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Crystallization and preliminary X-ray diffraction studies of the guanylate kinase-like domain of PSD-95 protein from rat

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The PSD-95 (postsynaptic density-95) protein, one of the members of the MAGUK (membrane-associated guanylate kinase) family, is composed of three PDZ domains, one SH3 domain and one guanylate kinase-like (GK) domain. The GK domain mediates the scaffolding function of PSD-95 by protein–protein interaction. Here, the GK domain was subcloned, expressed as an intein fusion protein, purified without the intein and then crystallized at room temperature by the hanging-drop vapour-diffusion method using PEG 8000 as a precipitant. The complete native data set was collected to a resolution of 2.35 Å using flash-cooling. The crystals belong to the primitive tetragonal space group $P4_3$ (or $P4_1$), with unit-cell parameters $a = b = 70.03$ (4), $c = 37.64$ (1) Å.

1. Introduction

PSD-95 (postsynaptic density-95) is a member of the membrane-associated guanylate kinase (MAGUK) family which is widely expressed in the brain and plays a critical role in regulating the synaptic structure and function by mediating specific interactions (Cho *et al.*, 1992; Kornau *et al.*, 1997; Craven & Bredt, 2000). MAGUK proteins are composed of three N-terminal PDZ domains, one SH3 domain and one GK (guanylate kinase-like) domain (Woods & Bryant, 1991; Kistner *et al.*, 1993). The molecular and cellular functions of PDZ domains are relatively well known (Sheng & Kim, 1996; Ziff, 1997). In contrast, those of the SH3 and GK domains are less clear. The binding partners of the SH3 domain, which generally mediates protein–protein interactions by binding to proline-rich sequences, are poorly characterized (Mayer & Gupta, 1998; Musacchio *et al.*, 1994), except for a report on the intramolecular interaction between the SH3 and GK domains (Shin *et al.*, 2000). The GK domain of PSD-95 has 37% amino-acid sequence identity to the yeast guanylate kinase, an enzyme that converts GMP to GDP with hydrolysis of ATP. However, it does not possess this enzymatic activity (Kistner *et al.*, 1995). Recently, several GK-binding proteins such as BEGAIN (brain-enriched guanylate kinase-associated protein) and GKAP (guanylate kinase-associated protein) have been identified (Deguchi *et al.*, 1998; Kim *et al.*, 1997). Also, it was found that the GK domain confers G-protein sensitivity to an inward-rectifier K^+ channel and that CASK (calcium/calmodulin-dependent serine protein kinase) interacts with Tbr-1, a T-box transcription factor that is involved in forebrain development, through its guanylate kinase domain (Hibino *et al.*, 2000; Hsueh *et al.*, 2000). We initiated a crystallographic analysis of the GK domain of rat PSD-95 to help in understanding the functional role of the GK domain. In this paper, we describe the overexpression, purification, crystallization and X-ray diffraction analysis of the GK domain of PSD-95 from *Rattus norvegicus*.

2. Materials and method

2.1. Cloning of GK domain of PSD-95

A DNA fragment encoding the GK domain (amino acids 502–712) was amplified by the polymerase chain reaction (PCR) technique from the cDNA of PSD-95 using a specific primer pair designed to introduce a convenient restriction site for cloning (*Nde*I and *Eco*RI). It was then subcloned into the vector pTYB1 (New England BioLabs, Inc.) and the recombinant DNA was transformed into *Escherichia coli* strain ER2566.

2.2. Protein expression and purification

The bacterial cells were induced with 0.3 m*M* IPTG (isopropyl-β-D-thiogalactopyranoside) for 8 h at 303 K and lysed using a French press. The supernatant separated by centrifugation was applied to IMPACT (intein-mediated purification with an affinity chitin-binding tag; New England Biolabs, Inc.) affinity chromatography beads. After batch binding and washing, the GK-domain protein was cleaved from the intein tag bound to chitin beads by incubating the beads with the elution buffer (20 m*M* Tris–HCl pH 8.0, 50 m*M* NaCl, 50 m*M* β-mercaptoethanol) for 24 h, was

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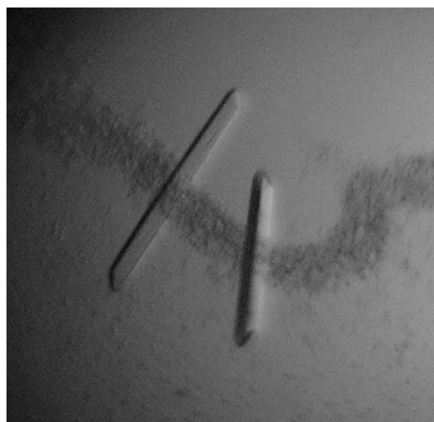

Figure 1
Rod-shaped crystals of the GK domain of about $0.3 \times 0.05 \times 0.05$ mm in size were used for diffraction data collection.

further purified to near-homogeneity by gel filtration on a Superdex 75 column (Hiload 16/60, Pharmacia) and was concentrated by ultrafiltration (Amicon, Centriprep 10) to 5 mg ml^{-1} for crystallization.

2.3. Crystallization

The crystallization conditions were screened using Crystal Screens I and II (Hampton Research). Initial microcrystals were grown in Crystal Screen I No. 9. The crystallization conditions were refined using the hanging-drop vapour-diffusion method at 294 ± 1 K. Crystals (Fig. 1) were grown on a siliconized cover slip by equilibrating a mixture containing 1 µl of the protein solution (5 mg ml^{-1} protein in 10 m*M* Tris–HCl pH 8.0, 25 m*M* NaCl, 0.05 m*M* EDTA, 10 m*M* β-mercaptoethanol) and 1 µl of the reservoir solution [100 m*M* sodium citrate pH 5.6, 26–30%(*w*/*v*) PEG 8000, 0.2 *M* sodium acetate, 16–20%(*v*/*v*) glycerol] against 500 µl of the reservoir solution.

2.4. X-ray data collection

X-ray diffraction data were collected using synchrotron radiation (1.00 Å wavelength) with an image-plate system at beamline 6A of the Photon Factory, the National Laboratory for High Energy Physics, Tsukuba, Japan and using Cu $K\alpha$ radiation from a Rigaku RU-300 rotating-anode generator operating at 50 kV and 90 mA with an R-AXIS IV image-plate system in a nitrogen-gas stream at 110 K (Oxford Cryosystems). The reservoir solution was used as the cryoprotectant in cryogenic experiments. Data sets were indexed and processed using the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1996).

Table 1
Summary of the native data set obtained at cryogenic temperature.

Values in parentheses are for the highest resolution shell.

Data set	Native
X-ray source	Photon Factory (BL-6A)
Space group	$P4_3$ or $P4_1$
Unit-cell parameters (Å)	$a = b = 70.03$, $c = 37.64$
Resolution of data (Å)	2.35
No. of data collected	26136
No. of unique data	7311
Completeness (%)	94.1 (84.1)
$R_{\rm merge}$† (%)	6.1
Mean $I/\sigma(I)$	13.8 (5.1)

† $R_{\rm merge} = \sum |I_h - I| / \sum I_h$, where I_h is the intensity of the reflections h and I is the average of measurements for reflection h.

3. Results

Autoindexing of native data with *DENZO* indicated that the crystal belongs to the primitive tetragonal space group $P4_3$ (or $P4_1$), with unit-cell parameters $a = b = 70.03$ (4), $c = 37.64$ (1) Å (Table 1). Diffraction patterns were observed to a limit of 2.35 Å. The data set consists of 26 136 total measurements with 7311 unique reflections (94.1% complete); $R_{\rm merge}$ was 6.1%. The unit volume was 184 594 Å^3 and the unit-cell volume per protein mass ($V_{\rm M}$) yielded a value of 2.17 Å^3 Da^{-1} for one molecule per asymmetric unit. The solvent content was 43.3% by volume. These values are well within the range previously observed for protein crystals (Matthews, 1968).

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