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Crystallization and preliminary X-ray diffraction analysis of human seminal plasma protein PSP94

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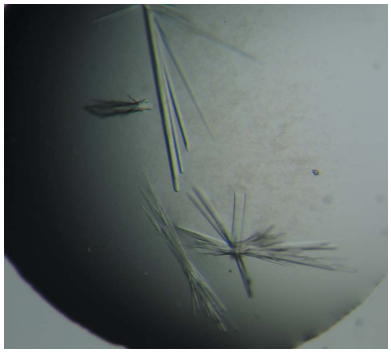
The human seminal plasma protein PSP94 is a small protein of 94 residues that contains ten cysteines. Since its discovery about 25 years ago, several potential biological functions have been reported for this protein. Many PSP94 homologues have also been identified since then from various species, but no crystal structure has been determined to date. PSP94 has been purified from human seminal plasma and crystallized. These crystals diffracted to ∼2.3 Å resolution and belonged to space group $P4_12_12$, with unit-cell parameters a = 107.9, b = 107.9, c = 92.1 Å. There are four molecules in the asymmetric unit. Structure solution by the heavy-atom method is currently in progress.

1. Introduction

The prostate-secretory protein of 94 residues (PSP94), also known as β-microseminoprotein (β-MSP), is one of the most abundant proteins in human semen (Lilja & Abrahamsson, 1988; Anahi Franchi *et al.*, 2008). It is a small nonglycosylated protein that is rich in cysteines. It is secreted at a high level in the seminal fluid by the epithelial cells of the prostate gland; however, the protein is not prostate-specific as its presence has been reported in several other tissues (Weiber *et al.*, 1990; Baijal-Gupta *et al.*, 2000). Although the exact biological function of PSP94 remains elusive, various systemic and confined roles for this protein have been reported, including as a modulator of follicle-stimulating hormone (FSH) level (Sheth *et al.*, 1984), as a binder of immunoglobulins (Liang *et al.*, 1992), as a motility inhibitor of sperm (Chao *et al.*, 1996), as a growth regulator and inducer of apoptosis in prostate cancer cells *in vitro* and *in vivo* (Garde *et al.*, 1999) and as a regulator of calcium levels during hypercalcaemia of malignancy (Shukeir *et al.*, 2003). Two proteins that bind to PSP94 in seminal fluid (CRISP-3; Udby *et al.*, 2005) and in blood (PSP94-binding protein; Reeves *et al.*, 2005) have also been identified, which is likely to drive further investigations in the search for more biological functions of this protein. PSP94 serum measurements have potential clinical utility in prostate-cancer management (Eeles *et al.*, 2008; Nam *et al.*, 2006).

In addition to humans, PSP94 homologues have been identified in many other mammals (Fernlund *et al.*, 1994; Makinen *et al.*, 1999; Xuan *et al.*, 1999), ostrich (Lazure *et al.*, 2001) and recently in the serum of a reptile, the Japanese viper (Aoki *et al.*, 2007, 2008). Interestingly, one of the PSP94 homologues (SSP-2) from this reptile binds to the snake-venom-derived Ca^{2+}-channel blocker (triflin), perhaps imparting self-protection from its own venom to the snake (Aoki *et al.*, 2007). There is a large degree of sequence diversity among different PSP94 homologues, but the positions of the ten cysteine residues are well conserved throughout, suggesting that the overall tertiary structures of this family of proteins should remain conserved. No crystal structures of any members of this family of proteins have been reported to date. However, two laboratories (Wang *et al.*, 2005; Ghasriani *et al.*, 2006) have reported the NMR structure of PSP94, showing that this small protein contains two domains that are bridged by a disulfide linkage. Interestingly, the secondary structures of the individual domains reported by the two laboratories were similar but the orientation of the two domains was



very different, giving rise to two distinct overall shapes for this small protein. These differences reportedly stemmed from a different interpretation of the ten specific inter-domain NOEs (nuclear Overhauser effects). It remains to be seen which of the two structures is correct and whether the differences are genuine and are of biological relevance.

2. Materials and methods

2.1. Protein purification and crystallization

PSP94 was purified from human seminal plasma as described previously (Thakur *et al.*, 1981; Jagtap *et al.*, 2007). Briefly, the seminal plasma was subjected to ammonium sulfate precipitation followed by hydrophobic interaction chromatography using a phenyl Sepharose column. The eluted PSP94 fraction was further purified using a reverse-phase column on an HPLC system. The purified protein was then lyophilized and stored at 253 K until further use. For crystallization purposes, the lyophilized protein was dissolved in water (10 mg ml^{-1}). Initial screening for crystallization conditions was performed using a CyBio-HTPC crystallization robot and several commercial screening kits from Molecular Dimensions (Structure Screen 1 and 2, JCSG+ and MemGold) and Jena Bioscience (JBScreen Basic 1, 2, 3 and 4). The crystallization trials were set up in 96-well plates (Greiner) using the sitting-drop vapour-diffusion method at 293 K. In these setups, 0.7 µl protein solution was mixed with 0.7 µl mother liquor and equilibrated against 75 µl reservoir solution within a sealed well. The crystallization leads obtained from these screens were further optimized in manual setups in a 24-well plate by varying the precipitants and their concentration, the pH and additives using the hanging-drop vapour-diffusion method.

2.2. X-ray diffraction data collection

X-ray diffraction data were collected on the X06DA (PXIII) beamline of the Swiss Light Source, Paul Scherrer Institut, Villigen, Switzerland. Prior to X-ray diffraction data collection, the crystals were flash-frozen in liquid nitrogen. The crystallization buffer, which contained ∼45%(*v*/*v*) PEG 400, was directly used for freezing without the addition of any further cryoprotectant solution. The diffraction data were recorded at 100 K using X-rays of wavelength 1.0 Å and a MAR Mosaic 225 mm CCD detector (MAR Research). A total of 180 consecutive diffraction images were collected with 1° oscillation per image, an exposure time of 3 s and a crystal-to-detector distance of 200 mm. The diffraction data were indexed, integrated and scaled using the programs *MOSFLM* (Leslie, 2006) and *SCALA* (Collaborative Computational Project, Number 4, 1994).

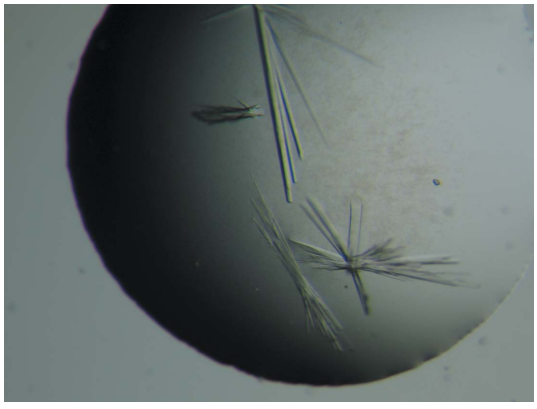
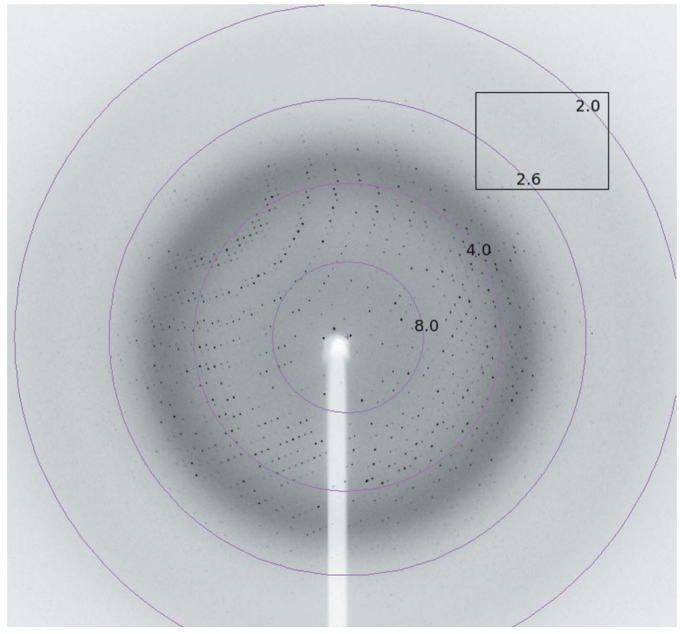


Figure 1
Typical crystals of PSP94 obtained by hanging-drop vapour diffusion at 293 K.

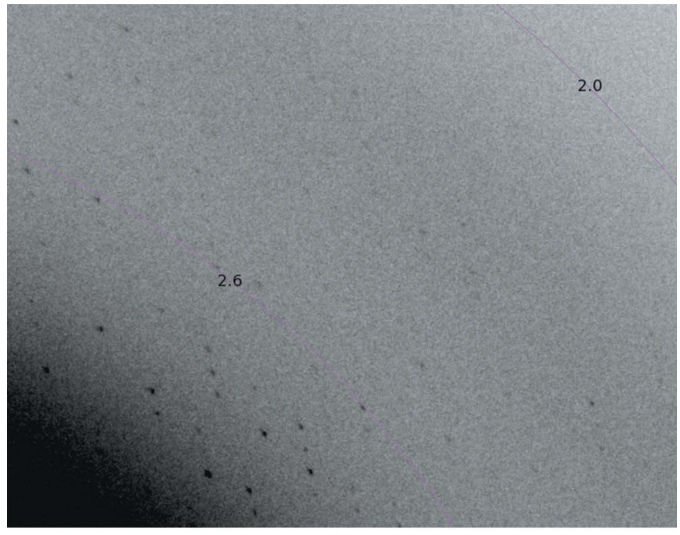
3. Results and discussion

Crystals suitable for X-ray diffraction were obtained at 293 K in manual setups from drops prepared by mixing 2 µl protein solution (10 mg ml^{-1} in water) with 2 µl reservoir solution [0.1 *M* sodium acetate pH 4.5, 0.2 *M* lithium sulfate and 44–47%(*v*/*v*) PEG 400] and 0.4 µl additive solution (γ-butyrolactone) and equilibrated against 1 ml reservoir solution in the well. The crystals appeared within one week and grew to typical dimensions of 0.03 × 0.03 × 1.0 mm (Fig. 1).

These crystals diffracted to 2.3 Å resolution on the synchrotron beamline (Fig. 2). The indexing results showed that the crystals have a tetragonal lattice with unit-cell parameters $a = b = 107.9$, $c = 92.1$ Å.



(*a*)



(*b*)

Figure 2
(*a*) Representative X-ray diffraction image from a PSP94 crystal. (*b*) A magnified view of a portion of the diffraction image. Resolution limits at the concentric circles are marked in Å.

Table 1
X-ray data-collection statistics.

Values in parentheses are for the highest resolution shell.

X-ray source	PXIII beamline, Swiss Light Source
Wavelength (Å)	1.00
Resolution range (Å)	50.0–2.3 (2.42–2.30)
Space group	$P4_12_12$
Unit-cell parameters (Å)	a = 107.9, b = 107.9, c = 92.1
Total No. of reflections	198419 (28313)
No. of unique reflections	24778 (3528)
Multiplicity	8.0 (8.0)
Completeness (%)	100.0 (100.0)
$\langle I/\sigma(I)\rangle$	16.1 (4.1)
$R_{\rm merge}$† (%)	10.4 (46.0)
Mosaicity (°)	0.50

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

Analysis of the reflection data revealed systematic absences consistent with space group $P4_12_12$, which was further verified using the *CCP*4 program *POINTLESS* (Evans, 2006). The Matthews coefficient ($V_{\rm M}$) is 3.17 Å^3 Da^{-1} for four protein molecules in the asymmetric unit, corresponding to a solvent fraction of 61.2%. The statistics of data collection are summarized in Table 1. Molecular replacement using NMR structures (PDB codes 1xhh, 2iz3 and 2iz4) as search models did not produce any solutions. Various heavy-atom derivatives are currently in preparation for solution of the phase problem.

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References

Anahi Franchi, N., Avendano, C., Molina, R. I., Tissera, A. D., Maldonado, C. A., Oehninger, S. & Coronel, C. E. (2008). *Reproduction*, **136**, 157–166.
Aoki, N., Matsuo, H., Deshimaru, M. & Terada, S. (2008). *Gene*, **426**, 7–14.
Aoki, N., Sakiyama, A., Deshimaru, M. & Terada, S. (2007). *Biochem. Biophys. Res. Commun.* **359**, 330–334.
Baijal-Gupta, M., Clarke, M. W., Finkelman, M. A., McLachlin, C. M. & Han, V. K. (2000). *J. Endocrinol.* **165**, 425–433.
Chao, C. F., Chiou, S. T., Jeng, H. & Chang, W. C. (1996). *Biochem. Biophys. Res. Commun.* **218**, 623–628.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Eeles, R. A. *et al.* (2008). *Nature Genet.* **40**, 316–321.
Evans, P. (2006). *Acta Cryst.* D**62**, 72–82.
Fernlund, P., Granberg, L. B. & Roepstorff, P. (1994). *Arch. Biochem. Biophys.* **309**, 70–76.
Garde, S. V., Basrur, V. S., Li, L., Finkelman, M. A., Krishan, A., Wellham, L., Ben-Josef, E., Haddad, M., Taylor, J. D., Porter, A. T. & Tang, D. G. (1999). *Prostate*, **38**, 118–125.
Ghasriani, H., Teilum, K., Johnsson, Y., Fernlund, P. & Drakenberg, T. (2006). *J. Mol. Biol.* **362**, 502–515.
Jagtap, D. D., Narahari, A., Swamy, M. J. & Mahale, S. D. (2007). *Biochim. Biophys. Acta*, **1774**, 723–731.
Lazure, C., Villemure, M., Gauthier, D., Naude, R. J. & Mbikay, M. (2001). *Protein Sci.* **10**, 2207–2218.
Leslie, A. G. W. (2006). *Acta Cryst.* D**62**, 48–57.
Liang, Z. G., Kamada, M., Mitsudo, S. M. & Koide, S. S. (1992). *Arch. Androl.* **28**, 159–164.
Lilja, H. & Abrahamsson, P. A. (1988). *Prostate*, **12**, 29–38.
Makinen, M., Valtonen-Andre, C. & Lundwall, A. (1999). *Eur. J. Biochem.* **264**, 407–414.
Nam, R. K., Reeves, J. R., Toi, A., Dulude, H., Trachtenberg, J., Emami, M., Daigneault, L., Panchal, C., Sugar, L., Jewett, M. A. & Narod, S. A. (2006). *J. Urol.* **175**, 1291–1297.
Reeves, J. R., Xuan, J. W., Arfanis, K., Morin, C., Garde, S. V., Ruiz, M. T., Wisniewski, J., Panchal, C. & Tanner, J. E. (2005). *Biochem. J.* **385**, 105–114.
Sheth, A. R., Arabatti, N., Carlquist, M. & Jornvall, H. (1984). *FEBS Lett.* **165**, 11–15.
Shukeir, N., Arakelian, A., Kadhim, S., Garde, S. & Rabbani, S. A. (2003). *Cancer Res.* **63**, 2072–2078.
Thakur, A. N., Vaze, A. Y., Dattatreyamurthy, B. & Sheth, A. R. (1981). *Indian J. Exp. Biol.* **19**, 307–313.
Udby, L., Lundwall, A., Johnsen, A. H., Fernlund, P., Valtonen-Andre, C., Blom, A. M., Lilja, H., Borregaard, N., Kjeldsen, L. & Bjartell, A. (2005). *Biochem. Biophys. Res. Commun.* **333**, 555–561.
Wang, I., Lou, Y. C., Wu, K. P., Wu, S. H., Chang, W. C. & Chen, C. (2005). *J. Mol. Biol.* **346**, 1071–1082.
Weiber, H., Andersson, C., Murne, A., Rannevik, G., Lindstrom, C., Lilja, H. & Fernlund, P. (1990). *Am. J. Pathol.* **137**, 593–603.
Xuan, J. W., Kwong, J., Chan, F. L., Ricci, M., Imasato, Y., Sakai, H., Fong, G. H., Panchal, C. & Chin, J. L. (1999). *DNA Cell Biol.* **18**, 11–26.