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Purification, crystallization and preliminary X-ray diffraction analysis of human phosphoserine phosphatase

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Phosphoserine phosphatase (PSP), a human enzyme involved in the L-serine biosynthesis pathway, has been crystallized using the hanging-drop vapour-diffusion method at 277 K. The crystals are orthorhombic, belonging to space group $C222_1$, with unit-cell parameters a = 49.03 Å, b = 130.25 Å, c = 157.29 Å. Calculation of the Matthews coefficient indicates that there are two molecules in the asymmetric unit. A complete native data set to a resolution of 1.53 Å has been collected at 100 K using synchrotron radiation.

1. Introduction

Phosphoserine phosphatase (PSP) is a 25 kDa enzyme responsible for the third and last step of the L-serine biosynthesis pathway. It catalyses the Mg^{2+}-dependent hydrolysis of L-phosphoserine. The reaction mechanism of many phosphatases or phosphotransferases involves the formation of a catalytic intermediate in which the phosphate derived from the substrate is bound to the side chain of either a serine, histidine, cysteine (Vincent *et al.*, 1992; Wo *et al.*, 1992) or aspartate residue present in the catalytic site.

PSP belongs to a recently identified class of phosphotransferases forming a phosphoaspartate intermediate during catalysis (Collet *et al.*, 1998). Other enzymes forming a phosphoaspartate intermediate include, for example, P-type ATPases (Post & Kume, 1973; Aravind *et al.*, 1998) and phosphomannomutases (Collet *et al.*, 1998). Amino-acid comparison revealed that PSP shares three statistically significant motifs with P-type ATPases, with other members of this new class of phosphotransferases and with haloacid dehalogenase (Koonin & Tatusov, 1994).

The first of these motifs contains an absolute conserved aspartate in a conserved D*X*D*X*-(T/V) motif, which covalently binds phosphate in PSP and P-type ATPases (Collet *et al.*, 1998). The second motif contains a conserved serine or threonine and the third motif contains a conserved lysine residue followed by less conserved residues and a strictly conserved aspartate. Mutation in all these motifs shows that they play an important role in catalysis (MacLennan *et al.*, 1992; Lingrel & Kuntzweiler, 1994; Collet *et al.*, 1999). Moreover, elucidation of the three-dimensional structure of haloacid dehalogenase indicates that those three motifs line up in the catalytic pocket (Hisano *et al.*, 1996).

In order to further explore the mechanism of phosphoenzyme formation and hydrolysis, it is of importance to determine the three-dimensional structure of human PSP.

2. Experimental and results

2.1. Purification

Human PSP was expressed in BL21(DE3)-pLysS bacteria harbouring the expression plasmid described in Collet *et al.* (1997). Bacterial extracts were prepared from cells derived from a 3 l culture in M9 medium as described elsewhere (Veiga da Cunha *et al.*, 1994), with a lysis buffer containing 20 m*M* HEPES pH 7.4, 5 m*M* EDTA, 1 m*M* dithiothreitol, 5 µg ml^{-1} leupeptin, 5 µg ml^{-1} antipain, 0.5 m*M* phenylmethylsulfonyl fluoride (PMSF) and 1 mg ml^{-1} hen egg lysozyme. The extract was centrifuged for 40 min (20 000*g* at 277 K) and 9 g of polyethylene glycol 6000 was dissolved in the resulting supernatant (150 ml), which contained 1515 U of PSP. The mixture was maintained for 15 min on ice and centrifuged for 15 min at 12 000*g*. PSP activity was found in the supernatant, to which 24 g of polyethylene glycol was then added. After 15 min on ice, the resulting mixture was centrifuged for 15 min at 12 000*g* and the pellet, containing 95% of the PSP activity, was resuspended in 60 ml buffer *A* (25 m*M* Tris pH 8.5, 1 m*M* dithiothreitol, 1 µg ml^{-1} leupeptin, 1 µg ml^{-1} antipain). The mixture was then applied to a DEAE-Sepharose column (1.6 × 10 cm). The column was washed with 100 ml buffer *A* and protein was eluted with a linear gradient of NaCl (0–400 m*M* in 2 × 150 ml buffer *A*). PSP eluted at a NaCl concentration of 250 m*M*. The active fractions were pooled and loaded onto a Q-Sepharose column (1.6 × 10 cm). The column was washed with 100 ml of buffer *B* (25 m*M* glycine, 1 m*M* dithiothreitol, 1 µg ml^{-1} leupeptin, 1 µg ml^{-1} antipain) and protein was eluted with a linear gradient of

NaCl (0–400 m*M* in 2 × 125 ml buffer *B*). After this step, PSP had a specific activity of 7.9 U mg^{-1} of protein and was nearly homogeneous as assessed by SDS–PAGE. Finally, the preparation was concentrated to 1 ml in an Amicon pressure cell equipped with a YM-10 membrane. It was then diluted with 9 ml of a buffer devoid of antipain and leupeptin and reconcentrated. This procedure was repeated twice to reduce the concentration of antipain and leupeptin by about 1000-fold.

2.2. Crystallization

To determine initial crystallization conditions, Hampton Crystal Screen I (Jancarik & Kim, 1991) was applied to a sample of the PSP. Crystallization was carried out by the hanging-drop vapour-diffusion technique at 277 K using Linbro multiwell tissue plates. Each well was filled with 700 µl of reservoir solution. Drops consisting of 1.5 µl protein solution and 1.5 µl of reservoir solution were placed on cover slips and set to equilibrate against these reservoir solutions.

Three crystallization conditions from this screening yielded crystals. Crystal form I was grown from 8% polyethylene glycol 4000 and 0.1 *M* acetate buffer pH 4.6, whereas form II crystals could be grown from 0.1 *M* HEPES buffer pH 7.5 with 1.5 *M* Li_2SO_4. However, both these crystal forms yielded unsatisfactory results in data collection. Diffraction was very poor (lower than 8 Å resolution) and all attempts to improve the crystal quality were unsuccessful. Crystal form III (Fig. 1*a*), grown from 18% polyethylene glycol 8000, 0.1 *M* cacodylate buffer pH 6.3 and 0.2 *M* calcium acetate, also initially gave bad results in data collection. However, the diffraction characteristics of these crystals could be improved substantially by optimizing the initial crystallization conditions: a solution containing 0.7 *M* $CaCl_2$, 0.1 *M* cacodylate buffer pH 6.3 and 20% polyethylene glycol 1500 yielded better crystals (Fig. 1*b*). The protein concentration used was 6.0 mg ml^{-1}. Furthermore, the removal of antipain and leupeptin from the protein sample was found to be essential for obtaining crystals with excellent diffraction properties.

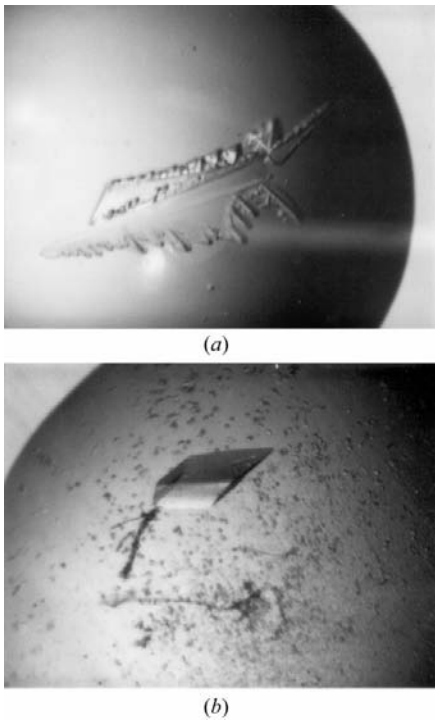

Figure 1
Type III crystals of PSP (*a*) before (*b*) and after optimization. The typical size of diffraction quality crystals is approximately 0.3 × 0.3 × 0.3 mm.

2.3. Data collection and processing

Diffraction data were collected at 100 K using synchrotron radiation (BW7B beamline of the DESY synchrotron, Hamburg) after soaking the PSP crystals for 24 h in a solution of the mother liquor with 22% polyethylene glycol 400. The crystals grow in space group $C222_1$, diffract to a resolution of 1.53 Å and have unit-cell parameters a = 49.03, b = 130.25, c = 157.29 Å. All data processing was performed using *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997). The final data set is 99.8% complete and is characterized by an R_{sym} of 3.5%. Further statistics are summarized in Table 1. According to Matthews coefficient calculations (Matthews, 1974), the asymmetric unit should consist of two molecules, with a corresponding V_M of 2.53 Å^3 Da^{-1} and a solvent content of 51%.

Table 1
Data-collection and reduction statistics for the PSP crystals.

Values in parentheses indicate data in the highest resolution shell.

Space group	$C222_1$
Wavelength used (Å)	0.8423
Resolution limit (Å)	1.53 (1.56–1.53)
Total observations	319890
Unique reflections	76323 (3735)
Completeness of all data (%)	99.8 (98.7)
Completeness of data ($I > 2\sigma$) (%)	95.2 (80.7)
Mean $I/\sigma(I)$†	15.0 (3.1)
R_{merge}‡ (%)	3.5 (18.7)
R_{meas}†§ (%)	3.8 (21.9)

† These values were obtained using *NOVEL_R* (Diederichs & Karplus, 1997*a*,*b*). ‡ $R_{merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$. § $R_{meas} = \sum_{hkl}[n_{hkl}/(n_{hkl}-1)] \times \sum_i^{n_{hkl}} |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i^{n_{hkl}} I_i(hkl)$.

3. Conclusions

With the crystallization procedure and preliminary X-ray analysis described here, an initial step has been made to explore the three-dimensional structure of PSP. The elucidation of this structure will hopefully contribute to a detailed understanding of the reaction mechanism of phosphoenzyme formation and hydrolysis performed by many phosphatases and phosphotransferases. A selenomethionyl derivative for phase determination is currently being prepared.

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References

Aravind, L., Galperin, M. Y. & Koonin, E. V. (1998). *Trends Biochem. Sci.* **23**, 127–129.
Collet, J. F., Gerin, I., Rider, M. H., Veiga da Cunha, M. & Van Schaftingen, E. (1997). *FEBS Lett.* **408**, 281–284.
Collet, J. F., Stroobant, V., Pirard, M., Delpierre, G. & Van Schaftingen, E. (1998). *J. Biol. Chem.* **273**, 14107–14112.
Collet, J. F., Stroobant, V. & Van Schaftingen, E. (1999). *J. Biol. Chem.* **274**, 33985–33990.
Diederichs, K. & Karplus, A. (1997). *Nature Struct. Biol.* **4**, 269–275.
Diederichs, K. & Karplus, A. (1997). *Nature Struct. Biol.* **4**, 592.
Hisano, T., Hata, Y., Fujii, T., Liu, J. Q., Kurihara, T., Esaki, N. & Soda, K. (1996). *J. Biol. Chem.* **271**, 20322–20330.
Jancarik, J. & Kim, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.
Koonin, E. V. & Tatusov, R. L. (1994). *J. Mol. Biol.* **244**, 125–132.
Lingrel, J. B. & Kuntzweiler, T. (1994). *J. Biol. Chem.* **269**, 19659–19662.
MacLennan, D. H., Clarke, D. M., Loo, T. W. & Skerjanc, I. S. (1992). *Acta Physiol. Scand. Suppl.* **607**, 141–150.
Matthews, B. W. (1974). *J. Mol. Biol.* **82**, 513–526.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Post, R. L. & Kume, S. (1973). *J. Biol. Chem.* **248**, 6993–7000.
Veiga da Cunha, M., Detheux, M., Watelet, N. & Van Schaftingen, E. (1994). *Eur. J. Biochem.* **225**, 43–51.
Vincent, J. B., Crowder, M. W. & Averill, B. A. (1992). *Trends Biochem. Sci.* **17**, 105–110.
Wo, Y. Y., Zhou, M. M., Stevis, P., Davis, J. P., Zhang, Z. Y. & Van Etten, R. L. (1992). *Biochemistry*, **31**, 1712–1721.