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Crystallization of *Pleurotus ostreatus* (oyster mushroom) lectin

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Crystals of *Pleurotus ostreatus* (oyster mushroom) lectin have been grown by the hanging-drop technique using ammonium sulfate as the precipitant at 293 K. Over a period of between two and three weeks, crystals of hexagonal bipyramidal morphology grew to maximum dimensions of $0.2 \times 0.2 \times 0.5$ mm. The crystals belong to space group $P6_122$ or $P6_522$, with unit-cell parameters $a = b = 155.9$, $c = 149.8$ Å, $V = 3\,153\,078$ Å^3, $Z = 12$ (assuming 50% solvent), and diffract to 4.1 Å at 293 K.

1. Introduction

Lectins are proteins which are able to cause agglutination in erythrocytes (and other cells). They are found in the seeds and vegetative parts of a wide variety of plants, and also in tissues of vertebrates, invertebrates and in bacteria as haemagglutinins. They are proteins of non-immune origin which reversibly bind oligosaccharides of glycoconjugates (glycoproteins or glycolipids) or polysaccharides without modifying their covalent structure (Kocourek & Horejsi, 1981; Barondes, 1988). Lectins can often be inhibited by simple sugars and consequently are often classified on the basis of their sugar-binding properties.

Lectins are employed in a number of important applications over a wide variety of immunological and biochemical fields. Moreover, they can be regarded as model systems for studying the molecular basis of protein–carbohydrate interactions as they occur in cell–cell recognition events (for reviews, see Lis & Sharon, 1986, 1998). The structures of several plant lectins, including some lectin–carbohydrate complexes, are known at high resolution (see Loris *et al.*, 1998, for a review). The X-ray structures of a number of animal lectins have also recently been reported (see Rini, 1995; Gabius, 1997 for reviews).

However, the specific mechanisms by which lectins can discriminate between different but closely related oligosaccharides are not well understood. In order to gain a detailed understanding of the mechanisms which govern their exquisite carbohydrate-binding specificity, further knowledge of their high-resolution three-dimensional structures and conformations is required.

The fruiting bodies of many mushrooms contain lectins, some of which have been isolated and characterized (Giollant *et al.*, 1993; Guillot *et al.*, 1991; Kawagishi & Mizuno, 1988; Kawagishi *et al.*, 1988; Kochibe & Matta, 1989; Rüdiger, 1988; Ticha *et al.*, 1988; Yagi & Tadera, 1988; Yatohgo *et al.*, 1988). The lectin from the edible oyster mushroom *Pleurotus ostreatus* was recently isolated and purified (Conrad & Rüdiger, 1994). It is a dimer of molecular mass 87 kDa. The *P. ostreatus* lectin is exceptional in its ability to enhance the activity of the endogenous enzyme phosphatase. The enzyme is significantly more active in the presence of the lectin – by up to 34% at high lectin concentration (Conrad & Rüdiger, 1994). The underlying mechanism of this property is not understood. It is also unusual in its *inability* to differentiate between α- and β-galactosides. This is in contrast to the lectins from the fungi *Ischnoderma resinosus* (Kawagishi & Mizuno, 1988) and *Auricularia polytricha* (Yagi & Tadera, 1988), which prefer β-galactosides (Kochibe & Matta, 1989), but is in accord with the blood-group unspecific lectin from *Agrocybe aegerita* (Lin & Chou, 1984), which binds galactose better than GalNAc and lactose better than galactose. *P. ostreatus* lectin, on the other hand, binds GalNAc better than galactose and galactose better than lactose. The amino-acid composition indicates a high content of acidic amino-acid residues or their amides and a particularly high arginine content compared with other *Polyporcaceae* lectins. There appear to be only two cysteine residues per molecule.

2. Methods and results

P. ostreatus lectin was purified from *P. ostreatus* fruiting bodies as described in detail elsewhere (Conrad & Rüdiger, 1994). Briefly, the fruiting bodies were sliced and homogenized with a threefold amount of 0.05 *M* Tris–HCl buffer pH 8 containing 1 m*M* $CaCl_2$, 1 m*M* $MgCl_2$ and 0.02%(*w*/*w*) NaN_3. Insoluble material was removed by centrifugation (14000*g*, 25 min). The lectin was isolated from the supernatant in a single step by adsorption to a column of desialylated hog gastric mucin

Table 1
Data-collection statistics.

Resolution range (Å)	R_{merge} (%)	$I/\sigma(I)$ (%)	Completeness	N_{hkl} (unique)	N_{hkl} (total)	M
20.0–6.56	3.8	22.6	78.0	1815	19602	10.8
6.56–5.23	8.6	10.1	91.6	1873	19292	10.3
5.23–4.48	7.2	9.4	92.8	2264	22414	9.9
4.48–4.09	9.8	8.3	88.1	1498	14081	9.4
4.09–3.74	19.2	4.5	86.3	2069	16966	8.2

immobilized to Sepharose 4B (Freier *et al.*, 1985) and desorption with lactose. The lectin fractions were collected and dialysed exhaustively against demineralized water. From 100 g of fruiting bodies, 20 mg of lectin was produced. In SDS–PAGE under denaturing conditions, the lectin splits into two bands of M_r = 44 and 41 kDa. On heating, only one band at $M_r \simeq 80$ kDa is present. In HPLC gel filtration of the native lectin, an M_r of 87 kDa is obtained. In isoelectric focusing, a broad band appears at about pH 9.5. It therefore seems reasonable to assume that the lectin is a pseudo-dimer composed of two structurally similar subunits with almost identical isoelectric points, which are held together by covalent forces.

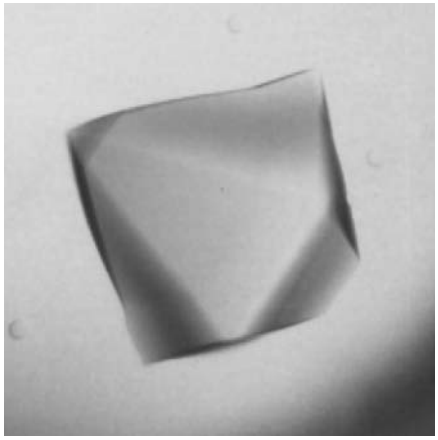

Figure 1
Crystals of the *P. ostreatus* lectin growing in sitting drops. The crystals are approximately 0.1–0.2 mm and are of hexagonal symmetry.

The lectin was crystallized in a series of trials using the Terasaki screening procedure (Freier *et al.*, 1985; Jancarik & Kim, 1991). A total of 98 different crystallization wells were set up in order to establish the optimum conditions for crystal growth. Crystals were obtained from a 5 µl hanging drop containing 10 mg ml^{-1} protein and a 500 µl well containing 2.0 *M* $(NH_4)_2SO_4$ precipitant, 0.1 *M* sodium citrate buffer pH 5.6 in the presence of 0.2 *M* potassium/sodium tartrate. The crystals appeared within two or three weeks and grew to dimensions of up to 0.5 mm, having a typical hexagonal development (Fig. 1) with hexagonal bipyramidal morphology and a strongly polar sixfold axial development typical of protein crystals. X-ray diffraction data of the crystals mounted in a quartz capillary were collected at 293 K using a rotating-anode source operating at 40 kV, 100 mA with Cu $K\alpha$ radiation and a MAR Research image-plate detector (Table 1). Data were collected to 4.0 Å resolution using the rotation method with 3° oscillations over a 90° range. Determination of unit-cell parameters and space group and integration of reflection intensities were performed using *DENZO* (Otwinowski, 1993) and the data were scaled with *SCALEPACK* (Otwinowski, 1993). The autoindexing procedure of *DENZO* (Otwinowski, 1993) indicated that the crystals belong to the hexagonal system with unit-cell parameters $a = b = 155.9$, $c = 149.8$ Å, $V = 3153078$ Å^3, $Z = 12$ (assuming 50% solvent content). The presence of 00l reflections for $l = 6n$ only establishes the space group of the crystals to be $P6_122$ or $P6_522$. An 88% complete data set to 4.1 Å was collected with an R_{merge} of 7.4%. A search for suitable heavy-atom derivative crystals is in progress.

References

Barondes, S. H. (1988). *Trends Biol. Sci.* **13**, 480–482.
Conrad, F. & Rüdiger, H. (1994). *Phytochemistry*, **36**, 277–283.
Freier, T., Fleischmann, G. & Rüdiger, H. (1985). *Biol. Chem. Hoppe-Seyler*, **336**, 1023–1028.
Gabius, H. J. (1997). *Eur. J. Biochem.* **243**, 543–576.
Giollant, M., Guillot, J., Damez, M., Dusser, M., Didier, P. & Didier, E. (1993). *Plant Physiol.* **101**, 513–517.
Guillot, J., Giollant, M., Damez, M. & Dusser, M. (1991). *J. Biochem.* **109**, 840–845.
Jancarik, J. & Kim, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.
Kawagishi, H. & Mizuno, T. (1988). *FEBS Lett.* **227**, 99–101.
Kawagishi, H., Nomura, A., Yumen, T., Mizuno, T., Hagiwara, T. & Nakamura, T. (1988). *Carbohydr. Res.* **183**, 150–158.
Kochibe, N. & Matta, K. L. (1989). *J. Biol. Chem.* **264**, 173–175.
Kocourek, J. & Horejsi, V. (1981). *Nature (London)*, **290**, 188.
Lin, J. Y. & Chou, T. B. (1984). *J. Biochem.* **96**, 35–38.
Lis, H. & Sharon, N. (1986). *Annu. Rev. Biochem.* **53**, 35–67.
Lis, H. & Sharon, N. (1998). *Chem. Rev.* **98**, 637–674.
Loris, R., Hamelryck, T., Bouckaert, J. & Wyns, L. (1998). *Biochem. Biophys. Acta*, **1383**, 9–36.
Otwinowski, Z. (1993). *Proceedings of the CCP4 Study Weekend. Data Collection and Processing*, edited by L. Sawyer, N. Isaacs & S. Bailey, pp. 56–62. Warrington: Daresbury Laboratory.
Rini, J. M. (1995). *Annu. Rev. Biophys. Biomol. Struct.* **24**, 551–577.
Rüdiger, H. (1988). *Advances in Lectin Research*, edited by H. Franz, Vol. 1, pp. 26–31. Hellerup, Denmark: Textop.
Ticha, M., Sychrova, H. & Kocourek, J. (1988). *Lectins: Biology, Biochemistry, Clinical Biochemistry*, edited by T. C. Bøg-Hansen & D. L. J. Freed, Vol. 6, pp. 383–390. Hellerup, Denmark: Sigma Press.
Yagi, F. & Tadera, K. (1988). *Agric. Biol. Chem.* **52**, 2077–2081.
Yatohgo, T., Nakata, M., Tsumuraga, Y., Hasimoto, Y. & Yamamoto, S. (1988). *Agric. Biol. Chem.* **52**, 1485–1489.