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# Crystallization and preliminary X-ray crystallographic analysis of quinolinate phosphoribosyltransferase of *Helicobacter pylori*

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Quinolinic acid phosphoribosyltransferase (NadC; EC 2.4.2.19) is the key enzyme of $NAD^+$ biosynthesis in both prokaryotes and eukaryotes. NadC catalyzes the decarboxylation of quinolinic acid (QA) to produce nicotinic acid mononucleotide (NAMN), an intermediate in NAD synthesis. NadCs of *Helicobacter pylori* appeared to be a hexamer during the purification procedure. Three different complexes of NadC, with QA, NAMN and phthalic acid (PA), an analogue of QA, were crystallized at 294 ± 1 K using the hanging-drop vapour-diffusion method. The QA complex crystal was found to belong to space group $P4_12_12$, with unit-cell parameters $a = b = 148.8$, $c = 145.7$ Å, $\alpha = \beta = \gamma = 90^\circ$. Diffraction data were collected from the NadC–substrate and NadC–substrate analogue complexes to resolutions of 2.3 Å (QA), 2.8 Å (PA) and 3.3 Å (NAMN) using synchrotron X-ray radiation.

## 1. Introduction

Quinolinic acid phosphoribosyltransferase (NadC; EC 2.4.2.19) is a key enzyme of $NAD^+$ biosynthesis in both prokaryotes and eukaryotes (Foster & Moat, 1980). Nicotinic acid mononucleotide (NAMN) is formed by NadC as an intermediate during NAD synthesis by decarboxylation of QA. Quinolinate is a degradation product of tryptophan in eukaryotes. The reaction mediated by NadC occurs mainly in the liver and kidney. In prokaryotes, QA is produced from dihydroxyacetone phosphate and L-aspartate by the *nadA* and *nadB* genes (Foster & Moat, 1980).

The three-dimensional structures of NadC from *Mycobacterium tuberculosis* and *Salmonella typhimurium* have been solved in complex with substrates (QA, NAMN) and substrate analogues (PA, PRPCP) (Eads *et al.*, 1997; Sharma *et al.*, 1998). In these structures, NadC was observed to be a dimer. Dimeric NadCs have also been observed from *M. tuberculosis* (Sharma *et al.*, 1998) and the caster bean (Mann & Stewart, 1974). In contrast, hexameric NadCs have only been reported in mammals such as hog (Iwai & Taguchi, 1974), rat (Okuno & Schwarcz, 1985) and human (Okuno *et al.*, 1988).

Here, we report the overexpression, purification and crystallization of *Helicobacter pylori* NadC in three different complexed forms, complexed with QA, NAMN and PA. Interestingly, the NadC of *H. pylori* purified as a hexamer during gel filtration under physiological conditions. In this paper, we report the first crystallographic studies on hexameric NadC.

## 2. Materials and methods

### 2.1. Expression and purification

Genomic DNA of *H. pylori* (ATCC 700392) was used as a template for PCR amplification and the plasmid vector pET-21a (+) (Novagen) was used to add a hexahistidine tag at the carboxy-terminus of the NadC protein for affinity purification. PCR products were purified using a PCR cleanup kit (Viogene) and digested with *Nde*I and *Xho*I (NEB). The digested PCR product was ligated into pET-21a(+). The resulting plasmid was used to transform *Escherichia coli* strain C41(DE3) to achieve high expression levels (Miroux & Walker, 1996). The overexpressed NadC protein was initially purified by Ni–NTA affinity chromatography (Peptron). Partially purified protein was further purified on a Superdex-200 gel-filtration column (Pharmacia). The selenomethionine derivative of NadC was expressed in the met(−) strain B834 (Novagen) grown in minimal medium supplemented with seleno-L-methionine (Sigma) and other nutrients and was purified using the same procedure as used for the native protein.

### 2.2. Crystallization and data collection

*H. pylori* NadC crystals were grown at 294 ± 1 K using the hanging-drop vapour-diffusion method (Hampel *et al.*, 1968). The best crystals were obtained from drops prepared by mixing 2 µl of protein solution (15 mg $ml^{-1}$ NadC in 20 m*M* HEPES–NaOH pH 7.5 and 100 m*M* KCl) and 2 µl of drop solution (0.1 *M* HEPES–NaOH pH 7.0, 1.1 *M* $Li_2SO_4$). Hanging drops were equilibrated

against a reservoir solution composed of 0.1 *M* HEPES–NaOH pH 7.0, 1.5 *M* $Li_2SO_4$. Large crystals grew to maximum dimensions of 0.8 × 0.6 × 0.6 mm over one week. 2 m*M* QA was added to the drop solution in order to prepare crystals of the QA complex.

The selenomethionine-labelled NadC–PA co-crystals and native NadC–NAMN co-crystals were grown under the same conditions, but 2 m*M* PA or 5 m*M* NAMN were added to the drop solution instead of QA, respectively. Crystals complexed with substrate (QA, NAMN) and substrate analogue (PA) grew over 7 d to a maximum dimension of 0.8 mm (Fig. 1).

For data collection, all crystals were transferred to a cryoprotection solution containing 40%(*v*/*v*) PEG 300, 100 m*M* HEPES–NaOH pH 7.5 and 200 m*M* sodium chloride. Data were collected at 100 ± 1 K using a Dual Image Processing System at the BL6B beamline of the Pohang Accelerator Laboratory. Data statistics are listed in Table 1. Data sets were processed and scaled using the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997). The data-collection statistics are summarized in Table 1.

**Table 1**
Summary of the data statistics.

Values in parentheses indicate statistics for the last resolution shell.

| | | PA complex (SeMet) | | | |
|---|---|---|---|---|---|
| | QA complex | Peak | Inflection | Remote | NAMN complex |
| X-ray source | BL6B (PLS) | BL6B (PLS) | BL6B (PLS) | BL6B (PLS) | BL6B (PLS) |
| Wavelength (Å) | 1.12714 | 0.9794 | 0.9796 | 0.9718 | 0.9794 |
| Resolution (Å) | 2.3 | 2.8 | 2.8 | 2.8 | 3.3 |
| Space group | $P4_12_12$ | $P4_12_12$ | $P4_12_12$ | $P4_12_12$ | $P4_12_12$ |
| Unit-cell parameters | | | | | |
| $a, b$ (Å) | 148.8 | 149.5 | 149.5 | 149.4 | 147.8 |
| $c$ (Å) | 145.6 | 146.1 | 146.2 | 146.0 | 144.1 |
| No. of reflections | 325942 | 243878 | 247545 | 257096 | 140384 |
| Unique reflections | 73432 | 40644 | 41016 | 40554 | 24834 |
| Completeness (%) | 93.6 | 96.4 (90.3) | 96.3 (89.8) | 96.7 (91.6) | 91.3 |
| $R_{sym}$† | 0.031 (0.139) | 0.072 (0.189) | 0.062 (0.189) | 0.063 (0.154) | 0.050 (0.169) |
| $I/\sigma(I)$ | 43.2 (8.7) | 19.0 (4.9) | 19.1 (4.5) | 20.7 (7.1) | 25.5 (5.9) |

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

## 3. Results

Attempts to determine the crystal structure of *H. pylori* NadC by molecular replacement (MR) using the known structures as starting models were unsuccessful owing to their lack of similarity. MR was performed with *CNS* (Brünger *et al.*, 1998) using the models from previously solved structures of the NadCs of *M. tuberculosis* and *S. typhimurium* at 2.4 and 2.8 Å resolution, respectively (PDB codes 1qap and 1qpo; Eads *et al.*, 1997; Sharma *et al.*, 1998). A MAD data set was collected from NadC complexed with PA using a Dual Image Processing System at the BL6B beamline of Pohang Accelerator Laboratory. The diffraction limit of the MAD data set was 2.8 Å and the unit-cell parameters were $a = b = 149.5$, $c = 146.1$ Å. Selenomethionine-labelled NadC was used for MAD phasing.

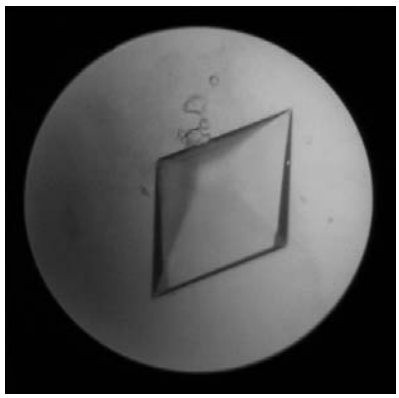

**Figure 1**
A crystal of NadC grown for 7 d using 1.5 *M* $Li_2SO_4$, 100 m*M* HEPES–NaOH pH 7.5 and 2 m*M* PA. Its approximate dimensions were 0.5 × 0.5 × 0.8 mm. NadC–QA and NadC–NAMN co-crystals were found to have the same morphology.

The QA complex data set of NadC indicates that the crystal belongs to space group $P4_12_12$ (or $P4_32_12$), with unit-cell parameters $a = b = 148.8$, $c = 145.7$ Å, $\alpha = \beta = \gamma = 90^\circ$. The initial phases of the QA complex crystal structure were obtained by the three-wavelength MAD phasing method using the *K* edge anomalous signal of the Se atoms. The space group was determined to be $P4_12_12$. 28 Se sites were found using the heavy-atom search routine and were refined in *SOLVE*/*RESOLVE* (Terwilliger & Berendzen, 1997). The figure of merit was 0.58 between 20 and 2.8 Å resolution. The initial model was built into the density using *O* (Jones *et al.*, 1991). The asymmetric unit of this unit cell contained three molecules and $V_M$ was calculated to be 4.36 $Å^3\,Da^{-1}$, corresponding to a solvent content of 71.8% (Matthews, 1968).

The NAMN complex data set was collected. The resolution limit of the data was 3.3 Å and the unit-cell parameters were $a = b = 147.8$, $c = 144.1$ Å. Crystals were soaked in cryoprotectant solution containing 40% PEG 300, 100 m*M* HEPES–NaOH pH 7.5 and 200 m*M* sodium chloride.

The MAD phased electron-density map was calculated at a resolution of 2.8 Å and showed electron density corresponding to the PA. The electron density of QA and the NAMN were also identified in the electron-density map. Models complexed with QA and NAMN were built from the initial model complexed with PA by rigid-body refinement using *CNS* (Brünger *et al.*, 1998) and *O* (Jones *et al.*, 1991). Currently, *H. pylori* NadC structures are being subjected to model building and refinement.

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## References

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.

Eads, J. C., Ozturk, D., Wexler, T. B., Grubmeyer, C. & Sacchettini, J. C. (1997). *Structure*, **5**, 47–58.

Foster, J. W. & Moat, A. G. (1980). *Microbiol. Rev.* **44**, 83–105.

Hampel, A., Labanauskas, M., Connors, P. G., Kirkegard, L., RajBhandary, U. L., Sigler, P. B. & Bock, R. M. (1968). *Science*, **162**, 1384–1387.

Iwai, K. & Taguchi, H. (1974). *Biochem. Biophys. Res. Commun.* **56**, 884–891.

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

Mann, S. O. & Stewart, C. S. (1974). *J. Gen. Microbiol.* **84**, 379–382.

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

Miroux, B. & Walker, J. E. (1996). *J. Mol. Biol.* **260**, 289–298.

Okuno, E. & Schwarcz, R. (1985). *Biochim. Biophys. Acta*, **841**, 112–119.

Okuno, E., White, R. J. & Schwarcz, R. (1988). *J. Biochem. (Tokyo)*, **103**, 1054–1059.

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

Sharma, V., Grubmeyer, C. & Sacchettini, J. C. (1998). *Structure*, **6**, 1587–1599.

Terwilliger, T. C. & Berendzen, J. (1997). *Acta Cryst.* D**53**, 571–579.