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Crystallization and preliminary X-ray crystallographic analysis of human PACSIN 1 protein

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PACSIN 1, which is mainly detected in brain tissue, is one of the PACSIN-family proteins involved in endocytosis and recruitment of synaptic vesicles. It binds to dynamin, synaptojanin 1 and N-WASP, and functions in vesicle formation and transport. However, the mechanisms of action of PACSIN 1 in these processes are largely unknown. Here, full-length and five C-terminal truncation constructs of human PACSIN 1 have been successfully expressed and purified in *Escherichia coli*. PACSIN 1 (1–344) was crystallized and diffracted to a resolution of 3.0 Å. The crystal belonged to space group *C*2, with unit-cell parameters a = 158.65, b = 87.38, c = 91.76 Å, α = 90.00, β = 113.61, γ = 90.00°. There were two molecules in the asymmetric unit and the solvent content was estimated to be about 70.47%.

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1. Introduction

Intracellular trafficking between different membranous organelles is important and many tubular and vesicular membrane carriers contribute to this process *via* budding and fusing (Farsad & De Camilli, 2003). These proteins can either penetrate the outer bilayer leaflet of the membrane or bind the bilayer of the membrane *via* hydrophilic interfacial interactions or *via* their property of assembling into curved scaffolds in order to result in curvature of the membrane for related biological functions (Itoh *et al.*, 2005). However, the precise mechanism of how invagination and fission of the membrane occurs is still elusive.

PACSIN 1, also called syndapin I, is a neurospecific protein which mainly comprises an N-terminal FCH (Fes/CIP4 homology) domain, a coiled-coil (CC) domain and a C-terminal SH3 domain. In synaptic vesicle endocytosis, PACSIN 1 binds to dynamin, synaptojanin 1 and N-WASP *via* its SH3 domain to participate in this process (Qualmann *et al.*, 1999). Dynamin I is a guanosine triphosphatase (GTPase) that is enriched in pre-synaptic nerve terminals (Powell & Robinson, 1995) and is important for vesicle fission in SVE (synaptic vesicle endocytosis; Sweitzer & Hinshaw, 1998). PACSIN 1 binds the overlapping proline-rich (PRD) regions of dynamin I and functions in SVE (Anggono & Robinson, 2007); phosphorylation of PACSIN 1 increases this interaction (Hilton *et al.*, 2001).

By electrical stimulation, PACSIN 1 may stabilize the plasma membrane and/or facilitate bulk endocytosis (Andersson *et al.*, 2008). The N-terminal F-BAR of PACSIN 1 has 22% sequence identity to the BAR domain of CIP4, which is dimerized and tubulates liposome (Frost *et al.*, 2008). Therefore, PACSIN 1 may be involved in invagination and fission in endocytosis.

PACSIN 1 is absent from synaptic varicosities in presymptomatic Huntington's disease brains (Modregger *et al.*, 2002). However, the precise functions of PACSIN 1 in all these processes remain elusive. In this study, we expressed, purified and crystallized full-length PACSIN 1 and five C-terminal truncation constructs and finally obtained preliminary X-ray crystallographic data for PACSIN 1 (1–344).

2. Methods

2.1. Protein purification

The cDNA of full-length human *pacsin1* was cloned into pET21-DEST expression vector with the N-terminal tag MASMTGGQQ-MGSSHHHHHSS using the Gateway cloning system (Invitrogen, USA). After confirmation by DNA sequencing, the expression plasmid was transformed into *Escherichia coli* strain BL21 (DE3) and plated on a Luria–Bertani broth (LB) agar plate with 100 mg l^{-1} ampicillin. A single colony was cultured in 200 ml LB medium supplemented with 100 mg l^{-1} ampicillin overnight at 310 K and then transferred into 6 l LB medium and incubated until the OD_{600} reached 0.6. The expression of PACSIN 1 was induced with 0.5 m*M* isopropyl β-D-1-thiogalactopyranoside (IPTG; Sigma) for 6 h at 303 K. Cells were harvested by centrifugation at 5000 rev min^{-1} for 10 min at 277 K and were suspended in binding buffer containing 50 m*M* HEPES pH 7.5, 500 m*M* NaCl and 5 m*M* imidazole. The cells were then sonicated and the lysate was centrifuged at 18 000 rev min^{-1} for 30 min at 277 K. The supernatant was collected, filtered through a 0.22 µm filter and applied onto a 5 ml Ni^{2+}-HiTrap affinity column (GE Healthcare, Sweden). The protein peak eluted at 500 m*M* imidazole. To further purify PACSIN 1, the peak fractions were concentrated to 2 ml and loaded onto a Superdex-75 column (GE Healthcare) which was equilibrated with buffer containing 10 m*M* HEPES pH 7.5, 500 m*M* NaCl.

Five truncates of PACSIN 1 (1–333, 1–342, 1–344, 1–346 and 1–384) were also designed based on the degradation of full-length PACSIN 1. The truncation constructs were amplified by PCR, ligated into pET28a vector (Novagen) with N-terminal MGSSHHHHHHSS tags and purified following the same protocol as described above.

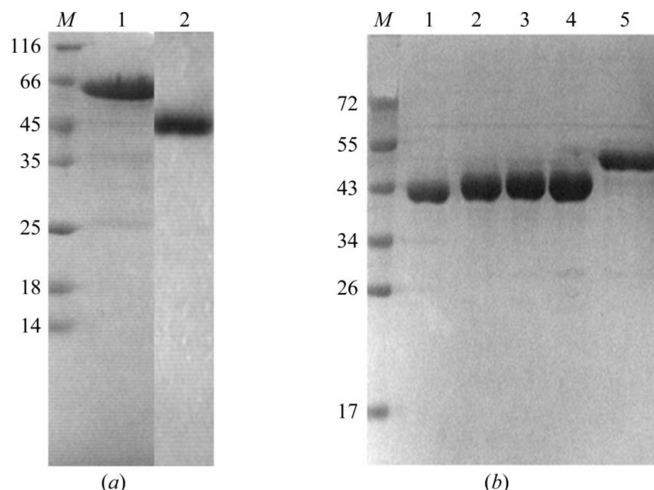

Figure 1
SDS–PAGE analysis of purified PACSIN 1. (*a*) Lane *M*, markers (kDa); lane 1, full-length PACSIN 1 with N-terminal tags immediately after purification; lane 2, degraded protein from a microcrystal. (*b*) Purified truncates of PACSIN 1. Lane *M*, markers (kDa); lanes 1–5 indicate PACSIN 1 truncation constructs 1–333, 1–342, 1–344, 1–346 and 1–384, respectively.

2.2. Protein crystallization

The protein solution was centrifuged at 14 000 rev min^{-1} for 30 min before crystallization setup. Initial and optimized crystallization experiments were performed at 293 K by the hanging-drop vapour-diffusion method.

Initial crystallization conditions were screened using kits from Hampton Research including PEG, Crystal Screen, Crystal Screen 2 and Index, with each drop containing 1 µl protein solution (8 mg ml^{-1} in 500 m*M* NaCl, 10 m*M* HEPES pH 7.5) mixed with 1 µl of reservoir solution and being equilibrated against 200 µl reservoir solution.

For further crystal optimization, five truncation constructs of PACSIN 1, 1–333, 1–342, 1–344, 1–346 and 1–384 (Fig. 1*b*), were designed. Drops were prepared by mixing 2 µl protein solution (8 mg ml^{-1} in 500 m*M* NaCl, 10 m*M* HEPES pH 7.5) with 2 µl reservoir solution and were equilibrated against 500 µl reservoir solution.

2.3. Diffraction data collection

For crystal data collection, crystals were transferred into the corresponding reservoir solution containing 20% glycerol as a cryoprotectant. The crystal was mounted in a cryoloop and flash-cooled in a nitrogen stream at 100 K. X-ray diffraction data were collected on a MAR 345 image-plate detector at Beijing Synchrotron Radiation Facility (beamline 3W1A), Institute of High Energy Physics, Chinese Academy of Life Sciences. The crystal-to-detector distance was 200 mm. A total of 180 frames of 1° oscillation were measured with 60 s exposure per frame. Data were processed with *MOSFLM* (Powell, 1999) and *CCP*4 (Collaborative Computational Project, Number 4, 1994).

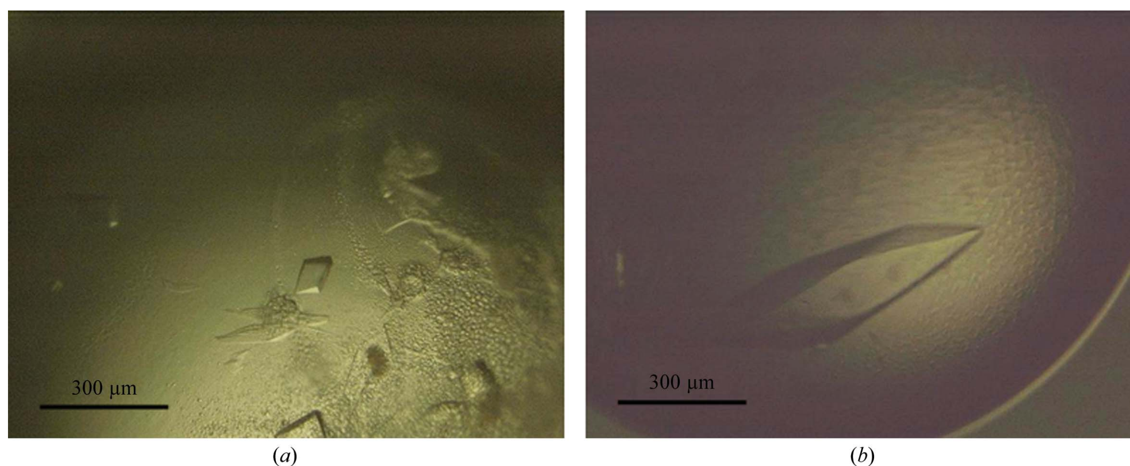

Figure 2
Crystals of PACSIN 1. (*a*) Crystal of full-length PACSIN 1. (*b*) Crystal of truncate PACSIN 1 (1–344).

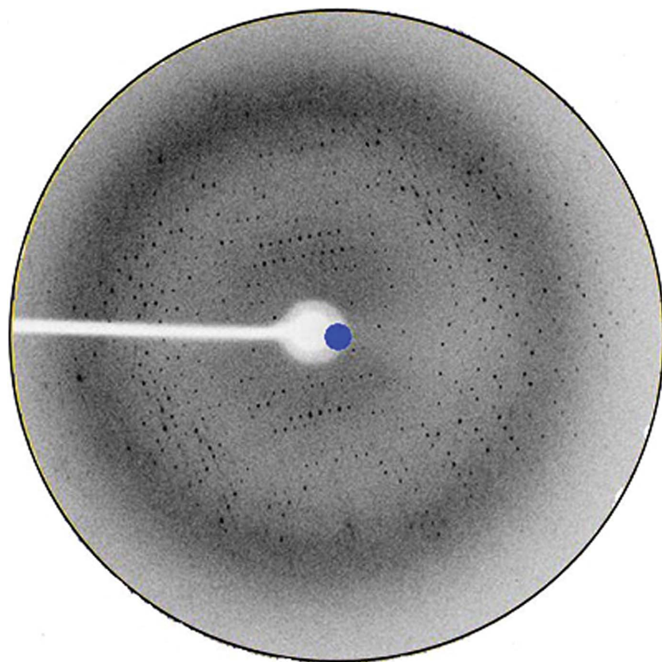

Figure 3
Diffraction pattern of a PACSIN 1 (1–344) crystal.

3. Results and discussion

Purified full-length PACSIN 1 (or truncated PACSIN 1) protein was expressed in *E. coli* with a typical measured final yield of 5 mg per litre of culture and a purity of over 95% (Figs. 1*a* and 1*b*).

PACSIN 1 microcrystals were obtained using 200 m*M* $NH_4H_2PO_4$ pH 7.9, 20%(*w*/*v*) PEG 3350. However, SDS–PAGE analysis showed that these microcrystals were a degraded form of full-length PACSIN 1 with a molecular weight of 38 kDa (Fig. 1*a*, lane 2). N-terminal sequencing analysis showed that the N-terminal amino acids of this degraded band were MSSSY, which corresponded to the first five N-terminal amino acids of PACSIN 1, indicating that the N-terminal tags degraded naturally and the microcrystals mainly degraded from the C-terminus.

The full-length PACSIN microcrystal shown in Fig. 2(*a*) diffracted to 4.2 Å resolution. Therefore, in order to improve the resolution, we designed and constructed five truncation constructs of PACSIN 1 and obtained a larger crystal of PACSIN 1 (1–344) (600 × 190 × 200 µm) within two weeks from 200 m*M* $NH_4H_2PO_4$ pH 7.5, 16%(*w*/*v*) PEG 3350 and 5% glycerol (Fig. 2*b*).

The truncated protein (1–344) diffracted to a higher resolution (3.0 Å; Fig. 3). The PACSIN 1 (1–344) crystal belonged to space group *C*2, with unit-cell parameters $a = 158.65$, $b = 87.38$, $c = 91.76$ Å, $\alpha = 90.00$, $\beta = 113.61$, $\gamma = 90.00°$. Because it mainly forms dimers in solution and the self-rotation map showed twofold noncrystallographic symmetry (NCS) rotation axes, we presumed there were two molecules in the asymmetric unit and this gave a V_M value of 4.16 Å^3 Da^{-1}. The crystallographic parameters and data-collection statistics are listed in Table 1.

Table 1
Crystallographic parameters and data-collection statistics.

Values in parentheses are for the last resolution shell.

Wavelength (Å)	1.072
Resolution (Å)	30–3.0 (3.12–3.0)
Completeness (%)	100 (99.7)
R_{merge}† (%)	16.3 (51.9)
$I/\sigma(I)$	8.6 (2.16)
Space group	*C*2
Unit-cell parameters (Å)	$a = 158.65$, $b = 87.38$, $c = 91.76$
No. of observed reflections	42008
No. of unique reflections	22946
Molecules per ASU	2
V_M (Å^3 Da^{-1})	4.16
Solvent content (%)	70.47

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

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