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Crystallization and preliminary X-ray diffraction data for a purple acid phosphatase from sweet potato

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Purple acid phosphatase from sweet potato is a homodimer of 110 kDa. Two forms of the enzyme have been characterized. One contains an Fe–Zn centre similar to that previously reported for red kidney bean purple acid phosphatase. Another isoform, the subject of this work, is the first confirmed example of an Fe–Mn-containing enzyme. Crystals of this protein have been grown from PEG 6000. They have unit-cell parameters $a = b = 118.4$, $c = 287.4$ Å and have the symmetry of space group $P6_522$, with one dimer per asymmetric unit. Diffraction data collected using a conventional X-ray source from a cryocooled crystal extend to 2.90 Å resolution. The three-dimensional structure of the enzyme will provide insight into the coordination of this novel binuclear metal centre.

1. Introduction

Purple acid phosphatases contain binuclear metal centres and utilize a broad range of phosphomonoesters as substrates (for a review, see Klabunde & Krebs, 1997). The characteristic purple colour of the enzyme arises from a charge-transfer transition between a conserved tyrosine residue and an Fe^{III} ion located in the active site. Purple acid phosphatases have been studied from a variety of animals and plants. In mammals (pig, human, cow and rat), purple acid phosphatase is a ~35 kDa monomer and the second metal ion is a redox-active iron (Fe^{III}/Fe^{II}). Metal-substitution studies on the pig enzyme have shown that this iron can be replaced by zinc without loss of activity (Beck *et al.*, 1984). Plant purple acid phosphatases differ from mammalian enzymes in both size and sequence (less than 20% identity; Klabunde *et al.*, 1995). The best characterized plant enzyme, from red kidney bean, is a homodimer of ~110 kDa with one binuclear metal centre per subunit. In this enzyme, the second metal ion is Zn^{II} (Beck *et al.*, 1986). Replacement of Zn^{II} by Fe^{II} yields an enzyme with full activity, whereas substitution with Mn^{II} yields an enzyme with only 20% activity (Beck *et al.*, 1988).

The three-dimensional structure of red kidney bean purple acid phosphatase has been determined to 2.65 Å resolution (Klabunde *et al.*, 1996) and recently the crystal structure of the pig enzyme has been determined to 1.55 Å resolution (Guddat *et al.*, 1999). Each subunit of the red kidney-bean purple acid phosphatase contains two domains: an N-terminal domain (residues 1–120) of unknown function and a C-terminal domain (residues 121–432) which contains the catalytic centre. This domain resembles the core structure of the pig enzyme (Guddat *et al.*, 1999). The seven amino acids providing ligands to the metal ions are also identical in the two enzymes. Magnetic and spectroscopic studies indicate that in both cases the metal ions in the binuclear centre are linked *via* a μ-hydroxo bridge (Day *et al.*, 1988; Gehring *et al.*, 1996).

Two isoforms of sweet potato purple acid phosphatase (both are homodimers of ~110 kDa) have been purified and partially characterized (Schenk, Ge *et al.*, 1999; Durmus *et al.*, 1999). One isoform, the subject of this study, has an antiferromagnetically coupled Fe–Mn centre (Schenk, Carrington *et al.*, 1999). EPR studies and magnetic susceptibility measurements indicate that the metals are probably in the Fe^{III} and Mn^{II} oxidation states. The strong exchange coupling is consistent with the presence of a μ-oxo bridge between the metal ions (Schenk, Carrington *et al.*, 1999). This is the first confirmed example of such a centre in a protein. Structural analysis of the Fe–Mn-containing sweet potato enzyme will thus provide insights into the structure of this novel metal centre.

2. Materials and methods

2.1. Purification and preparation

Purple acid phosphatase was extracted from sweet potato as described elsewhere (Schenk, Ge *et al.*, 1999). In brief, the enzyme was purified in several steps involving juice extraction, acetone and ammonium sulfate fractionations, DEAE-cellulose chromatography (pH 7.0) and gel filtration on a Sephadex G-150 Superfine column (pH 4.90). The visible absorption spectrum had a peak at

Table 1
Data-collection statistics for sweet-potato purple acid phosphatase crystals.

Values in parentheses are for the outer shell (3.0–2.9 Å).

Temperature (K)	100
Resolution (Å)	50.0–2.90
Space group	$P6_522$
Unit-cell parameters (Å)	$a = b = 118.4$, $c = 287.4$
Solvent (%)	54
Number of dimers in asymmetric unit	1
Crystal dimensions (mm)	0.6 × 0.3 × 0.2
Data collection	
Number of observations [$I > 0\sigma(I)$]	96869 (3939)
Unique reflections [$I > 0\sigma(I)$]	22172 (1948)
R_{sym}†	0.132 (0.318)
Completeness (%)	89.8 (73.6)
$I/\sigma(I)$	7.9 (2.2)

† $R_{sym} = \sum|I - \langle I\rangle|/\sum\langle I\rangle$.

λ_{max} = 560 nm with a corresponding ε_{max} = 3207 M^{-1} cm^{-1} (per Fe ion). Samples for this study had a purity of >95% as judged by SDS–PAGE. Metal analysis indicated the presence of 1.04 Fe, 0.58 Mn, 0.18 Zn and 0.11 Cu per subunit (Schenk, Ge *et al.*, 1999).

2.2. Crystallization

The purified enzyme in 0.1 *M* acetate buffer pH 4.90 was concentrated to 22 mg ml^{-1} using an Amicon Centricon YM-10 microconcentrator. Crystals were obtained by the hanging-drop vapour-diffusion method, incubating at 293 K. The well solution contained 0.1 *M* citric acid (pH 3.5–4.0) and 7.5–10% PEG 6000. The hanging drops contained equal volumes of well and protein solution. After 4–5 d, purple diamond-shaped crystals reached their maximum size (0.6 mm in the largest dimension; Fig. 1). After 10 d, the edges of the crystals were no longer sharp and after one month the purple colour faded before the crystals eventually dissolved. Presumably, this loss of colour was a consequence of the release of one or both metal ions from the active site. This release of metals may result in a conformational change which ultimately leads to the degradation of the crystals. For optimum X-ray diffraction, data had to be collected on crystals that were 3–5 d old.

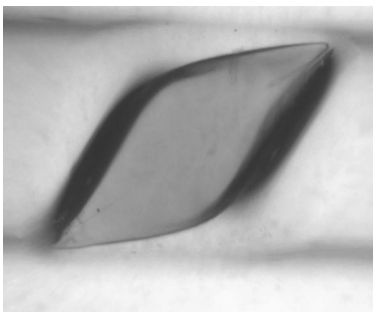

Figure 1
A crystal of sweet potato purple acid phosphatase (0.6 × 0.5 × 0.3 mm). The intense colour disappeared after several weeks indicating loss of metal from the protein.

2.3. Cryocooling and data collection

Data were initially collected at 288 K, but after several hours of exposure to X-rays the intensity of reflections had decreased by >50%. In order to collect a complete data set, a cryocooling strategy was used. Crystals were soaked for 15 s in a stepwise manner in crystallization buffer containing 10–35% glycerol (5% increments). The crystals were then placed directly into the nitrogen stream (Oxford Cryosystems Cryostream) for data collection at 100 K.

X-ray diffraction experiments were carried out using a Rigaku RU200 Cu $K\alpha$ rotating-anode generator (equipped with Yale focusing mirrors) operating at 46 kV and 60 mA. The X-ray diffraction data were recorded on an R-AXIS IIC imaging-plate area detector and were integrated and scaled with the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997).

3. Results and discussion

Initial crystallization conditions were based on a previously reported protocol for a manganese-containing purple acid phosphatase from sweet potato (0.01 *M* phosphate buffer pH 6.0 in the presence of 40% sucrose; Sugiura *et al.*, 1981). These conditions proved unsuccessful for the crystallization of the Fe–Mn isoform, which required a pH < 4.0 (see §2). It is most likely that the enzyme isolated by the Japanese group from Kintoki sweet-potato tubers represents a third isoform, since its amino-acid composition differs significantly from that of the other two isoforms. The 'Kintoki' enzyme also has a different metal composition (only manganese) and different spectral properties in the visible range (λ_{max} at 515 nm with a corresponding ε_{max} of 1230 M^{-1} cm^{-1}; Sugiura *et al.*, 1981; Schenk, Carrington *et al.*, 1999).

Based on the diffraction image, the unit-cell parameters for our crystals are $a = b = 118.4$, $c = 287.4$ Å and the space group is either $P6_122$ or its enantiomorph $P6_522$. Based on the assumption of one dimer per asymmetric unit, the solvent content of the crystals was calculated to be 54%, with a corresponding Matthews coefficient of $V_m = 2.64$ Å^3 Da^{-1}. Both values are within the usual range for protein crystals (Matthews, 1968). Table 1 summarizes the data-collection statistics. The resolution limit (2.9 Å) for these crystals is similar to that initially obtained for red kidney-bean purple acid phosphatase (Sträter *et al.*, 1995). Preliminary molecular-replacement studies using *X-PLOR* (Brünger, 1990) with the red kidney bean enzyme as a search model (sequence similarity ∼75%) yielded a solution with an R_{free} (Brünger, 1992) of 0.449 and an R factor of 0.448 for the space group $P6_122$ and an R_{free} of 0.379 and an R factor of 0.381 for the space group $P6_522$, thus confirming the space group to be $P6_522$. Inspection of the molecular-replacement solution using the graphics program *O* (Jones *et al.*, 1991) did not reveal any interpenetration of symmetry molecules. Refinement and model building of this structure are currently in progress.

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