Acta Crystallographica Section F
Structural Biology and Crystallization Communications
ISSN 1744-3091

Crystallization and preliminary X-ray diffraction analysis of rat protein tyrosine phosphatase η

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Received 30 May 2006
Accepted 7 August 2006

The rat protein tyrosine phosphatase η (rPTPη) is a cysteine-dependent phosphatase which hydrolyzes phosphoester bonds in proteins and other molecules. rPTPη and its human homologue DEP-1 are involved in neoplastic transformations. Thus, expression of the protein is reduced in all oncogene-transformed thyroid cell lines and is absent in highly malignant thyroid cells. Moreover, consistent with the suggested tumour suppression role of PTPη, inhibition of the tumorigenic process occurs after its exogenous reconstitution, suggesting that PTPη might be important for gene therapy of cancers. In this study, the catalytic domain of rPTPη was produced in *Escherichia coli* in soluble form and purified to homogeneity. Crystals were obtained by the hanging-drop vapour-diffusion method. Diffraction data were collected to 1.87 Å resolution. The crystal belongs to space group $P2_12_12_1$, with unit-cell parameters a = 46.46, b = 63.07, c = 111.64 Å, and contains one molecule per asymmetric unit.

1. Introduction

The dephosphorylation of tyrosyl residues by protein tyrosine phosphatases plays a major role in controlling cell activities such as embryogenesis, proliferation, differentiation, fertilization and neoplastic transformation *in vivo* (Chagnon *et al.*, 2004; den Hertog, 1999; Mustelin *et al.*, 2002). The PTPs represent a diverse family of enzymes that exist in both soluble cytosolic and receptor-like tyrosine phosphatase (RPTP) forms. In humans, the classical tyrosine-specific PTPs are encoded by 38 genes. Generally, the RPTPs contain one or two conserved intracellular catalytic domains of approximately 240 amino acids with a conserved motif [(I/V)HC*X*AG*XX*R(S/T)G], a single transmembrane domain and a highly variable external segment. These cysteine-dependent phosphatases utilize the conserved C(X)$_5$R sequence motif to hydrolyze phosphoester bonds in proteins and non-protein substrates (Alonso *et al.*, 2004; Kolmodin & Åqvist, 2001). The tertiary structure of the catalytic domains of all crystallized RPTPs revealed an architecture comprised of a globular fold that consists of an eight-stranded twisted β-sheet flanked by four α-helices on one side and another on the opposite side (Jia *et al.*, 1995; Stuckey *et al.*, 1994; Barford *et al.*, 1998). The PTP-signature motif is conservatively located at the bottom of the catalytic site cleft.

rPTPη is a ubiquitous gene that is highly homologous to human *DEP-1*, also known as *RPTP*η, *PTPRJ* and *CD148*, as well as mouse protein phosphatase η, *Ptprj* (Zhang *et al.*, 1997; Honda *et al.*, 1994; Ostman *et al.*, 1994; Ruivenkamp *et al.*, 2002). The protein structure of PTPη contains only one intracellular phosphatase domain, a single transmembrane domain and eight fibronectin type III-like repeats in the extracellular region (Krueger *et al.*, 1990; Fischer *et al.*, 1991; Saito, 1993). Similar to thyroid-specific genes, rPTPη expression is induced by TSH and is positively regulated by thyrotropin through the protein kinase A pathway and negatively regulated by protein kinase C activation (Martelli *et al.*, 1998). Further evidence has demonstrated the involvement of rPTPη and human DEP-1 in neoplastic transformations of rat and human cells, respectively. A reduction in expression of the protein is observed in all oncogene-transformed thyroid cell lines and expression is absent in highly malignant thyroid cells (Okazaki & Sagato, 1995). Moreover, the

malignant phenotype can be reverted when *PTPη* gene expression is re-established. The mechanism involved in this process includes increasing levels of the cell-cycle inhibitor $p27^{kip1}$ protein and dephosphorylation of PLCγ1, a substrate of DEP-1/HPTPη (Trapasso *et al.*, 2000). Recently, it has been shown that the PTPη protein is capable of binding to c-Src in living cells. The dephosphorylation of the negative regulatory tyrosine (Tyr529 of the c-Src family protein tyrosine kinases) increases c-Src tyrosine kinase activity in malignant rat thyroid cells stably transfected with rPTPη (Ardini *et al.*, 2000). Additionally, studies have also implicated the mouse homologue of *rPTPη*, *Ptprj*, in susceptibility to mouse colon cancer, reinforcing the idea that restoration of PTPη function could be a useful tool for gene therapy of human cancers (Ruivenkamp *et al.*, 2002).

In order to better understand the molecular mechanism of the catalytic activity and substrate specificity of rPTPη, we have expressed the catalytic domain (CD) of rPTPη in *Escherichia coli*, purified it to homogeneity and crystallized it. Here, we describe the crystallization and preliminary X-ray crystallographic analysis of rPTPηCD.

2. Materials and methods

2.1. Expression and purification of recombinant rPTPη phosphatase domain

BL21 (DE3) cells harbouring the plasmid containing the rPTPη intracellular domain insert were grown at 303 K in 2×YT media plus kanamicin with shaking until the absorbance at 600 nm reached 0.6–0.8. At this point, 0.5 m*M* isopropyl β-D-thiogalactopyranoside (IPTG) was added to induce rPTPη expression and cells were incubated for 4 h. The induced bacteria were harvested by centrifugation at 6000*g* in a Sorvall RC-5C Plus centrifuge at 277 K for 20 min. The bacterial pellets from 2.5 l culture were resuspended in 100 ml lysis buffer (50 m*M* sodium phosphate buffer pH 7.8, 100 m*M* NaCl, 10% glycerol, 10 m*M* imidazole, 2 m*M* β-mercaptoethanol) containing 1 m*M* PMSF and 0.5 mg ml^{-1} lysozyme (Sigma). The suspension was incubated on ice for 30 min to lyse cells. The lysate was further disrupted by sonication on ice with a 550 Sonic Dismembrator (Fisher Scientific) to reduce the viscosity. Centrifugation was performed at 14 000*g* for 1 h and the clear supernatant obtained constituted the crude protein preparation. The supernatant from the above step was mixed with 20 ml Talon Superflow resin (Clontech) pre-equilibrated with equilibration buffer (50 m*M* sodium phosphate buffer pH 7.8, 300 m*M* NaCl, 10% glycerol, 10 m*M* imidazole, 2 m*M* β-mercaptoethanol) and left rotating at 277 K for 1 h. The mixture of resin and supernatant was poured into a c16/10 glass column (Amersham Biosciences) connected to a HPLC ÄKTA purifier (Amersham Biosciences) and the tightly bound proteins were eluted with elution buffer (50 m*M* sodium phosphate buffer pH 7.8, 50 m*M* NaCl, 10% glycerol, 300 m*M* imidazole, 2 m*M* β-mercaptoethanol). The protein was further purified to >96% by size-exclusion chromatography on a Superdex 200HL 26/60 column (Amersham Biosciences) using HEPES buffer (20 m*M* HEPES pH 7.8, 200 m*M* NaCl, 5% glycerol, 1 m*M* DTT) as eluent. All purification procedures were carried out at 277 K. The purified protein fractions were visualized on 15% SDS–PAGE. Soluble His_6-rPTPη (molecular weight 43 kDa) was concentrated to 1 mg ml^{-1} and incubated, according to the manufacturer's recommendation, with 0.5 U ml^{-1} bovine thrombin protease for 1–18 h at 291 K followed by dialysis against HEPES buffer. The thrombin-cleaved rPTPη was then frozen in liquid nitrogen and stored at 193 K (Santos *et al.*, 2005).

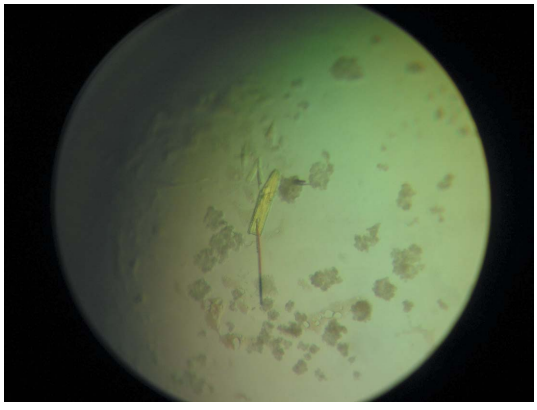

Figure 1
Crystal of rPTPηCD. Typical dimensions are approximately 0.2 × 0.4 × 0.2 mm.

2.2. Crystallization

Crystallization conditions were screened by the sparse-matrix method with hanging-drop vapour diffusion using Hampton Crystal Screen 1 and 2 and Nextal Suites. Suitable crystals appeared using Nextal PEGs Suite condition No. 35 (20% PEG 10 000, 0.1 *M* MES pH 6.5) after 30 d (Fig. 1).

2.3. Data collection and processing

A single crystal was harvested in a nylon loop and transferred to a cryoprotectant solution containing 20% PEG 10 000, 0.1 *M* MES pH 6.5 and 15%(*v*/*v*) ethylene glycol for one minute. The crystal was then flash-cooled to 100 K in a nitrogen stream for data collection. Data collection was carried out at the MX-1 beamline of the Brazilian National Synchrotron Light Laboratory (LNLS, Campinas, Brazil; Polikarpov, Oliva *et al.*, 1997; Polikarpov *et al.*, 1998) using synchrotron radiation of wavelength 1.42 Å to optimize both the diffraction

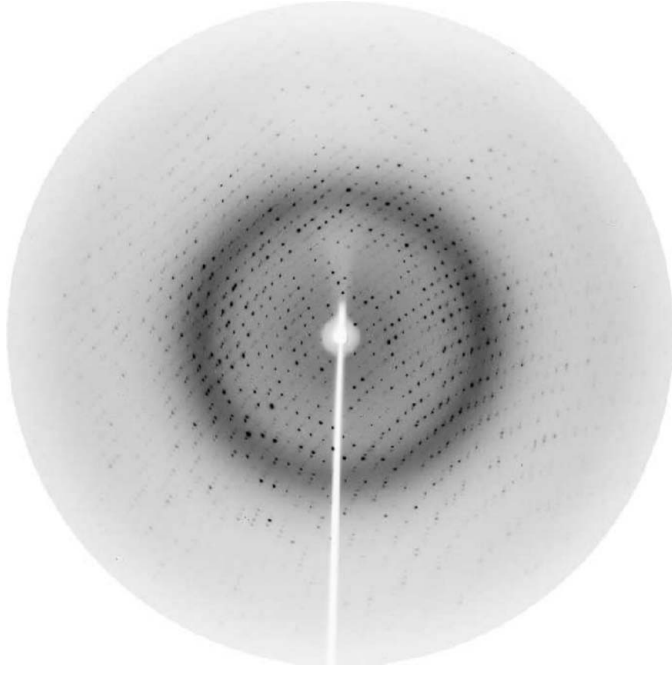

Figure 2
Diffraction pattern of the rPTPηCD crystal collected on the MX-1 beamline at LNLS. The maximum resolution at the edge of the image is 1.87 Å.

Table 1
X-ray data-collection statistics.

Values in parentheses are for the highest resolution shell (1.97–1.87 Å).

Wavelength (Å)	1.42
Resolution range (Å)	35.7–1.87
Space group	$P2_12_12_1$
Unit-cell parameters (Å, °)	a = 46.46, b = 63.07, c = 111.64
Completeness (%)	99.4 (99.4)
Redundancy	3.6 (3.8)
$R_{\rm merge}$† (%)	6.0 (19.3)
Average $I/\sigma(I)$	7.5 (3.7)
Total reflections	242862
Unique reflections	27816

† $R_{\rm merge} = \sum |I_i - \langle I \rangle| / \sum I$, where I_i is the intensity of the ith observation and $\langle I \rangle$ is the mean intensity of the reflections.

efficiency of the crystal and the synchrotron-radiation flux of the LNLS storage ring (Polikarpov, Teplyakov *et al.*, 1997; Teplyakov *et al.*, 1998). 100 images were recorded with an oscillation of 1° per image on a MAR CCD detector (Fig. 2). The data set was integrated and scaled using *MOSFLM* (Leslie, 1992) and *SCALA*. Data-collection statistics are given in Table 1.

3. Results and discussion

Initial screening of crystallization conditions resulted in a crystal appropriate for data collection, which diffracted to 1.9 Å resolution at a synchrotron beamline. Initial analysis of the solvent content by determining the Matthews coefficient (Matthews, 1968) suggested that the asymmetrical unit could accommodate one molecule with 46% solvent content. Molecular replacement using *MOLREP* (Vagin & Teplyakov, 1997) and chain *A* of the crystal structure of the catalytic domain of human tyrosine-protein phosphatase β (PDB code 2ahs) as a search model resulted in a clear solution with a single molecule in the asymmetric unit. The top solution after rotation function had a value of 10.64σ, in contrast to 4.36σ for the second solution. The best solution after translation function had a score of 0.427 and an R factor of 0.569. Simulated annealing performed with *CNS* (Brünger *et al.*, 1998) using this solution and following a slow-cooling protocol led to a structure with an R factor of 0.361 and $R_{\rm free}$ = 0.396. Structural refinement is in progress.

SCALA and *MOLREP* are programs from the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994).

This work was supported in part by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) grant Nos. 04/08070-9 and 06/00182-8 and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). The authors thank Laboratório Nacional de Luz Síncrotron (LNLS) for technical support during data collection.

References

Alonso, A., Sasin, J., Bottini, N., Friedberg, I., Friedberg, I., Osterman, A., Godzik, A., Hunter, T., Dixon, J. & Mustelin, T. (2004). *Cell*, **117**, 699–711.
Ardini, E., Agresti, R., Tagliabue, E., Greco, M., Aiello, P., Yang, L. T., Ménard, S. & Sap, J. (2000). *Oncogene*, **19**, 4979–4987.
Barford, D., Das, A. K. & Egloff, M. P. (1998). *Annu. Rev. Biophys. Biomol. Struct.* **27**, 133–164.
Brünger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, J.-S., Kuszewski, J., Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T. & Warren, G. L. (1998). *Acta Cryst.* D**54**, 905–921.
Chagnon, M. J., Uetani, N. & Tremblay, M. L. (2004). *Biochem. Cell Biol.* **82**, 664–675.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
den Hertog, J. (1999). *Mech. Dev.* **85**, 3–14.
Fischer, E. H, Charbonneau, H. & Tonks, N. K. (1991). *Science*, **253**, 401–406.
Honda, H., Inazawa, J., Nishida, J., Yazaki, Y. & Hirai, H. (1994). *Blood*, **15**, 4186–4194.
Jia, Z., Barford, D., Flint, A. J. & Tonks, N. K. (1995). *Science*, **268**, 1754–1758.
Kolmodin, K. & Åqvist, J. (2001). *FEBS Lett.* **498**, 208–213.
Krueger, N. X., Streuli, M. & Saito, H. (1990). *EMBO J.* **9**, 3241–3252.
Leslie, A. G. W. (1992). *Jnt CCP4/ESF–EAMCB Newsl. Protein Crystallogr.* **26**.
Martelli, M. L., Trapasso, F., Bruni, P., Berlingieri, M. T., Battaglia, C., Vento, M. T., Belletti, B., Iuliano, R., Santoro, M., Viglietto, G. & Fusco, A. (1998). *Exp. Cell Res.* **245**, 195–202.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Mustelin, T., Abraham, R. T., Rudd, C. E., Alonso, A. & Merlo, J. J. (2002). *Front. Biosci.* **7**, 918–969.
Okazaki, K. & Sagato, N. (1995). *EMBO J.* **14**, 5048–5059.
Ostman, A., Yang, Q. & Tonks, K. (1994). *Proc. Natl Acad. Sci. USA*, **91**, 9680–9684.
Polikarpov, I., Oliva, G., Castellano, E. E., Garratt, R. C., Arruda, P., Leite, A. & Craievich, A. (1997). *Nucl. Instrum. Methods A*, **405**, 159–164.
Polikarpov, I., Perles, L. A., de Oliveira, R. T., Oliva, G., Castellano, E. E., Garratt, R. & Craievich, A. (1998). *J. Synchrotron Rad.* **5**, 72–76.
Polikarpov, I., Teplyakov, A. & Oliva, G. (1997). *Acta Cryst.* D**53**, 734–737.
Ruivenkamp, C. A. L. *et al.* (2002). *Nature Genet.* **31**, 295–300.
Saito, H. (1993). *Cell Biol.* **4**, 379–387.
Santos, M. A. M., Santos, S. M., Matozo, H. C., Portugal, R. V., Iuliano, R., Fusco, A. & Polikarpov, I. (2005). *Protein Expr. Purif.* **41**, 113–120.
Stuckey, J. A., Schubert, H. L., Fauman, E. B., Zhang, Z. Y., Dixon, J. E. & Saper, M. A. (1994). *Nature (London)*, **370**, 571–575.
Teplyakov, A., Oliva, G. & Polikarpov, I. (1998). *Acta Cryst.* D**54**, 610–614.
Trapasso, F., Iuliano, R., Boccia, A., Stella, A., Visconti, R., Bruni, P., Baldassarre, G., Santoro, M., Viglietto, G. & Fusco, A. (2000) *Mol. Cell. Biol.* **20**, 9236–9246.
Vagin, A. & Teplyakov, A. (1997). *J. Appl. Cryst.* **30**, 1022–1025.
Zhang, L., Martelli, M. L., Battaglia, C., Trapasso, F., Cerutti, J., Tramontano, D., Viglietto, G., Porcellini, A., Santoro, M. & Fusco, A. (1997). *Exp. Cell Res.* **235**, 62–70.