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

B. Gopal,[a,b] K. G. Papavinasasundaram,[b] M. J. Colston,[b] G. Dodson[a] and Lesley F. Haire[a]*

[a]Division of Protein Structure, The National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, England, and [b]Division of Mycobacterial Research, The National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, England

Correspondence e-mail: lhaire@nimr.mrc.ac.uk

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N-utilizing substance A (NusA) is a protein which performs several roles as a cofactor of DNA-dependent RNA polymerase. Its acts as an elongation factor and facilitates pausing, termination and the formation of a complex assembly that mediates transcription antitermination in eubacteria. Biochemical and biophysical data in the literature suggest that this protein performs these functions by binding to the core RNA polymerase, other protein factors and certain RNA fragments having specific signal sequences. The NusA of *Mycobacterium tuberculosis* has been cloned and overexpressed in *Escherichia coli* and crystallized using the hanging-drop vapour-diffusion method. The space group is $P3_121$, with unit-cell parameters $a = b = 78.1$, $c = 180.3$ Å. A native data set complete to 1.7 Å resolution has been collected from a single crystal.

1. Introduction

DNA-dependent RNA polymerases make use of several protein cofactors to facilitate transcription (Landick, 1999). These proteins serve to guide transcription by recognizing signal sequences along the DNA template/RNA and thus modulate a host of transcriptional processes. A key protein which is involved in this process is NusA, which binds to RNA polymerase as transcription enters the elongation stage (Richardson & Greenblatt, 1996). NusA has been shown to interact with the same surface of the RNA polymerase as the sigma factor sigA (Traviglia *et al.*, 1999). We chose to characterize this protein structurally in order to identify the specific role that it plays in the ribonucleoprotein complex effecting transcription antitermination. This complex consists of at least five protein components, RNA polymerase, NusA, NusB, NusE and NusG, which assemble on an RNA sequence called the *nut* site (Mogridge *et al.*, 1998). This complex allows prokaryotic RNA polymerases to over-read transcription termination sites, thus enabling genes located further downstream to be expressed. These factors were first characterized in phage λ infection of *E. coli*, where the λ N protein solicits the assistance of these factors to facilitate antitermination; hence the term 'Nus' for N-utilizing substances. 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). We have previously shown (Gopal *et al.*, 2000, 2001) *M. tuberculosis* NusB to be a dimer, as opposed to the *E. coli* homologue which is a monomer, and that NusE does not have substantial tertiary structure. Here, we report the cloning, expression, purification, crystallization and data-collection statistics of NusA.

2. Materials and methods

2.1. Cloning, expression and purification

The gene corresponding to the NusA protein of *M. tuberculosis* was PCR amplified using the following primers: *Nde*I-NusA, CTCTATTTAT**CATATG**AACATCGACAT-GGCTGCTCTG; *Bam*HI-NusA, TTAT-**GGATCC**GCCCGATTGCCCGGCTATCT. This was ligated into the pET15b expression vector between the *Nde*I and the *Bam*HI sites, the corresponding sites in the insert having been introduced by the two primers. These are highlighted in the primers above. The plasmid was transformed into the *E. coli* strain BL21(DE3)PlysS (Novagen Inc). The cells were initially grown in Terrific Broth at 310 K and were induced with IPTG when the culture density reached $A_{600} = 0.8$. They were grown for another 6 h at 298 K before harvesting. The cells were lysed in lysis buffer (50 m*M* phosphate buffer pH 7.5 containing 250 m*M* NaCl). As the protein seemed to be very susceptible to proteolysis, two protease inhibitor cocktail tablets (Complete, EDTA-free, Roche) were added every 2 h through the initial stages. 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 7.5, washed with the same buffer containing 5 m*M* imidazole and eluted using 300 m*M* imidazole. We eluted the protein in a single step as opposed to a gradient, as this substantially reduced the time frame involved and the

protein was less likely to be proteolytically degraded. 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 7.5, 200 m*M* NaCl. The protein eluted as a single peak at an elution volume corresponding to a molecular weight of 65 kDa. While this suggests it to be an oligomer, similar observations have been reported on *E. coli* NusA (Gill *et al.*, 1991); these have been demonstrated to be an artefact associated with an elongated molecule.

The purity of the protein was analyzed by SDS–PAGE on a 10% NuPAGE bis-tris gel with MES–SDS running buffer (Invitrogen). The protein solution was concentrated by ultrafiltration using a Centricon-10 microconcentrator (Amicon Inc.) to a final concentration of 8 mg ml^{-1}. Protein concentration was estimated by measuring the absorbance at 280 nm employing the extinction coefficient 17 780 M^{-1} cm^{-1}.

3. Results and discussion

3.1. Crystallization

Crystallization trials were carried out using both the automated microbatch technique (Chayen *et al.*, 1990) and the vapour-diffusion method. Various commercially available screens were used in addition to in-house screens. Microbatch droplets (0.5 µl screen solution plus 0.5 µl protein solution) were dispensed under paraffin oil using a computer-controlled microdispenser *IMPAX* (Douglas Instruments). The hanging drops consisted of 1 µl of the protein solution at a concentration of 8 mg ml^{-1} plus 1 µl of the reservoir solution equilibrated against 750 µl of the reservoir solution at 291 K.

Crystals of NusA were obtained using either vapour diffusion or the microbatch technique in conditions containing ammonium sulfate as precipitant over the pH range 6–7. Lithium or magnesium sulfate could be substituted for the ammonium salt. The hanging-drop method using 1.1 *M* ammonium sulfate and 0.1 *M* MES pH 6.1 as the reservoir solution yielded crystals suitable for X-ray diffraction (Fig. 1). The crystals were removed from the drop with a mounted loop and were transferred through a series of cryoprotectant solutions of increasing glycerol concentration (5–35%) before they were frozen. All these manipulations were carried out using a humidifier to prevent evaporation.

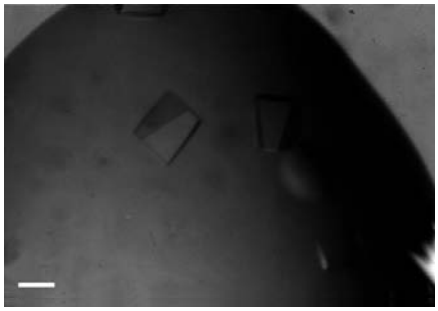

Figure 1
Crystals of NusA as grown by the hanging-drop method. The average dimensions of these crystals are 150 × 100 × 80 µm. The scale bar is 100 µm in length.

3.2. Data collection and processing

Diffraction data was collected from cryocooled (100 K) crystals on a Quantum ADSC (Area Detector Systems Corporation) CCD detector on beamline ID14 at the European Synchrotron Radiation Facility at Grenoble. Data were collected at 0.25° oscillation with the crystal-to-detector distance set to 180 mm. Data were processed using the *HKL* program package (Otwinowski & Minor, 1997). The space group was assigned as $P3_121$ or $P3_221$ based on the inspection of systematic absences. Two NusA molecules per asymmetric unit yields a Matthews coefficient of 2.4 Å^3 Da^{-1} and an estimated solvent content of 56% (Matthews, 1968). The data are 95.2% complete to 1.7 Å resolution, with a total number of 34 188 reflections; the refined value of the mosaicity is 0.35°. 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%. Fig. 2 shows an oscillation X-ray photograph obtained from a crystal of NusA. As repeated attempts to obtain selenomethionine-substituted NusA have thus far been unsuccessful, MIR (multiple isomorphous replacement) or SAD (single wavelength anomalous diffraction) experiments on crystals soaked with heavy-atom derivatives are under way to facilitate the phasing.

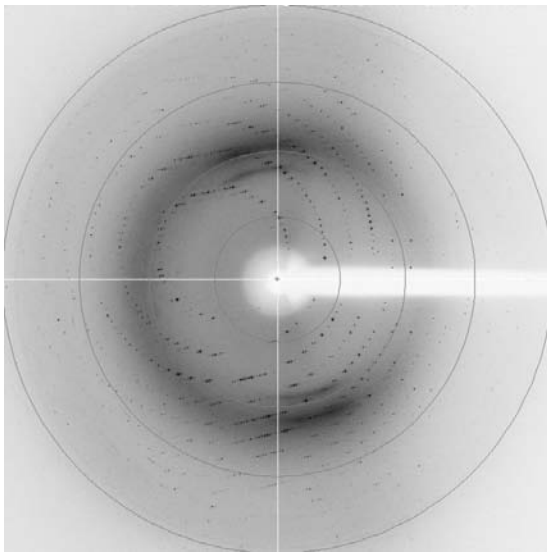

Figure 2
0.25° oscillation image collected on the CCD at beamline ID14, ESRF, Grenoble. The crystal-to-detector distance was set to 180 mm and the exposure time for each frame was 1 min. The circles in order of decreasing radii represent resolutions of 2.0, 2.6, 3.9 and 7.9 Å, respectively.

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