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Crystallization and preliminary X-ray analysis of NAD kinase from *Mycobacterium tuberculosis* H37Rv

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NAD kinase from *Mycobacterium tuberculosis* H37Rv utilizes ATP or inorganic polyphosphate [poly(P)] as a phosphoryl donor for the phosphorylation of NAD. The enzyme overexpressed in *Escherichia coli* was purified and crystallized by means of the hanging-drop vapour-diffusion method with polyethylene glycol 4000 as the precipitant. Preliminary X-ray analysis of the resultant crystals revealed they belonged to the monoclinic space group *C*2 and had unit-cell parameters $a = 140.0$, $b = 69.3$, $c = 106.3$ Å, $\beta = 130.1^\circ$. The molecular weight of the NAD kinase is 35 kDa; assuming that a crystal contains two subunits of the NAD kinase in an asymmetric unit, the solvent content V_{sol} is 0.62. X-ray diffraction data to 2.99 Å have been collected from the native crystal.

1. Introduction

In living organisms, NADP is formed through the phosphorylation of NAD, which is catalyzed by NAD kinase (E.C. 2.7.1.23) in the presence of ATP (Moat & Foster, 1987). NAD kinase has been regarded to be the only enzyme that produces NADP and, therefore, to be the key enzyme in the synthesis of NADP and the NADP-dependent anabolic/biosynthetic pathway in cells (Magni *et al.*, 1999). However, the occurrence of another type of NAD kinase that utilizes ATP and inorganic polyphosphate [poly(P)] has been reported in some bacteria, especially in the genera *Micrococcus* and *Brevibacterium* (Murata *et al.*, 1980).

We recently identified Rv1695 from *M. tuberculosis* H37Rv as the NAD kinase gene and showed that the NAD kinase of *M. tuberculosis* H37Rv could utilize poly(P) or ATP as a phosphoryl donor for the phosphorylation of NAD (Kawai *et al.*, 2000). The NAD kinase specifically and completely phosphorylated NAD to NADP most efficiently at pH 6.5 [for poly(P)-dependent activity] and pH 8.0 (for ATP-dependent activity). Poly(P) [tetrapolyphosphate, poly$(P)_4$] and ATP were utilized for the phosphorylation of NAD with similar efficiencies in the presence of bivalent metal ions such as Mg^{2+} and Mn^{2+} (Kawai *et al.*, 2000). The NAD kinase is a tetramer of identical subunits, each with a molecular weight of ~35 kDa. In contrast to the NAD kinase from *M. tuberculosis* H37Rv, the enzymes from *E. coli* and *Saccharomyces cerevisiae* use only ATP as a phosphoryl donor (Kawai, Mori *et al.*, 2001; Kawai, Suzuki *et al.*, 2001). The amino-acid sequence of *M. tuberculosis* H37Rv NAD kinase shows a high similarity to those of *E. coli* (51% similarity) and *S. cerevisiae* (49% similarity). However, the amino-acid sequences of these NAD kinases show little homology with those of other kinases whose structures have been determined.

It is a well accepted idea that poly(P) may have been the primitive 'energy source' for the generation of phosphate-group potential. As speculated in the case of poly(P)/ATP-glucokinase (Phillip *et al.*, 1993), the poly(P)/ATP-NAD kinase of *M. tuberculosis* H37Rv might be an 'intermediate enzyme' located between the 'fossil poly(P)-dependent NAD kinase' and 'present ATP-dependent NAD kinase' such as the *E. coli* and *S. cerevisiae* kinases. Therefore, the determination of the crystal structure of NAD kinase is indispensable for the clarification of the mechanisms underlying not only the NAD kinase reaction, but also the energy-carrier evolution. However, no crystal structures of NAD kinase have been reported.

We purified the NAD kinase, which was designated poly(P)/ATP-NAD kinase, from recombinant *E. coli* cells, crystallized it and determined its preliminary X-ray data. This is the first report on the crystallographic data of an NAD kinase.

2. Crystallization

Rv1695 was cloned from *M. tuberculosis* H37Rv genomic DNA by the PCR method and was expressed in *E. coli* using the vector pET3a (Novagen). The recombinant NAD kinase was purified from the cell extract of the *E. coli* by

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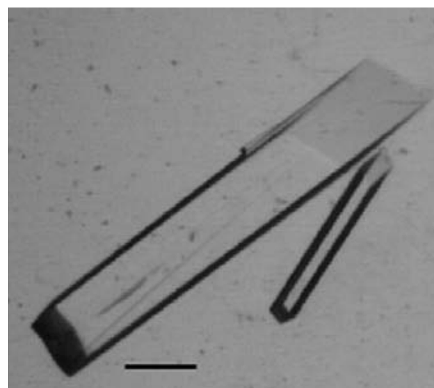

Figure 1
Crystal of NAD kinase from *M. tuberculosis* H37Rv. The scale bar is 0.1 mm long.

the same procedures as described previously (Kawai *et al.*, 2000). The purified NAD kinase was dissolved in 10 m*M* potassium phosphate pH 7.0 containing 0.2 m*M* NAD, 1 m*M* ethylenediaminetetraacetic acid and 0.5 m*M* dithiothretiol, and the solution was concentrated by ultrafiltration with a Centrisalts (Saltrius) to a final concentration of 6 mg ml^{-1}. Protein concentrations were determined by the method of Bradford (1976).

The crystallization of the NAD kinase was achieved by the hanging-drop vapour-diffusion method on Linbro tissue-culture plates. A hanging-drop (6 µl), prepared by mixing 3 µl each of the reservoir solution (1.8% polyethylene glycol 4000, 0.1 *M* sodium 2-morpholinoethanesulfonic acid buffer pH 6.0) and the protein solution described above, on a siliconized cover slip over 0.5 ml of the reservoir solution described above, produced prismatic colourless crystals in about 7 d at 293 K, which grew to a maximum dimension of 0.5 mm (Fig. 1).

Table 1
Data-collection statistics for NAD kinase crystal.

Values in parentheses refer to data in the highest resolution shell.

X-ray source	Cu $K\alpha$
Wavelength (Å)	1.54
Resolution (Å)	25.5–2.99 (3.17–2.99)
Space group	*C*2
Unit-cell parameters (Å, °)	a = 140.0, b = 69.3, c = 106.3, $\alpha = \gamma$ = 90, β = 130.01
Total observations	42821
Independent reflections	15802 (2527)
Completeness (%)	98.9 (96.6)
$I/\sigma(I)$	2.73 (1.85)
$R_{\rm sym}$ (%)	4.3 (35.0)

3. X-ray analysis

A crystal was mounted in a thin-walled glass capillary for X-ray analysis. Both ends of the capillary were filled with the reservoir solution and then sealed with wax. The diffraction data for a native crystal were collected to 2.99 Å with a Bruker Hi-Star multiwire area detector at 293 K using Cu $K\alpha$ radiation generated by a MacScience M18XHF rotating-anode generator and were processed with the *SADIE* and *SAINT* software packages (Bruker).

Preliminary characterization of the NAD kinase indicated the monoclinic space group *C*2, with unit-cell parameters a = 140.01, b = 69.3, c = 106.3 Å, β = 130.1°. From the 33 857 reflections observed, 12 763 independent reflections were obtained, with an $R_{\rm sym}$ value of 4.3%. The data exhibited a completeness of 98.9% to 2.99 Å resolution. Data-collection statistics for the NAD kinase crystal are summarized in Table 1. Two subunits of the NAD kinase per asymmetric unit give a $V_{\rm M}$ value of 3.28 Å^3 Da^{-1} and a solvent content of 62%. The $V_{\rm M}$ value and solvent content lie within the range usually found for protein crystals (Matthews, 1968).

A search for heavy-atom derivatives for phasing with the multiple isomorphous replacement method is now in progress.

References

Bradford, M. M. (1976). *Anal. Biochem.* **72**, 248–254.

Kawai, S., Mori, S., Mukai, T., Hashimoto, W. & Murata, K. (2001). In the press.

Kawai, S., Mori, S., Mukai, T., Suzuki, S., Yamada, T., Hashimoto, W. & Murata, K. (2000). *Biochem. Biophys. Res. Commun.* **276**, 57–63.

Kawai, S., Suzuki, S., Mori, S. & Murata, K. (2001). In the press.

Magni, G., Amici, A., Emanuelli, M. & Raffaelli, N. (1999). *Adv. Enzymol. Relat. Areas Mol. Biol.* **73**, 135–182.

Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.

Moat, G. A. & Foster, J. W. (1987). *Pyridine Nucleotide Coenzymes*, Part A, edited by D. Dolphin, R. Poulson & O. Avramovic, p. 2. New York: John Wiley & Sons.

Murata, K., Uchida, T., Tani, K., Kato, J. & Chibata, I. (1980). *Agric. Biol. Chem.* **44**, 61–68.

Phillip, N. F. B., Horn, P. J. & Wood, H. G. (1993). *Arch. Biochem. Biophys.* **300**, 309–319.