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Cloning, purification, crystallization and preliminary X-ray studies of HMO2 from *Saccharomyces cerevisiae*

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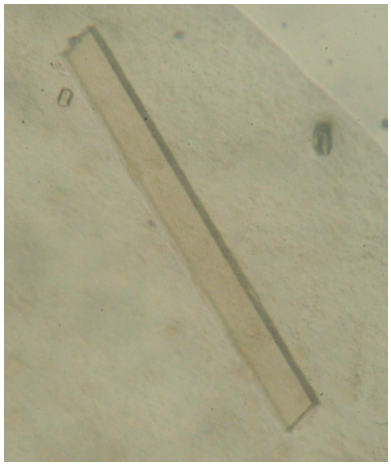

The high-mobility group protein (HMO2) of *Saccharomyces cerevisiae* is a component of the chromatin-remodelling complex INO80, which is involved in double-strand break (DSB) repair. HMO2 can also bind DNA to protect it from exonucleolytic cleavage. Nevertheless, little structural information is available regarding these functions of HMO2. Since determination of three-dimensional structure is a powerful means to facilitate functional characterization, X-ray crystallography has been used to accomplish this task. Here, the expression, purification, crystallization and preliminary crystallographic analysis of HMO2 from *S. cerevisiae* are reported. The crystal belonged to space group *P*222, with unit-cell parameters a = 39.35, b = 75.69, c = 108.03 Å, and diffracted to a resolution of 3.0 Å. The crystals are most likely to contain one molecule in the asymmetric unit, with a V_M value of 3.19 Å^3 Da^{-1}.

1. Introduction

High-mobility group box (HMGB) proteins are a set of nuclear non-histone proteins that are found in a variety of eukaryotic species (Bustin, 2001). The mobility proteins are composed of two homologous conserved HMGB domains, Box A and Box B, which bind nonspecifically to the minor groove of DNA. Both of the Box domains contain about 80 amino acids and are contiguous with each other, being located at the N- and C-terminus, respectively, and form an L-shaped structure with three α-helices (Thomas, 2001; Bianchi & Beltrame, 2000; Churchill *et al.*, 2010). Initially regarded as structural components of chromatin, studies over the past decade have drastically changed the view of their cellular role. HMGB proteins have been implicated in transcriptional regulation, DNA repair, recombination, differentiation and extracellular signalling (Thomas, 2001; Scaffidi *et al.*, 2002).

Saccharomyces cerevisiae contains several HMGB proteins, including NHP6A/B, HMO1 and HMO2. The single-domain HMGB proteins NHP6A/B participate in transcription by RNA polymerases II and III (Kruppa *et al.*, 2001; Moreira & Holmberg, 2000; Lopez *et al.*, 2001; Kassavetis & Steiner, 2006). NHP6A folds into an L-shaped pattern which comprises three helices that are held together by two hydrophobic cores for binding to DNA (Murphy *et al.*, 1999). HMO1 and HMO2 are similar to mammalian HMGB proteins in containing two HMGB domains (Box A and Box B). HMO1 has been shown to play a role in plasmid maintenance, normal growth, ribosomal RNA transcription and ribosomal protein expression (Lu *et al.*, 1996; Gadal *et al.*, 2002; Hall *et al.*, 2006; Merz *et al.*, 2008; Xiao & Grove, 2009; Berger *et al.*, 2007). HMO2, also known as NHP10, is a component of the chromatin-remodelling complex INO80, which is involved in the repair of DNA double-strand breaks (DSBs) and chromatin remodelling (Morrison *et al.*, 2004). HMO2 has also been reported to be responsible for interaction with the damage-induced phosphorylated histone H2A (γ-H2AX). The inactivation of H2A results in reduced INO80 recruitment to the DSBs, which does not impair chromatin remodelling (Morrison *et al.*, 2004; Au *et al.*, 2011; van Attikum *et al.*, 2007). In addition, HMO2 binds to DNA ends (blunt ends and cohesive ends) and protects DNA ends from exonucleolytic cleavage, which is essential to minimize loss of genetic information before repair (Morrison *et al.*, 2004; Ray & Grove, 2009). HMO2 is also thought to arrive early at DSBs in its complex with the INO80

components, suggesting that HMO2 may play a role in DSB repair beyond the recruitment of INO80 (Ray & Grove, 2009). Recently, Ray and Grove reported that HMO2 preferentially binds to distorted DNA, suggesting that HMO2 may also participate in directing the INO80 complex to sites such as stalled replication forks (Ray & Grove, 2009).

Nevertheless, little structural information is available regarding these functions of HMO2. In order to further investigate the biochemical and physiological functions of HMO2, we have initiated the determination of its three-dimensional structure by X-ray crystallography. Here, we report the expression, purification, crystallization and preliminary X-ray crystallographic study of HMO2 from *S. cerevisiae*. Structure determination will be pursued using experimental phasing methods. Determination of the three-dimensional structure of HMO2 will help in understanding how this widespread protein exerts its multitudinous functions.

2. Materials and methods

2.1. Cloning and expression

The full-length gene encoding HMO2 (NP_010282) was amplified from yeast genomic DNA by the polymerase chain reaction using primers containing *Nde*I and *Xho*I restriction sites. Both the PCR products and the vector pET-22b were digested with the *Nde*I and *Xho*I restriction enzymes. The ligation mixture was transformed into chemically competent *Escherichia coli* DH5α cells and the insertion was verified by PCR and DNA sequencing. Recombinant full-length yeast HMO2 with an N-terminal His tag (pET-22b-HMO2) was transformed into chemically competent *E. coli* B834 cells for expression. Cultures of bacteria carrying pET-22b-HMO2 were grown overnight in 10 ml LB medium supplemented with 20 mg l^{-1} ampicillin. The bacteria were then used to inoculate 4.0 l LB medium and were cultured at 310 K until the OD_{600} reached ∼0.5. Protein expression was induced by adding isopropyl β-D-1-thiogalactopyranoside (IPTG) to a final concentration of 0.2 m*M*. The culture was allowed to grow at 293 K for a further 15 h before the cells were harvested by centrifugation.

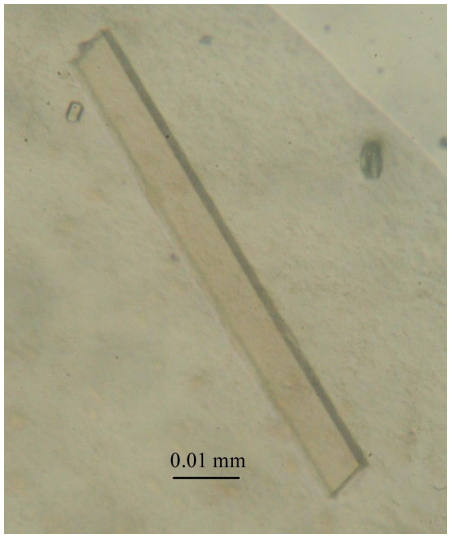

Figure 1
A crystal of HMO2 of *S. cerevisiae* grown by the hanging-drop method.

2.2. Purification

The cells were resuspended in lysis buffer (20 m*M* Tris–HCl pH 8.0, 300 m*M* NaCl) and disrupted by sonication. The cell lysate was centrifuged at 15 000*g* for 40 min at 277 K and the cell debris was discarded. The recombinant protein in the supernatant was applied onto an Ni^{2+}–NTA (Qiagen) column pre-equilibrated with lysis buffer. Nonspecifically bound proteins were washed from the affinity column with 300 ml lysis buffer containing 30 m*M* imidazole. The recombinant protein was then eluted from the column with 20 ml elution buffer (20 m*M* Tris–HCl pH 8.0, 200 m*M* imidazole). The affinity-column eluate was then loaded onto a DEAE Sepharose Fast Flow column (GE Healthcare) pre-equilibrated with 20 m*M* Tris–HCl pH 8.0 for further purification. The recombinant protein was eluted with a linear gradient of 0–300 m*M* NaCl in 20 m*M* Tris–HCl pH 8.0. For crystallization, fractions containing HMO2 were identified using SDS–PAGE and the target fractions were concentrated and buffer-exchanged into the final buffer (5 m*M* Tris–HCl pH 8.0, 50 m*M* NaCl) using a Millipore Amicon concentrator with a 5 kDa cutoff membrane. The concentration of the final purified recombinant protein was about 80 mg ml^{-1} and its purity was determined to be about 95% by SDS–PAGE.

2.3. Crystallization

Preliminary screening of crystallization conditions was performed by the sitting-drop vapour-diffusion method with the Crystal Screen, PEG/Ion and Index kits (Hampton Research). The crystallization experiments consisted of 1.0 µl protein solution (80 mg ml^{-1}) mixed with 1.0 µl reservoir solution and were equilibrated against 100 µl reservoir solution at 293 K. After about 20 d, small crystals of HMO2 were observed using a reservoir condition consisting of 20%(*w*/*v*) PEG 3350, 0.2 *M* ammonium formate pH 6.6. Further optimization of the conditions using PEGs of different molecular weights at various concentrations gave good diffraction-quality crystals with 18–25%(*w*/*v*) PEG 3350, 0.2 *M* ammonium formate in the presence of 0.1 *M* bis-tris pH 6.3–7.0 by the hanging-drop vapour-diffusion method. Crystals

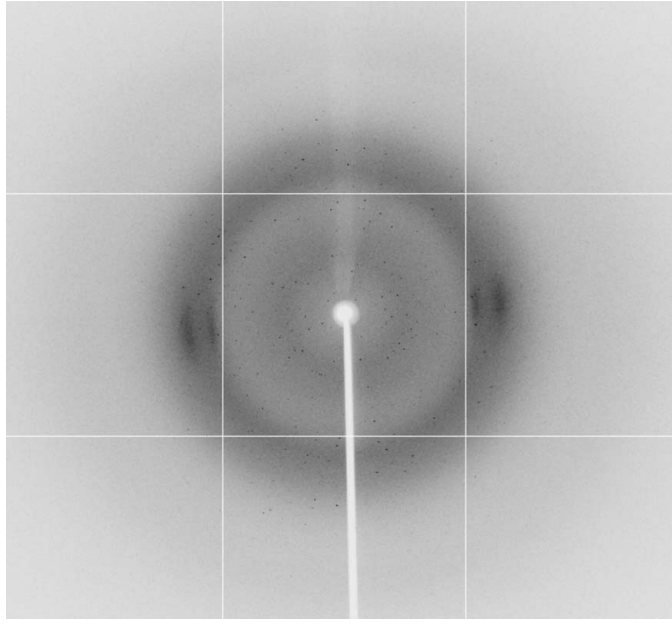

Figure 2
An X-ray diffraction pattern of a native crystal of HMO2 of *S. cerevisiae* recorded using a MAR CCD detector.

Table 1
Data-collection and processing statistics.

Values in parentheses are for the highest resolution shell.

Space group	*P*222
Unit-cell parameters (Å)	a = 39.35, b = 75.69, c = 108.03
Wavelength (Å)	0.9792
Resolution (Å)	40–3.0 (3.16–3.00)
No. of observed reflections	39572
No. of unique reflections	6870
Completeness (%)	99.5 (89.0)
$\langle I/\sigma(I)\rangle$	4.6 (3.6)
$R_{\rm merge}$† (%)	10.4 (31.4)

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

were obtained after 20 d equilibration against the crystallization solution and grew to full size (0.008 × 0.005 × 0.1 mm) in two months (Fig. 1).

2.4. Data collection and processing

X-ray diffraction data were collected from a single crystal of yeast HMO2 protein using an ADSC Quantum 315r on beamline BL17U1 at the Shanghai Synchrotron Radiation Facility (SSRF). The crystals were mounted in a nylon-fibre loop and flash-cooled in a liquid-nitrogen gas stream at 110 K without additional cryoprotectant. 180 images were collected at a wavelength of 0.9792 Å using an oscillation angle of 1° and an exposure time of 15 s per frame (Fig. 2). The images were processed and scaled to 3.0 Å using *iMosflm* (Battye *et al.*, 2011) and *SCALA* from the *CCP*4 suite (Winn *et al.*, 2011). The final data-collection and processing statistics are given in Table 1.

3. Results and discussion

A complete diffraction data set was collected to 3.0 Å resolution from a single crystal and the data-collection statistics are reported in Table 1. A total of 39 572 measured reflections in the resolution range 30–3.0 Å were merged into 6870 unique reflections with an $R_{\rm merge}$ of 10.4%. Analysis of the diffraction intensities indicated that the most plausible space group was orthorhombic *P*222, with unit-cell parameters a = 39.35, b = 75.69, c = 108.03 Å. Based on the molecular weight of yeast HMO2 (25.2 kDa) and space group *P*222, it was assumed that the crystal contained one molecule per asymmetric unit. The assumption gives a $V_{\rm M}$ value of 3.19 Å^3 Da^{-1} and a solvent content of 61.49% (Matthews, 1968). Molecular replacement using *AMoRe* (Navaza, 2001), *Phaser* (McCoy *et al.*, 2005) and *MOLREP* (Vagin & Teplyakov, 2010) was carried out using the structure of the sequence-nonspecific HMGB protein NHP6A as a search model (PDB entry 1lwm; Masse *et al.*, 2002). The model has about 34% sequence identity to the C-terminal domain (amino-acid sequence 90–162) of HMO2 of *S. cerevisiae*. However, efforts to determine the structure using molecular replacement with NHP6A have proved to be unsuccessful. We are now in the process of producing selenomethionylated HMO2 protein in order to use multi-wavelength anomalous dispersion methods for structure determination.

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