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

Crystallization and crystallographic analysis of human NUDT16

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Received 17 March 2008
Accepted 4 June 2008

Human NUDT16, a decapping enzyme belonging to the Nudix superfamily, plays a pivotal role in U8 snoRNA stability. Recombinant NUDT16 expressed in *Escherichia coli* was crystallized using the hanging-drop vapour-diffusion method. The crystals, which diffracted to 2.10 Å resolution, belonged to space group $P2_12_12_1$, with unit-cell parameters a = 44.47, b = 79.32, c = 97.20 Å. The Matthews coefficient and the solvent content were calculated to be 1.92 Å^3 Da^{-1} and 35.84%, respectively, for two molecules per asymmetric unit.

1. Introduction

Members of the superfamily of Nudix hydrolases are widespread in bacteria, viruses, eukaryotes and archaea. They catalyze the hydrolysis of a nucleoside diphosphate linked to another moiety *X*. These enzymes are characterized by a highly conserved 23-amino-acid Nudix motif, GX_5EX_7REUXEEXGU, where *U* is an aliphatic or hydrophobic residue (Bessman *et al.*, 1996). This motif is involved in catalysis and the binding of Mg^{2+}, Mn^{2+} or other divalent cations (Mildvan *et al.*, 2005). The substrates of these enzymes are Np_nN, (d)NTP and capped RNAs (Ito *et al.*, 2005; Fisher *et al.*, 2004; Hori *et al.*, 2005). NUDT16 is the human homologue of *Xenopus laevis* X29 protein, which binds and decaps U8 snoRNA and other nuclear RNAs *in vitro* (Ghosh *et al.*, 2004; Tomasevic & Peculis, 1999). The varying divalent metal can determine the RNA-substrate specificity and the efficiency of the decapping activity of NUDT16 and X29 (Peculis *et al.*, 2007). The results of Peculis and coworkers showed that U8 snoRNA is involved in the processing of 5.8S and 28S rRNAs and that the decapping of U8 snoRNA leads to its degradation. Thus, X29 may be involved in the maturation of the large ribosomal subunit through regulating the stability of U8 snoRNA (Ghosh *et al.*, 2004). NUDT16 shares 51% identity with X29, but its structure and function is unclear.

Several structures of Nudix hydrolases have been determined, *e.g.* those of *X. laevis* X29 (Scarsdale *et al.*, 2006), *Schizosaccharomyces pombe* Dcp2p (She *et al.*, 2006) and *Escherichia coli* ADPRase (Gabelli *et al.*, 2001). Structural similarity is only observed in the Nudix domain of these proteins, which is the catalytic centre responsible for catalysis and the binding of divalent cations. There are several important regions that determine the substrate specificity of Nudix hydrolases (Mildvan *et al.*, 2005). The nucleotide diphosphatase activity of X29 is metal-dependent and requires the presence of a significant part of the RNA substrate, but no known RNA-binding motif has been observed. However, from its crystal structure we can observe a plausible RNA-binding channel on the positive face of the protein created by a striking surface dipolarity and unique structural features (Scarsdale *et al.*, 2006). A high-resolution crystal structure would provide much valuable information on the catalytic mechanism of NUDT16 and other Nudix hydrolases.

In this study, we report the cloning and expression of NUDT16 in *E. coli*. Purified recombinant NUDT16 protein was crystallized using

the hanging-drop vapour-diffusion method. Finally, we performed a preliminary crystallographic analysis of human NUDT16.

2. Materials and methods

2.1. Cloning and expression

Human kidney cDNA library was used as a template to amplify the full-length coding sequence of NUDT16 with the specific primers 5′-GGAATTCCATATGATGGCTGGTGCTCGTCGTCTGGAGCTAGGCGAG-3′ (forward) and 5′-ATAGTTTAGCGGCCGCCTAGTGGTGGTGGTGGTGATGAGCTGGAATC-3′ (reverse). The amplified NUDT16 DNA fragment was inserted into pET30a+ vector with *Nde*I/*Nco*I sites. The positive clone was confirmed by direct DNA sequencing and double-enzyme digestion.

The plasmid was transformed into *E. coli* strain BL21 (DE3) competent cells. For NUDT16 expression, a single colony was incubated in Luria–Bertani (LB) medium with 50 µg ml^{-1} kanamycin sulfate (Amresco USA) for overnight growth at 310 K. The overnight culture was then transferred into 2 l fresh LB medium. When the culture density (OD_{600}) reached about 0.7, IPTG (isopropyl β-D-1-thiogalactopyranoside; Sigma, USA) was added to a final concentration of 0.1 m*M* and the culture was kept at 310 K for 4 h. The expressed NUDT16 contains a C-terminal His tag introduced by the primer.

2.2. Protein purification

The cells were harvested and resuspended in chilled lysis buffer (20 m*M* Tris–HCl pH 8.0, 150 m*M* NaCl, 5% glycerol) and homogenized by sonication. The supernatant was mixed with Ni–NTA beads (Qiagen) for 2 h at 277 K. The loaded beads were washed three times with lysis buffer. NUDT16 was then eluted with lysis buffer supplemented with 150 m*M* imidazole. The elution fractions were further purified using a Superdex 75 gel-filtration column (GE Healthcare). The protein peak was collected and concentrated to about 20 mg ml^{-1} by ultrafiltration (Millipore) for crystallization.

2.3. Crystallization and X-ray diffraction analysis

Cystallization attempts were carried out with Crystal Screens I and II (Hampton Research) at 291 and 277 K using the hanging-drop vapour-diffusion method. The drops were prepared by mixing 1 µl protein solution and 1 µl reservoir solution and were equilibrated against 200 µl reservoir solution.

X-ray diffraction data were collected in-house on a Rigaku MicroMax007 rotating-anode X-ray generator operated at 40 kV and 20 mA (Cu $K\alpha$; λ = 1.5418 Å) equipped with an R-AXIS VII^{++} image-plate detector. A single crystal was transferred to cryoprotectant solution containing 20% glycerol and 0.6 *M* imidazole pH 7.0, picked up in a nylon loop and flash-cooled in a cold nitrogen-gas stream at 100 K using an Oxford Cryosystem. *DENZO* and *SCALEPACK* from the *HKL*-2000 program suite were used to index, integrate and scale the data (Otwinowski & Minor, 1997).

3. Results and discussion

Human NUDT16 was expressed in *E. coli* as a soluble protein with a His tag at the C-terminus. The purified protein migrated with a molecular weight of 22 kDa on SDS–PAGE. Recombinant NUDT16 possesses the activity of decapping U8 snoRNA (data not shown).

NUDT16 crystals grew at 291 K after two weeks from a solution consisting of 1.0 *M* imidazole pH 7.0. Diffraction data were collected to 2.10 Å resolution. The crystals belonged to space group $P2_12_12_1$, with unit-cell parameters a = 44.47, b = 79.32, c = 97.20 Å. Detailed data statistics are given in Table 1. As in the case of *X. laevis* X29 (Peculis *et al.*, 2004), the crystallographic asymmetric unit contains two molecules. The Matthews coefficient and solvent content were calculated to be 1.92 Å^3 Da^{-1} and 35.84% (Matthews, 1968), respectively.

Table 1
Summary of data-collection statistics.

Space group	$P2_12_12_1$
Unit-cell parameters	
a (Å)	44.47
b (Å)	79.32
c (Å)	97.20
$\alpha = \beta = \gamma$ (°)	90.00
Resolution (Å)	44.47–2.10 (2.38–2.10)
Observations	54884
Unique reflections	15094
Average redundancy	3.64 (3.56)
Completeness (%)	94.9 (97.9)
R_{merge}†	0.062 (0.303)
Reduced χ^2	0.94 (0.93)
Average $I/\sigma(I)$	12.9 (5.4)

† $R_{merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of an individual measurement of a reflection and $\langle I(hkl)\rangle$ is the mean value for all eaquivalent measurements of this reflection.

The molecular-replacement method was performed with the program *AMoRe* (Navaza, 1994) using the crystal structure of *X. laevis* X29 as a search model (PDB code 1u20). The $R_{working}$ and R_{free} values fell to 28% and 34%, respectively, and refinement is in progress. Attempts are also being made to crystallize the NUDT16–substrate complex.

This work was completed in the laboratory of Professor George F. Gao at the Institute of Microbiology, Chinese Academy of Sciences and was supported by the National Natural Science Foundation of China (NSFC 30670091).

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