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Purification, crystallization and X-ray diffraction study of basic 7S globulin from soybean

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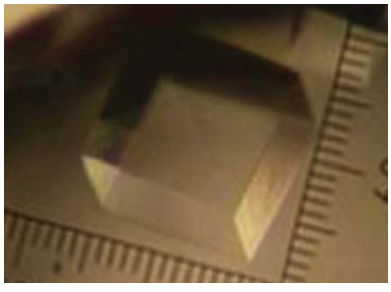


Basic 7S globulin (Bg7S) is expressed by soybeans in response to biotic or abiotic stress. Bg7S is capable of binding to a 4 kDa protein which is supposedly involved in cell proliferation. Bg7S is widely found not only in legumes, but also in other plants; however, its function is still unclear. Here, Bg7S was successfully crystallized. Orthorhombic and monoclinic crystals of Bg7S were obtained under different conditions and belonged to space groups $P2_12_12$, with unit-cell parameters a = 111.9, b = 130.1, c = 287.8 Å, and $P2_1$, with unit-cell parameters a = 85.3, b = 137.6, c = 162.1 Å, β = 91.2°, respectively.

1. Introduction

Plants express various proteins in response to biotic or abiotic stress. When mature soybean seeds are immersed in hot water at 323–333 K, many proteins are released into the water. The major of these proteins is basic 7S globulin (Bg7S), which has a molecular mass of 43 kDa and consists of 27 and 16 kDa chains linked by disulfide bonds. Bg7S binds a 4 kDa protein from soybean called leginsulin, which is supposedly involved in cell proliferation. Thus, Bg7S is also called 43 kDa protein or leginsulin-binding protein (LBP; Hirano *et al.*, 1992). Normally, Bg7S is localized in plasma membranes and cell walls (Nishizawa *et al.*, 1994). Carrot callus tissue cultured with the 4 kDa protein grows more rapidly than in medium lacking the 4 kDa protein (Yamazaki *et al.*, 2003), suggesting that the 4 kDa protein may regulate growth, differentiation and cell proliferation. In previous work, the three-dimensional structure of the 4 kDa protein has been determined by NMR spectroscopy (Yamazaki *et al.*, 2003). The 4 kDa protein adopts a T-knot motif that relies on disulfide bonds to maintain its structure. The T-knot motif is shared by disulfide-rich small proteins with diverse functions, such as growth factors in animals, protease inhibitors, antimicrobial peptides in plants and toxin in insects (Lin & Nussinov, 1995). These small proteins bind to their target proteins to regulate or inhibit their functions.

Proteins that are homologous to the 4 kDa protein have only been discovered in legumes. In contrast, homologues of Bg7S are widely present in plants such as wheat, tomato and *Arabidopsis* (York *et al.*, 2004). It has been reported that several proteins that are homologous to Bg7S inhibit the activity of endoglucanases that belong to glycoside hydrolase families 11 or 12 (GH11 or GH12). GH11 and GH12 cleave xyloglucan or xylan, which are major components of the cell wall. It is therefore suggested that the homologues of Bg7S play important roles in plant defence by inhibition of the GH11 and GH12 endoglucanases secreted by invading phytopathogens. Functional studies of homologues from wheat (TAXI-I and TAXI-II; Gebruers *et al.*, 2004; Bourgois *et al.*, 2007), tomato (XEGIP; Qin *et al.*, 2003), tobacco (NEC4; Naqvi *et al.*, 2005), lupin (γ-conglutin; Scarafoni *et al.*, 2010) and carrot (EDGP; Shang *et al.*, 2005) have been reported. Of these, crystal structures of TAXI-I and TAXI-II, which specifically inhibit GH11 enzymes, have been determined (Sansen *et al.*, 2004; Pollet *et al.*, 2009). Bg7S is predicted to have diverse functions as a receptor-like protein in signal transduction in combination with the 4 kDa protein and as a defensive factor against plant-pathogen endoglucanases. However, the details of the function of Bg7S are still unclear. In the present paper, we describe the purification and crystallization of Bg7S and initial diffraction analysis of the crystals.

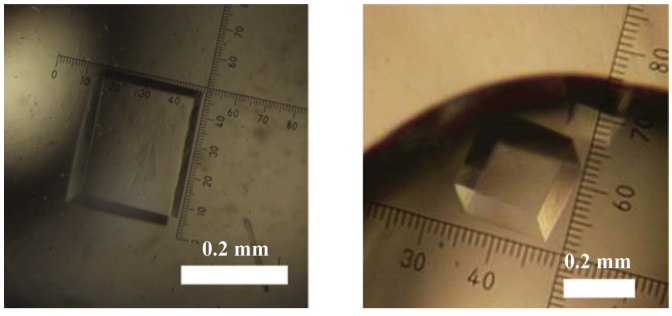
2. Materials and results

2.1. Preparation of Bg7S for crystallographic study

Soybean seeds (*Glycine max* L. Merrill cv. Miyagishirome) were ground in a food processor (Cuisinart) with 5 ml water per gram of soybean seeds at room temperature. The soybean paste was homogenized using a Polytron homogenizer (Kinematica) with an additional 5 ml water per gram of seeds at 277 K and then filtered using Miracloth (Merck Biosciences). The residue was further homogenized in 10 ml buffer (20 m*M* potassium phosphate pH 7.4 and 0.5 *M* NaCl) per gram of soybean seeds for 30 min at 277 K and centrifuged for 30 min at 277 K (43 667*g*). The supernatant was applied onto an Ni Sepharose column equilibrated with buffer consisting of 20 m*M* potassium phosphate pH 7.4 and 500 m*M* KCl. The bound proteins were eluted with a linear gradient from 0 to 100 m*M* imidazole. Fractions containing Bg7S were applied onto a HiTrap SP HP column (GE Healthcare) equilibrated with 20 m*M* potassium phosphate pH 7.4 and the bound proteins were eluted with a linear gradient from 0 to 400 m*M* KCl. Fractions containing Bg7S were applied onto an Econo-Pac CM column (Bio-Rad) equilibrated with 20 m*M* potassium phosphate pH 7.4 and the bound proteins were eluted with a linear gradient from 0 to 300 m*M* KCl. Fractions containing Bg7S were concentrated to 20 mg ml^{-1} in 20 m*M* potassium phosphate pH 7.4 using Amicon Ultra 30 kDa cutoff filter units (Millipore). The purity of Bg7S was confirmed by SDS–PAGE with Coomassie Brilliant Blue stain. About 30 g mature soybean seeds yielded approximately 10 mg purified Bg7S.

2.2. Crystallization and initial crystallographic study of Bg7S

Screening of crystallization conditions for Bg7S was performed by the sitting-drop vapour-diffusion method using a Hydra II Plus One (Matrix) and 1150 different reservoir solutions from commercially available screening kits (Hampton Research, Molecular Dimensions, Emerald BioSystems and Qiagen). Crystals were obtained using several reservoir solutions, including Crystal Screen condition No. 42 (50 m*M* KH_2PO_4 and 20% PEG 800) and Wizard II condition No. 46 [1.0 *M* $(NH_4)_2HPO_4$, 0.1 *M* imidazole pH 8.0 and 0.2 *M* NaCl]. The buffer, the pH and the salt and precipitant concentrations of these crystallization conditions were optimized using the hanging-drop vapour-diffusion method. Two types of Bg7S crystal were obtained in different conditions (Figs. 1*a* and 1*b*). The orthorhombic crystal of Bg7S (form I) was obtained using a reservoir solution consisting of 50 m*M* KH_2PO_4, 100 m*M* sodium citrate pH 5.3, 6.5% PEG 8000 and 20% ethylene glycol after several days. Prior to the X-ray experiment, the form I crystal was transferred to a cryoprotectant solution consisting of 50 m*M* KH_2PO_4, 100 m*M* sodium citrate pH 5.3, 6.5% PEG 8000 and 30% ethylene glycol with a nylon loop and then cooled in a



(*a*) (*b*)

Figure 1
Crystal forms I (*a*) and II (*b*) of Bg7S.

Table 1
Data-collection statistics for Bg7S crystals.

Values in parentheses are for the highest resolution shell.

	Form I	Form II
Wavelength (Å)	1.0000	1.0000
Resolution range (Å)	50.0–2.90 (3.00–2.90)	50.0–2.60 (2.69–2.60)
Measured reflections	643183 (38025)	364026 (36799)
Unique reflections	90761 (6671)	105418 (10514)
Multiplicity	7.1 (5.7)	3.5 (3.4)
Completeness (%)	96.2 (71.7)	88.7 (89.0)
Mean $I/\sigma(I)$	13.9 (5.4)	12.3 (3.3)
$R_{\rm merge}$† (%)	8.8 (30.2)	10.1 (43.5)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$.

N_2-gas stream at 100 K. X-ray diffraction data were collected using a Quantum 315 CCD detector (Area Detector Systems Corp.) on beamline BL-41XU at SPring-8. Diffraction data were integrated, scaled and averaged with *HKL*-2000 (Otwinowski & Minor, 1997). The form I crystal belonged to space group $P2_12_12$, with unit-cell parameters a = 111.9, b = 130.1, c = 287.8 Å. Data-collection statistics are summarized in Table 1. The asymmetric unit of the form I crystal is estimated to contain 8–12 molecules ($V_{\rm M}$ = 3.04–2.02 Å^3 Da^{-1}). The monoclinic crystal (form II) was obtained using reservoir solution consisting of 1.0 *M* $(NH_4)_2HPO_4$ and 100 m*M* imidazole pH 8.0 after one week. For cryoprotection, the crystal was transferred into a cryoprotectant solution consisting of 1.0 *M* $(NH_4)_2HPO_4$, 100 m*M* imidazole pH 8.0 and 2.5 *M* K_2HPO_4. X-ray diffraction data were collected from the form II crystal using a Quantum 315 CCD detector (Area Detector Systems Corp.) on beamline BL-5A at the Photon Factory (PF). The form II crystal belonged to space group $P2_1$, with unit-cell parameters a = 85.3, b = 137.6, c = 162.1 Å, β = 91.2°. The asymmetric unit of the form II crystal is estimated to contain 8–10 molecules ($V_{\rm M}$ = 2.96–2.15 Å^3 Da^{-1}). The preparation of heavy-atom derivatives and structure determination of Bg7S by the isomorphous replacement method are now in progress.

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