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

Expression, purification, crystallization and preliminary X-ray analysis of an NAD-dependent glyceraldehyde-3-phosphate dehydrogenase from *Helicobacter pylori*

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Received 24 February 2008
Accepted 2 July 2008

Helicobacter pylori is a dangerous human pathogen that resides in the upper gastrointestinal tract. Little is known about its metabolism and with the onset of antibiotic resistance new treatments are required. In this study, the expression, purification, crystallization and preliminary X-ray diffraction of an NAD-dependent glyceraldehyde-3-phosphate dehydrogenase from *H. pylori* are reported.

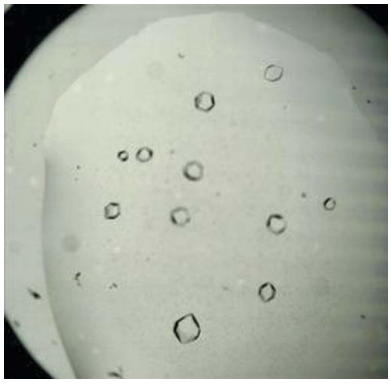
1. Introduction

Helicobacter pylori, a micro-aerophilic Gram-negative rod-shaped bacteria, colonizes the human upper gastrointestinal tract. *H. pylori* infection is more common in developing countries than in developed countries, with 70–90% of the population of developing countries carrying the bacteria, compared with 25–50% of the population of developed countries (Dunn *et al.*, 1997). Infection with *H. pylori* is implicated as being the cause of a variety of diseases including gastric and peptic ulcers, gastric cancer and mucosal-associated lymphoma (Kuipers *et al.*, 1995). Patients infected with *H. pylori* have a 20% lifetime risk of developing peptic ulcer disease and a 2% risk of developing gastric cancer (Ernst, 2000; Kuipers, 1999).

Treatment of *H. pylori* infection consists of a triple therapy comprising of treatment with two antibiotics, typically metronidazole and amoxicillin, coupled with proton-pump inhibitors (Cavallaro *et al.*, 2006). With high incidences of antibiotic resistance, quadruple therapies involving bismuth salts and three antibiotics are commonly being used (Gisbert & Pajares, 2002). These treatments have a variety of side effects (Perri *et al.*, 2001). A combination of these side effects and the prolonged and intense nature of treatment often results in patients not completing treatment, resulting in further incidences of antibiotic resistance and making eradication of the bacteria increasingly difficult (Egan *et al.*, 2007). Therefore, new alternative methods of treatment are required.

Analysis of the genome of *H. pylori* suggested that its only carbohydrate source was glucose (Tomb *et al.*, 1997). This was confirmed by the *in vitro* growth requirements of the bacteria (Mendz *et al.*, 1993) and the fact that the only carbohydrate transporter is glucose permease (Alm *et al.*, 1999). Genomic analysis also suggested that the main carbohydrate catabolic pathway, glycolysis, is incomplete (Alm *et al.*, 1999; Tomb *et al.*, 1997). However, the early steps in glycolysis can be bypassed by the Entner–Doudoroff pathway (which is constitutively induced in *H. pylori*; Mendz *et al.*, 1994). The entry point of the Entner–Doudoroff pathway into glycolysis is through glyceraldehyde-3-phosphate, which is also a key substrate for the pentose-phosphate pathway (required for the synthesis of five-carbon sugars for nucleotide biosynthesis and for the generation of NADPH for oxidative-stress prevention). In glycolysis and gluconeogenesis, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) catalyses the reversible oxidative phosphorylation of glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate.

Two GAPDH genes have been identified in *H. pylori*: *gapA* (gi:6626253) and *gapB* (gi:2494645). The expression and crystallization of *gapA* is described in an accompanying paper (Elliott *et al.*,

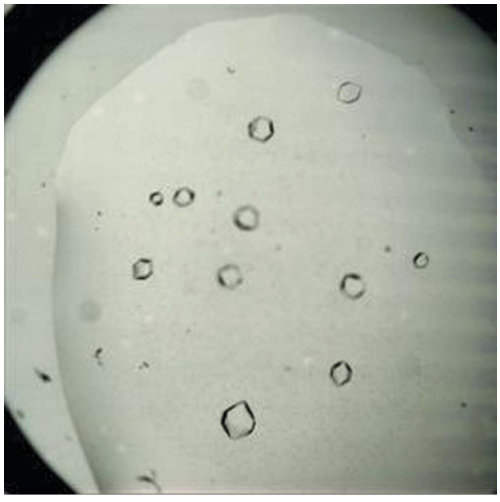


2008). Here, we report the cloning of *gapB*, its expression, and the purification and preliminary X-ray analysis of its gene product GAPDHB. Solution work to be published elsewhere confirms that GAPDHB is an NAD-dependent GAPDH that is able to utilize both glyceraldehyde-3-phosphate and erythrose-4-phosphate in a phosphate-dependent manner.

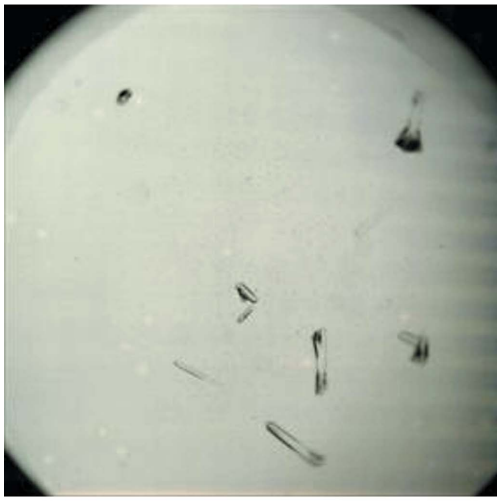
2. Materials and methods

2.1. Cloning and overexpression

The full nucleotide sequences encoding the *gapB* sequence (gi:2494645, NCBI, NIH) were cloned into TOPO pET151/D (Invitrogen) containing an N-terminal His_6 tag linked by a TEV protease site. The primer sequences for the forward and reverse amplification of *gapB* were CACCATGAAAATTTTTATCATTGGATTG and TTAATAATGATACATAACTGG, respectively. Dideoxy sequencing confirmed the presence of the full-length *gapB* sequence. *Escherichia coli* strain Rosetta DE3 transformed with pET151/D-GAPDHB was grown at 303 K in the rich medium 2YT supplemented with 100 µg ml^{-1} ampicillin and 35 µg ml^{-1} chloramphenicol. Upon reaching an OD_{600} of 0.6, the cultures were cooled to 291 K and isopropyl β-D-1-thiogalactopyranoside was added to a final concentration of 100 µ*M*. Cells were left overnight at 291 K, harvested by centrifugation approximately 20 h after induction and immediately frozen.



(*a*)



(*b*)

Figure 1
Two distinct crystal forms of GAPDHB were obtained after 48 h in 100 m*M* acetate pH 4.0, 38% 2-methyl-2,4-pentanediol. The form *A* crystals in (*a*) diffracted to 2.8 Å resolution, whilst the form *B* crystals in (*b*) did not diffract beyond 11 Å. 500 µl drops are shown.

2.2. Purification

Purification of His_6-tagged GAPDHB took place as follows. Frozen cell pellets were thawed in buffer *A* (20 m*M* Na_2HPO_4, 500 m*M* NaCl, 50 m*M* imidazole pH 7.4 supplemented with protease-inhibitor cocktail VII; Calbiochem). The suspension was sonicated and cell debris was removed by centrifugation. The supernatant was loaded onto a pre-equilibrated 5 ml Hi-Trap Nickel Sepharose (Amersham Biosciences) column and eluted with a linear gradient of buffer *B* (20 m*M* Na_2HPO_4, 500 m*M* NaCl, 500 m*M* imidazole pH 7.4). Fractions containing enzymatic activity were pooled and dialysed extensively against buffer *C* (20 m*M* Na_2HPO_4, 50 m*M* NaCl, 1 m*M* DTT pH 7.2). The sample was loaded onto a 5 ml Hi-Trap Sulfopropyl Sepharose cation-exchange column and eluted with a linear gradient of buffer *D* (20 m*M* Na_2HPO_4, 1 *M* NaCl, 1 m*M* DTT pH 7.2). A single peak was collected at 550 m*M* NaCl and was judged to be ∼95% pure by SDS–PAGE analysis. GAPDHB was concentrated to 8 mg ml^{-1} using an Amicon Ultra-15 centrifugal filter unit (10 kDa molecular-weight cutoff; Millipore) and the buffer was exchanged to 20 m*M* MES, 100 m*M* NaCl, 1 m*M* DTT pH 6.5 prior to crystallization.

2.3. Crystallization and data collection

Crystallization trials for the hexahistidine-tagged GAPDHB were performed in the presence of 1 m*M* NAD. A total of 192 crystal-growth conditions were screened by vapour diffusion using 100 nl drops of protein solution mixed with 100 nl precipitant from the

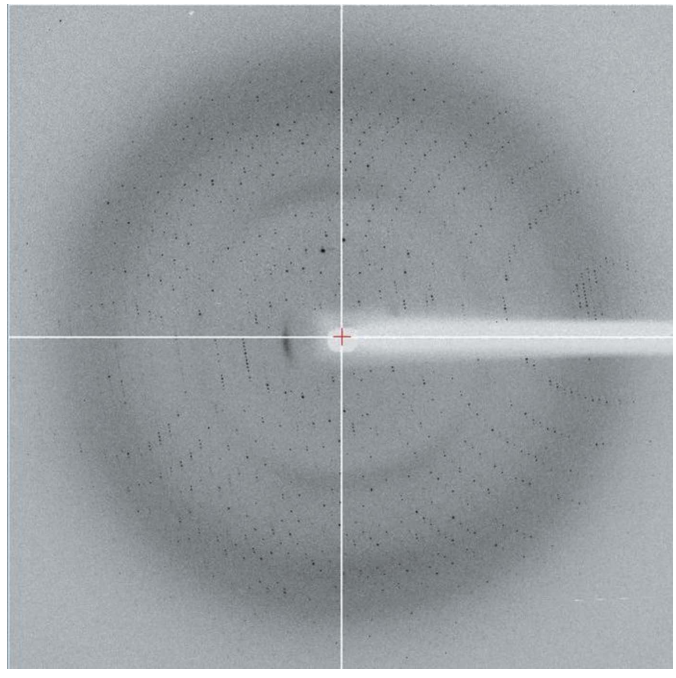


Figure 2
Typical diffraction pattern of crystal form *A*. Data were collected with a crystal-to-detector distance of 269.6 mm. Images of 1° oscillation were collected over 120° at a fixed wavelength of 0.931 Å.

Table 1
Summary of data-collection statistics for GAPDHB.

Values in parentheses are for the highest resolution shell.

Space group	$P6_522$
Unit-cell parameters (Å, °)	$a = b = 166.1$, $c = 253.1$, $\alpha = \beta = 90.0$, $\gamma = 120.0$
Resolution limits	83.1–2.8 (2.95–2.80)
No. of observations	393489 (57245)
No. of unique observations	50370 (7302)
Completeness	98.4 (99.6)
$\langle I/\sigma(I)\rangle$	17.0 (6.1)
$R_{\rm merge}$†	0.094 (0.32)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of an individual measurement of the reflection with Miller indices hkl and $\langle I(hkl)\rangle$ is the mean intensity of that reflection.

crystal screen kits Wizard I and II and Cryo I and II (Emerald Biosciences) dispensed with a Genomics Solutions Cartesian Honeybee 8+1 (Harvard Bioscience) onto 96-well MRC plates (Innovadyne) with reservoirs containing 80 µl of precipitant in a humidity chamber. Plates were sealed with transparent tape and monitored for crystal growth using CrystalProHT (TriTek) plate-storage and imaging systems at 277 and 293 K.

Several crystallization hits were recorded approximately 4 d after the plates were sealed. Crystallization conditions were predominately from the Cryo screens (Emerald Biosciences), with a preference for low pH and small-molecular-weight PEGs as a precipitant. Conditions yielding crystals were optimized using a Tecan 75 liquid-handling robot (Tecan) and 500 nl drops of protein and precipitant were dispensed from the Cartesian Honeybee 8+1 (Harvard Bioscience). Suitable crystals of diffraction quality grew from 8 mg ml^{-1} GAPDHB containing 1 m*M* NAD mixed with an equal volume of reservoir containing 100 m*M* acetate pH 4.0 and 38%(*v*/*v*) 2-methyl-2,4-pentanediol. Crystals grew after 48 h at 277 K, with two distinct crystal forms present in the drops (Figs. 1*a* and 1*b*).

Crystals were prepared for cryocrystallography by harvesting the crystals directly into a stream of boiled-off liquid nitrogen at 110 K. The crystallization condition acted as a suitable cryoprotectant and no further rounds of optimization were required.

Diffraction data were collected on the ID14-3 beamline at the ESRF, Grenoble at 0.931 Å on an ADSC Q4R CCD detector; a typical diffraction pattern is shown in Fig. 2. Intensities were measured using *MOSFLM* (Leslie, 1992), with the autoindexing routines giving a solution consistent with a primitive hexagonal cell. The data were scaled using *SCALA* (Evans, 2006) and were consistent with the Laue group 622. Analysis of the distribution of intensities along the principal axes indicated the presence of either a 6_1 or a 6_5 screw axis. Molecular replacement using GAPDH from *Bacillus stearothermophilus* (PDB code 1gd1; Skarzynski *et al.*, 1987) as a search model in *Phaser* (Read, 2001; the sequence identity between the two enzymes is 43%) gave a solution that was only consistent with space group $P6_522$ (data-collection statistics are given in Table 1).

3. Results and discussion

GAPDHB has been cloned, expressed and purified. Crystals of GAPDHB grew with the hexahistidine tag present and diffracted to 2.8 Å resolution. Preliminary electron-density analysis of GAPDHB confirmed that NAD was bound. Solution work, which will be presented elsewhere, demonstrated that GAPDHB catalyses the oxidative phosphorylation of glyceraldehyde-3-phosphate and erythrose-4-phosphate. Work is currently in progress to improve the diffraction quality of the crystals and to produce ternary complex analogues with glyceraldehyde-3-phosphate and with erythrose-4-phosphate in order to understand the apparent dual substrate-specificity.

We would like to thank the beamline scientists at ID14-3 of ESRF. PRE was supported by an MRC studentship.

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