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Preliminary X-ray crystallographic analysis of a Ca^{2+}-binding protein human S100A1

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S100A1, a Ca^{2+}-binding protein from the S100 protein family, has been crystallized by the vapour-diffusion method using polyethylene glycol 4000 as the precipitant at pH 8.5. The crystal belongs to space group $P6_3$. The unit-cell parameters are $a = b = 57.3$, $c = 104.7$ Å. There appear to be two S100A1 molecules in the asymmetric unit. The crystals were stable during exposure to X-rays and diffract to 2.6 Å resolution in-house.

1. Introduction

The S100 protein family is a group of calcium-binding proteins, between approximately 9 and 13 kDa in size, which contain two EF-hand calcium-binding domains and share approximately 50% homology in amino-acid sequence. Each member of the family exhibits a unique pattern of expression, with some cells expressing multiple members of the family (Donato, 1999). S100 proteins can regulate a diverse group of cellular functions, including cell–cell communication, cell growth, cell structure and energy metabolism contraction (Zimmer *et al.*, 1995). S100A1, a member of the S100 protein family, plays an important role in cell metabolism. Increased S100A1 levels have been documented in renal carcinoma and the level of S100A1 expressed in renal carcinoma is directly related to the prognosis of the disease. S100A1 can bind to tubulin and inhibit brain microtubule assembly in a calcium-dependent manner at pH 6.7 and can stimulate microtubule disassembly at pH 7.5 (Donato, 1991). It has been reported to interact with aldolase to regulate energy metabolism and with glycogen phosphorylase to regulate the mobilization of glucose. In addition, S100A1 can modify both Ca^{2+} and cAMP signal transduction (Fano *et al.*, 1989). S100A1 was recently reported to interact with S100A4, a metastasis-promoting gene, but the biological significance of this is still unclear (Wang *et al.*, 2000; Tarabykina *et al.*, 2000).

S100 proteins all have a highly conserved calcium-binding loop consisting of 12 amino acids which is flanked by two α-helices, *i.e.* the EF-hand, common to all EF-hand proteins. The low-affinity Ca^{2+}-binding site near the N-terminal end is S100-specific, containing 14 amino-acid residues (Schafer & Heizmann, 1996). Three-dimensional structures of several members of the S100 protein family have been reported. These include the solution structures of apo S100B (Kilby *et al.*, 1996) and apo calcyclin and the crystal structures of holo and apo calbindin D9k (Szebenyi & Moffat, 1986; Svensson *et al.*, 1992; Kordel *et al.*, 1993; Skelton *et al.*, 1995). These studies have provided interesting data on calcium-dependent conformation change and the effects of mutation in the Ca^{2+}-binding loops on function. To obtain more detailed information about the interaction of S100A1 with its targets (such as S100A4, tubulin, aldolase) and the significance of the 14-residue amino-terminal calcium-binding loop, we have obtained crystals of S100A1 and are analyzing its three-dimensional structure.

2. Material and methods

2.1. Preparation of the sample

The cDNA of human S100A1, from a yeast two-hybrid library of human breast cancer (Wang *et al.*, 2000), was inserted into plasmid pET16b (Novagen) and high levels of S100A1 were produced in *Escherichia coli* with a 6×His tag at the N-terminus. Because of an initial failure of crystallization, the S100A1 cDNA was subcloned into pET25b (Novagen) and expressed without any tag after transformation into *E. coli* strain BL21 (DE3). Protein

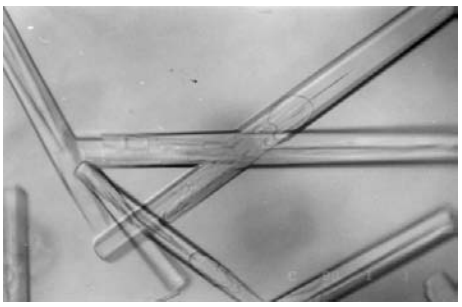

Figure 1
Crystals of S100A1. These crystals grew from buffer containing 47% PEG 4000, 0.1 *M* Tris pH 8.5. Crystal dimensions are about 0.5 × 0.1 × 0.05 mm.

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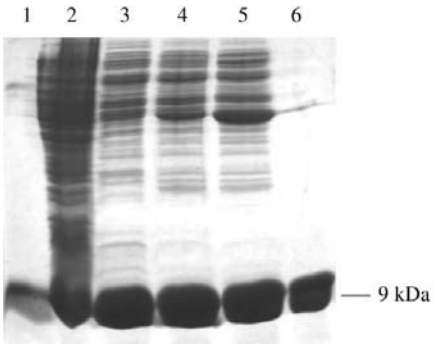

Figure 2
SDS–PAGE (20% acrylamide). Lane 1 and lane 6, fraction after phenyl-Sepharose column; lane 2, crude *E. coli* extract; lane 3–5, fractions after DEAE-cellulose anion-exchange column chromatography.

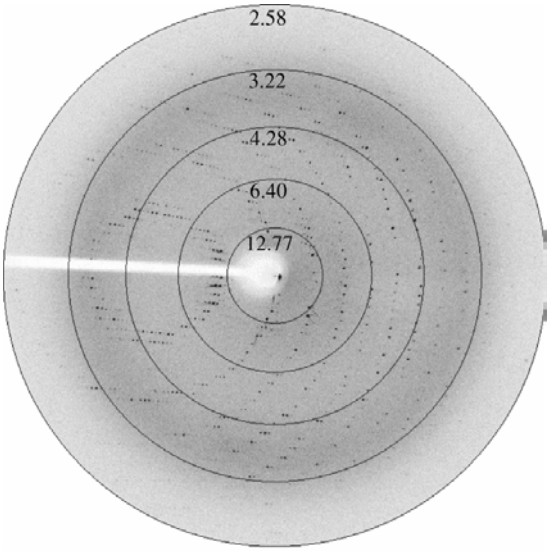

Figure 3
Typical diffraction pattern of a crystal of S100A1.

Table 1
X-ray data statistics.

Values in parentheses are the corresponding values for the highest resolution shell (2.60–2.69 Å).

Space group	$P6_3$
Unit-cell parameters (Å)	$a = b = 57.3$, $c = 104.7$
Completeness	99.5 (99.7)
No. of unique reflections	3939 (396)
Mean redundancy	3.2
R_{merge}	0.068 (0.319)
$I/\sigma(I)$	12.8 (4.5)
No. of molecules per asymmetric unit	2
Matthews coefficient	2.5
Solvent content	About 50%

overexpression was induced at OD_{595} = 0.6–0.8 by addition of 1 m*M* isopropyl-β-D-thiogalactopyranoside (IPTG) and was continued for 5–6 h. Harvested cells were suspended in 25 m*M* Tris–HCl pH 7.6, 150 m*M* NaCl and were lysed by sonication. The soluble cell lysate obtained by centrifugation at 15 000 rev min^{-1} for 30 min was loaded onto a DEAE-Sepharose fast-flow anion-exchange chromatography column (APB, Milton Keynes, England) equilibrated with 25 m*M* Tris–HCl pH 7.6, 150 m*M* NaCl. After washing away the unbound protein with 2 bed volumes of buffer, a linear gradient of 0.15–0.6 *M* NaCl in the same buffer was applied. S100A1 eluted at approximately 0.35 *M* NaCl. 2 m*M* $CaCl_2$ was added to the collected fractions, which were then centrifuged for 30 min at 15 000 rev min^{-1}. The supernatant was absorbed onto a phenyl-Sepharose column (APB) equilibrated with 25 m*M* Tris–HCl, 0.1 m*M* $CaCl_2$ pH 7.6. Bound proteins were eluted with the same buffer containing 5 m*M* EGTA.

2.2. Crystallization and X-ray diffraction analysis

The purified protein was concentrated using a 5K ultrafiltration membrane (Filtron) to 40 mg ml^{-1} in a solution containing 150 m*M* NaCl and 2 m*M* $CaCl_2$. Initial crystallization conditions were established by the hanging-drop vapour-diffusion method. 1.5 µl of protein solution were mixed with 1.5 µl of reservoir solution and equilibrated against 0.6 ml of the reservoir solution. The reservoir solution contained 47%(*w*/*v*) PEG 4000, 0.1 *M* Tris–HCl pH 8.5. Crystals grew in 3 d at 291 K and reached their final size in about a week (Fig. 1).

The preliminary X-ray diffraction analysis of S1000A1 crystals was performed at room temperature on an in-house MAR 345 image plate with a Rigaku rotating Cu anode X-ray generator at 48 kV and 98 mA (λ = 1.5418 Å). All diffraction data were processed using the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997).

3. Result and discussion

3.1. Protein purification and crystal growth

After purification by DEAE-Sepharose fast flow and phenyl-Sepharose chromatography, one 9 kDa band corresponding to the molecular weight of S100A1 was visible in 20% SDS–PAGE analysis (Fig. 2).

A PEG 4000 screening kit (prepared by our laboratory) was used for preliminary screening. Throughout preliminary screening, multiple crystals were obtained in buffers containing 41–61% PEG 4000, 0.1 *M* Tris at pH 7.0–9.0. Based on the preliminary results, further refinement was carried out. The precipitant was changed from PEG 400 to PEG 10 000 including PEG 400, PEG 1000, PEG 2000, PEG 4000, PEG 8000 and PEG 10 000. The pH of the 0.1 *M* Tris buffer ranged from 7.5 to 8.5 including pH 7.5, pH 7.8, pH 8.0, pH 8.2 and pH 8.5. Some high-quality crystals were produced in a buffer containing 47% PEG 4000, 0.1 *M* Tris pH 8.5 (Fig. 1).

3.2. Data collection and analysis

X-ray diffraction data were collected to 2.6 Å resolution at room temperature (Fig. 3) in-house. The crystal-to-detector distance was 250 mm. The data were collected with 1° oscillation per frame over a 180° oscillation range. The crystal belongs to space group $P6_3$, with unit-cell parameters $a = b = 57.3$, $c = 104.7$ Å. There are two S100A1 molecules in an asymmetric unit. The X-ray data statistics are summarized in Table 1. Final structure determination is in progress.

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