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Crystallization and preliminary X-ray analysis of the C-terminal RNase III domain of human Dicer

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Human Dicer protein contains two RNase III domains (RNase IIIa and RNase IIIb) which are involved in the production of short interfering RNAs (siRNAs). The C-terminal RNase III domain (RNase IIIb) of human Dicer was expressed, purified and crystallized by the sitting-drop vapour-diffusion method. The crystals belonged to space group $C222_1$, with unit-cell parameters a = 88.6, b = 199.7, c = 119.6 Å, and diffracted X-rays to 2.0 Å resolution. The asymmetric unit contained three molecules of the RNase IIIb and the solvent content was 67%.

1. Introduction

In many eukaryotic cells, small RNAs trigger gene silencing in a sequence-specific manner. The Dicer protein is responsible for the processing of long double-stranded RNAs (dsRNAs) into short interfering RNAs (siRNAs) and for the processing of precursor miRNAs (pre-miRNAs) into microRNAs (miRNAs). siRNAs and miRNAs induce gene silencing known as RNA interference (RNAi; reviewed in Hannon & Rossi, 2004; Tomari & Zamore, 2005).

Dicer homologues have been found in all eukaryotes studied except for *Saccharomyces cerevisiae*. In zebrafish and mouse, the Dicer protein is essential for normal development (Bernstein *et al.*, 2003; Wienholds *et al.*, 2003). Dicers are approximately 200 kDa multidomain proteins which consist of an N-terminal DEXH-box helicase/ATPase domain, a domain of unknown function (DUF283), a PAZ domain, two RNase III nuclease domains (RNase IIIa and RNase IIIb) and a dsRNA-binding domain (dsRBD). The single processing-centre model for human Dicer suggested that Dicer functions through intramolecular dimerization of its two RNase III domains (Zhang *et al.*, 2004). Here, we describe the expression, purification, crystallization and preliminary X-ray diffraction analysis of the RNase IIIb domain of human Dicer.

2. Protein expression and purification

A cDNA clone (KIAA0928, NCBI accession No. AB023145) coding for human Dicer was obtained from the Kazusa DNA Research Institute (Chiba, Japan). The region encoding the RNase IIIb domain (residues 1660–1852) was inserted into the bacterial expression vector pET26b to obtain the RNase III domain with a C-terminal His tag. *Escherichia coli* BL21 star (DE3) (Invitrogen) was transformed with the plasmid. The *E. coli* cells were grown in Luria–Bertani broth containing kanamycin (30 µg ml^{-1}) until mid-log phase and protein expression was induced by the addition of 0.5 m*M* isopropyl β-D-thiogalactopyranoside (IPTG) at 293 K. The cells were harvested by centrifugation at 5000*g* for 15 min and resuspended in lysis buffer containing 50 m*M* sodium phosphate pH 8.0, 300 m*M* NaCl, 1.0 m*M* DTT (dithiothreitol) and 10 m*M* imidazole.

The cells were disrupted by sonication and insoluble materials were removed by centrifugation at 40 000*g* for 60 min. The supernatant containing the RNase IIIb protein was applied onto an Ni–NTA agarose column (Qiagen) and the RNase IIIb was eluted with

250 m*M* imidazole dissolved in 50 m*M* sodium phosphate pH 8.0, 300 m*M* NaCl and 1.0 m*M* DTT. The fraction containing the RNase IIIb was dialyzed against 20 m*M* Tris–HCl pH 8.0, 1.0 m*M* EDTA, 1.0 m*M* DTT and 10% glycerol. The solution was then applied onto a Resource-Q 6 ml anion-exchange column (Amersham Biosciences) and eluted with a linear gradient of 0–1.0 *M* NaCl. The RNase IIIb eluted at approximately 300 m*M* NaCl. The fraction containing the RNase IIIb was dialyzed against 20 m*M* Tris–HCl pH 8.0 and 1.0 m*M* DTT and then concentrated to 28 mg ml^{-1} using an Apollo ultrafiltration concentrator (Orbital Biosciences). The purified RNase IIIb domain with a His tag was used for crystallization experiments. The protein concentration was determined using the Bradford Protein Assay (Bio-Rad).

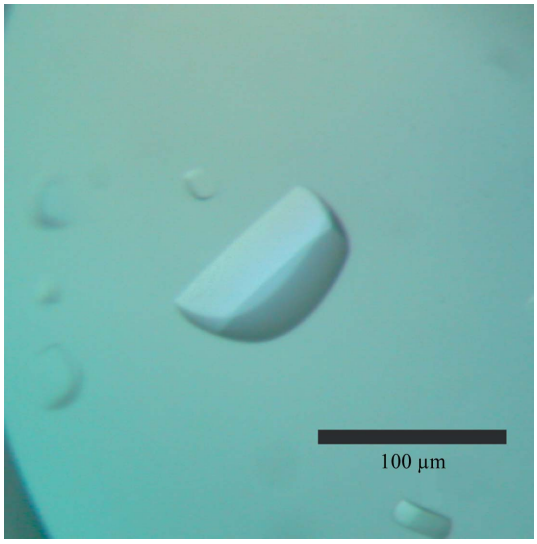

Figure 1
A crystal of the RNase IIIb domain with approximate dimensions 80 × 40 × 100 µm.

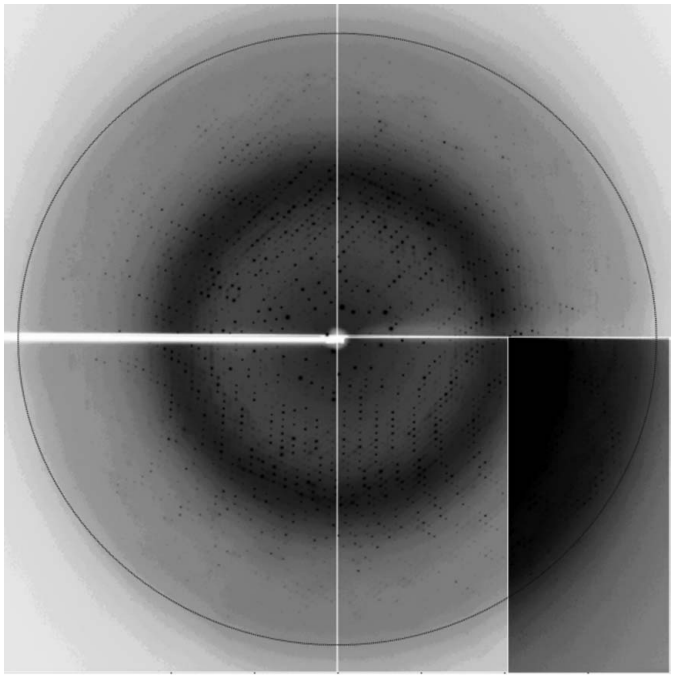

Figure 2
A diffraction image (0.5° oscillation) of the RNase IIIb crystal. The diffraction extends to 2.0 Å resolution (indicated by the circle).

Table 1
Data-collection statistics.

Values in parentheses are for the highest resolution shell.

X-ray source	PF-AR NW12
Wavelength (Å)	1.000
Space group	$C222_1$
Unit-cell parameters (Å)	a = 88.6, b = 199.7, c = 119.6
Resolution range (Å)	50.0–2.00 (2.07–2.00)
Observed reflections	645201
Unique reflections	72223
Completeness (%)	99.7 (100.0)
$R_{\rm merge}$† (%)	6.2 (37.2)
$\langle I\rangle/\langle\sigma(I)\rangle$	20.8 (6.2)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle|/\sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the ith intensity measurement of reflection hkl, including symmetry-related reflections, and $\langle I(hkl)\rangle$ is its average.

3. Crystallization

The sparse-matrix screening kits Crystal Screens I and II, Crystal Screen Lite (Hampton Research) and Wizards I and II (Emerald Biostructures) were used for initial crystallization trials using the sitting-drop vapour-diffusion method. Drops consisting of 2 µl protein solution and 2 µl reservoir solution were equilibrated against 500 µl reservoir solution in Cryschem plates (Hampton Research) and incubated at 278 K.

Thin crystals were observed using a reservoir solution consisting of 0.1 *M* Tris–HCl pH 8.5 and 1.0 *M* $(NH_4)_2SO_4$. The crystallization conditions were optimized by varying the precipitant concentration and the pH. Thicker crystals were obtained with a reservoir solution consisting of 0.1 *M* Tris–HCl pH 8.0 and 1.0 *M* $(NH_4)_2SO_4$. The conditions were further optimized using Additive Screens 1–3 (Hampton Research). A significant improvement was observed by the addition of 10 m*M* $MgCl_2$. The crystals used for data collection were obtained with a reservoir solution containing 0.1 *M* Tris–HCl pH 8.0, 1.0 *M* $(NH_4)_2SO_4$ and 20 m*M* $MgCl_2$ using 28 mg ml^{-1} protein solution. The crystals had typical dimensions of 80 × 40 × 100 µm (Fig. 1).

4. Data collection and processing

Diffraction experiments were conducted at beamline NW12 of the Photon Factory-Advanced Ring (PF-AR), Tsukuba, Japan. The crystals were transferred into a cryoprotectant solution consisting of 0.07 *M* Tris–HCl pH 8.0, 0.7 *M* $(NH_4)_2SO_4$, 14 m*M* $MgCl_2$ and 25% glycerol for approximately 30 s. The crystals were then mounted in nylon loops (Hampton Research) and flash-frozen in a nitrogen stream at 95 K. Diffraction data were collected using a Quantum 210 CCD X-ray detector (Area Detector Systems Corporation) in 0.5° oscillation steps and the crystal diffracted X-rays to 2.0 Å resolution (Fig. 2). The data set was processed and scaled using the program *HKL*2000 (Otwinowski & Minor, 1997). The crystals belonged to space group $C222_1$, with unit-cell parameters a = 88.6, b = 199.7, c = 119.6 Å. The data-collection statistics are given in Table 1. If it is assumed that the asymmetric unit contains three molecules of RNase IIIb, the calculated solvent content is 67% and the Matthews coefficient is 3.7 Å^3 Da^{-1} (Matthews, 1968). The structure determina-

tion of RNase IIIb by molecular replacement using the structure of *Aquifex aeolicus* RNase III (Blaszczyk *et al.*, 2001; PDB code 1jfz) as a search model is currently under way.

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References

Bernstein, E., Kim, S. Y., Carmell, M. A., Murchison, E. P., Alcorn, H., Li, M. Z., Mills, A. A., Elledge, S. J., Anderson, K. V. & Hannon, G. J. (2003). *Nature Genet.* **35**, 215–217.
Blaszczyk, J., Tropea, J. E., Bubunenko, M., Routzahn, K. M., Waugh, D. S., Court, D. L. & Ji, X. (2001). *Structure*, **9**, 1225–1236.
Hannon, G. J. & Rossi, J. J. (2004). *Nature (London)*, **431**, 371–378.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Tomari, Y. & Zamore, P. D. (2005). *Genes Dev.* **19**, 517–529.
Wienholds, E., Koudijs, M. J., van Eeden, F. J., Cuppen, E. & Plasterk, R. H. (2003). *Nature Genet.* **35**, 217–218.
Zhang, H., Kolb, F. A., Jaskiewicz, L., Westhof, E. & Filipowicz, W. (2004). *Cell*, **118**, 57–68.