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# Crystallographic characterization of the N-terminal domain of PEX1

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Peroxisomal enzymes are responsible for several primary metabolism pathways, including $\beta$-oxidation and lipid biosynthesis. PEX1 and PEX6 are hexameric AAA-type ATPases and both are necessary for the import of more than 50 peroxisomal resident proteins from the cytosol into peroxisomes. In this study, PEX1 N-terminal domain crystals have been prepared. The crystals belong to space group $P3_1$ or $P3_2$, with unit-cell parameters $a = b = 63.5$ Å, $c = 33.5$ Å, and contain one protein molecule per crystallographic asymmetric unit. An intensity data set was collected to a resolution of 2.05 Å.

## 1. Introduction

Peroxisomes are metabolic organelles with enzymatic content that are found in virtually all cells and are involved in $\beta$-oxidation of fatty acids, in hydrogen-peroxide-based respiration and in defence against oxidative stress (Wanders, 2000; Wanders *et al.*, 2000). Peroxisome biogenesis involves peroxins, which are proteins encoded by PEX genes (Kunau, 1998; Brown & Baker, 2003; Maxwell *et al.*, 2002). Peroxins are required for the recognition, targeting and import of peroxisomal proteins containing peroxisome-transport signals (PTS). PEX1 and PEX6 are two membrane-associated ATPases that belong to the AAA (ATPase associated with diverse cellular activities) family and are the only known ATPases required for matrix-protein import. AAA ATPases contain a highly conserved AAA domain of 230 amino acids which contains Walker homology sequences and imparts ATPase activity. Biochemical and genetic interaction studies of the *P. pastoris*, *H. polymorpha* and human isoenzymes has demonstrated that PEX1 and PEX6 physically interact in an ATP-dependent manner (Kiel *et al.*, 1999, 2000; Tamura *et al.*, 1998). A common disease-causing PEX1 mutation disrupts this interaction; therefore, the PEX1–PEX6 interaction is required for normal peroxisome biogenesis. PEX1 stabilizes the PTS receptor, PEX5, although the stabilization mechanism is not understood (Titorenko & Rachubinski, 2000; Oliveira *et al.*, 2003).

Although the tandem AAA ATPase domains found in the central regions of PEX1 and PEX6 are highly similar, the N-terminal sequences of these proteins are unique. Mouse PEX1 is a type II AAA ATPase of 1124 amino acids in length, which consists of two tandem AAA domains at positions 436–901, while approximately 400 N-terminal residues have not been annotated to date. Preliminary bioinformatic analysis using the program *FORTE*, a fold-recognition method, indicated that the N-terminal region of PEX1 is related to the N-domains of VCP (PDB code 1e32; Zhang *et al.*, 2000) and NSF (PDB code 1qcs; Yu *et al.*, 1999) with high *Z* scores of 6.4 and 5.0, respectively (Tomii & Akiyama, 2004). Here, we report the first crystallization and crystallographic studies of the N-terminal domain of PEX1.

## 2. Methods and results

### 2.1. Expression and purification

Mouse PEX1 (residues 3–180) was cloned into a pGEX-4T3-PRESAT plasmid (Goda *et al.*, 2004) and overexpressed in BL21(DE3)star cells or B834(DE3) as a fusion protein with glutathione *S*-transferase (GST) in LBG or LeMaster media, respectively. The cells were disrupted by sonication at 277 K in a solution containing 150 m*M* NaCl, 1% Triton X-100, 20 m*M* sodium phosphate buffer pH 7.5 and 1 m*M* EDTA. The supernatant was applied onto a glutathione Sepharose 4B affinity column (Amersham Biosciences). The bound fusion protein was digested with 40 units ml$^{-1}$ bovine thrombin (Amersham Biosciences) for 7 h at 295 K; this procedure degrades GST and releases recombinant PEX1 from the affinity matrix. Cleaved proteins were collected and further purified by gel filtration using HiPrep 26/60 Sephacryl S-100 (Amersham Biosciences). Protein purification was monitored using 12.5% SDS–PAGE; gels were stained with Coomassie brilliant blue. Analysis of a matrix-assisted laser desorption/ionization–time-of-flight mass spectrum (MALDI–TOF MS; Bruker) validated the purity of the protein.

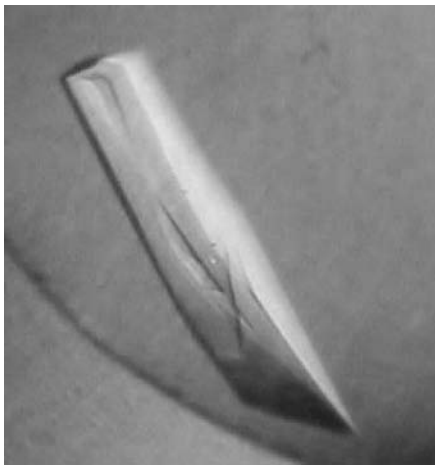

**Figure 1**
A PEX1 N-terminal domain crystal. The approximate dimensions of the crystal are 0.1 × 0.1 × 0.5 mm.

### 2.2. Crystallization

Purified protein in 25 m*M* HEPES buffer pH 7.5, 5% glycerol and 0.5 m*M* DTT was concentrated to about 10 mg ml$^{-1}$ using a Centricon-3 ultrafiltration membrane (Amicon) at 277 K. All crystallization experiments were conducted using the hanging-drop vapour-diffusion method in 24-well tissue-culture plates (Sumitomo Bakelite Co.). Crystallization conditions were screened using reagents from Hampton Research Crystal Screens 1 and 2. Thin plate-like crystals which grew within a few days at 293 K were obtained using solution No. 31 [0.2 *M* ammonium sulfate and 30%(*w*/*v*) polyethyleneglycol (PEG) 4000] from Crystal Screen 1. A solution of 0.2 *M* ammonium sulfate, 30% PEG 4000 and 5% glycerol was found to improve the quality and the size of the crystals. Crystals suitable for high-resolution X-ray crystallography (Fig. 1) were obtained when drops containing equal volumes (1.0 µl) of protein (10 mg ml$^{-1}$) and reservoir solution were equilibrated with 0.5 ml reservoir solution at 293 K for a few days.

### 2.3. Data collection and analysis

Crystals were soaked in the crystallization buffer with 15% glycerol as the cryoprotectant for X-ray data collection. X-ray diffraction data were collected from a native crystal (0.1 × 0.1 × 0.5 mm) to 2.05 Å resolution at 100 K using an FR-D rotating-anode X-ray generator with an R-AXIS IV$^{++}$ imaging plate (Rigaku). Data from an SeMet crystal were collected at Photon Factory BL-6A with a Quantum R4 CCD detector (ADSC). Diffraction data were processed using the program *MOSFLM* (Leslie, 1999) and scaled using the program *SCALA* (Collaborative Computational Project, Number 4, 1994). The crystals belonged to space group $P3_1$ or $P3_2$, with unit-cell parameters $a$ = 63.5, $b$ = 63.5, $c$ = 33.5 Å. Given one PEX1 N-terminal domain of molecular weight 20.0 kDa per asymmetric unit, the corresponding crystal volume per protein weight, $V_M$, is 1.95 Å$^3$ Da$^{-1}$ and the corresponding solvent content is 37%.The resolution range is 30–2.05 Å (2.15–2.05 Å for the outer shell). The values for $V_M$ and the solvent content are within the ranges typically found for protein crystals (Matthews, 1968). The total number of observed reflections is 52 572, with 9465 unique reflections. The value for $R_{merge}$ is 7.3% (25.1% for the outer shell), with a completeness of 99.8% (99.8% for the outer shell). The redundancy for the reflections is 5.6 (5.5 for the outer shell). The mean $I/\sigma(I)$ is 5.4 (2.3 for the outer shell). The sequence identities of the N-terminal domain of mouse PEX1 to the 'N-domain' of VCP (PDB code 1e32, residues 1–196) and NSF (PBD code 1qcs, residues 1–202) were 15.3 and 12.5%, respectively. As a result, molecular replacement using either the 1e32 or 1qcs coordinates as a search model was not successful. MAD phasing was attempted using the SeMet derivative, but was not sufficient to solve the structure. Preparation of heavy-atom derivatives is in progress.

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