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Crystallization and preliminary X-ray diffraction studies on the N-utilizing substance-B (NusB) from *Mycobacterium tuberculosis*

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N-utilizing substance B (NusB) is a protein which forms part of a complex assembly in transcriptional antitermination in *Mycobacterium tuberculosis*. It forms a heterodimer with the product of the *NusE* gene (identical to the ribosomal protein S10) and mediates the process of transcriptional antitermination by forming the core complex with the *nut* site of the ribosomal RNA along with other protein factors. NusB has been cloned and overexpressed in *Escherichia coli* and crystallized using the hanging-drop vapour-diffusion method. The space group is $P2_12_12_1$, with unit-cell parameters $a = 46.6$, $b = 64.2$, $c = 90.1$ Å. A native data set complete to 1.6 Å resolution has been collected from a single crystal.

1. Introduction

Modulation of the efficiency of transcription termination appears to be one of the mechanisms employed by the bacteriophage λ to switch between the lysogenic and the lytic cycle (Richardson & Greenblatt, 1996). This phenomenon is mediated by the phage protein N and also involves host protein factors, collectively designated nus for N-utilizing substance. The ribonucleoprotein complex effecting transcription antitermination appears to require at least four host proteins: NusA, NusB, NusE and NusG, which assemble on the *nut* site of RNA (Mogridge *et al.*, 1998). NusB and NusE from *E. coli* have been shown to form a heterodimer *in vitro*. Although the role of the Nus proteins in bacterial cells is less well understood, antitermination mediated by these proteins has been reported to play a regulatory role in rRNA biosynthesis (Keener & Nomura, 1996).

2. Materials and methods

2.1. Cloning, expression and purification

The gene corresponding to the NusB protein of *M. tuberculosis* was PCR amplified using the following primers: *Nde*1–NusB, CCTATACTA CATATGTCGGACAGAAAGCC; *Bam*H1–NusB, CCTCGGATCCTAGGCGGCGACAC CAC. This was ligated into the Pet15b expression vector between the *Nde*1 and the *Bam*H1 sites, the corresponding sites in the insert having been introduced by the two primers. The plasmid was transformed into the *E. coli* strain BL21(DE3)pLysS (Novagen Inc). The cells were induced with IPTG when the culture density reached $A_{600} = 0.8$ and were grown for another 6 h before they were harvested. The protein was extracted from inclusion bodies using urea. The first step of purification utilizing the His-tag in the N-terminus was performed using metal affinity chromatography with TALON resin (Clontech Inc). The protein was bound at pH 8.0 and eluted at pH 6.0 using the buffer compositions suggested by the manufacturer. Refolding was achieved by slow dialysis to remove the urea in steps of 1 *M* from 8.0 *M* to a final solution containing 50 m*M* Tris buffer pH 7.5, 200 m*M* NaCl. The protein was further purified using size-exclusion chromatography on a Superdex S-75 column (Pharmacia Inc) equilibrated in 50 m*M* sodium phosphate pH 6.5, 200 m*M*

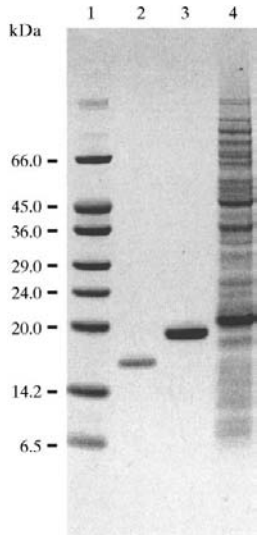

Figure 1
Electrophoretic analysis of NusB. Lane 1, low-range molecular-weight markers (6.5–66 kDa; Sigma); lane 2, native NusB, no His-tag; lane 3, native NusB with His-tag; lane 4, crude cell lysate of induced NusB.

NaCl. The protein eluted as a single peak at the elution volume corresponding to its molecular weight of 18.7 kDa. The purity of the protein was analyzed by SDS–PAGE (Fig. 1) on a 10% NuPAGE Bis–Tris gel with MES–SDS running buffer (Novex-Electrophoresis GmbH). The protein solution was concentrated by ultrafiltration using a Centricon-10 microconcentrator (Amicon Inc.) to a final concentration of 17 mg ml^{-1}. Protein concentration was estimated by measuring the absorbance at 280 nm and employing the extinction coefficient 12 660 M^{-1} cm^{-1}.

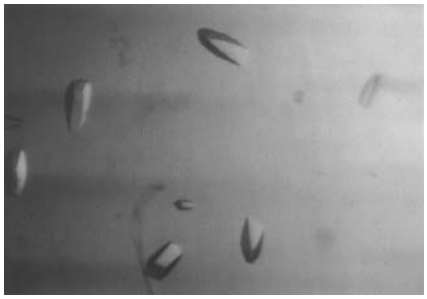

Figure 2
Crystals of NusB as grown by the hanging-drop method. The average dimensions of these crystals were 100 × 60 × 40 µm.

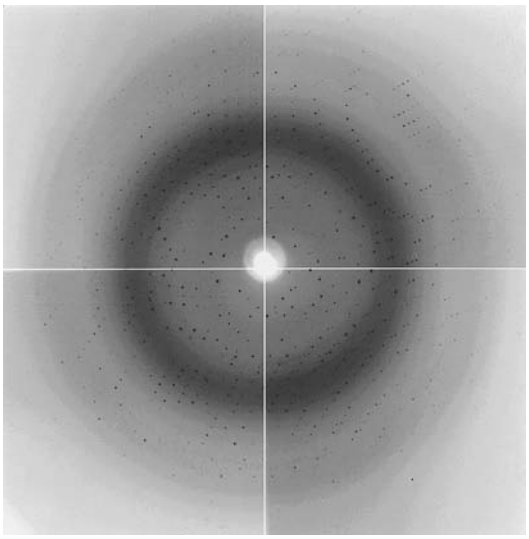

Figure 3
0.5° oscillation image collected on the CCD at beamline 9.6, SRS, Daresbury. The crystal-to-detector distance was set to 160 mm and the exposure time for each frame was 1 min.

3. Results and discussion

3.1. Crystallization

All crystallization experiments were performed using the hanging-drop vapour-diffusion method in 24-well tissue-culture plates (ICN Flow) at 291 K. Drops contained 1 µl of the respective reservoir solution and 1 µl of protein solution.

The initial crystallization trials were carried out using reservoirs consisting of 1 ml of Hampton Research Crystal Screen (Jancarik & Kim, 1991). Small clusters of crystals were obtained under condition number 39 (2.0 *M* ammonium sulfate, 0.1 *M* Na HEPES buffer pH 7.5, 2% PEG 400). The protein concentration used was 9 mg ml^{-1}. Further trials optimizing these conditions yielded crystals which appeared amenable to diffraction studies. However, crystals grown under these conditions exhibited anisotropic diffraction patterns upon flash-freezing using 20% glycerol in mother liquor as cryoprotectant. Improved crystals (Fig. 2) were obtained by increasing the pH of the crystallization solution to 8.0 with 0.1 *M* Tris–HCl buffer. These crystals were used to collect native data and were also employed in the search for heavy-atom derivatives. Crystals could not be obtained when the protein was in any buffer other than sodium phosphate pH 6.5 with 200 m*M* NaCl, thus implying that a trace of phosphate may be crucial for crystallization.

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               10        20        30        40        50        60
MtNusB MSDRKPVRGRHQARKRAVALLFEAEVRGISAAEVVDTRAALAEAKPDIARLHPYTAAVAR
          ::. .:..::. ::  :.  ..   . :.:     :  ..: :.  :  :   .
EcNusB    MKPA-ARRRARECAVQALYSWQLSQNDIADVEYQFLAEQDVK-DVDVL--YFRELLA
                  10        20        30        40            50

               70        80        90       100       110       120
MtNusB GVSEHAAHIDDLITAHLRGWTLDRLPAVDRAILRVSVWELLHAADVPEPVVVDEAVQLAK
       ::. ..:..: :.  .: .  :..:  :..:.::....:: . .:::  :...::..:::
EcNusB GVATNTAYLDGLMKPYL-SRLLEELGQVEKAVLRIALYELSKRSDVPYKVAINEAIELAK
            60        70        80        90       100       110

              130       140       150
MtNusB ELSTDDSPGFVNGVLGQVM-LVTPQLRAAAQAVRGGA
        ....::  :::::: ..  .. :.
EcNusB SFGAEDSHKFVNGVLDKAAPVIRPNKK
            120       130
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Figure 4
Sequence comparison of the *M. tuberculosis* NusB and *E. coli* NusB illustrating the extent of similarity between the two proteins (33.6% identity in a 140 amino-acid overlap).

3.2. Data collection and processing

Diffraction data was collected from cryo-cooled (100 K) crystals on a Quantum ADSC (Area Detector Systems Corporation) CCD detector on beamline 9.6 at the Daresbury Synchrotron Radiation Source. Data were collected at 0.5° oscillation with the crystal-to-detector distance set to 160 mm. Data were processed using the *HKL* program package (Otwinowski & Minor, 1997). The space group has been assigned as $P2_12_12_1$ based on the inspection of systematic absences. Two NusB molecules per asymmetric unit yields a Matthews coefficient of 2.00 Å^3 Da^{-1} and an estimated solvent content of 38% (Matthews, 1968). The data are 95.2% complete to 1.6 Å resolution, having a total number of 34 188 reflections. The completeness in the 1.7–1.6 Å shell is 75%. $R_{\rm merge}$ for the entire data set collected on a single crystal is 5.2% (22.5% for the outer shell). Fig. 3 shows an oscillation X-ray photograph obtained from a crystal of NusB.

Molecular-replacement trials performed using *AMoRe* (Navaza, 1994) with the NMR structure of *E. coli* NusB (Huenges *et al.*, 1998) as the starting model failed to identify a solution (SWISS-PROT database references: *M. tuberculosis* NusB, P95020; *E. coli* NusB, P04381; Fig. 4). Therefore, MIR (multiple isomorphous replacement)/MAD (multiwavelength anomalous diffraction) experiments are being planned to facilitate the phasing. A search for heavy-atom derivatives and production of selenomethionine-substituted protein are in progress.

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