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Crystallization and preliminary X-ray crystallographic studies of the sixth PDZ domain of glutamate-receptor interacting protein 1 (GRIP1) from *Rattus norvegicus*

Seong Ho Park,[a] Young Jun Im,[a] Seong Hwan Rho,[a] Jun Hyuck Lee,[a] Soyoung Yang,[b] Eunjoon Kim[b] and Soo Hyun Eom[a]*

[a]Department of Life Science, Kwangju Institute of Science and Technology, Gwangju 500-712, South Korea, and [b]Department of Biological Science, Korea Advanced Institute of Science and Technology, Daejon 305-701, South Korea

Correspondence e-mail: eom@kjist.ac.kr

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The sixth PDZ domain from GRIP1 and its complex with the octapeptide of the liprin-α1 C-terminus were crystallized at 294 K by the hanging-drop vapour-diffusion method. The native crystal belongs to space group $P6_122$ (or $P6_522$), with unit-cell parameters $a = b = 40.3$, $c = 222.9$ Å. The complex crystal belongs to space group $R32$, with unit-cell parameters $a = b = 117.8$, $c = 102.0$ Å. Native and peptide-complex diffraction data were collected to resolutions of 1.5 and 1.8 Å, respectively, using synchrotron X-rays.

1. Introduction

PDZ domains, named after three structurally related proteins PSD-95 (postsynaptic density protein, 95 kDa), Dlg (*discs-large* protein) and ZO-1 (zonula occludens-1), are molecular-recognition elements that mediate protein–protein interactions (Garner *et al.*, 2000). They are associated with the localization of channels, signalling enzymes and adhesion molecules to sites of cell–cell contact (Craven & Bredt, 1998). The PDZ domain is a globular domain containing about 80–100 amino acids and is a conserved motif with two α-helices and six β-strands which has been identified in many intracellular proteins. Most of them bind selectively to the C-termini of the interacting proteins in the complexes of signalling molecules, membrane-associated receptors and channels (Ponting *et al.*, 1997; Songyang *et al.*, 1997; Schultz *et al.*, 1998).

The synaptic PDZ domain-containing protein GRIP (glutamate-receptor interacting protein) specifically interacts with the C-termini of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionate) receptors (Dong *et al.*, 1997). GRIP is a distinctive member of the PDZ-domain-containing protein family and has seven PDZ domains and no catalytic domain. GRIP appears to serve as an adapter protein that links AMPA receptors to other proteins and may be critical for the clustering of AMPA receptors at excitatory synapses in the brain. GRIP has two subtypes, GRIP1 and GRIP2, and GRIP2 is also expressed to ABP (AMPA-receptor-binding protein) by an alternative splicing (Dong *et al.*, 1999).The sixth PDZ domain of the seven PDZ domains of GRIP1 (GRIP1-PDZ6) binds to the C-terminus of liprin-α, which binds to the LAR family of receptor tyrosine phosphatases (Wyszynski, Kim and Sheng, unpublished work). LAR family transmembrane protein tyrosine phosphatases function in axon guidance and mammary-gland development (Serra-Pages *et al.*, 1998). Like the action of GRIP on the AMPA receptor, GRIP proteins may provide a scaffold for the assembly of the developmental signalling complex (Dong *et al.*, 1999; Wyszynski *et al.*, 1999). As the first step toward its structural elucidation, we report the overexpression, purification and crystallization of the GRIP1-PDZ6 domain and its complex with the liprin-α1 C-terminal octapeptide as well as its preliminary X-ray characterization.

2. Materials and methods

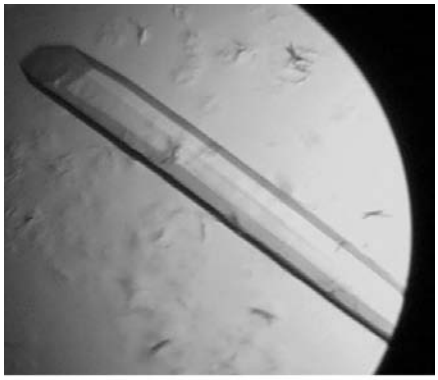
2.1. Expression and purification

The DNA for the GRIP1-PDZ6 domain (amino acids 665–761) was subcloned between the *Eco*RI and *Bam*HI sites of the expression vector pGEX4T-1 (Pharmacia Biotech) and transformed into *Escherichia coli* strain C41(DE3) (Miroux & Walker, 1996). The bacterial cells were induced by 1 m*M* isopropyl β-D-thiogalactopyranoside at 310 K for 2 h and harvested by centrifugation at 4500*g* for 20 min. Cells were resuspended in the lysis buffer (1× PBS; phosphate-buffered saline) and lysed in a French press; the lysate was centrifuged at 16 000*g* for 30 min. Glutathione-Sepharose 4B affinity resin (Peptron) was loaded onto a gravity-flow column (Bio-Rad) and pre-equilibrated with lysis buffer (2 ml bed volume per litre of culture). The supernatant was added to the column and the matrix was washed with ten bed volumes of lysis buffer. The glutathione-S-transferase (GST) fusion protein was eluted by addition of 50 m*M* Tris–HCl pH 8.0, 10 m*M* glutathione. The GST protein was cleaved off by thrombin and

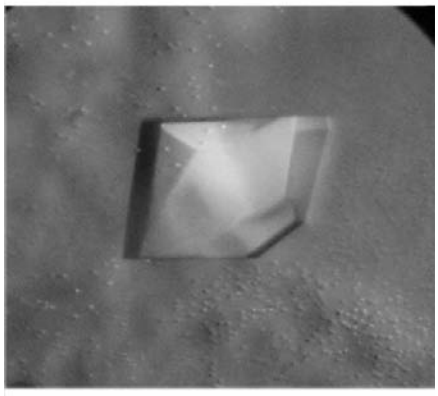
GRIP1-PDZ6 domain was further purified by gel filtration on a Superdex 75 column (Pharmacia Biotech) pre-equilibrated with 50 m*M* Tris–HCl pH 7.4. The fractions containing the GRIP1-PDZ6 domain were collected and concentrated by ultrafiltration to 18 mg ml^{-1} (Amicon Centricon 3). The octapeptide (ATVRTYSC) of the liprin-α1 C-terminus (Serra-Pages *et al.*, 1998) was synthesized (Anygen).

2.2. Crystallization and data collection

GRIP1-PDZ6 was crystallized at room temperature (294 ± 1 K) using the hanging-drop vapour-diffusion method. Crystals (Fig. 1*a*) were grown on a siliconized cover slip by equilibrating a mixture containing 2 µl of protein solution (18 mg ml^{-1} protein in 50 m*M* Tris–HCl pH 7.4) and 2 µl of reservoir solution [22%(*w*/*v*) PEG 8000, 100 m*M* CHES–NaOH pH 9.0–9.5] against 0.5 ml of reservoir solution. Crystals of the GRIP1-PDZ6–peptide complex were grown by equilibrating a mixture containing 2 µl of a protein solution containing the synthesized peptide (18 mg ml^{-1} protein in 50 m*M* Tris–HCl pH 7.4 plus 4 m*M* peptide) and 2 µl of reservoir solution [100 m*M* sodium citrate pH 5.5, 14%(*w*/*v*) PEG 4000 and 20%(*v*/*v*) MPD] against 0.5 ml of reservoir solution using the hanging-drop vapour-diffusion method at 294 ± 1 K.



(*a*)



(*b*)

Figure 1
(*a*) Crystal of GRIP1-PDZ6 grown for 2 d using 22% PEG 8000 as a precipitating agent (pH 9.0–9.5). Its approximate dimensions are 0.2 × 0.2 × 0.8 mm. (*b*) Crystal of GRIP1-PDZ6 complexed with the liprin-α1 C-terminal octapeptide grown for 2 d using 14% PEG 4000 as a precipitating agent (pH 5.5). Its approximate dimensions are 0.2 × 0.2 × 0.3 mm.

Table 1
Summary of the data statistics.

Values in parentheses indicate the statistics for the last resolution shell.

	Native I	Native II	NaBr	Complex
X-ray source	Rigaku RU-300	X8C (NSLS)	X8C (NSLS)	BL-18B (PF)
Wavelength (Å)	1.54	0.90	0.9192	λ = 1.00
Resolution range (Å)	50–2.5	15–1.5	50–1.9	99–1.8
Space group	$P6_122$ (or $P6_522$)	$P6_122$ (or $P6_522$)	$P6_122$ (or $P6_522$)	$R32$
Unit-cell parameters (Å)	$a = b = 40.3$, $c = 223.2$	$a = b = 40.3$, $c = 222.9$	$a = b = 40.4$, $c = 223.1$	$a = b = 117.8$, $c = 102.0$
No. of observations	35812	151976	90315	191677
Unique reflections	4312	19920	9461	25271
Completeness (%)	99.3 (99.0)	98.3 (93.3)	99.0 (91.9)	99.6 (97.3)
Mean $I/\sigma(I)$	14.7 (11.4)	11.5 (3.6)	12.7 (6.1)	10.1 (4.5)
$R_{\rm merge}$† (%)	8.3 (31.1)	4.3 (36.0)	5.3 (17.9)	8.8 (56.6)

† $R_{\rm merge} = \sum_h \sum_i |I(h)_i - \langle I\rangle| / \sum_h \sum_i I(h)_i$, where $I(h)$ is the intensity of reflection h, $\sum_h$ is the sum over all reflections and $\sum_i$ is the sum over i measurements of reflection h.

For the cryogenic experiments, a suitable cryoprotectant was determined to be reservoir solution plus 20%(*v*/*v*) glycerol. Successful flash-freezing was achieved when the crystals were transferred directly from the drop to cryoprotection solution and were allowed to equilibrate for 30 s. The first set of native data was collected on an R-AXIS IV image-plate system attached to a Rigaku rotating-anode generator (RU-300) providing Cu $K\alpha$ radiation and running at 50 kV and 90 mA with a 0.3 mm focus cup in a nitrogen-gas stream at 110 K (Oxford Cryosystems).

The second native data set was collected to 1.5 Å resolution at 100 K with an ADSC Quantum 4R CCD detector at beamline X8C at the National Synchrotron Light Source, Brookhaven National Laboratory, USA. A multiwavelength anomalous dispersion (MAD) experiment was carried out. Since the bromine is a convenient anomalous scatterer for MAD phasing, the crystals were soaked for 30 s in the cryoprotection solution containing 1 *M* NaBr (Dauter *et al.*, 2000). MAD data were collected to 1.9 Å using X-rays at three wavelengths (0.9202, 0.9192 and 0.9000 Å). Data sets were processed and scaled using the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997).

The data set from the complex crystal was collected to 1.8 Å at 100 K with an ADSC Quantum 4R CCD detector at beamline BL-18B at the Photon Factory, Japan. Successful flash-freezing was achieved when the crystals were mounted directly in the liquid-nitrogen stream. The data set was processed and scaled with the program *MOSFLM* (Leslie, 1994).

3. Results

The first native data set from the GRIP1-PDZ6 domain, collected using Cu $K\alpha$ X-rays and an R-AXIS IV system, indicated that the crystal belongs to the primitive hexagonal space group $P6_122$ (or $P6_522$), with unit-cell parameters $a = b = 40.3$, $c = 223.2$ Å. Assuming one molecule per asymmetric unit, the Matthews coefficient ($V_{\rm M}$) was calculated to be 2.55 Å^3 Da^{-1}, corresponding to a solvent content of 51.8% (Matthews, 1968). Initially, molecular-replacement calculations were carried out with *AMoRe* (Navaza & Saludjian, 1997) using search models based on nNOS (PDB code 1qav; Hillier *et al.*, 1999), hCASK (PDB code 1kwa; Daniels *et al.*, 1998) and the third PDZ domain of PSD-95 (PDB code 1be9; Doyle *et al.*, 1996), which have sequence identities of 39, 30 and 37%, respectively, but no distinct solution appeared.

The second native and the MAD data set from the GRIP1-PDZ6 domain were collected using an ADSC Quantum 4R CCD detector with synchrotron radiation at beamline X8C of the National Synchrotron Light Source, Brookhaven National Laboratory. The diffraction limit of the native data set was improved to 1.5 Å and the unit-cell parameters were $a = b = 40.3$, $c = 222.9$ Å. The resolution limit of the MAD data was 1.9 Å and the unit-cell parameters were $a = b = 40.4$, $c = 223.1$ Å. The bromine positions were located using the program *SOLVE* (Terwilliger & Berendzen, 1997). The initial phases were calculated using the program *SOLVE* and further improved using the program *DM*, with a figure of merit of 0.78 (Collaborative

Computational Project, Number 4, 1994). The structure determination of the GRIP1-PDZ6 domain is ongoing.

Crystals complexed with peptide grew over 2 d to a maximum dimension of 0.3 mm (Fig. 1*b*). Crystals belong to the rhombohedral space group *R*32, with unit-cell parameters $a = b = 117.8$, $c = 102.0$ Å. Assuming two molecules per asymmetric unit, the Matthews coefficient (V_M) was calculated to be 3.05 Å^3 Da^{-1}, corresponding to a solvent content of 59.7%. The statistics of the data collection are summarized in Table 1.

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