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Crystallization and preliminary X-ray diffraction studies of NP24-I, an isoform of a thaumatin-like protein from ripe tomato fruits

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NP24 is a 24 kDa (207-amino-acid) antifungal thaumatin-like protein (TLP) found in tomato fruits. An isoform of the protein, NP24-I, is reported to play a possible role in ripening of the fruit in addition to its antifungal properties. The protein has been isolated and purified and crystallized by the hanging-drop vapour-diffusion method. The crystals belong to the tetragonal space group $P4_3$, with unit-cell parameters $a = b = 61.01$, $c = 62.90$ Å and one molecule per asymmetric unit. X-ray diffraction data were processed to a resolution of 2.45 Å and the structure was solved by molecular replacement.

1. Introduction

A number of stress-related proteins are produced by plants that play a part in their defence mechanism by resisting disease-causing microorganisms. These proteins are collectively referred to as pathogenesis-related (PR) proteins and have been classified into different distinct groups (Van Loon *et al.*, 1994). One of these groups, the PR-5 family, consists of the thaumatin-like proteins (the TLPs), thus named because of their similarity in amino-acid sequence (Cornelissen *et al.*, 1986) and structure (Batalia *et al.*, 1996; Koiwa *et al.*, 1999) to the sweet-tasting protein thaumatin from *Thaumatococcus daniellii* fruits.

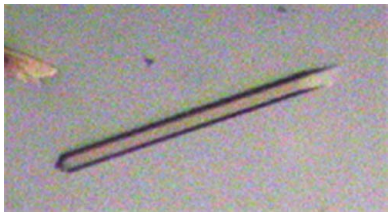
NP24, a basic protein, is found to increase in the roots of tomato plants in particular when they are stressed with NaCl (King *et al.*, 1986). This protein was first discovered in extracts of the ripe tomato fruit by Pressey (1997). Two isoforms, NP24-I and NP24-II, differing in two of the 21 N-terminal amino-acid residues, have been isolated.

Although the exact mechanism of action of the TLPs is as yet unknown, it is believed that at least some TLPs are involved in plant defence (Van Damme *et al.*, 2002). Both isoforms of NP24 were found to inhibit the growth of six different fungi. Some TLPs, including NP24, have been shown to possess glucanase activity (Grenier *et al.*, 1999), which may be correlated with their antifungal properties.

The present study involves the crystallographic characterization of NP24-I, which was isolated and purified from locally available ripe tomato fruits. The increase in NP24-I during ripening suggests a possible role of the protein in fruit development and ripening (Pressey, 1997). It would be interesting to understand on a structural basis the role in fruit ripening, the antifungal properties and the glucanase activity of this salt-induced protein. Furthermore, although the crystal structures of three TLPs are currently known, that of one more TLP might shed some light on the as yet unresolved question of why the thaumatin-like proteins lack the sweet taste of thaumatin.

2. Purification

NP24-I was purified from ripe tomatoes following a procedure essentially derived from that reported by Pressey (1997). In brief, 1 kg of outer pericarp tissue from ripe tomatoes was homogenized with cold autoclaved water. The homogenate was adjusted to pH 3 by adding 1 *M* HCl and solid NaCl was then added to yield a concentration of 0.15 *M*. The pH was further lowered to 1.6 and this crude extract was centrifuged at 8 000*g* for 20 min. A suspension of 40 ml S-Sepharose, previously equilibrated to pH 1.6, was mixed with the supernatant. The slurry was stirred for 30 min, allowed to settle and



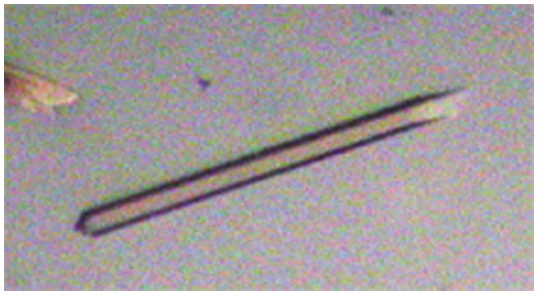


Figure 1
A representative crystal of NP24-I.

loaded onto a 1.50 × 20 cm glass column after discarding the supernatant solution. The column was first washed with 100 ml water and then with 200 ml 0.02 *M* MES pH 6.0. Subsequent elution using a 200 ml linear gradient of 0–0.5 *M* NaCl in 0.02 *M* MES pH 6.0 separated the two isoforms of NP24. Fractions were collected and analyzed by SDS–PAGE and those containing NP24-I were pooled, dialysed against 0.02 *M* MES pH 6.0 and concentrated by ultrafiltration using an Amicon YM-10 (Millipore) membrane. The protein was further purified on a Sephadex G-75 column using the same buffer and concentrated prior to crystallization. However, crystals of NP24-I were not obtained during purification as reported by Pressey (1997).

3. Crystallization

Crystals were obtained by the hanging-drop vapour-diffusion method in 7 d from drops containing 4 µl protein solution (15 mg ml^{-1} in 0.02 *M* MES pH 6.0) and 2 µl well solution suspended over 500 µl well solution at room temperature. Initial crystals were obtained under condition No. 4 of the PEG/Ion Screen (Hampton Research Inc.). This condition was optimized and crystals suitable for X-ray diffraction studies were grown from 0.1 *M* $LiCl_2$ and 20%(*w*/*v*) PEG 3350 pH 6.7 (Fig. 1).

4. Data collection and processing

X-ray diffraction data were collected in-house at room temperature with a MAR345 imaging-plate system using Cu $K\alpha$ radiation generated by a Bruker–Nonius FR591 rotating-anode generator running at 50 kV and 80 mA. Data were collected from a crystal of dimensions 0.92 × 0.08 × 0.08 mm mounted in a glass capillary tube over 100 frames with a crystal-to-detector distance of 150 mm, an oscillation range of 1° and an exposure time of 6 min per image. Diffraction data were collected to a resolution of 2.45 Å and were processed and scaled using the *AUTOMAR* program suite (http://www.marresearch.com/automar/automar/run.htm). The NP24-I crystals belong to the tetragonal space group $P4_3$, with unit-cell parameters $a = b = 61.01$, $c = 62.90$ Å, and had a mosaicity of 0.19°. Assuming the presence of one molecule of NP24-I in the asymmetric unit, the Matthews coefficient (Matthews, 1968) is 2.4 Å^3 Da^{-1} and the solvent content is 48.4%. Crystal data and data-collection statistics are given in Table 1.

Table 1
Crystallographic data for NP24-I.

Values in parentheses are for the highest resolution shell.

Space group	$P4_3$
Unit-cell parameters (Å)	$a = b = 61.01$, $c = 62.90$
Resolution range (Å)	100–2.45 (2.51–2.45)
Rotation per exposure (°)	1
Time per image (s)	360
Observed reflections	35092
Unique reflections	8574
Mosaicity (°)	0.19
Completeness (%)	99.9 (99.8)
Multiplicity	4.06 (4.04)
Mean $I/\sigma(I)$	3.9 (1.5)
R_{merge}† (%)	9.10 (33.69)

† $R_{merge} = [\sum(I - \langle I\rangle)^2/\sum(I^2)]^{1/2}$.

5. Structure analysis

Molecular-replacement calculations were performed using the program *AMoRe* implemented in the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994). Crystal structures of thaumatin (PDB code 1ly0) and three other TLPs, tobacco PR5d (1aun), maize zeamatin (1du5) and osmotin (1pcv), were used as templates. The best results were obtained with osmotin as the model using data from 10 to 4 Å. The *R* factor and correlation factors were 32.6 and 71.1%, respectively. Rigid-body refinement with this solution using *CNS* (Brünger *et al.*, 1998) gave $R = 30.8\%$ and $R_{free} = 29.6\%$ with 5% data in the test set. Refinement is in progress.

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