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Nucleoside diphosphate kinase from the hyperthermophilic archaeon *Methanococcus jannaschii*: overexpression, crystallization and preliminary X-ray crystallographic analysis

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Nucleoside diphosphate (NDP) kinase is a key enzyme in maintaining cellular pools of all nucleoside triphosphates. NDP kinase from the hyperthermophilic archaebacterium *Methanococcus jannaschii* has been overexpressed in *Escherichia coli* and crystallized at 297 K using polyethylene glycol 4000 as precipitant. The crystal is hexagonal, belonging to the space group $P6_3$, with unit-cell parameters $a = b = 72.89$, $c = 100.87$ Å. The asymmetric unit contains two subunits of NDP kinase, with a corresponding crystal volume per protein mass (V_M) of 2.38 Å^3 Da^{-1} and a solvent content of 48.3%. Native X-ray diffraction data to 2.30 Å resolution have been collected using synchrotron X-rays.

1. Introduction

The main function of nucleoside diphosphate (NDP) kinases is to exchange γ-phosphates between nucleoside triphosphates and diphosphates using adenosine triphosphate (ATP) as a major phosphate donor (Parks & Agarwal, 1973). The reaction has a ping-pong mechanism with a high-energy phosphohistidine intermediate. As the enzyme accepts all common nucleotides as substrates, it plays a key role in maintaining cellular pools of all nucleoside triphosphates. Regulatory functions have also been attributed to NDP kinases in differentiation (Biggs *et al.*, 1990; Okabe-Kado *et al.*, 1992), development (Wallet *et al.*, 1990; Rosengard *et al.*, 1989) and apoptosis (Venturelli *et al.*, 1995). Certain NDP kinases specifically bind DNA and act as a transcription factor (Hildebrandt *et al.*, 1995; Postel *et al.*, 1993).

NDP kinases are oligomeric proteins composed of four or six identical subunits of molecular weight 17–22 kDa. Eukaryotic NDP kinases are homohexamers, whereas bacterial NDP kinases are homotetramers. The amino-acid sequences of NDP kinases are highly conserved and the subunit structures are very similar, with a characteristic $\beta\alpha\beta\beta\alpha\beta$ fold. The crystal structures of NDP kinases from prokaryotic *Myxococcus xanthus* (Williams *et al.*, 1993) and eukaryotes including *Dictyostelium discoideum* (Dumas *et al.*, 1992), *Drosophila melanogaster* (Chiadmi *et al.*, 1993), bovine retina (Abdulaev *et al.*, 1998) and human (Moréra *et al.*, 1995; Webb *et al.*, 1995) have been determined. However, no three-dimensional structure of any NDP kinase from archaebacteria or hyperthermophilic organisms has been reported.

In order to provide missing structural information on archaebacterial NDP kinases, we have initiated the structure determination of NDP kinase from the hyperthermophilic archaeon *M. jannaschii*. Its polypeptide chain comprises 140 amino-acid residues (subunit M_r 16 224). Its amino-acid sequence (Bult *et al.*, 1996) shows a minimum of 44% identity with NDP kinases from both prokaryotes and eukaryotes whose structures have been determined. As a first step toward its structural elucidation, we report here its overexpression, crystallization and preliminary X-ray crystallographic analysis. The crystal structure of NDP kinase from *M. jannaschii* will permit a detailed comparison with other known NDP kinase structures and an understanding of the structural determinants for high thermostability.

2. Experimental

2.1. Overexpression and purification

The gene encoding NDP kinase (MJ1265) was amplified by the polymerase chain reaction using the genomic DNA of *M. jannaschii* as a template. The forward and reverse oligonucleotide primers were designed using the published sequence (Bult *et al.*, 1996). The PCR product was inserted into *Nde*I/*Bam*HI-digested expression vector pET-22b(+) (Novagen). *E. coli* ER2566 cells transformed with the pET22b-MjNDK plasmid were grown at 310 K in LB medium containing 5 µg ml^{-1} ampicillin. The enzyme was highly overexpressed in soluble form upon induction with 0.4 m*M* isopropyl-β-D-thiogalactoside (IPTG). Cells were grown in LB medium for 5 h after

Table 1
Data-collection statistics.

Values in parentheses refer to the highest resolution shell (2.34–2.30 Å).

Space group	$P6_3$
Unit-cell parameters (Å)	$a = b = 72.89$, $c = 100.87$
Resolution range (Å)	20–2.30
No. of unique reflections	13004 (665)
Multiplicity of data	5.36 (3.74)
$I/\sigma(I)$	17.2 (3.3)
Data completeness (%)	96.3 (97.9)
Data with $I > 3\sigma(I)$ (%)	64.9
$R_{\rm merge}$† (%)	8.0 (20.8)

† $R_{\rm merge} = \sum_h \sum_i I(hkl)_i - \langle I(hkl)_i \rangle / \sum_h \sum_i I(hkl)_i$, where $I(hkl)$ is the intensity of reflection hkl, $\sum_h$ is the sum over all reflections and $\sum_i$ is the sum over i measurements of reflection hkl.

IPTG induction and were harvested. Cultured cells were resuspended in the lysis buffer (25 m*M* Tris–HCl pH 8.5, 1 m*M* EDTA, 1 m*M* phenylmethylsulfonyl fluoride) and were homogenized by sonication. The solution was centrifuged at 25 000*g* for 30 min and the cell debris was discarded. The cell extract was heated and kept between 353 and 355 K for 10 min. This enzyme was found to be stable and active after heat treatment (unpublished data). It was then centrifuged again at 25 000*g* for 30 min. The supernatant was subjected to column chromatography using Q-Sepharose (Pharmacia), employing a linear gradient of 0–0.4 *M* NaCl in buffer *A* (25 m*M* Tris–HCl pH 8.5). The fractions containing NDP kinase were concentrated by ultrafiltration (Amicon, YM 30) and loaded onto a Superdex 200 gel-filtration column (HiLoad 16/60, Pharmacia) equilibrated with buffer *A* containing 100 m*M* NaCl. The purified protein was homogeneous on SDS–PAGE. Dynamic light-scattering measurements were performed on a Model DynaPro-801 instrument from Protein Solutions (Charlottesville, Virginia, USA) at room temperature using protein dissolved to about 1 mg ml^{-1} concentration in buffer *A* containing 100 m*M* NaCl.

2.2. Crystallization

The purified enzyme was concentrated to about 20 mg ml^{-1} concentration using a YM30 ultrafiltration membrane (Amicon). The protein concentration was estimated by measuring the absorbance at 280 nm, employing a calculated extinction coefficient $\varepsilon_{280{\rm nm},0.1\%,1{\rm cm}}$ (cm^2 mg^{-1}) of 0.237. Crystallization was performed by the hanging-drop vapour-diffusion method using 24-well tissue-culture VDX plates (Hampton Research) at 297 K. Each hanging drop was prepared by mixing 2 µl each of the protein solution and the reservoir solution and was placed over 1.0 ml of the reservoir solution. Initial searches for the crystallization conditions were performed using Crystal Screen I (Jancarik & Kim, 1991), Crystal Screen II and MembFac screening solutions (Hampton Research).

2.3. X-ray diffraction experiments

X-ray diffraction data were collected at 100 K in the Weissenberg mode using a screenless Weissenberg camera for macromolecular crystallography at the BL-6B experimental station of the Photon Factory, Tsukuba, Japan (Sakabe *et al.*, 1997). Before flash-freezing the crystal in the nitrogen-gas stream at 100 K, it was dipped for a few seconds into a solution containing 10%(*v*/*v*) glycerol in addition to the reservoir solution. The wavelength of the synchrotron X-rays was 1.000 Å and a 0.2 mm collimator was used. Two image plates (40 × 80 cm, Fuji BASIII) were placed at a distance of 573 mm from the crystal. The oscillation range per frame was 5.5°, with a speed of 2.0° s^{-1} and a coupling constant of 1.0° mm^{-1}. An overlap of 0.5° was allowed between contiguous image plates. The total oscillation range was 180°. The diffraction patterns recorded on the image plates were digitized by the off-line scanner IPR4080 (Rigaku). The raw data were processed and integrated using the *DENZO*/*SCALEPACK* programs (Otwinowski & Minor, 1997).

3. Results

Recombinant NDP kinase from *M. jannaschii* has been overexpressed in *E. coli* as a soluble fraction under the control of T7 promoter to the level of about 20 mg per litre of culture (Fig. 1). The native molecular mass of the recombinant NDP kinase was estimated to be 89 kDa, with a polydispersity of 17%, from dynamic light-scattering measurements. This indicates that the protein is likely to exist as a homohexamer. It was crystallized under the reservoir conditions 0.10 *M* Tris–HCl pH 8.5, 18–20%(*w*/*v*) polyethylene glycol 4000 and 4–6%(*v*/*v*) 2-propanol. Needle-shaped crystals grew to dimensions of 0.1 × 0.1 × 1.3 mm within 3 d (Fig. 2).

The native crystals diffracted to 2.30 Å resolution using synchrotron X-rays. A total of 69 730 reflections were measured, which were merged to 13 004 unique reflections with an $R_{\rm merge}$ (on intensity) of 8.0%. The merged data set is 96.3% complete to 2.30 Å resolution, with the shell completeness between 2.34 and 2.30 Å resolution being 97.9%. The symmetry of the diffraction intensity and the systematic absences indicate that the crystals belong to the hexagonal space group $P6_3$, with unit-cell parameters $a = b = 72.89$ (7), $c = 100.87$ (5) Å, where the estimated standard deviations are given in parentheses. Table 1 summarizes the statistics for data collection. The presence of two subunits in each asymmetric unit gives a crystal volume per protein mass ($V_{\rm M}$) of 2.38 Å^3 Da^{-1} and a solvent content of 48.3%. These values are within the frequently observed ranges for protein crystals (Matthews, 1968). The presence of two subunits in the asymmetric unit is also consistent with the homohexameric structure of the enzyme. This is because each asymmetric unit must contain one or more homotetramers, as dictated by the space-group symmetry, if it is assumed that the enzyme is a homotetramer. The structure will be solved by molecular replacement.

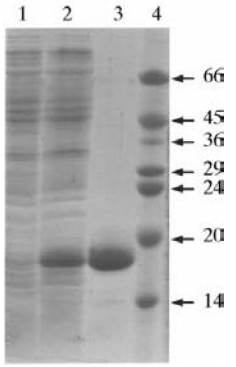

Figure 1
Expression and purification of *M. jannaschii* NDP kinase. SDS–PAGE of the cell extracts of *E. coli* ER2566 cells transformed with the plasmid pET22b-MjNDK (lane 1, before IPTG induction; lane 2, after induction). Lane 3, purified enzyme. Lane 4, size markers with the indicated molecular weights (kDa).

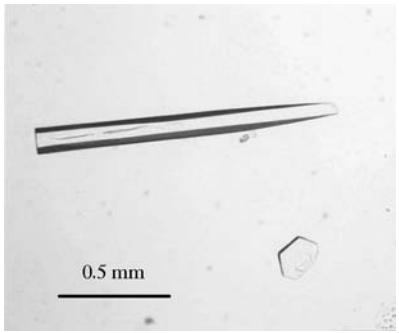

Figure 2
A needle-shaped crystal of NDP kinase from *M. jannaschii*. Its approximate dimensions are 0.1 × 0.1 × 1.3 mm.

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