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Purification, crystallization and preliminary X-ray diffraction studies of a Ca^{2+}-binding protein, human S100P

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S100P, a Ca^{2+}-binding protein, is a member of the S100 family. Its presence is associated with the development of prostate cancer, but its cellular function is not known. Recombinant human S100P has been expressed and purified in bacterial cells and crystals of human S100P in the calcium-bound state have been grown using the vapour-diffusion technique with PEG 4000 as precipitant. Diffraction data have been obtained to a resolution of 2.0 Å from a single frozen S100P crystal which belongs to the space group $P4_12_12$, with unit-cell parameters $a = b = 60.8$, $c = 47.6$ Å.

1. Introduction

Calcium is responsible for the control of vital biochemical pathways. These Ca^{2+}-dependent signal transduction pathways are achieved through the interaction of calcium ions with Ca^{2+}-binding proteins (CaBPs). A large class of CaBPs share a common Ca^{2+}-binding structural motif, the EF-hand (Kretsinger, 1976; Kawasaki & Kretsinger, 1995). The S100 family is possibly the largest multigenic family and its proteins are characterized by a low molecular weight (Schafer & Heizmann, 1996) among the EF-hand proteins characterized by calcium binding. Owing to their relative abundance, the S100 proteins are thought to be involved in a variety of intracellular activities in a Ca^{2+}-dependent (and in some cases Zn^{2+}-, Cu^{2+}- or Mg^{2+}-dependent) manner, including protein phosphorylation, enzyme activities, cell proliferation (including neoplastic transformation) and differentiation, the dynamics of cytoskeleton constituents, intracellular Ca^{2+} homeostasis and inflammation, modulation of cell proliferation or regulation of macrophage activation (Donato, 1999). Another characteristic feature of S100 proteins is their cell-type-specific expression and their association with both neurological and neoplastic human diseases (Hilt & Kligman, 1991; Schafer & Heizmann, 1996). Expression and an altered cellular localization of S100P are related to the development of prostate cancer (Averboukh *et al.*, 1996). Thus, for the S100 proteins, different cell-type-specific expression patterns may indicate their different functional roles.

Detailed studies of S100 proteins have revealed some important structural properties. S100 proteins are thought to function as Ca^{2+}-sensor proteins that, with a few exceptions, undergo Ca^{2+}-dependent conformational changes resulting in the exposure of a binding surface (Donato, 1999). Commonly, they interact with their target proteins as homodimers or/and heterodimers such as $(S100A1)_2$, $(S100B)_2$, $(S100A6)_2$ and S100A/S100B under non-reducing conditions or even reducing conditions (Teigelkamp *et al.*, 1991; Potts *et al.*, 1995; Drohat *et al.*, 1996; Kilby *et al.*, 1996; Matsumura *et al.*, 1998; Brodersen *et al.*, 1998). Thus, the members of the S100 protein family have some similar structural properties in common, but their specific roles and their localization in cells are likely to reflect subtle and possibly unique structure features associated with their interaction with particular target proteins.

Human S100P, a 95-residue protein, was first isolated from human placenta (Becker *et al.*, 1992; Emoto *et al.*, 1992) and may be the least known member of the S100 family. Studies of the structure of S100P at low resolution show that S100P undergoes a significant rearrangement of its tertiary structure with changes in Ca^{2+} concentration and that the oligomer–dimer equilibrium of the S100P molecule moves towards oligomer upon the addition of Ca^{2+} ions (Gribenko & Makhatadze, 1998). Few details of S100P are known. The elucidation of the cellular functions and biochemical properties of S100P requires detailed structure information. Here, we report (i) a detailed protocol of protein preparation for crystallization, (ii) successful crystallization procedures and (iii) X-ray diffraction studies of S100P protein.

2. Experimental

2.1. Expression and purification

Production of recombinant human S100P from *Escherichia coli* strain BL21 was induced when the culture had grown to an optical

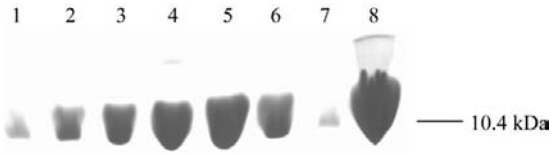

Figure 1
Purity determination of S100P by non-reducing 16.5% SDS–PAGE. Lanes 1–7, fractions after Superdex 75 (120 ml column volume); lane 8, protein concentrated for crystallization.

density of OD_{595} = 0.6–0.8 by the addition of 1 m*M* isopropyl-β-D-thiogalactopyranoside and the culture was grown for a further 2 h at 310 K. Cells harvested by centrifugation were suspended in 25 m*M* Tris–HCl pH 7.6, 50 m*M* NaCl and lysed by sonication. The supernatant obtained by centrifugation of the extract 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, 50 m*M* NaCl. After washing out unbound protein with two bed volumes of buffer, a linear gradient of 0.05–0.6 *M* NaCl in the same buffer was applied. S100P was eluted at approximately 0.25 *M* NaCl. The collected fractions were brought to 2 m*M* with respect to $CaCl_2$ and 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 pH 7.6. Bound proteins were eluted with the same buffer containing 10 m*M* EGTA. The eluted fractions were concentrated and then subjected to HPLC/gel filtration on a Superdex G75 column with 120 ml column volume equilibrated and eluted with 5 m*M* Tris–HCl pH 8.0 containing 50 m*M* NaCl, 0.5 m*M* $CaCl_2$. The collected protein sample was then concentrated for crystallization. After purification according to the above steps, the purity of S100P is suitable for crystallization as determined by non-reducing 16.5% SDS–PAGE analysis (Fig. 1).

2.2. Crystallization and X-ray diffraction analysis

Recombinant S100P was purified from 1 l *E. coli* cell extracts as described above and concentrated to about 50 mg ml^{-1} in a solution containing 5 m*M* Tris–HCl pH 8.0, 50 m*M* NaCl and 0.5 m*M* $CaCl_2$. Crystallization experiments were performed at 291 K using the hanging-drop vapour-diffusion method. Crystals appeared in 2 d when 1.2 µl of S100P protein in buffer was mixed with 1.2 µl reservoir solution (30–34% PEG 4000, 0.1 *M* sodium citrate pH 6.5) and equilibrated against 0.3 ml reservoir solution (Fig. 2).

The preliminary X-ray diffraction analysis of the S100P crystal was performed at 110 K on an in-house MAR 345 image plate with a Rigaku rotating-anode Cu $K\alpha$ X-ray generator operating at 48 kV and 98 mA (λ = 1.5418 Å). The space group has been determined to be $P4_12_12$, with unit-cell parameters $a = b = 60.8$, $c = 47.6$ Å. X-ray diffraction data were collected to 2.0 Å resolution. All diffraction data were processed using the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997). The final structure determination is in progress.

3. Results and discussion

3.1. Protein purification and crystal growth

After purification by DEAE-Sepharose Fast Flow, phenyl-Sepharose and Sephadex G75 chromatography, one 10.4 kDa band corresponding to the molecular weight of S100P was visible on non-reducing 16.5% SDS–PAGE analysis. The weak band should correspond to the S100P dimer. On running reducing SDS–PAGE, the weak band disappears (Fig. 1).

In order to determine the preliminary crystallization conditions, we employed screening crystallization kits (Hampton Research) using different protein concentrations and obtained crystals from some conditions containing ammonium salts. However, these crystals diffracted X-rays as salt crystals. At the same time, some other conditions produced multiple crystals. Further refinements were carried out with no useful results. We continued to screen our home PEG kit. A full range of PEG 4000 and pH 6.0–8.5 was used for different crystallization trials. Some needle-shaped crystals were produced in buffers containing 22–28% PEG 4000, 0.1 *M* Tris–HCl pH 7.0 and 7.5, and 22–37% PEG 4000, 0.1 *M* sodium citrate pH 6.5; larger crystals appear in buffer containing 22–28% PEG 4000, 0.1 *M* Tris–HCl pH 8.0 and 8.5 when the protein concentration was below about 40 mg ml^{-1}, but they diffracted weakly with a resolution below 7 Å. Based on the preliminary results, several further rounds of refinement were carried out, including altering the concentration of protein and precipitant and adding additives in the pH range 6.5–8.5. The crystal from buffer consisting of 20–40% PEG 4000 pH 7.0 and 7.5, 50–100 mg ml^{-1} protein diffracted to a resolution below 3.5 Å. This result shows no apparent improvement from buffer at pH 8.0 and 8.5. The crystals still diffracted weakly or as multiple crystals even though the crystals appeared to be good quality. The crystal diffracted to 2.0 Å when protein concentration was increased to 50 mg ml^{-1} or above and PEG 4000 concentration was increased to 30–34% at pH 6.5. In conclusion, we think that it is crucial to increase the concentration of protein and precipitant simultaneously at low pH for successful crystallization and to obtain a high-quality crystal.

Figure 2
Crystals of human S100P. Crystals from buffer containing 30% PEG 4000, 0.1 *M* sodium citrate pH 5.0.

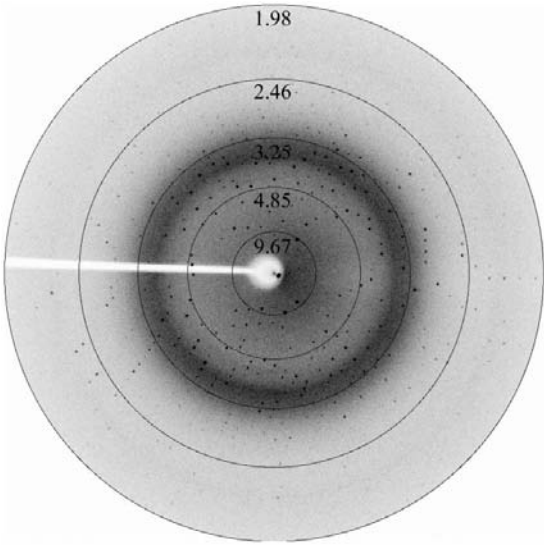

Figure 3
Typical diffraction pattern of a crystal of S100P.

Table 1
X-ray data statistics.

Values in parentheses are for the highest resolution shell (2.07–2.0 Å).

Space group	$P4_12_12$
Unit-cell parameters (Å)	$a = b = 60.8$, $c = 47.6$
Completeness (%)	100 (100)
No. of unique reflections	6442 (615)
Mean redundancy	8.9 (8.1)
$R_{\rm merge}$ (%)	10.4 (40.3)
$I/\sigma(I)$	14.9 (10.9)
No. of molecules per asymmetric unit	1
Matthews coefficient (Å^3 Da^{-1})	2.12
Solvent content (%)	∼40

3.2. Data collection and analysis

X-ray diffraction data were collected to 2.0 Å resolution at 110 K in-house without additional cryoprotectant (Fig. 3). The crystal-to-detector distance was 117 mm. The data were collected with 1.5° per frame over a 120° oscillation range. The crystal belongs to space group $P4_12_12$, with unit-cell parameters $a = b = 60.8$, $c = 47.6$ Å. There is one S100P molecule in an asymmetric unit. The Matthews coefficient is 2.12 Å^3 Da^{-1} and the solvent content is about 40% (Matthews, 1968). The X-ray data statistics are summarized in Table 1. The final structure determination is in progress.

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References

Averboukh, L., Liang, P., Kantoff, P. W. & Pardee, A. B. (1996). *Prostate*, **29**, 350–354.
Becker, T., Gerke, V., Kube, E. & Weber, K. (1992). *Eur. J. Biochem.* **207**, 541–547.
Brodersen, D. E., Etzerodt, M., Madsen, P., Celis, J. E., Thögersen, H. C., Nyborg, J. & Kjildgaard, M. (1998). *Structure*, **6**, 477–489.
Donato, R. (1999). *Biochem. Biophys. Acta*, **1450**, 191–231.
Drohat, A. C., Amburgey, J. C., Abildgaard, F., Starich, M. R., Baldisseri, D. & Weber, D. (1996). *Biochemistry*, **35**, 11577–11588.
Emoto, Y., Kobayashi, R., Akatsuka, H. & Hidaka, H. (1992). *Biochem. Biophys. Res. Commun.* **182**, 1246–1253.
Gribenko, A. V. & Makhatadze, G. I. (1998). *J. Mol. Biol.* **283**, 679–694.
Hilt, D. C. & Kligman, D. (1991). *Novel Calcium Binding Proteins*, pp. 65–104. Berlin/Heidelberg: Springer-Verlag.
Kawasaki, H. & Kretsinger, R. H. (1995). *Protein Profile*, **2**, 297–490.
Kilby, P. M., Van Eldik, L. J. & Roberts, G. C. K. (1996). *Structure*, **4**, 1041–1052.
Kretsinger, R. H. (1976). *Annu. Rev. Biochem.* **45**, 239–266.
Matsumura, H., Shiba, T., Inoue, T., Harada, S. & Kai, Y. (1998). *Structure*, **6**, 233–241.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Potts, B. C. M., Smith, J., Akke, M., Macke, T. J., Okazaki, K., Hidaka, H., Case, D. A. & Chazin, W. J. (1995). *Nature Struct. Biol.* **2**, 790–796.
Schafer, B. W. & Heizmann, C. W. (1996). *Trends Biochem. Sci.* **21**, 134–140.
Teigelkamp, S., Bhardwayj, R. S., Roth, J., Meinardus-Hager, G., Karas, M. & Sorg, C. (1991). *J. Biol. Chem.* **266**, 13462–13467.