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

The rat protein tyrosine phosphatase η (rPTP η) is a cysteine-dependent phosphatase which hydrolyzes phosphoester bonds in proteins and other molecules. rPTP η 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 PTP η , inhibition of the tumorigenic process occurs after its exogenous reconstitution, suggesting that PTP η might be important for gene therapy of cancers. In this study, the catalytic domain of rPTP η 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)HCXAGXXR(S/T)G], a single transmembrane domain and a highly variable external segment. These cysteine-dependent phosphatases utilize the conserved C(X)₅R 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.

rPTP η 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 , Ptp η (Zhang *et al.*, 1997; Honda *et al.*, 1994; Ostman *et al.*, 1994; Ruivenkamp *et al.*, 2002). The protein structure of PTP η 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, rPTP η 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 rPTP η 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



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malignant phenotype can be reverted when *PTP η* 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 PLC γ 1, a substrate of DEP-1/HPTP η (Trapasso *et al.*, 2000). Recently, it has been shown that the PTP η 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 rPTP η (Ardini *et al.*, 2000). Additionally, studies have also implicated the mouse homologue of rPTP η , *Ptp η* , in susceptibility to mouse colon cancer, reinforcing the idea that restoration of PTP η 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 rPTP η , we have expressed the catalytic domain (CD) of rPTP η in *Escherichia coli*, purified it to homogeneity and crystallized it. Here, we describe the crystallization and preliminary X-ray crystallographic analysis of rPTP η CD.

2. Materials and methods

2.1. Expression and purification of recombinant rPTP η phosphatase domain

BL21 (DE3) cells harbouring the plasmid containing the rPTP η intracellular domain insert were grown at 303 K in 2 \times YT media plus kanamycin with shaking until the absorbance at 600 nm reached 0.6–0.8. At this point, 0.5 mM isopropyl β -D-thiogalactopyranoside (IPTG) was added to induce rPTP η expression and cells were incubated for 4 h. The induced bacteria were harvested by centrifugation at 6000g 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 mM sodium phosphate buffer pH 7.8, 100 mM NaCl, 10% glycerol, 10 mM imidazole, 2 mM β -mercaptoethanol) containing 1 mM 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 000g 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 mM sodium phosphate buffer pH 7.8, 300 mM NaCl, 10% glycerol, 10 mM imidazole, 2 mM β -mercapto-

ethanol) 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 mM sodium phosphate buffer pH 7.8, 50 mM NaCl, 10% glycerol, 300 mM imidazole, 2 mM 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 mM HEPES pH 7.8, 200 mM NaCl, 5% glycerol, 1 mM DTT) as eluent. All purification procedures were carried out at 277 K. The purified protein fractions were visualized on 15% SDS-PAGE. Soluble His₆-rPTP η (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 rPTP η was then frozen in liquid nitrogen and stored at 193 K (Santos *et al.*, 2005).

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

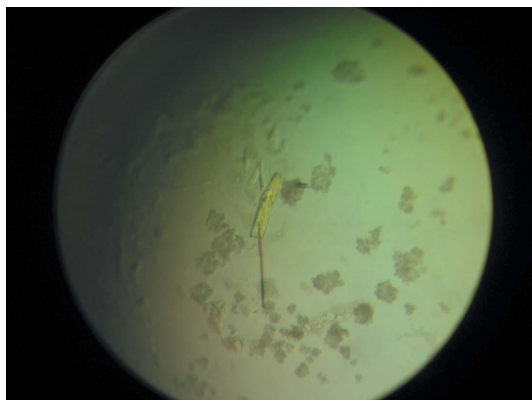


Figure 1
Crystal of rPTP η CD. Typical dimensions are approximately 0.2 \times 0.4 \times 0.2 mm.

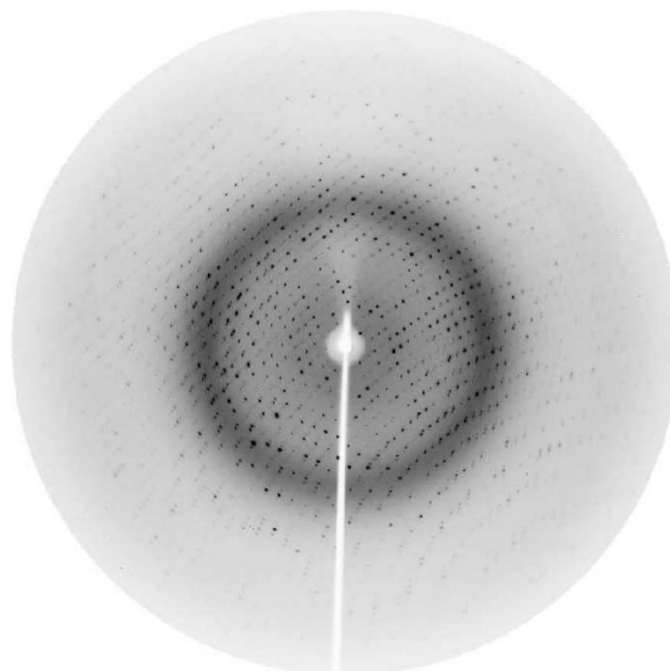


Figure 2
Diffraction pattern of the rPTP η CD crystal collected on the MX-1 beamline at LNLS. The maximum resolution at the edge of the image is 1.87 Å.

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_{merge}^\dagger (%)	6.0 (19.3)
Average $I/\sigma(I)$	7.5 (3.7)
Total reflections	242862
Unique reflections	27816

$^\dagger R_{\text{merge}} = \sum |I_i - \langle I \rangle| / \sum I_i$, where I_i is the intensity of the i th 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 $R_{\text{free}} = 0.396$. Structural refinement is in progress.

SCALA and *MOLREP* are programs from the *CCP4* suite (Collaborative Computational Project, Number 4, 1994).

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