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Crystallization and preliminary X-ray crystallographic analysis of dioscorin from *Dioscorea japonica*

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Dioscorin, the major tuber storage protein in yam, has been reported to possess carbonic anhydrase, trypsin inhibitor, angiotensin-converting enzyme (ACE) inhibitor, free-radical scavenger, dehydroascorbate reductase and monodehydroascorbate reductase activities. Recent research has also found that dioscorin can enhance immune modulation *via* the toll-like receptor 4 (TLR-4) signal transduction pathway in RAW 264.7 cells, murine bone-marrow cells and human monocytes *ex vivo*. Resolving the structure of dioscorin would help in better understanding its activities and would provide clues to understanding the mechanism of its multiple functions. The full-length protein (residues 1–246) with an additional His_6 tag at the N-terminus was expressed in *Escherichia coli* Rosetta (DE3) cells. After His-tag cleavage and purification, the protein was crystallized by the sitting-drop vapour-diffusion method at 278 K. An X-ray diffraction data set was collected to a resolution of 2.11 Å using a synchrotron X-ray source. The crystal belonged to space group $C222_1$, with unit-cell parameters a = 83.5, b = 156.8, c = 83.6 Å, and was estimated to contain two protein molecules per asymmetric unit.

1. Introduction

Yam, the tuber of *Dioscorea* spp., is a staple food in West Africa, South Asia and the Caribbean islands (Conlan *et al.*, 1995). In Asia, yam has traditionally been used as a health food and a herbal medicine. It has also received much attention owing to its functional properties and pharmaceutical potential (Akanbi *et al.*, 1996; Omonigho & Ikenebomeh, 2000), and some of these functions have been attributed to the storage protein dioscorin (Araghinknam *et al.*, 1996; Hou, Liu *et al.*, 1999; Hou *et al.*, 2000; Hsu *et al.*, 2002).

Dioscorin is the major storage protein in the yam tuber, in which it accounts for approximately 80–85% of the total soluble protein (Harvey *et al.*, 1983). This protein has been reported to exhibit carbonic anhydrase, trypsin inhibitor (Hou, Liu *et al.*, 1999), dehydroascorbate reductase and monodehydroascorbate reductase activities (Hou, Chen *et al.*, 1999). Recent research has also found that dioscorin is able to scavenge DPPH and hydroxyl free radicals (Hou *et al.*, 2001), to control high blood pressure (Hsu *et al.*, 2002) and to enhance immune modulation (Fu *et al.*, 2006; Liu *et al.*, 2009). To unveil the distinct biological properties of dioscorin, a number of studies have focused on determining its secondary structure and exploring its functional properties (Liao *et al.*, 2006; Hou, Chen *et al.*, 1999; Liu *et al.*, 2007). However, very little is known about the relevance of the tertiary structure of dioscorin. Therefore, high-resolution crystal structure analysis will be needed in order to clarify the mechanisms that are responsible for the multiple activities and functions of dioscorin.

We have already cloned the gene of and characterized dioscorin from *D. japonica* (Xue *et al.*, 2012). In the present work, we report the preparation, crystallization and preliminary X-ray crystallographic analysis of recombinant *D. japonica* dioscorin. Resolving the

structure of dioscorin may help us to better understand its structure–function relationship.

2. Materials and methods

2.1. Protein preparation

According to a previously described method (Sawano *et al.*, 2008; Xue *et al.*, 2012), full-length dioscorin (residues 1–246) was cloned into the *Nde*I and *Xho*I sites of plasmid pET28a (Novagen) and was expressed in *Escherichia coli* Rosetta (DE3) cells (Novagen) with an additional His_6 tag at the N-terminus. After harvesting, the cells were disrupted by sonication on ice and cell debris was removed by centrifugation at 40 000*g* for 30 min at 277 K. After affinity purification using 3 ml Ni Sepharose 6 Fast Flow resin (GE Healthcare) and on-column His-tag cleavage using 50 units of thrombin (GE Healthcare), the target protein was eluted and was further purified by anion-exchange chromatography (Resource Q column; GE Healthcare) using an ÄKTA FPLC system (GE Healthcare). The purity of the protein was checked by SDS–PAGE. It was concentrated to 20 mg ml^{-1} in 20 m*M* Tris–HCl pH 8.0, 1 m*M* DTT and was stored at 193 K for crystallization trials.

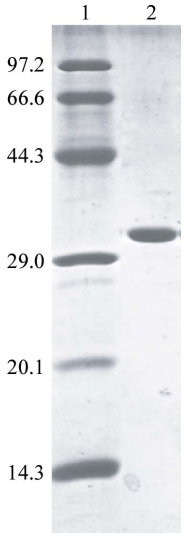

Figure 1
12% SDS–PAGE analysis of dioscorin following ion-exchange chromatography. Lane 1, molecular-weight markers (labelled in kDa); lane 2, purified dioscorin (approximately 31 kDa).

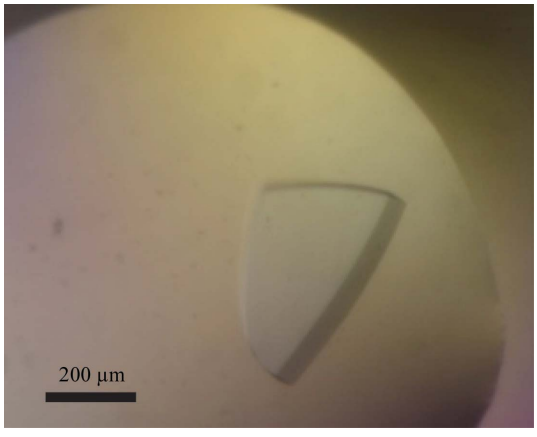

Figure 2
A dioscorin crystal obtained using the optimized crystallization conditions.

2.2. Crystallization

Initial crystallization trials of the purified protein were performed manually by the sitting-drop vapour-diffusion method at 278 K using the screening kits Crystal Screen HT, Index HT (Hampton Research) and Wizard I and II (Jena Bioscience), with drops consisting of 0.5 µl protein solution and 0.5 µl reservoir solution equilibrated against 40 µl reservoir solution in 96-well Intelli-Plates. Dioscorin crystals were observed under several crystallization screening conditions. Several rounds of optimization were performed with respect to buffer composition, pH, precipitant concentration and protein concentration.

2.3. Data collection and processing

Crystals were cryoprotected with a mixture of 30% ethylene glycol and 70% reservoir solution, mounted on nylon loops (Hampton Research) and immediately exposed to a stream of liquid nitrogen at 95 K to flash-cool the crystals. Preliminary X-ray data were collected on the NW12 synchrotron-radiation beamline at Photon Factory (Tsukuba, Japan). 180° of data were collected as 360 diffraction images using a 0.5° oscillation angle and an exposure time of 2 s. The

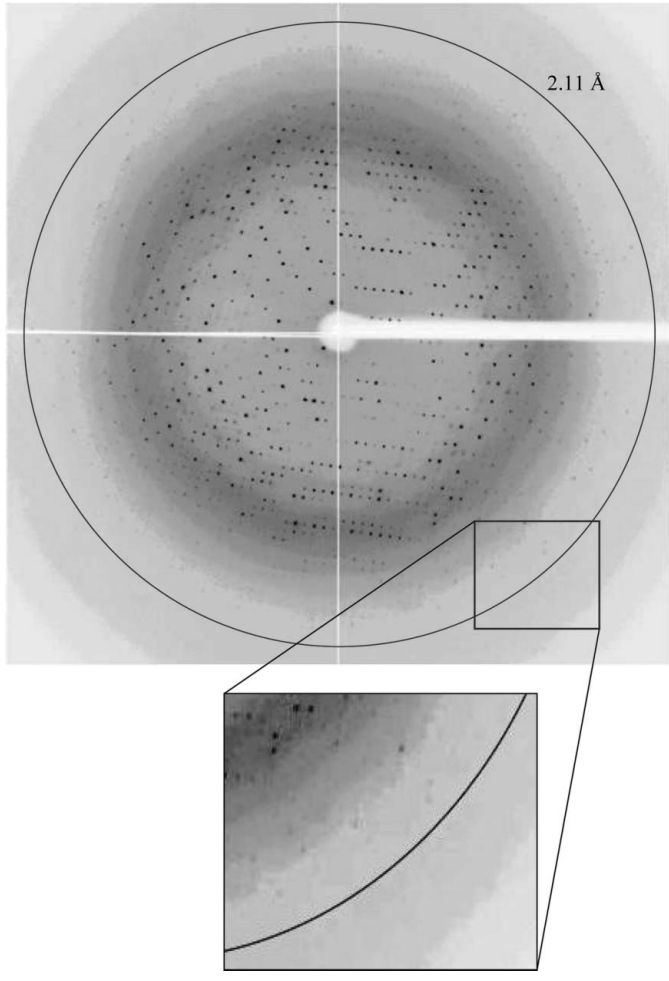

Figure 3
An X-ray diffraction image (0.5° oscillation) collected from a crystal of dioscorin. The circle indicates a resolution of 2.11 Å.

Table 1
Crystal parameters and data-collection statistics.

Values in parentheses are for the highest resolution shell.

Beamline	PF-AR NW12A
Wavelength (Å)	1.0000
Space group	$C222_1$
Unit-cell parameters (Å)	a = 83.5, b = 156.8, c = 83.6
Resolution range (Å)	20.0–2.11 (2.16–2.11)
No. of observed reflections	222696 (10400)
No. of unique reflections	31706 (2203)
Average multiplicity	7.02 (4.72)
Completeness (%)	99.3 (95.2)
$R_{\rm merge}$†	0.033 (0.212)
Average $I/\sigma(I)$	39.32 (6.61)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ and $\langle I(hkl)\rangle$ are the observed intensity and mean intensity of symmetry-related reflections, respectively.

wavelength was set to 1.0 Å and the crystals diffracted X-rays beyond 2.11 Å resolution. The crystal-to-detector distance was 212.5 mm. The diffraction images were integrated and scaled using the *XDS* package (Kabsch, 2010).

3. Results and discussion

Recombinant dioscorin was expressed in *E. coli* and purified by affinity-resin and anion-exchange chromatography. The yield was 1.2 mg per litre of *E. coli* culture. As shown in Fig. 1, the purity of dioscorin was confirmed by SDS–PAGE. Crystal screening was performed for the recombinant dioscorin. The best initial crystallization conditions were Wizard I solution C9 (0.1 *M* CAPS pH 10.5, 0.2 *M* lithium sulfate, 2.0 *M* ammonium sulfate). Tiny stick-shaped crystals were detected under these conditions. After several rounds of optimization, a single crystal with dimensions of 0.5 × 0.3 × 0.06 mm was obtained after 6 d using a reservoir solution consisting of 0.1 *M* CAPS pH 10, 0.2 *M* lithium sulfate, 1.8 *M* ammonium sulfate at 278 K (Fig. 2). The concentration of the protein for the final crystallization was 12 mg ml^{-1}. X-ray diffraction data were collected at a resolution of 2.11 Å (Fig. 3). The crystal belonged to the centred orthorhombic space group $C222_1$, with unit-cell parameters a = 83.5, b = 156.8, c = 83.6 Å. A total of 222 696 reflections in the resolution range 20.0–2.11 Å were collected with 99.3% completeness and an $R_{\rm merge}$ of 3.3%. The data-collection and processing statistics are summarized in Table 1. Matthews analysis suggested that there were two molecules per asymmetric unit in the dioscorin crystal (Matthews coefficient of 2.43 Å^3 Da^{-1}, solvent content 49.3%; Matthews, 1968).

We are currently attempting phase determination by the molecular-replacement method using the crystal structure of carbonic anhydrase from *Neisseria gonorrhoeae* (PDB entry 1kop; Huang *et al.*, 1998) as a search model with the *MOLREP* program (Vagin & Teplyakov, 2010) included in the *CCP*4 program package (Winn *et al.*, 2011). A single correct solution was found and complete structure determination and refinement are in progress.

The synchrotron-radiation experiments were performed on beamline NW12 at Photon Factory with the approval of the High Energy Accelerator Research Organization (Proposal No. 2011G605). This work was supported by the Targeted Proteins Research Program (TPRP) of the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References

Akanbi, C. T., Gureje, P. O. & Adeyemi, I. A. (1996). *J. Food Eng.* **28**, 45–54.
Araghinknam, M., Chung, S., White, T. N., Eskelson, E. & Waston, R. R. (1996). *Life Sci.* **59**, 147–157.
Conlan, R. S., Griffiths, L. A., Napier, J. A., Shewry, P. R., Mantell, S. & Ainsworth, C. (1995). *Plant Mol. Biol.* **28**, 369–380.
Fu, S.-L., Hsu, Y.-H., Lee, P.-Y., Hou, W.-C., Hung, L.-C., Lin, C.-H., Chen, C.-M. & Huang, Y.-J. (2006). *Biochem. Biophys. Res. Commun.* **339**, 137–144.
Harvey, P. J. & Boulter, D. (1983). *Phytochemistry*, **22**, 1687–1693.
Hou, W.-C., Chen, H.-J. & Lin, Y.-H. (1999). *Plant Sci.* **149**, 151–156.
Hou, W.-C., Chen, H.-J. & Lin, Y.-H. (2000). *Bot. Bull. Acad. Sin.* **41**, 191–196.
Hou, W.-C., Lee, M.-H., Chen, H.-J., Liang, W.-L., Han, C.-H., Liu, Y.-W. & Lin, Y.-H. (2001). *J. Agric. Food Chem.* **49**, 4956–4960.
Hou, W.-C., Liu, J.-S., Chen, H.-J., Chen, T.-E., Chang, C.-F. & Lin, Y.-H. (1999). *J. Agric. Food Chem.* **47**, 2168–2172.
Hsu, F.-L., Lin, Y.-H., Lee, M.-H., Lin, C.-L. & Hou, W.-C. (2002). *J. Agric. Food Chem.* **50**, 6109–6113.
Huang, S., Xue, Y., Sauer-Eriksson, E., Chirica, L., Lindskog, S. & Jonsson, B. H. (1998). *J. Mol. Biol.* **283**, 301–310.
Kabsch, W. (2010). *Acta Cryst.* D**66**, 125–132.
Liao, Y.-H., Tseng, C.-Y. & Chen, W. (2006). *J. Phys. Conf. Ser.* **28**, 119–122.
Liu, Y.-W., Liu, J.-C., Huang, C.-Y., Wang, C.-K., Shang, H.-F. & Hou, W.-C. (2009). *J. Agric. Food Chem.* **57**, 9274–9279.
Liu, Y.-W., Shang, H.-F., Wang, C.-K., Hsu, F.-L. & Hou, W.-C. (2007). *Food Chem. Toxicol.* **45**, 2312–2318.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Omonigho, S. E. & Ikenebomeh, M. J. (2000). *Food Chem.* **71**, 215–220.
Sawano, Y., Hatano, K., Miyakawa, T., Komagata, H., Miyauchi, Y., Yamazaki, H. & Tanokura, M. (2008). *Plant Physiol.* **146**, 1909–1919.
Vagin, A. & Teplyakov, A. (2010). *Acta Cryst.* D**66**, 22–25.
Winn, M. D. *et al.* (2011). *Acta Cryst.* D**67**, 235–242.
Xue, Y.-L., Miyakawa, T., Sawano, Y. & Tanokura, M. (2012). *Plant Sci.* **183**, 14–19.