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Crystallization and preliminary X-ray crystallographic analysis of rat calcineurin B homologous protein 1

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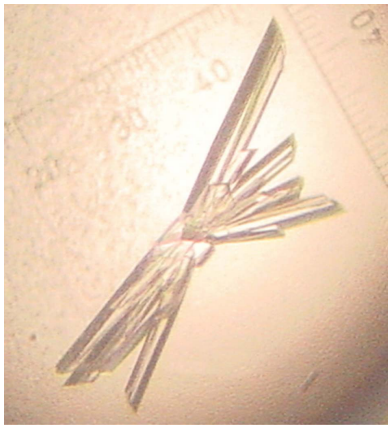

Calcineurin B homologous protein 1 (CHP1), also known as p22, is a calcium-binding protein that plays a role in membrane trafficking and binds to multiple effector proteins, including Na^+/H^+ exchangers, serine/threonine protein kinase and calcineurin, potentially modulating their function. CHP1 has been crystallized at 277 K using polyethylene glycol as a precipitant. The crystal belongs to space group $P2_1$, with unit-cell parameters $a = 55.5$, $b = 38.5$, $c = 90.0$ Å, $\beta = 90.7^\circ$. A full set of diffraction data was collected to 2.2 Å resolution at 100 K using the Photon Factory synchrotron-radiation source.

1. Introduction

Calcineurin B homologous protein 1 (CHP1) is a calcium-binding EF-hand protein and shows substantial sequence similarity (∼39% identity) with the regulatory B subunit of the protein phosphatase calcineurin (CNB). CHP1 was initially identified as a protein, p22, involved in vesicular transport (Barroso *et al.*, 1996). CHP1 was also identified as an accessory protein that associates tightly with Na^+/H^+ exchangers (NHEs; Lin & Barber, 1996), which catalyze H^+ extrusion coupled to Na^+ influx across the biological membranes (for a review, see Baumgartner *et al.*, 2004). Further studies have shown that CHP1 serves as an essential cofactor required for at least three NHE isoforms, NHE1–3, and interacts with the intracellular region of NHE1, regulating intracellular pH-sensitivity (Pang *et al.*, 2001, 2004).

CHP1 has been reported to possess multiple cellular functions. CHP1 binds to the catalytic A subunit of calcineurin (CNA) directly and inhibits its phosphatase activity, suggesting that CHP1 is an endogeneous inhibitor of calcineurin (Lin *et al.*, 1999). CHP1 also significantly reduces the kinase activity of death-associated protein (DAP) kinase-related apoptosis-inducing protein kinase 2 (DRAK2). DRAK2 possesses a CHP1-binding region at the C-terminal region of the kinase domain (Matsumoto *et al.*, 2001). The inhibitory effect of CHP1 is dependent on the presence of calcium, whereas the interaction between CHP1 and DRAK2 is not calcium-dependent (Matsumoto *et al.*, 2001; Kuwahara *et al.*, 2003). CHP1 interacts with kinesin family 1Bβ2 (KIF1Bβ2) in a calcium-dependent manner (Nakamura *et al.*, 2002).

To clarify the multiple functional mechanisms of CHP1, we have attempted to determine the CHP1 structure by X-ray crystallographic analysis. Here, we report the crystallization and preliminary X-ray crystallographic analysis of rat CHP1.

2. Materials and methods

2.1. Expression and purification

Recombinant rat CHP1 corresponding to amino-acid residues 1–195 was expressed as a fusion protein with a histidine tag in *Escherichia coli* BL21 (DE3) Codon Plus RIL cells transformed with plasmid pET21b (Novagen). The cells were disrupted by sonication at 277 K. The supernatant was applied onto an Ni–NTA agarose affinity column (Qiagen) equilibrated with equilibration buffer (50 m*M* Tris–HCl pH 7.5, 0.1 *M* NaCl, 1 m*M* $CaCl_2$). The column was washed with equilibration buffer containing 1 *M* NaCl and 5 m*M* imidazole. The adsorbed protein was eluted with equilibration buffer containing

250 m*M* imidazole. The eluted protein was diluted with 50 m*M* Tris–HCl pH 7.5, 1 m*M* $CaCl_2$ and was applied onto a Hitrap Q HP column equilibrated with 50 m*M* Tris–HCl pH 7.5 containing 25 m*M* NaCl, 1 m*M* $CaCl_2$, 1 m*M* dithiothreitol (DTT) using an ÄKTA FPLC system (Amersham Biosciences). The adsorbed protein was eluted with a linear gradient of 0.025–1 *M* NaCl. The eluted protein was applied onto a HiLoad Superdex 75 26/60 gel-filtration column (Amersham Biosciences). The purified protein was eluted in 5 m*M* Tris–HCl pH 7.5 containing 0.1 *M* NaCl, 1 m*M* $CaCl_2$, 1 m*M* DTT and was concentrated to 10 mg ml^{-1} at 277 K in an Amicon Ultra-15 10000 MW cutoff (Millipore) for crystallization. The homogeneity of the protein was confirmed by SDS–PAGE.

2.2. Crystallization

Crystallization was performed by the hanging-drop vapour-diffusion method. The drops were prepared by mixing 1 µl CHP1 solution with 1 µl reservoir solution. Preliminary screening of crystallization conditions was performed using Hampton Research Crystal Screen kits and Emerald Biostructure Screen kits. Small crystals were obtained with Hampton Screen PEG/Ion condition No. 24 containing 0.2 *M* lithium acetate, 20%(*w*/*v*) polyethylene glycol 3350 (PEG 3350) pH 7.8 at 277 K. Refinement of the crystallization conditions to 27.5% PEG 3350, 0.4 *M* lithium acetate and 0.1 *M* 3-(cyclohexylamino)-2-hydroxy-1-propanesulfonic acid (CAPSO) pH 9.0 greatly improved the quality and size of the crystals. The crystals tend to be clustered (Fig. 1) and single crystals for data collection were dissected from the clusters.

2.3. Data collection and processing

As a cryoprotectant prior to the X-ray experiment we used a reservoir containing 30% PEG 3350, 0.1 *M* CAPSO pH 9.0, 0.4 *M* lithium acetate, 10 m*M* $CaCl_2$. X-ray diffraction data from the native crystal were collected at 100 K on the ADSC Quantum 315 system installed at BL5 at Photon Factory (Tsukuba, Japan). The data were processed using *HKL*2000 (Otwinowski & Minor, 1997).

3. Results

Crystals suitable for high-resolution X-ray crystallographic analysis were obtained at 277 K in a few weeks by the hanging-drop vapour-diffusion method. The reservoir contained 27.5% PEG 3350, 0.4 *M* lithium acetate and 0.1 *M* CAPSO pH 9.0.

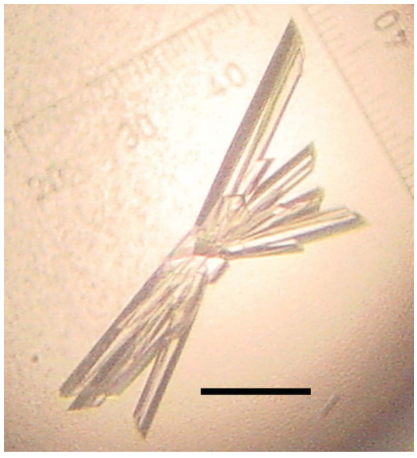

Figure 1
A crystal of CHP1. The black scale bar is 200 µm long.

Table 1
Data-collection statistics.

Values in parentheses are for the highest resolution shell (2.28–2.20 Å).

X-ray source	PF BL5
Wavelength (Å)	1.0000
Space group	$P2_1$
Unit-cell parameters (Å, °)	a = 55.5, b = 38.5, c = 90.0, β = 90.7
Resolution range (Å)	50.00–2.20
$R_{\rm merge}$† (%)	6.1 (22.1)
Average $I/\sigma(I)$	15.3
No. observations	71429
No. unique reflections	19708
Data completeness (%)	98.9 (97.3)
Redundancy	3.6 (3.6)
Crystal mosaicity (°)	0.368

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

The crystals belong to space group $P2_1$. The presence of two CHP1 molecules in the asymmetric unit gives a crystal volume per protein weight $V_{\rm M}$ of 2.0 Å^3 Da^{-1} and a solvent content of 38.5%, which lie within the ranges usually found for protein crystals (Matthews, 1968). A full set of X-ray diffraction data was collected to 2.2 Å resolution with a completeness of 98.9%. Table 1 summarizes the data-collection statistics of the native crystal. CHP1 shows 39% sequence identity with human CNB (PDB code 1aui; Kissinger *et al.*, 1995). Molecular replacement using human CNB as a search model has been attempted with *AMoRe* (Navaza, 1994), but so far without success. Structural analysis by isomorphous replacement combined with anomalous diffraction is in progress.

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