,[a] Thomas Hansen,[b] Winfried Meining,[a] Peter Schönheit[b] and Rudolf Ladenstein[a]*

[a]Center for Structural Biochemistry, Department of Biosciences at Novum, Karolinska Institute, S-141 57 Huddinge, Sweden, and [b]Institut für Allgemeine Mikrobiologie, Christian-Albrecht-Universität Kiel, 24118 Kiel, Germany

Correspondence e-mail: rudolf.ladenstein@biosci.ki.se

Received 28 April 2005
Accepted 17 May 2005
Online 1 June 2005



Methanocaldococcus jannaschii nucleoside kinase (*Mj*NK) is an ATP-dependent non-allosteric phosphotransferase that shows high catalytic activity for guanosine, inosine and cytidine. *Mj*NK is a member of the phosphofructokinase B family, but participates in the biosynthesis of nucleoside monophosphates rather than in glycolysis. *Mj*NK was crystallized as the apoenzyme as well as in complex with an ATP analogue and Mg^{2+}. The latter crystal form was also soaked with fructose-6-phosphate. Synchrotron-radiation data were collected to 1.70 Å for the apoenzyme crystals and 1.93 Å for the complex crystals. All crystals exhibit orthorhombic symmetry; however, the apoenzyme crystals contain one monomer per asymmetric unit whereas the complex crystals contain a dimer.

1. Introduction

The phosphofructokinase B family (PFK-B family; Prosite PS00583 and PS00584; Pfam PF00294) is a diverse protein family within the ribokinase superfamily (SCOP 53613) present in all three domains of life: archaea, bacteria and eukarya. The PFK-B protein family includes a variety of non-allosteric ATP-dependent kinases, *e.g.* inosine-guanosine kinases, tagatose-6-phosphate kinases, ribokinases, fructokinases and 1-phosphofructokinases, as well as the minor 6-phosphofructokinase (PFK-B) from *Escherichia coli* and the 6-phosphofructokinases from the archaea *Desulfurococcus amylolyticus* and *Aeropyrum pernix* (Bork *et al.*, 1993; Hansen & Schönheit, 2000, 2001). The latter PFKs are not related to the ATP-dependent 6-phosphofructokinases from the phosphofructokinase A family (Pfam PF00365; Wu *et al.*, 1991; Ronimus & Morgan, 2001), which represent key regulatory enzymes in the Embden–Meyerhof pathways of various bacteria and eukarya (Wu *et al.*, 1991; Ronimus & Morgan, 2001; Hansen *et al.*, 2002). Moreover, ADP-dependent 6-phosphofructokinases and glucokinases constitute a separate protein family within the ribokinase superfamily (Pfam PF04587; Ito *et al.*, 2001).

Seven structures of enzymes from the PFK-B family have been described so far. These include *E. coli* ribokinase (PDB code 1rkd), human and *Toxoplasma gondii* adenosine kinases (1bx4 and 1dgm), the 2-keto-3-deoxygluconate kinases from *Thermotoga maritima* (1j5v) and *Thermus thermophilus* (1v1s), sheep pyridoxal kinase (1lhp) and putative 1-phosphofructokinase from *Thermotoga maritima* (1o14) (Sigrell *et al.*, 1998; Mathews *et al.*, 1998; Schumacher *et al.*, 2000; Ohshima *et al.*, 2004; Li *et al.*, 2002; Joint Center For Structural Genomics, unpublished results). A structure of an archaeal member of the PFK-B family has not yet been reported. The structures of the ADP-dependent glucokinases from *Thermococcus litoralis* (PDB code 1gc5) and *Pyrococcus furiosus* (1ua4) were the first archaeal representatives within the ribokinase superfamily (Ito *et al.*, 2001). For a better understanding of the evolution of the ribokinase superfamily as well as of sugar kinases in general, additional structures of members of the PFK-B family are necessary.

In 1996, when the complete genome of *Methanocaldococcus jannaschii* was released, Bult and coworkers described the gene product of MJ0406 as a hypothetical PFK-B sugar kinase (Bult *et al.*,

1996). Owing to sequence similarity to the *A. pernix* PFK-B 6-phosphofructokinase (Hansen & Schönheit, 2001), it has been suggested that MJ0406 might also encode a PFK-B. However, characterization of the respective heterologous protein revealed that MJ0406 catalyzes the ATP-dependent phosphorylation of several substrates. The enzyme, which was designed as a nucleoside kinase (*Mj*NK), showed the highest catalytic activity with guanosine, inosine and cytidine, but used fructose-6-phosphate only to a very low extent (Hansen & Schönheit, unpublished work). Thus, *Mj*NK plays a role in the nucleoside metabolism rather than in sugar degradation in *M. jannaschii*.

Moreover, the three-dimensional structure of *Mj*NK will help in understanding the wide substrate range of the enzyme as well as the evolution and non-allosteric nature of the PFK-B family and will provide additional data on the structural factors that give rise to thermostability of proteins.

Here, we describe the expression, purification, crystallization and preliminary X-ray analysis of *M. jannaschii* nucleoside kinase.

2. Materials and methods

2.1. Expression

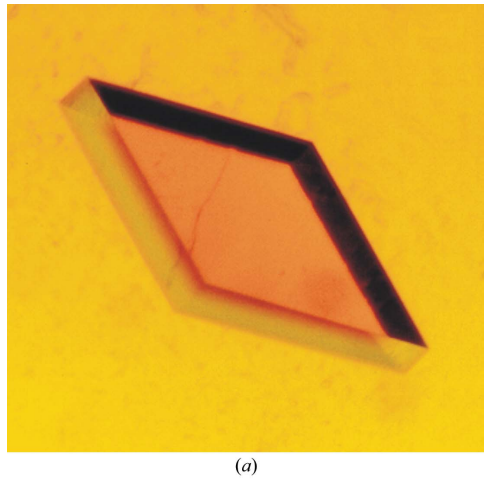
E. coli BL 21 (DE3) pLys S cells were transformed with a pET-17b plasmid which contained the *mjnk* gene (Hansen & Schönheit, unpublished work). Transformed cells were grown in 400 ml Luria–Bertani medium with 100 µg ml^{-1} carbenicillin and 34 µg ml^{-1} chloramphenicol at 310 K. When the cells reached an optical density at 600 nm of 0.8, protein expression was induced by the addition of 0.8 m*M* IPTG. After further 4 h of growth ($OD_{600} \simeq 3.2$–3.6), the cells were harvested by centrifugation at 277 K and then washed in a buffer containing 50 m*M* Tris–HCl pH 7.0 and 50 m*M* NaCl.

2.2. Purification

All chromatographic steps were carried out at 277 K. Cell extracts were prepared by French press treatment (1.3×10^8 Pa) of cell suspensions in buffer *A* (50 m*M* Tris–HCl pH 8.4). After ultracentrifugation (100 000*g* for 60 min), the solution was heat-precipitated at 353 K for 45 min, centrifuged again (15 000*g* for 30 min), dialyzed three times against a 30-fold excess of 50 m*M* Tris–HCl pH 8.0 (buffer *B*) and applied onto a DEAE Sepharose column (60 ml) previously equilibrated with buffer *B*. The protein was eluted at a flow rate of 3 ml min^{-1} with 180 ml 50 m*M* piperazine pH 6.5 at 298 K (buffer *C*) as well with a combination of fixed NaCl concentration and linear gradients from 0 to 2 *M* NaCl in buffer *B*: 0–0.1 *M* (120 ml), 0.1–1 *M* (180 ml), 1–2 *M* (120 ml) and 2 *M* NaCl (120 ml). Fractions containing the highest nucleoside kinase activity (160 ml, 0.2–1 *M* NaCl) were pooled and diluted with 3 *M* ammonium sulfate in buffer *B* to a final ammonium sulfate concentration of 2 *M*. Subsequently, the solution was applied onto a phenyl-Sepharose column (15 ml) previously equilibrated with 2 *M* ammonium sulfate in buffer *B*. The protein was eluted with a linear ammonium sulfate gradient (2–0 *M*, 150 ml) followed by washing the column with 60 ml 50 m*M* Tris–HCl pH 8.0 and 60 ml water. Fractions containing nucleoside kinase activity (75 ml, 1.2–0.2 *M* ammonium sulfate) were checked for purity with respect to protein and DNA impurities. *Mj*NK, which was almost pure (45 ml, 0.8–0.2 *M* ammonium sulfate, protein and DNA contamination <5%), was used for further purification. The protein was concentrated by ultrafiltration (exclusion size 10 kDa) and then applied onto a Superdex 200 gel-filtration column (120 ml) previously equilibrated with buffer *D* (150 m*M* NaCl, 50 m*M* Tris–HCl pH 7.5); pure *Mj*NK was obtained (72–86 ml) from this step.

2.3. Crystallization

M. jannaschii nucleoside kinase was dialyzed against 20 m*M* Tris–HCl pH 8.0 containing 0.02% sodium azide, concentrated to a protein concentration of 13 mg ml^{-1} and kept at 277 K. For crystallization of the complex, *Mj*NK was mixed with the ATP analogue AMPPNP and $MgCl_2$ in the ratio 1:10:20 and incubated for at least 15 h. In order to find crystallization conditions for both the apo form (*Mj*NK-apo) and the complex with AMPPNP (*Mj*NK-A), homemade screening sets identical to commercially available screens [Crystal Screen I (Jancarik & Kim, 1991), Crystal Screen Lite (Jancarik & Kim, 1991; McPherson, 1990), Natrix (Scott *et al.*, 1995) and PEG/Ion Screen from Hampton Research] were used. Promising conditions were optimized using a finer grid search. All crystallization experiments were carried out at 293 K using the sitting-drop or hanging-drop vapour-diffusion methods; the latter method was preferred in order to prevent crystals from sticking to the plate. 2.5 µl mother liquor was added to a fresh drop of an equal volume of protein solution and the drops were allowed to equilibrate against 500 µl mother liquor. A total concentration of 0.02% azide was used in all crystallization drops. Some of the complex crystals were further soaked with 10 m*M*



(*a*)

(*b*)

Figure 1
Orthorhombic crystals of *M. jannaschii* nucleoside kinase showing (*a*) the apo form (*Mj*NK-apo) and (*b*) the enzyme in complex with the ATP analogue AMPPNP and $MgCl_2$. The crystal sizes for *Mj*NK-apo and *Mj*NK-A were $0.1 \times 0.3 \times 0.3$ and $0.2 \times 0.2 \times 0.2$ mm, respectively.

Table 1
Crystallographic data analysis.

Values in parentheses are for the highest resolution shell.

	*Mj*NK-apo	*Mj*NK-A	*Mj*NK-AF
Space group	$P2_12_12$	$P2_12_12_1$	$P2_12_12_1$
Unit-cell parameters (Å, °)	a = 64.1, b = 148.1, c = 41.1, $\alpha = \beta = \gamma = 90$	a = 64.2, b = 83.2, c = 147.0, $\alpha = \beta = \gamma = 90$	a = 64.0, b = 83.1, c = 146.8, $\alpha = \beta = \gamma = 90$
Resolution (Å)	50–1.70 (1.73–1.70)	20–1.93 (1.96–1.93)	20–1.93 (1.96–1.93)
No. of observations	694623	1132735	800362
Unique reflections	44110	58677	58526
Completeness (%)	100 (100)	97.4 (83.5)	97.0 (85.8)
$\langle I\rangle/\langle\sigma(I)\rangle$	41.5 (3.3)	19.3 (1.2)	19.9 (1.4)
$R_{\rm merge}$† (%)	4.3 (65.4)	5.9 (55.7)	6.0 (65.7)
Subunits per AU	1	2	2
$V_{\rm M}$ (Å^3 Da^{-1})	2.88	2.90	2.88

† $R_{\rm merge} = \sum|I - \langle I\rangle|/\sum I$.

fructose-6-phosphate in mother liquor for at least 24 h. The soaked crystals are referred to as *Mj*NK-AF. Suitable cryoconditions were screened by the addition of polyethylene glycol 400, glycerol and ethylene glycol. The cryoprotectant solutions for each crystal form contained the mother-liquor composition with the addition of 30% ethylene glycol for *Mj*NK-apo and 20% ethylene glycol for *Mj*NK-A and *Mj*NK-AF.

2.4. Data collection and preliminary X-ray analysis

Prior to data collection, the crystals were soaked for 1 min in the cryoprotectant solution and flash-cooled in liquid nitrogen. Synchrotron-radiation data for *Mj*NK-apo were collected on beamline I711 at MAX-lab (Lund, Sweden) at a wavelength of 1.134 Å using a MAR Research imaging-plate detector. Data for the two complex forms were collected at beamline X11 at the EMBL Outstation, DESY (Hamburg, Germany) at a wavelength of 0.8098 Å using a MAR CCD detector. For all three crystal forms, both high- and low-resolution data sets were collected. The data were indexed and scaled using the *HKL* package programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997).

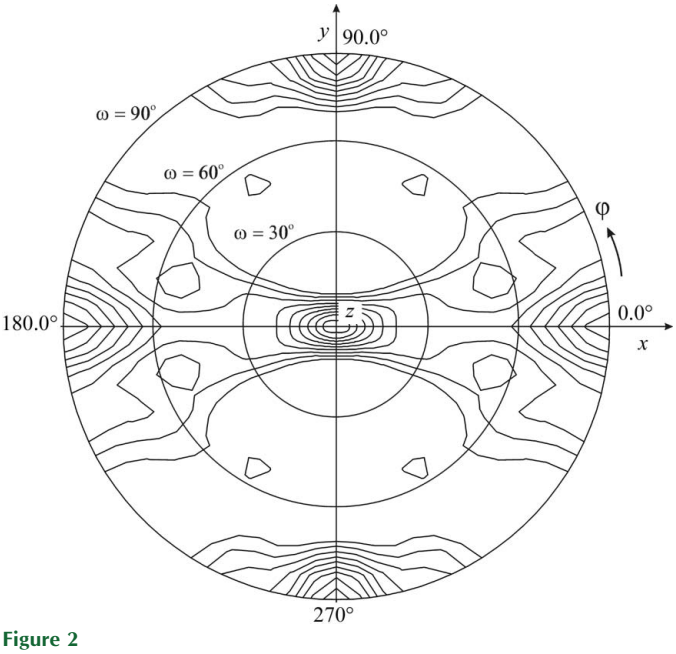


Figure 2
Stereographic plot of the self-rotation function at the section $\kappa = 180°$ calculated from the *Mj*NK-AF data. The integration radius is between 15 and 5 Å and the resolution limits are 10–4 Å. Contour lines are drawn in steps of 10%. Only high peaks arising from crystallographic symmetry can be seen, which indicates that the twofold non-crystallographic axis is parallel to a crystallographic axis. The plot was produced using *POLARRFN* (Collaborative Computational Project, Number 4, 1994).

3. Results and discussion

M. jannaschii nucleoside kinase has been crystallized in two forms: an apo form (*Mj*NK-apo) and a form with the protein in complex with AMPPNP and $MgCl_2$ (*Mj*NK-A). Some of the *Mj*NK-A crystals were further soaked with fructose-6-phosphate (*Mj*NK-AF).

*Mj*NK-apo crystals were obtained from a solution containing 10%(*w*/*v*) PEG 3500 and 0.15 *M* magnesium acetate. *Mj*NK-A crystallized from a solution containing 15% PEG 4000, 0.10 *M* $MgCl_2$ and 0.1 *M* Tris–HCl pH 8.5. Colourless orthorhombic crystals (Fig. 1) appeared within 1–4 d.

Data-collection parameters and statistics of the three crystal forms of *M. jannaschii* nucleoside kinase are summarized in Table 1. Pseudo-precession photographs (Lu, 1999) of the diffraction data evaluated in space group *P*222 showed systematic extinctions at $h = 2n + 1$, $k = 2n + 1$, $l = 2n$ for *Mj*NK-apo and $h = 2n + 1$, $k = 2n + 1$, $l = 2n + 1$ for *Mj*NK-A and *Mj*NK-AF. The space groups were thus assigned as $P2_12_12$ for *Mj*NK-apo and $P2_12_12_1$ for the two complex forms. Moreover, the unit-cell parameters of the apo form and the complexes are almost identical with one exception. The *b* axes of the complexes (b = 83.2 Å for *Mj*NK-A and b = 83.1 Å for *Mj*NK-AF) are twice as long as the *c* axis for *Mj*NK-apo (c = 41.1 Å). This results in a unit-cell volume twice the size for the complex crystals and thus the unit cell of *Mj*NK-apo contains half the number of subunits compared with *Mj*NK-A and *Mj*NK-AF. In addition, the calculated Matthews coefficient ($V_{\rm M}$; Matthews, 1968) of 2.88 Å^3 Da^{-1} for four monomers in the unit cell of *Mj*NK-apo indicated that the crystal contains one subunit per asymmetric unit. This corresponds to a solvent content of 56.9%. Gel-filtration data reveals that *Mj*NK exists as a dimer in solution (Hansen & Schönheit, unpublished results). Therefore, it is concluded that the biological dimer is generated by a twofold crystallographic symmetry operation in the apo crystals. Owing to the different unit-cell volumes and according to the calculated Matthews coefficients (Table 1), *Mj*NK-A and *Mj*NK-AF contain one dimer per asymmetric unit, with solvent contents of 57.2 and 56.9%, respectively. The self-rotation function obtained from the *Mj*NK-A and *Mj*NK-AF data, calculated with the programs *MOLREP* (Vagin & Teplyakov, 1997), *AMoRe* (Navaza, 1994) and *POLARRFN* (Collaborative Computational Project, Number 4, 1994) in the *CCP*4 suite, shows no additional twofold symmetry apart from crystallographic symmetry (Fig. 2). Furthermore, the native Patterson map calculated with *FFT* (Read & Schierbeek, 1988) within

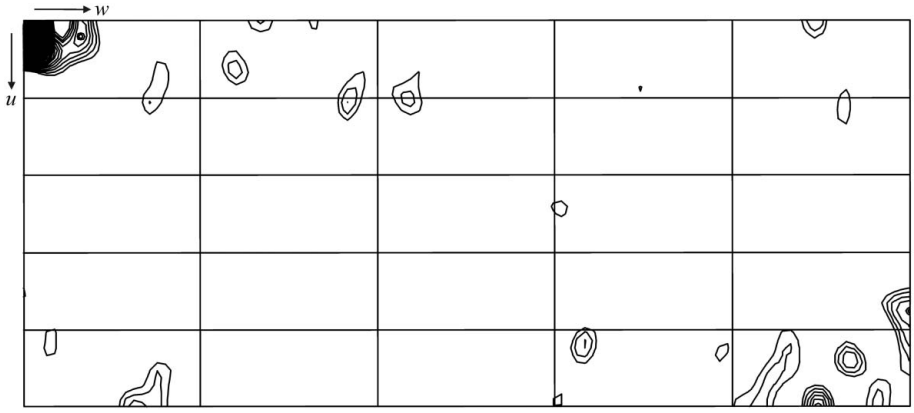


Figure 3
Native Patterson function calculated from all data from the *Mj*NK-AF complex. The plot shows fractional coordinates of *u* and *w* ranging from 0.0 to 0.5 at Harker section $v = 0.5$. An intense pseudo-origin peak appears at $(u, w) = (0.0, 0.0)$. Grid lines are drawn every 0.10 units and contours are drawn every 0.5% of the origin peak hight, starting at 1.5%. The figure was prepared with the programs *NPO* and *PLTDEV* from the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994).

the *CCP*4 suite of programs reveals a pseudo-origin peak at $(u, v, w) = (0.0, 0.5, 0.0)$ (Fig. 3) with an intensity of 25% of the Patterson origin peak. The results from the self-rotation function and the native Patterson indicate that the twofold non-crystallographic symmetry axis relating the two subunits in the asymmetric unit is parallel to the *y* axis in the unit cell.

We gratefully acknowledge MAX-lab (Lund, Sweden) and the EMBL Outstation at DESY (Hamburg, Germany) for provision of synchrotron-radiation facilities. We thank the European Community Research Infrastructure Action under the FP6 'Structuring the European Research Area Programme' contract No. HPRI/1999/CT/00017 for travel expenses and NorFA (Norway) for financial support under the 'Biology of Thermophiles' project.

References

Bork, P., Sander, C. & Valencia, A. (1993). *Protein Sci.* **2**, 31–40.
Bult, C. J. *et al.* (1996). *Science*, **273**, 1058–1073.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Hansen, T., Musfeldt, M. & Schönheit, P. (2002). *Arch. Microbiol.* **177**, 401–409.
Hansen, T. & Schönheit, P. (2000). *Arch. Microbiol.* **173**, 103–109.
Hansen, T. & Schönheit, P. (2001). *Arch. Microbiol.* **177**, 62–69.
Ito, S., Fushinobu, S., Yoshioka, I., Koga, S., Matsuzawa, H. & Wakagi, T. (2001). *Structure*, **9**, 205–214.
Jancarik, J. & Kim, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.
Li, M. H., Kwok, F., Chang, W. R., Lau, C. K., Zhang, J. P., Lo, S. C., Jiang, T. & Liang, D. C. (2002). *J. Biol. Chem.* **277**, 46385–46390.
Lu, G. (1999). *J. Appl. Cryst.* **32**, 375–376.
McPherson, A. (1990). *Eur. J. Biochem.* **189**, 1–23.
Mathews, I. I., Erion, M. D. & Ealick, S. E. (1998). *Biochemistry*, **37**, 15607–15620.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Navaza, J. (1994). *Acta Cryst.* A**50**, 157–163.
Ohshima, N., Inagaki, E., Yasuike, K., Takio, K. & Tahirov, T. (2004). *J. Mol. Biol.* **340**, 477–489.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Read, R. J. & Schierbeek, A. J. (1988). *J. Appl. Cryst.* **21**, 490–495.
Ronimus, R. S. & Morgan, H. W. (2001). *Extremophiles*, **5**, 357–373.
Schumacher, M. A., Scott, D. M., Mathews, I. I., Ealick, S. E., Roos, D. S., Ullman, B. & Brennan, R. G. (2000). *J. Mol. Biol.* **298**, 875–893.
Scott, W. G., Finch, J. T., Grenfell, R., Fogg, J., Smith, T., Gait, M. J. & Klug, A. (1995). *J. Mol. Biol.* **250**, 327–332.
Sigrell, J. A., Cameron, A. D., Jones, T. A. & Mowbray, S. L. (1998). *Structure*, **6**, 183–193.
Vagin, A. & Teplyakov, A. (1997). *J. Appl. Cryst.* **30**, 1022–1025.
Wu, L.-F., Reizer, A., Reizer, J., Cai, B., Tomich, J. M. & Saier, M. H. Jr (1991). *J. Bacteriol.* **173**, 3117–3127.