Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Purification, crystallization and preliminary X-ray analysis of the N-terminal domain of NO38, a nucleolar protein from *Xenopus laevis*

V. M. Haridasan Namboodiri,[a] Marion S. Schmidt-Zachmann,[b] James F. Head[a] and Christopher W. Akey[a]*

[a]Department of Physiology and Biophysics, Boston University School of Medicine, 700 Albany Street, Boston, Massachusetts 02118-2526, USA, and [b]Division of Cell Biology, German Cancer Research Center, Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany

Correspondence e-mail: cakey@bu.edu

Received 13 May 2004
Accepted 23 September 2004

NO38 is a multidomain protein that belongs to the nucleoplasmin (Np) family. Previous studies have suggested that acidic chaperones such as Np may function as histone-storage platforms. Here, the purification and crystallization of the N-terminal domain of NO38 in two crystal forms is reported. The *C*2 crystal form diffracts to 1.9 Å and contains two pentamers in the asymmetric unit, while the *P*1 crystals diffract to 1.7 Å and contain a non-crystallographic decamer with 522 symmetry. By analogy with Np, the NO38 decamer may represent the active form of this chaperone.

1. Introduction

NO38 is a multidomain protein that is localized in the granular component of *Xenopus laevis* nucleoli. There are two homologous gene forms of NO38 in the tetraploid frog *X. laevis*, with 296 and 299 residues, respectively, and a monomer molecular weight of ~33 kDa. NO38 runs anomalously on SDS–PAGE owing to its low pI and thus was named nucleolar protein of 38 kDa (NO38; Schmidt-Zachmann *et al.*, 1987). Based on its sequence, NO38 has been classified as a member of the nucleoplasmin (Np) family of acidic chaperones (Schmidt-Zachmann *et al.*, 1987; Dutta *et al.*, 2001), which bind core histones and under some conditions will assemble nucleosomes *in vitro* (Laskey *et al.*, 1978; Okuwaki *et al.*, 2001*b*). Members of this family have a conserved N-terminal domain with a small tract of acidic residues (termed the A1 tract). The N-terminal domain (the 'core') associates to form pentamers in Np and the *Drosophila* nucleoplasmin-like protein (dNLP; Dutta *et al.*, 2001; Namboodiri *et al.*, 2003). The C-terminal domain is rather variable in the family and may contain one additional acidic tract, as in dNLP and NO29, or multiple acidic tracts, as in family members such as NO38 and p62.

Xenopus NO38 is also known as B23 or nucleophosmin and belongs to an Np-family subgroup that also contains p62 from *Lytechinus pictus* (sea urchin) and ANO39 from *Asterina pectinifera* (starfish). The function of NO38 in the nucleolus is not clear, although it has been extensively used as a marker owing to its abundance. It has been suggested that the N-domain of NO38 may chaperone proteins in the nucleolus, while the C-domain has been implicated in nucleic acid binding (Hingorani *et al.*, 2000; Wang *et al.*, 1994) and may function as a nucleolar-targeting sequence (Peculis & Gall, 1992; Zirwes *et al.*, 1997; Warner & Sloboda, 1999). In addition, phosphorylation of the C-terminal region in B23 and p62 may lower the affinity of this domain for nucleic acids before the onset of mitosis (Okuwaki *et al.*, 2002). B23 has also been shown to stimulate the replication of adenovirus chromatin *in vitro*. This property may accrue from the ability of B23 to bind core histones (Okuwaki *et al.*, 2001*a*,*b*).

We have previously reported the crystal structures of Np and dNLP cores determined at 2.2 and 1.5 Å resolution, respectively (Dutta *et al.*, 2001; Namboodiri *et al.*, 2003). These structures revealed a conserved subunit fold comprised of an eight-stranded β-barrel with a jelly-roll topology. In addition, these structures suggested that oligomerization into thermostable pentamers may be driven by the formation of an extensive hydrophobic subunit interface (Dutta *et al.*, 2001; Namboodiri *et al.*, 2003). Based on these structures and other data, we suggested a model for how large complexes are formed in the presence of core histones. In this model, two pentameric chaperones dimerize to form a decamer with 522 symmetry and this oligomer may then bind core histones. Moreover, a β-hairpin is present at the interface between adjacent subunits in the pentamer and may play an important role in recognizing histone dimers (Dutta *et al.*, 2001; Namboodiri *et al.*, 2003).

In order to attain a better understanding of NO38 function, we have purified and crystallized the conserved N-terminal core domain. Here, we report a preliminary analysis of two crystal forms of the NO38 core.

2. Purification of NO38 core

A full-length cDNA for NO38 (Schmidt-Zachmann *et al.*, 1987) was used to amplify the

© 2004 International Union of Crystallography
Printed in Denmark – all rights reserved

Table 1
X-ray diffraction data-collection statistics for the NO38-core crystals.

	Ca form	Mg form
Wavelength (Å)	1.54	1.54
Space group	*C*2	*P*1
Unit-cell parameters		
a (Å)	198.9	59.0
b (Å)	64.5	59.0
c (Å)	97.5	87.2
α (°)	90.0	77.0
β (°)	113.7	88.2
γ (°)	90.0	60.8
Unit-cell volume (Å^3)	1145980	248826
No. molecules per AU	Two pentamers	One decamer
V_M† (Å^3 Da^{-1})	2.17	1.9
Solvent content (%)	43	35
Resolution range	91–1.9	90–1.7
Total reflections	442030	502140
Unique reflections	89372	109907
R_{merge} (%)	4.0 (22.1)	3.0 (18.3)
Completeness (%)	94.0 (95.8)	79.7 (88.0)
Average $\langle I/\sigma(I)\rangle$	14.5 (3.24)	9.6 (4.2)

† Matthews coefficient (Matthews, 1968).

N-terminal domain (Gln16–Asp123; Swiss-Prot accession No. P07222) by PCR.

Appropriate primers were used to introduce *Bam*H1 and *Eco*RI sites at the 5′ and 3′ ends, respectively, and the fragment was cloned into a pPep-T expression vector (Brandenberger *et al.*, 1996) containing an N-terminal 6×His tag, a laminin-oligomerization domain and a thrombin clip site that can also be cut by thermolysin. The vector was transformed into BL21 (DE3) cells and protein expression was induced with 1 m*M* isopropyl-β-D-1-thiogalactopyranoside for 5 h. After pelleting, the cells were suspended in 25 m*M* Tris–HCl pH 9.0, 100 m*M* NaCl, 1 m*M* PMSF, 1 m*M* EDTA and lysed by the sequential addition of lysozyme, deoxycholic acid and DNase I (Dutta *et al.*, 2001). The lysate was incubated in a shaking waterbath at 353 K for 10 min to precipitate less stable proteins. After centrifugation, the supernatant contained thermostable NO38 core which was ∼90% pure. The 6×His tag and the laminin-oligomerization domain were cleaved from the NO38 core with thermolysin at a 20:1(*w*:*w*) protein-to-protease ratio for 2 h at 310 K. The reaction was terminated by the addition of 10 m*M* EDTA. The NO38 core was precipitated by the addition of ammonium sulfate to ∼80% saturation, followed by lowering the pH to ∼4.0 by the dropwise addition of 1 *M* HCl. The resulting pellet was dissolved in 25 m*M* Tris–HCl pH 7.5 and 100 m*M* NaCl buffer (TN buffer). After size-exclusion chromatography on a Superose-12HR column, the protein was concentrated to 15 mg ml^{-1} in TN buffer using a Centriprep YM10 concentrator (Amicon).

3. Crystallization

Crystallization was carried out by the hanging-drop vapour-diffusion method with silanized plastic cover slips and NO38 core at ∼15 mg ml^{-1}.

Initial crystals were obtained at 296 K with conditions Nos. 14 and 23 from Hampton Crystal Screen I (Hampton Research) using 1 µl each of precipitant and protein solutions. Two crystal forms were obtained after refining these conditions. The first crystal form was obtained in 18–20%(*v*/*v*) PEG 400, 100 m*M* $CaCl_2$ and 100 m*M* Tris–HCl pH 7.5 (Fig. 1*a*). The second form was crystallized in the presence of 26%(*v*/*v*) PEG 400, 5%(*v*/*v*) ethylene glycol, 100 m*M* Tris–HCl pH 7.5 and 20 m*M* $MgCl_2$ (Fig. 1*b*). The crystals grew to maximum dimensions of ∼0.3 × 0.2 × 0.1 mm (Ca form) and 0.4 × 0.2 × 0.1 mm (Mg form).

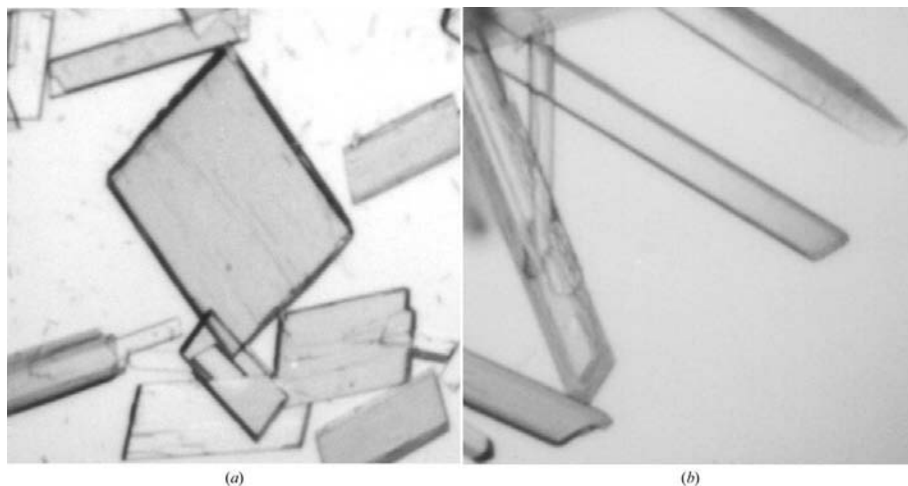

Figure 1
Images of the NO38-core crystals. (*a*) A drop containing the Ca-form crystals is shown. (*b*) This drop contains NO38-core crystals grown in $MgCl_2$.

4. Data collection

Data sets for both crystal forms were collected at 100 K on an R-AXIS IV^{++} image-plate detector using Cu $K\alpha$ X-rays (λ = 1.54 Å) from a Rigaku RU-300 generator, which were focused with Osmic mirrors (MSC). For the Ca form, the crystals were transferred with a cryoloop into a solution containing 5%(*v*/*v*) ethylene glycol, 20%(*v*/*v*) PEG 400, 100 m*M* $CaCl_2$ and 100 m*M* Tris–HCl pH 7.5 for ∼10 s and subsequently flash-cooled in a cold nitrogen-gas stream from an X-stream cryosystem (MSC). A data set was collected to 1.9 Å (Table 1). A crystal of the Mg form was looped in its mother liquor and directly frozen in the cryostream. This crystal diffracted to 1.7 Å resolution (Table 1). The data sets were indexed and integrated using *DENZO* and scaled using *SCALEPACK* from the *HKL* program suite (Otwinowski & Minor, 1997).

Details of the data collection are given in Table 1.

5. Phasing

5.1. Ca crystal form

The Np-core pentamer (PDB code 1k5j) was used as the initial search model to calculate a molecular-replacement solution for the Ca crystal form with *EPMR* (Kissinger *et al.*, 1999). The best solution gave a correlation coefficient of 55% for two pentamers in the asymmetric unit related by non-crystallographic symmetry (Fig. 2*a*; pentamers labeled *A* and *B*). The Np-core sequence was mutated to the NO38 sequence in *O* (Jones *et al.*, 1991) and then subjected to rigid-body refinement in *CNS* (Brünger *et al.*, 1998) to give a starting model with an R_{free} of 0.344. Electron-density maps at this stage showed the entire NO38-core subunit except for the A1 tract. Initial rounds of model building using $2F_o - F_c$ and $F_o - F_c$ maps and refinement cycles were performed in *O* (Jones *et al.*, 1991) and *CNS* (Brünger *et al.*, 1998), respectively. The model is currently being refined, but it is already clear that the N-terminal domain of NO38 can associate to form a thermostable pentamer, like Np and dNLP. Remarkably, the *A* and *B* pentamers in the asymmetric unit are not in direct lateral contact, nor are their fivefold axes parallel with each other. Instead, they make

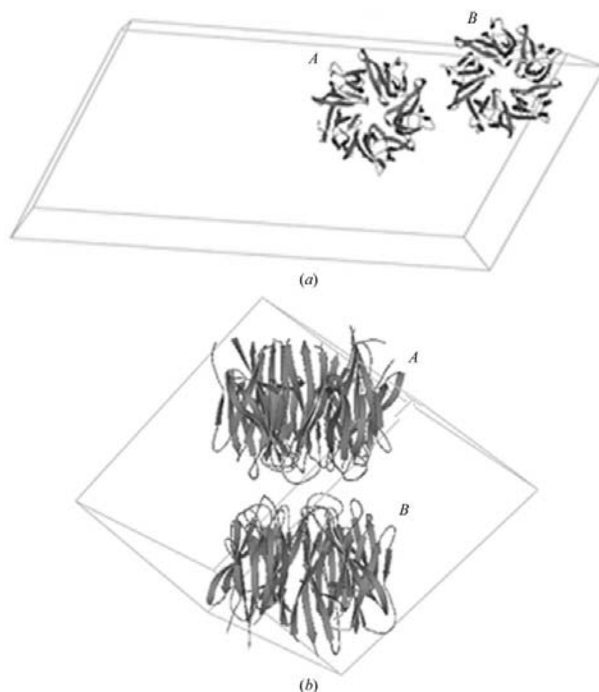

Figure 2
Overview of the NO38-core pentamer in two crystal forms. (*a*) Two pentamers (labelled *A* and *B*) are present in the asymmetric unit of the *C*2 crystal (Ca form). (*b*) A decamer is present in the unit cell of the *P*1 crystal (Mg form). The decamer is formed from two pentamers (labelled *A* and *B*) and has 522 symmetry. The crystal packing was visualized with *PyMOL*.

contacts to symmetry-related molecules located above and below them in the lattice. However, none of these crystal contacts creates a decamer with 522 symmetry.

5.2. Mg crystal form

The structure of the Mg crystal form was solved by molecular replacement with *EPMR* at 2.0 Å resolution using a partially refined pentamer from the Ca crystal form as a search model. The *EPMR* solution gave a correlation coefficient of ~40% and an *R* factor of ~35% for the packing shown in Fig. 2(*b*). In the *P*1 unit cell, a face-to-face decamer with 522 symmetry is formed from two NO38-core pentamers, whose collinear fivefold axes are aligned roughly along the body diagonal of the unit cell. An initial inspection reveals that the pentamer–pentamer interface in the NO38-core decamer is similar, though not identical, to that observed in a *C*2 crystal form of the Np core (Dutta *et al.*, 2001). This difference arises from a rotational offset of ~8° for each of the NO38-core pentamers relative to Np. Further refinement of the NO38-core decamer structure and histone-binding studies should provide insights into the function of this nucleolar chaperone.

References

Brandenberger, R., Kammerer, R. A., Engel, J. & Chiquet, M. (1996). *J. Cell Biol.* **135**, 1583–1592.

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.

Dutta, S., Akey, I. V., Dingwall, C., Hartman, K. L., Laue, T., Nolte, R. T., Head, J. F. & Akey, C. W. (2001). *Mol. Cell*, **8**, 841–853.

Hingorani, K., Szebeni, A. & Olson, M. O. (2000). *J. Biol. Chem.* **275**, 24451–24457.

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

Kissinger, C. R., Gehlhaar, D. K. & Fogel, D. B. (1999). *Acta Cryst.* D**55**, 484–491.

Laskey, R. A., Honda, B. M., Mills, A. D. & Finch, J. T. (1978). *Nature (London)*, **275**, 416–420.

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

Namboodiri, H. V. M., Dutta, S., Akey, I. V., Head, J. F. & Akey, C. W. (2003). *Structure*, **11**, 175–186.

Okuwaki, M., Iwamatsu, A., Tsujimoto, M. & Nagata, K. (2001*a*). *J. Mol. Biol.* **311**, 41–55.

Okuwaki, M., Iwamatsu, A., Tsujimoto, M. & Nagata, K. (2001*b*). *FEBS Lett.* **506**, 272–276.

Okuwaki, M., Tsujimoto, M. & Nagata, K. (2002). *Mol. Biol. Cell*, **13**, 2016–2030.

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

Peculis, B. A. & Gall, J. G. (1992). *J. Cell Biol.* **116**, 1–14.

Schmidt-Zachmann, M. S., Hugle-Dorr, B. & Franke, W. W. (1987). *EMBO J.* **6**, 1881–1890.

Wang, D., Baumann, A., Szebeni, A. & Olson, M. O. (1994). *J. Biol. Chem.* **269**, 30994–30998.

Warner, A. K. & Sloboda, R. D. (1999). *Cell Motil. Cytoskeleton*, **44**, 68–80.

Zirwes, R. F., Kouzmenko, A. P., Peters, J. M., Franke, W. W. & Schmidt-Zachmann, M. S. (1997). *Mol. Biol. Cell*, **8**, 231–248.