Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary X-ray crystallographic analysis of nicotinic acid mononucleotide adenylyltransferase from *Pseudomonas aeruginosa*

Hye-Lee Kim,[a]‡ Hye-Jin Yoon,[a]‡ Jun Yong Ha,[a] Byung Il Lee,[a] Hyung Ho Lee,[a] Bunzo Mikami[b] and Se Won Suh[a]*

[a]Department of Chemistry, College of Natural Sciences, Seoul National University, Seoul 151-742, South Korea, and [b]Laboratory of Quality Design and Exploitation, Division of Agronomy and Horticultural Science, Graduate School of Agriculture, Kyoto University, Gokasho, Uji, Kyoto 611-0011, South Korea

‡ These authors contributed equally to this work.

Correspondence e-mail: sewonsuh@snu.ac.kr

Received 2 January 2003
Accepted 10 March 2004

The enzyme nicotinic acid mononucleotide adenylyltransferase (NaMN AT; EC 2.7.7.18) is essential for the synthesis of nicotinamide adenine dinucleotide and is a potential target for antibiotics. It catalyzes the transfer of an adenyl group from ATP to nicotinic acid mononucleotide to form nicotinic acid adenine dinucleotide. NaMN AT from *Pseudomonas aeruginosa* was overexpressed in *Escherichia coli* and crystallized at 291 K using 100 m*M* bis–Tris propane pH 7.0, 700 m*M* trisodium citrate and 15%(*v*/*v*) glycerol. X-ray diffraction data have been collected to 1.70 Å. The crystals are tetragonal, belonging to space group $P4_122$ (or $P4_322$), with unit-cell parameters $a = b = 65.02$, $c = 109.80$ Å. The presence of one monomer in the asymmetric unit gives a reasonable V_M of 2.15 Å^3 Da^{-1}, with a solvent content of 42.7%.

1. Introduction

Nicotinamide adenine dinucleotide (NAD) is an essential molecule in all living cells. It is directly involved in numerous oxidation or reduction reactions as an electron acceptor or donor. It is also important as a cofactor for a number of crucial enzymes such as NAD-dependent DNA ligases (Petit & Ehrlich, 2000) and as a precursor for intracellular calcium-mobilizing agents such as cyclic ADP-ribose and nicotinic acid adenine dinucleotide phosphate (Ziegler, 2000). NAD is synthesized *via* a multistep *de novo* pathway or *via* a pyridine-salvage pathway in bacteria. These two pathways converge at the enzyme nicotinic acid mononucleotide adenylyltransferase (NaMN AT; EC 2.7.7.18). NaMN AT catalyzes the transfer of an adenyl group from ATP to NaMN to form nicotinic acid adenine dinucleotide (NaAD), which is converted to NAD by NAD synthetase. The *nadD* gene has been demonstrated to be essential for bacterial cell survival (Hughes *et al.*, 1983), thus making its gene product NaMN AT a potential target for antibacterial drugs (Gerdes *et al.*, 2002). NaMN AT possesses the (H/T)*X*GH nucleotidyl transferase consensus-sequence motif (D'Angelo *et al.*, 2000; Olland *et al.*, 2002). Sequence alignment of eubacterial *nadD*-encoded NaMN ATs with the eukaryotic enzymes or archaeal enzymes is difficult outside the region surrounding the (H/T)*X*GH signature motif. Crystal structures of *Escherichia coli* and *Bacillus subtilis* NaMN ATs have been described (Zhang *et al.*, 2002; Olland *et al.*, 2002).

Pseudomonas aeruginosa is a ubiquitous environmental Gram-negative bacterium that is one of the top three causes of opportunistic human infections. A major factor in its prominence as a pathogen is its intrinsic resistance to antibiotics and disinfectants. Infection with this pathogen is life-threatening in cystic fibrosis patients. The *nadD* gene of *P. aeruginosa* encodes a 214-residue protein (23 801 Da). *P. aeruginosa* NaMN AT shows an amino-acid sequence identity of 38 and 31% to the *E. coli* and *B. subtilis* enzymes, respectively. As the first step toward structure determination of *P. aeruginosa* NaMN AT, we have overexpressed it in *E. coli* and crystallized it. Its crystallization conditions and preliminary X-ray crystallographic data are reported here.

2. Experimental

2.1. Protein expression and purification

The *nadD* gene (PA4006; http://www.tigr.org/tigr-scripts/CMR2/CMRHomePage.spl) was amplified by the polymerase chain reaction (PCR) using the genomic DNA of *P. aeruginosa* strain PAO1 as a template. The forward and reverse oligonucleotide primers designed using the published genome sequence (Stover *et al.*, 2000) are 5′-CAT G**CC ATG G**GC AAA CGT ATC GGC CTG T-3′ and 5′-CCG CCG **CTC GAG** GTG TGG CGC ACG ATA CAG GTG-3′, respectively. The underlined bases denote *Nco*I and *Xho*I cleavage sites. The PCR product was digested with *Nco*I and *Xho*I and was then inserted into the *Nco*I/*Xho*I-digested expression vector pET-28b(+) (Novagen). This construction adds a hexahistidine-containing tag to the N- and C-termini of the recombinant protein. The protein was overexpressed in *E. coli* C41(DE3) cells. The cells were grown at 310 K to an OD_{600} of 0.7 in Luria–Bertani medium containing 50 µg ml^{-1} kanamycin and protein expression was induced with 1.0 m*M*

isopropyl-β-D-thiogalactopyranoside (IPTG). Cell growth continued at 288 K for 22 h after IPTG induction and the cells were harvested by centrifugation at 4200*g* (6000 rev min^{-1}; Sorvall GSA rotor) for 10 min at 277 K. The cell pellet was resuspended in ice-cold lysis buffer (20 m*M* Tris–HCl pH 7.9, 500 m*M* sodium chloride, 5 m*M* imidazole and 1 m*M* phenylmethylsulfonyl fluoride) and was homogenized with an ultrasonic processor. The crude cell extract was centrifuged at 36 000*g* (18 000 rev min^{-1}; Hanil Supra 21K rotor) for 30 min at 277 K and the recombinant protein in the supernatant fraction was purified by two chromatographic steps. The first step utilized the N- and C-terminal hexahistidine tags by metal-chelate chromatography on Ni–NTA resin (Qiagen). Next, gel filtration was performed on a HiLoad XK 16 Superdex 200 prep-grade column (Amersham-Pharmacia) previously equilibrated with buffer *A* (50 m*M* Tris–HCl pH 8.0) containing 100 m*M* sodium chloride. In the third step, the protein was loaded onto a Mono-Q HR5/5 ion-exchange column (Amersham-Pharmacia) previously equilibrated with buffer *A* and the protein was eluted with a linear gradient of 0–1.0 *M* sodium chloride. The homogeneity of the purified protein was assessed by SDS–PAGE (Laemmli, 1970). The protein solution was concentrated using a YM10 ultrafiltration membrane (Millipore-Amicon). The protein concentration was estimated by measuring the absorbance at 280 nm, employing the calculated extinction coefficient of 26 600 M^{-1} cm^{-1} (SWISS-PROT; http://www.expasy.ch/). The AT activity was measured by a coupled assay using the EnzChek pyrophosphate assay kit from Molecular Probes, Inc. (Eugene, OR, USA) according to Olland *et al.* (2002).

2.2. Crystallization

Crystallization trials were carried out at 291 K using the hanging-drop vapour-diffusion method with 24-well VDX plates (Hampton Research). Each hanging drop was prepared on a siliconized cover slip by mixing 2 µl each of the protein solution (at 13.1 mg ml^{-1} concentration in a buffer consisting of 50 m*M* Tris–HCl pH 8.0 and 150 m*M* sodium chloride) and the reservoir solution and was placed over 1.0 ml reservoir solution. Screening of crystallization conditions was carried out using commercial kits from Hampton Research (Crystal Screens I, II and MembFac) and Emerald Biostructures Inc. (Wizard I and II). The best crystals were grown with a reservoir solution consisting of 100 m*M* bis–Tris propane pH 7.0, 700 m*M* trisodium citrate and 15%(*v*/*v*) glycerol. Square pillar-shaped crystals grew to approximate dimensions of 0.5 × 0.15 × 0.15 mm within 1 d (Fig. 1).

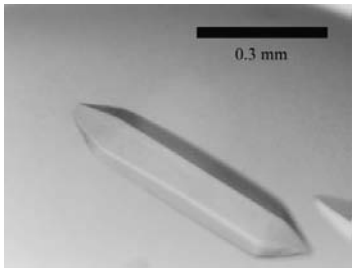

Figure 1
Crystals of NaMN AT from *P. aeruginosa*.

2.3. X-ray diffraction experiment

Crystals were flash-frozen with a cryoprotectant solution consisting of 100 m*M* bis–Tris propane pH 7.0, 700 m*M* trisodium citrate and 30%(*v*/*v*) glycerol. Crystals were soaked in 5 µl cryoprotectant solution for 10 s before being flash-frozen in liquid nitrogen. X-ray diffraction data were collected at 100 K on an ADSC Quantum 4R CCD detector at the BL38B1 experimental station, SPring-8, Japan. The crystal was rotated through a total of 120°, with a 1.0° oscillation range per frame. Intensity data were processed and scaled using the program *HKL*2000 (Otwinowski & Minor, 1997).

3. Results

The recombinant *P. aeruginosa* NaMN AT fused with both N- and C-terminal tags was highly overexpressed in soluble form in *E. coli* cells, with a yield of ∼75 mg purified enzyme per litre of culture. The purified enzyme showed a high AT activity against NaMN but not against nicotinamide mononucleotide. Despite the presence of both a 20-residue N-terminal tag (MGSSHHHH-HHSSGLVPRGSH) and an eight-residue C-terminal tag (LEHHHHHH), the recombinant enzyme readily formed well diffracting crystals. The best crystals were grown with a reservoir solution consisting of 100 m*M* bis–Tris propane pH 7.0, 700 m*M* trisodium citrate and 15%(*v*/*v*) glycerol. Square pillar-shaped crystals grew to approximate dimensions of 0.5 × 0.15 × 0.15 mm within 1 d (Fig. 1). A complete data set was measured to 1.70 Å resolution. Table 1 summarizes the data-collection statistics. The crystallographic asymmetric unit is likely to contain a monomer of the recombinant NaMN AT, giving a crystal volume per protein weight (V_M) of 2.15 Å^3 Da^{-1} and a solvent content of 42.7% (Matthews, 1968). When we attempted to solve the structure by molecular replacement using the *E. coli* NaMN AT (PDB code 1k4k, chain *A*) as a search model, promising solutions for both the rotation and translation functions were obtained but the model could not be refined below an *R* factor of 40% for the 20–2.3 Å data. Therefore, we plan to solve the structure by the mutiwavelength anomalous diffraction method.

Table 1
Data-collection statistics.

Values in parentheses refer to the highest resolution shell.

X-ray wavelength (Å)	1.000 (SPring-8 BL38B1)
Temperature (K)	100
Space group	$P4_122$ (or $P4_322$)
Unit-cell parameters (Å)	
$a = b$	65.02
c	109.80
Resolution range (Å)	20.0–1.70 (1.76–1.70)
Total/unique reflections	596148/26720
$R_{\rm merge}$† (%)	9.0 (34.6)
Data completeness (%)	99.5 (100.0)
Average $I/\sigma(I)$	50.8 (12.6)

† $R_{\rm merge} = \sum_h \sum_i |I(h)_i - \langle I(h)\rangle| / \sum_h \langle I(h)\rangle$, where $I(h)_i$ is the intensity of the *i*th measurement of reflection *h* and $\langle I(h)\rangle$ is the mean value of $I(h)$ for all *i* measurements.

This work was supported by a grant from the Korea Ministry of Science and Technology (NRL-2001, grant No. M10318000132) to SWS and a grant from the Korea Science and Engineering Foundation (R03-2003-000-10031-0) to HJY. HRK, JYH, BIL and HHL are supported by BK21 Fellowships.

References

D'Angelo, I., Raffaelli, N., Dabusti, V., Lorenzi, T., Magni, G. & Rizzi, M. (2000). *Structure*, **8**, 993–1004.
Gerdes, S. Y., Scholle, M. D., D'Souza, M., Bernal, A., Baev, M. V., Farrell, M., Kurnasov, O. V., Daugherty, M. D., Mseeh, F., Polanuyer, B. M., Campbell, J. W., Anantha, S., Shatalin, K. Y., Chowdhury, S. A., Fonstein, M. Y. & Osterman, A. L. (2002). *J. Bacteriol.* **184**, 4555–4572.
Hughes, K. T., Ladika, D., Roth, J. R. & Olivera, B. M. (1983). *J. Bacteriol.* **155**, 213–221.
Laemmli, U. K. (1970). *Nature (London)*, **227**, 680–685.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–493.
Olland, A. M., Underwood, K. W., Czerwinski, R. M., Lo, M. C., Aulabaugh, A., Bard, J., Stahl, M. L., Somers, W. S., Sullivan, F. X. & Chopra, R. (2002). *J. Biol. Chem.* **277**, 3698–3707.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Petit, M. A. & Ehrlich, S. D. (2000). *Nucleic Acids Res.* **28**, 4642–4648.
Stover, C. K. *et al.* (2000). *Nature (London)*, **406**, 959–964.
Zhang, H., Zhou, T., Kurnasov, O., Cheek, S., Grishin, N. V. & Osterman, A. (2002). *Structure*, **10**, 69–79.
Ziegler, M. (2000). *Eur. J. Biochem.* **267**, 1550–1564.