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X-ray crystallographic characterization and phasing of a fucose-specific lectin from *Aleuria aurantia*

Masahiro Fujihashi,[a]† Diane H. Peapus,[a]‡ Eijiro Nakajima,[b] Tsuyoshi Yamada,[b] Jun-ichi Saito,[a]§ Akiko Kita,[a] Yoshiki Higuchi,[a]¶ Yoko Sugawara,[c] Akikazu Ando,[b] Nobuo Kamiya,[d] Yoshiho Nagata[b] and Kunio Miki[a,d]*

[a]Department of Chemistry, Graduate School of Science, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan, [b]Department of Bioresources Chemistry, Faculty of Horticulture, Chiba University, Matsudo 271-8510, Japan, [c]School of Science, Kitasato University, Kitasato 1-15-1, Sagamihara, Kanagawa 228-8555, Japan, and [d]RIKEN Harima Institute/SPring-8, Koto 1-1-1, Mikazukicho, Sayo-gun, Hyogo 679-5148, Japan

† Present address: Division of Molecular and Structural Biology, Ontario Cancer Institute, University Health Network, 610 University Avenue, Toronto, Ontario M5G 2M9, Canada.
‡ Present address: Paleontological Research Institution, 1259 Trumansburg Road, Ithaca, NY 14850, USA.
§ Present address: Pharmaceutical Research Institute, Kyowa Hakko Kogyo Co. Ltd, Shimotogari 1188, Nagaizumi-cho, Sunto-gun, Shizuoka 411-8731, Japan.
¶ Present address: Graduate School of Science, Himeji Institute of Technology, Koto 3-2-1, Kamigori, Hyogo 678-1297, Japan.

Correspondence e-mail: miki@kuchem.kyoto-u.ac.jp

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A fucose-specific lectin from *Aleuria aurantia* was crystallized in its native form and was also cocrystallized with $HgCl_2$. Crystallization was performed using the sitting-drop vapour-diffusion method with ammonium sulfate as a precipitant. Both the Hg-free native crystals and the Hg cocrystals belong to the hexagonal space group $P6_522$. The unit-cell parameters for the Hg-free form are $a = 84.0$, $c = 250.1$ Å and for the Hg cocrystals are $a = 83.9$, $c = 254.3$ Å. Both forms of the crystals diffract X-rays to 2.3 Å resolution and are suitable for high-resolution crystal structure determination. Initial phasing was successfully performed by the MAD method using the Hg cocrystals and the electron density obtained was good enough for model building.

1. Introduction

Lectins are a group of proteins that bind carbohydrates and are widely used as specific probes for investigating the structure and function of carbohydrate chains. The carbohydrate moieties of glycoproteins and glycolipids on a cell surface have been shown to be involved in a variety of biological recognition processes, including cell–cell and cell–substratum interactions and the metastasis of tumour cells (Drickamer & Taylor, 1993).

Fucosylated sugars are widely distributed in cell-surface sugar chains. In many cases, their residues constitute parts of important antigens such as the blood-group antigen H (Hakomori & Kobata, 1974) and stage-specific embryonic antigens (Stelck *et al.*, 1999). Of the various lectins, three families of fucose-specific lectins have been found. Members of the first family have been purified from several species of legumes, such as *Lotus tetragonolobus* (LTA; Cheng *et al.*, 1998) and *Ulex europeus* (UEA-I; Konami *et al.*, 1991). A member of the second family has been isolated from the serum of an eel, *Anguilla japonica* (Honda *et al.*, 2000) and a member of the third family from an ascomycete mushroom, *Aleuria aurantia* (AAL; Kochibe & Furukawa, 1980). The crystal structure of a legume lectin from *Ulex europeus* (UEA-I) has been determined, showing a two-layered β-sheet structure (Audette *et al.*, 2000). The folding of this lectin is similar to that of other legume lectins, including non-fucose-specific lectins (Audette *et al.*, 2000; Vijayan & Chandra, 1999). Recently, the crystal structure of an eel serum lectin from *Anguilla anguilla* agglutinin has been determined, showing a β-barrel structure with jelly-roll topology (Bianchet *et al.*, 2002). The amino-acid sequences of the three types of fucose-specific lectins are completely different from each other, suggesting that the three-dimensional structures and the recognition mechanisms are very different. Of the three families of fucose-specific lectins, only the structure of the third type, the mushroom type such as AAL, is not available.

Because AAL exhibits a higher affinity toward fucosylated sugars than the other two types of fucose-specific lectin, it has commonly been used as a specific probe for fucose-containing oligosaccharides (Liljeblad *et al.*, 2001; Ohkura *et al.*, 1994). This lectin was isolated from the fruiting bodies of an orange-peel mushroom, *A. aurantia*, and is known to exist as a dimer under physiological conditions (Kochibe & Furukawa, 1980). The monomer molecule consisted of 312 amino acids and its molecular mass is approximately 33 400 (Fukumori *et al.*, 1990). The deduced amino-acid sequence includes six internal homologous regions. To investigate the structure and specific recognition mechanism of the third type of fucose-specific lectins, we have crystallized AAL and have succeeded in initial phasing for crystal structure determination.

2. Crystallization and data collection

AAL was overexpressed in *Escherichia coli* (Fukumori *et al.*, 1989) and purified using a fucose–starch column as described previously (Nagata *et al.*, 1991). Crystals obtained by the batch method were too small for the collection

of X-ray diffraction data sets (Nagata *et al.*, 1991). We improved the crystallization conditions using the sitting-drop vapour-diffusion method at 297 K. The AAL solutions were prepared at 5 mg ml^{-1} in phosphate-buffered saline (PBS; 8 m*M* Na_2HPO_4, 1.5 m*M* KH_2PO_4, 137 m*M* NaCl, 2.7 m*M* KCl pH 7.2) with 20 m*M* β-mercaptoethanol or 1 m*M* $HgCl_2$. The presence of either β-mercaptoethanol or $HgCl_2$ was effective in improving the crystallinity. These reagents may prevent two cysteine residues (Cys80 and Cys193) from forming S—S bonds. The reservoir solutions contain 40% saturated ammonium sulfate in PBS. A mixture of equal volumes of the protein and reservoir solutions was equilibrated against reservoir solution. Hexagonal bipyramidal crystals grew to a maximum dimension of 0.7 mm (Fig. 1). The native crystals were obtained in the presence of β-mercaptoethanol.

The native crystals were mounted and sealed in a glass capillary with a trace amount of reservoir solution and diffraction data were collected at room temperature at BL18B at the Photon Factory, KEK, Japan. The X-ray was monochromated to 1.000 Å with a Si(111) monochromator system. Oscillation photographs were taken on 400 × 800 mm imaging plates (Fuji Photo Film) on a screenless Weissenberg camera for macromolecular crystallography equipped with a cylindrical cassette with a radius of 429.7 mm. The image data frames were read using an IPR4080 drum-type imaging-plate reader (Sakabe *et al.*, 1995). The Hg cocrystals were used for the multiple anomalous dispersion (MAD) data collection at beamline BM14 at the European Synchrotron Radiation Facility, France. Based on X-ray absorption fluorescence spectroscopy (XAFS), three wavelengths were chosen for MAD data collection at 1.0090, 1.0039 and 0.9183 Å for the edge, peak and remote data sets, respectively. Diffraction data were recorded on a 300 mm diameter MAR Research area detector at a crystal-to-detector distance of 350 mm. Because of significant radiation damage, data were taken from one crystal in 15° blocks with inverse mates at all three wavelengths. All data were processed and reduced using the programs

Table 1
Data-collection and phasing statistics.

	Native	Hg cocrystal		
Crystal data				
Space group	$P6_522$	$P6_522$		
Unit-cell parameters *a*, *c* (Å)	84.0 (2), 250.1 (3)	83.9 (2), 254.3 (3)		
Data collection				
Wavelength	1.0000	1.0090 (edge)	1.0039 (peak)	0.9183 (remote)
f'/f'' (Hg)		−18.49/10.19	−12.44/10.10	−7.281/8.650
Resolution range†	100–2.24 (2.29–2.24)	200–2.49 (2.55–2.49)	200–2.48 (2.54–2.48)	200–2.31 (2.36–2.31)
Reflections, observed/unique	192074/23690	219413/18642	219442/18997	257489/23013
R_{merge}†‡ (%)	6.1 (30.1)	5.4 (19.6)	6.4 (16.9)	6.7 (29.0)
Completeness† (%)	91.2 (81.2)	95.5 (79.2)	96.2 (95.0)	95.4 (93.0)
$I/\sigma(I)$	31.3	37.4	31.5	27.2
MAD phasing				
No. of sites		2	2	2
R_{iso}§ (%)		0.0	2.8	5.4
Phasing power (acentric/centric)¶			1.31/0.86	1.51/1.05
Cullis *R* (acentric/centric)††			0.75/0.77	0.69/0.68
Cullis $R_{anomalous}$‡‡			0.40	0.43
Mean figure of merit		0.696		

† Values in parentheses are for the outermost resolution shell. ‡ $R_{merge} = \sum |I_i - \langle I_i \rangle| / \sum \langle I_i \rangle$, where I_i is the observed intensity and $\langle I_i \rangle$ is the average intensity over symmetry-equivalent measurements. § $R_{iso} = \sum \big| |F_{PH}| - |F_P| \big| / \sum |F_P|$, where F_{PH} and F_P are the derivative and 'edge' structure factors, respectively. ¶ Phasing power = $\langle |F_H| / \big| |F_{PH}| - |F_P + F_H| \big| \rangle$, where F_H, F_{PH} and F_P are the heavy-atom, derivative and 'edge' structural factors, respectively. †† Cullis $R = \langle \big| |F_{PH}| - |F_P + F_H| \big| \rangle / \langle \big| |F_{PH}| - |F_P| \big| \rangle$. ‡‡ Cullis $R_{anomalous} = \langle |F_{PH}(+) - F_{PH}(-)| - 2F_H \sin \alpha_P \rangle / \langle |F_{PH}(+) - F_{PH}(-)| \rangle$, where α_P is the protein phase.

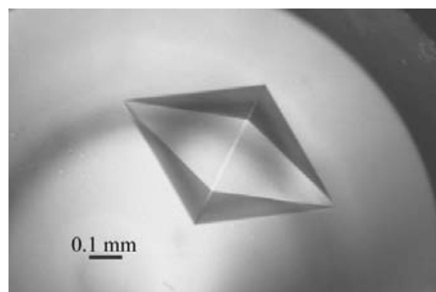

Figure 1
An Hg derivative crystal of lectin from *A. aurantia*. Hg-free native crystals and Hg cocrystals were grown in a similar way.

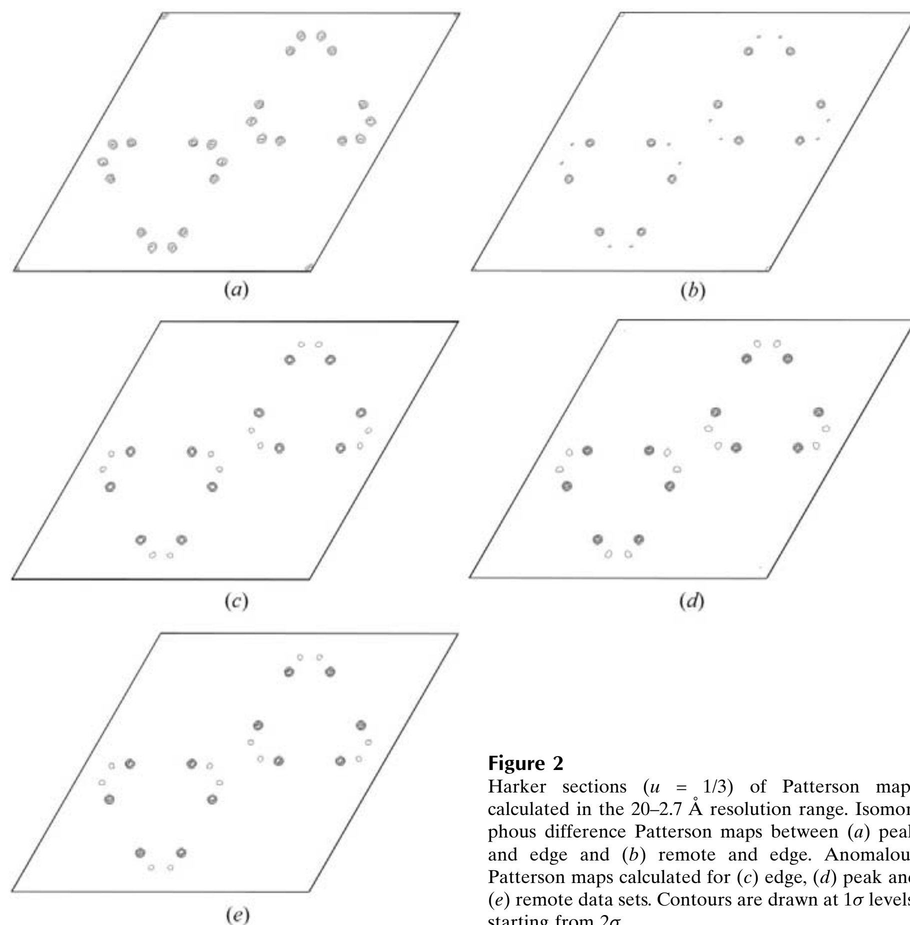

Figure 2
Harker sections (u = 1/3) of Patterson maps calculated in the 20–2.7 Å resolution range. Isomorphous difference Patterson maps between (*a*) peak and edge and (*b*) remote and edge. Anomalous Patterson maps calculated for (*c*) edge, (*d*) peak and (*e*) remote data sets. Contours are drawn at 1σ levels, starting from 2σ.

DENZO, *SCALEPACK* (Otwinowski & Minor, 1997) and *TRUNCATE* from the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994).

3. Phasing

The crystals of native AAL diffract X-rays to 2.24 Å resolution. They belong to the hexagonal space group $P6_122$ or $P6_522$, with unit-cell parameters a = 84.0, c = 250.1 Å. The Hg cocrystals diffract X-rays to 2.31 Å resolution. They belong to the same space group, with unit-cell parameters a = 83.9, c = 254.3 Å. These unit-cell parameters indicate that the native crystal and the Hg cocrystal were not isomorphous. Statistics of the intensity data are shown in Table 1.

Because of the difference in the c-axis length between the native crystal and the Hg cocrystal, the multiple isomorphous replacement (MIR) method could not be applied. Phasing was accomplished by the MAD method using data sets collected from the Hg cocrystal. Isomorphous-difference Patterson maps (between edge and peak and between edge and remote) and anomalous Patterson maps at three wavelengths show several clear peaks on the Harker sections (Fig. 2). The first Hg position could be deduced from the 12 relatively strong peak positions of these Patterson maps. Based on the first mercury position, phases were initially calculated using the program *MLPHARE* from *CCP*4 (Collaborative Computational Project, Number 4, 1994). The edge data, where f' is the lowest in the three data sets, were used as the reference data set (Table 1). Although there is no peak for the second Hg position in the isomorphous-difference Fourier maps, anomalous Fourier maps show clear peaks for the second Hg atom. The second position is consistent with the minor peaks on the Patterson maps shown in Fig. 2. Phases were recalculated on the basis of these two positions of the Hg atoms by the MAD method. The experimental phases were solvent flattened using the program *DM* from *CCP*4 (Collaborative Computational Project, Number 4, 1994). The electron-density map calculated in space group $P6_522$ was good enough for model building, whereas the map drawn in space group $P6_122$ could not be interpreted reasonably, indicating that the crystals belong to the space group $P6_522$. In the electron-density map, only one monomer was found in the crystallographic asymmetric unit. In this case, the Matthews coefficient V_M was calculated to be 3.9 $Å^3\,Da^{-1}$, indicating a large solvent content (68%) compared with known protein crystals (Matthews, 1968). The initial phases applied to the native diffraction data also gave reasonable electron-density maps.

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References

Audette, G. F., Vandonselaar, M. & Delbaere, L. T. (2000). *J. Mol. Biol.* **304**, 423–433.
Bianchet, M. A., Odom, E. W., Vasta, G. R. & Amzel, L. M. (2002). *Nature Struct. Biol.* **9**, 628–634.
Cheng, W., Bullitt, E., Bhattacharyya, L., Brewer, C. F. & Makowski, L. (1998). *J. Biol. Chem.* **273**, 35016–35022.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Drickamer, K. & Taylor, M. E. (1993). *Annu. Rev. Cell. Biol.* **9**, 237–264.
Fukumori, F., Takeuchi, N., Hagiwara, T., Ito, K., Kochibe, N., Kobata, A. & Nagata, Y. (1989). *FEBS Lett.* **250**, 153–156.
Fukumori, F., Takeuchi, N., Hagiwara, T., Ohbayashi, H., Endo, T., Kochibe, N., Nagata, Y. & Kobata, A. (1990). *J. Biochem. (Tokyo)*, **107**, 190–196.
Hakomori, S. & Kobata, A. (1974). *The Antigens*, Vol. 2, edited by M. Sela, pp. 79–140. New York: Academic Press.
Honda, S., Kashiwagi, M., Miyamoto, K., Takei, Y. & Hirose, S. (2000). *J. Biol. Chem.* **275**, 33151–33157.
Kochibe, N. & Furukawa, K. (1980). *Biochemistry*, **19**, 2841–2846.
Konami, Y., Yamamoto, K. & Osawa, T. (1991). *J. Biochem. (Tokyo)*, **109**, 650–658.
Liljeblad, M., Ryden, I., Ohlson, S., Lundblad, A. & Pahlsson, P. (2001). *Anal. Biochem.* **288**, 216–224.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Nagata, Y., Fukumori, F., Sakai, H., Hagiwara, T., Hiratsuka, Y., Kochibe, N. & Kobata, A. (1991). *Biochim. Biophys. Acta*, **1076**, 187–190.
Ohkura, T., Hada, T., Higashino, K., Ohue, T., Kochibe, N., Koide, N. & Yamashita, K. (1994). *Cancer Res.* **54**, 55–61.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Sakabe, N., Ikemizu, S., Sakabe, K., Higashi, T., Nakagawa, A., Watanabe, N., Adachi, S. & Sasaki, K. (1995). *Rev. Sci. Instrum.* **66**, 1276–1281.
Stelck, S., Robitzki, A., Willbold, E. & Layer, P. G. (1999). *Glycobiology*, **9**, 1171–1179.
Vijayan, M. & Chandra, N. (1999). *Curr. Opin. Struct. Biol.* **9**, 707–714.