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# Crystal structures of two lectin isomers from the roots of pokeweed

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## Introduction

Pokeweed lectin is a lectin specific for *N*-acetylglucosamine-containing saccharides, and stimulates peripheral lymphocytes to undergo mitosis by binding to their cell surfaces. Several lectins have been isolated from pokeweed roots and shown that they are homologous proteins but have different molecular sizes and biological properties [1]. They are composed of several chitin-binding domains homologous to wheat germ agglutinin. In these proteins, PL-Ds composed of two domains are the smallest one in the group and have two isomers, PL-D1 and PL-D2. PL-D1 consists of 84 amino acid residues ($M_r$ = 9,317), while PL-D2 has an identical sequence with PL-D1 except lack of the C-terminal Leu83-Thr84 [2]. Both PL-Ds showed the agglutinating activity toward trypsin-treated erythrocytes. Although PL-D2 presents mitogen activity toward lymphocyte cell, PL-D1 does not. We determined the crystal structures of PL-D1 and PL-D2 in order to elucidate the structural basis for mitogen activity of pokeweed lectine.

## Experimental

Crystals of PL-D2 was obtained by batch method using seeding technique with reservoir solution of 18% PEG8K, 0.2M Calcium acetate, and 0.1M Na acetate buffer pH4.6 at 25°C. Diffraction data for PL-D2 was collected on a screenless Weissenberg camera with imaging plates (400 mm 200 mm size) at the BL18B station of the Photon Factory. The data collection of PL-D2 was performed at 291K with two crystals with different orientation sealed in a glass capillary. The data were processed, scaled and merged with programs DENZO and SCALEPACK. The crystal belongs to space group $P2_1$ with unit cell dimensions of $a$ = 23.24 Å, $b$ = 56.95 Å, $c$ = 29.62Å, β = 109.3° containing one molecule in the asymmetric unit.

The structure of PL-D2 was determined by molecular replacement method using program MOLREP of program suit CCP4. The fourth-domain of wheat germ agglutinin isolectin 3 was employed as a starting model. The refinement was carried out using programs X-PLOR and CNS. The refinement of the current model was converged to an *R*-factor of 17.6% and the free *R*-factor of 20.7% at 1.5 Å.

Crystals of PL-D1 were grown by a hanging drop vapor diffusion method with reservoir solution of 30% PEG8K, 0.2M Calcium acetate, and 0.1M Na phosphate buffer pH6.5 at 25°C. Diffraction data for PL-D1 was collected on an ADSC Quantum 4 CCD detector at the BL6A station of the Photon Factory. The data collection of PL-D1 was performed at 100K with a flash-cooled crystal in the liquid nitrogen gas stream. The data were processed, scaled and merged with program DPS/MOSFLM and CCP4 program suit. The crystal belongs to space group $P2_12_12$ with unit cell dimensions of $a$ = 48.67 Å, $b$ = 49.01 Å, $c$ = 29.93 Å containing one molecule in the asymmetric unit.

The structure of PL-D1 was determined by molecular replacement method using program MOLREP of program suit CCP4 using PL-D2 model as a starting model. The refinement using program CNS was carried out. The refinement of the current model was converged to an *R*-factor of 17.5% and the free *R*-factor of 20.2% at 1.6 Å.

## Results and Discussion

The polypeptide folds of PL-Ds consists of two hevein-type domains, which have no secondary structure. On the comparison with PL-D2, the inclination of the arrangement of the domains in PL-D1 is about 10°. These facts indicates that the flexibility between two domains in PL-Ds. In the crystal structure of PL-D2, calcium ion binds the C-terminal regions. The β-carboxyl group of Asp57, α-carboxyl group of Asp82, β-carboxyl group of Asp48 in neighboring molecule, and three water molecules coordinate to the ion. Although these interactions apparently participate in the formation of crystal lattice, the physiological meaning of these interactions is unknown.

The electron density of the excess elongating C-terminal region, Leu83 and Thr84, of PL-D1 is not observed in the present crystal. One simple hypothesis could be supposed that the region around C-terminus of PL-D2 is the binding site for target protein which would cause mitogen activation. The flexible elongated C-terminal two amino-acid residues of PL-D1 may prevent the interaction.

## References

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