

Crystallisation of a low molecular weight phosphotyrosine protein phosphatase from bovine liver

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Abstract

Single crystals of a low molecular weight phosphotyrosine protein phosphatase from bovine liver have been grown. The crystals belong to space group $P2_12_12_1$, have cell dimensions $a = 46.3 \text{ \AA}$, $b = 62.2 \text{ \AA}$, $c = 62.7 \text{ \AA}$ and diffract to better than 2.0 \AA resolution. The crystals are well suited for high resolution X-ray studies.

Key words: Phosphotyrosine protein phosphatase; Protein crystallisation; X-ray diffraction; Structure determination

1. Introduction

Specific phosphorylation of tyrosine side chains is an universal mechanism for the control of cell proliferation. Some products of oncogenes are characterised by unregulated protein tyrosine kinase activity that leads to uncontrolled cell growth. Phosphotyrosine protein phosphatases (PTPases) have been demonstrated to be important in counterbalancing the tyrosine kinase activity and thereby in growth control [1]. PTPases can be classified in three families: one receptor-like family the members of which contain an extra cellular domain, a transmembrane spanning region and, typically, two repeated phosphatase domains; a second non-receptor-like family the members of which feature a single catalytic phosphatase domain; a low molecular weight PTPases (low- M_r PTPases) family the members of which are characterised by a single peptide chain. The catalytic domains of the first two families display significant sequence homology to each other. The low- M_r PTPases, however, do not have extensive sequence homology to the other two classes of proteins but do contain a CXXXXXR motif [2] which is the active site signature of the other two families of PTPases. The arginine and cysteine residues have been shown to be essential for catalytic activity in the low- M_r PTPase [2], as well as in the other PTPases

[3], and most likely all PTPases share a common catalytic mechanism. This mechanism accounts for a covalent PTPase(cysteinyI)-phosphate intermediate which has been experimentally confirmed [2,4]. The catalytic domain of the three families of PTPases has possibly evolved from the same origin and could therefore be expected to have related three-dimensional structures.

A crystallographic study of a low- M_r PTPase from bovine liver [5] could then, in addition to revealing information on the structural basis for substrate recognition and the catalytic mechanism, also serve as a model for the other two classes of PTPases.

2. Materials and methods

Bovine liver low- M_r PTPase was purified according to the method previously described [6] with minor modifications. All the chemicals used for the crystallisation experiments were purchased from Merck and were of highest purity available. Crystallisation conditions for the protein were initially searched by a random screening protocol [7], using hanging drop vapour diffusion set-ups in cell culture plates. Promising conditions were subsequently reproduced and optimised by a finer grid search on Petri dishes. All experiments were carried out at 20°C .

3. Results and discussion

Crystals of the low- M_r PTPase form at different ammonium sulphate and protein concentration over a broad pH range. The best crystals were grown in 0.1 M sodium acetate buffer, pH 5.3–5.9, in the presence of 50% saturated ammonium sulphate using a protein con-

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Abbreviations: PTPase, phosphotyrosine protein phosphatase.

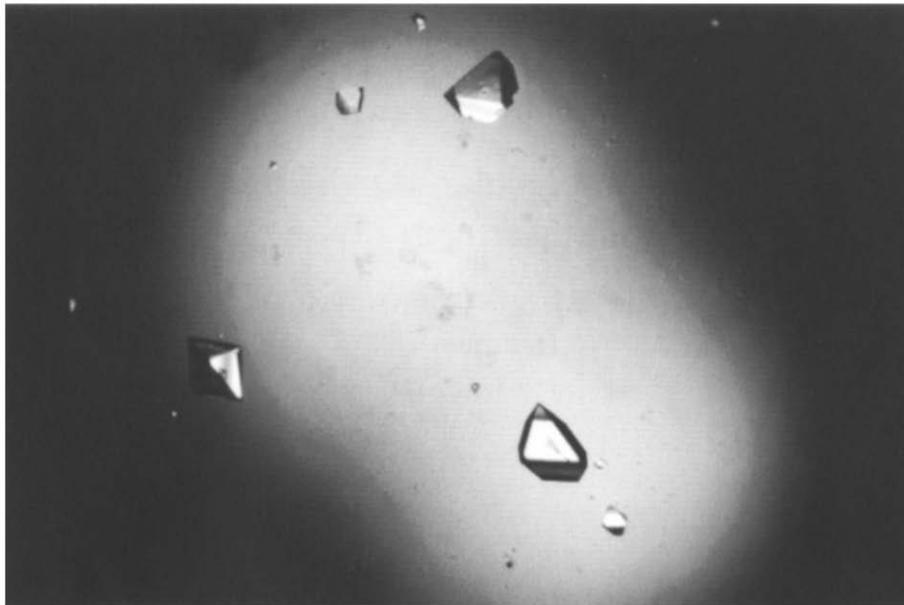


Fig. 1. Crystals of the low- M_r PTPases in a hanging drop.

centration of 3–6 mg/ml. This condition yields regular cottage shaped crystals, approximately 0.1 mm in all dimensions, within one week. The crystal size can be increased if the crystals are moved to pre-equilibrated hanging drops containing fresh protein, the same buffer as in the original experiment in the presence of 40% saturated ammonium sulphate. This macro seeding procedure yields crystals of approximately $0.2 \times 0.3 \times 0.5$ mm (Fig. 1). Similar crystals were grown in the presence of 0.1 M phosphate buffer at pH 5.6–6.5. The crystal parameters are described in Table 1. The volume of the unit cell agrees with the assumption that there is one PTPase molecule per asymmetric unit. The V_m value is $2.5 \text{ \AA}^3/\text{Da}$ which is in the normal range for protein crystals. The crystals diffract to better than 2.0 \AA when using an in-house rotating anode X-ray source and a graphite monochromator. A native PTPase data set, extending to 2.5 \AA resolution, has been collected using a Siemens Area detector system mounted on a Rigaku rotating anode. The data collected from one crystal was processed with the program XDS [8], giving an R -merge of 6% and a completeness of 90%.

Table 1
Main parameters for PTPase crystals

Space group	Cell dimensions (\AA)	V_m ($\text{\AA}^3/\text{Da}$)	Diffraction limit (\AA)
$P2_12_12_1$	46.3, 62.2, 62.7	2.5	< 2.0

Two unit cell-dimensions of the crystals are of similar magnitude which could in some cases introduce twinning problems. We have calculated Patterson functions as well as self rotation functions, using the native data set, to look for suspicious peaks. No such peaks were seen in these maps indicating that there is no high order twinning in the crystal lattice.

We conclude that the crystals of the low- M_r PTPase, presented in this paper, are well suited for a high-resolution crystallographic study.

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