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Crystallization and preliminary X-ray study of the common edible mushroom (*Agaricus bisporus*) lectin

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The lectin from the common edible mushroom *Agaricus bisporus* (ABL) belongs to the group of proteins that have the property of binding the Thomsen–Friedenreich antigen (T-antigen) selectively and with high affinity, but does not show any sequence similarity to the other proteins that share this property. The ABL sequence is instead similar to those of members of the saline-soluble fungal lectins, a protein family with pesticidal properties. The presence of different isoforms has been reported. It has been found that in order to be able to grow diffraction-quality crystals of the lectin, it is essential to separate the isoforms, which was performed by preparative isoelectric focusing. Using standard procedures, it was possible to crystallize the most basic of the forms by either vapour diffusion or equilibrium dialysis, but attempts to grow crystals of the other more acidic forms were unsuccessful. The ABL crystals belong to the orthorhombic space group $C222_1$, with unit-cell parameters $a = 93.06$, $b = 98.16$, $c = 76.38$ Å, and diffract to a resolution of 2.2 Å on a conventional source at room temperature. It is expected that the solution of this structure will yield further valuable information on the differences in the T-antigen-binding folds and will perhaps help to clarify the details of the ligand binding to the protein.

1. Introduction

The lectin from the common edible mushroom *Agaricus bisporus* (ABL) is a member of the remarkable group of proteins that share the property of binding the Thomsen–Friedenreich antigen, or T-antigen, selectively and with high affinity. The T-antigen is a disaccharide, Galβ1-3GalNAc, that is α-linked to either serines or threonines on cell-surface glycoproteins and is masked in healthy cells but overexpressed in a high percentage of human epithelial carcinomas (Springer, 1984, 1997). The proteins that bind this structure have received considerable attention because of their potential diagnostic and/or therapeutic value and several have been studied by X-ray diffraction analysis of crystals of the apoprotein and of complexes with the disaccharide. This group includes peanut agglutinin (*Arachis hypogaea* agglutinin; PNA; Adhikari *et al.*, 2001), amaranthin (*Amaranthus caudatus* agglutinin; Transue *et al.*, 1997), *Maclura pomifera* agglutinin (MPA; Lee *et al.*, 1998) and jacalin (*Artocarpus integrifolia* lectin; Jeyaprakash *et al.*, 2002) as well as a heat-labile *Escherichia coli* enterotoxin (LT-I; van den Akker *et al.*, 1996). A comparison of the amino-acid sequence of these proteins with the ABL sequence using the program *CLUSTALW* (Thompson *et al.*, 1994) revealed very low identity percentages: 18% for amaranthin and MPA, 14% for PNA, 13% for jacalin and 16% for LT-I. Similar results were obtained using the program *ALIGN* (Pearson *et al.*, 1997), but when the ABL sequence was compared with those of the saline-soluble fungal lectins, a protein family with pesticidal properties, significant levels of sequence similarity were observed (Trigueros *et al.*, 2003). The effect of the T-antigen-binding lectins on cell proliferation, while always being very pronounced, can be very different: PNA stimulates the proliferation of human intestinal epithelial cells (Ryder *et al.*, 1992), while jacalin has the opposite effect, *i.e.* strong inhibition of cell growth (Yu *et al.*, 2001). The common edible mushroom lectin, ABL, has been shown to reversibly inhibit the proliferation of epithelial cell lines without any apparent cytotoxicity (Yu *et al.*, 1993). This effect is believed to be the result of the activity of the lectin in blocking nuclear localization sequence (NLS) dependent protein import, which is essential for cell functioning (Yu *et al.*, 1999, 2000). More recently, it has been shown that the lectin binds in the cytoplasm to a truncated form of the oxygen-regulated protein 150 (Orp 150), which expresses the sialylated form of the ABL ligand sialyl-2,3-galactosyl-β1,3-*N*-acetyl-galactosamine-α and appears to be essential for NLS-dependent nuclear protein import (Yu *et al.*, 2002). ABL is a homotetramer of molecular weight 64 000 Da

and has a sequence that does not show any significant similarities to any of the other T-antigen-binding lectins that have been studied by X-ray diffraction (Crenshaw *et al.*, 1995). Four different isolectins have been isolated (Sueyoshi *et al.*, 1985) but the structural differences between them are unclear, although they can be attributed to differential glycosylation since the four isoforms share the same identical first 21 amino acids in the sequence (Crenshaw *et al.*, 1995). The structural requirements of the carbohydrates and glycotopes that bind to the lectin have also been studied in great detail (Irazoqui *et al.*, 1999; Wu *et al.*, 2003). Although the agglutinating activity of common mushroom extracts was first demonstrated in 1912 (Sage & Connett, 1969) and despite the fact that the lectin ABL has been characterized fairly well at the molecular level, no X-ray diffraction studies have been reported so far.

We report here the crystallization and preliminary X-ray results of the common edible mushroom *A. bisporus* lectin. In order to be able to grow crystals of this protein, we found that it was essential to separate its isoforms and we were only able to crystallize one of them. We do not anticipate that this structure will resemble those of the T-antigen-binding lectins for which three-dimensional structures are already known and we therefore believe that the establishment of the fold, a detailed study of the T-antigen-binding site and a comparison with the binding mode in the other lectins will be of particular value.

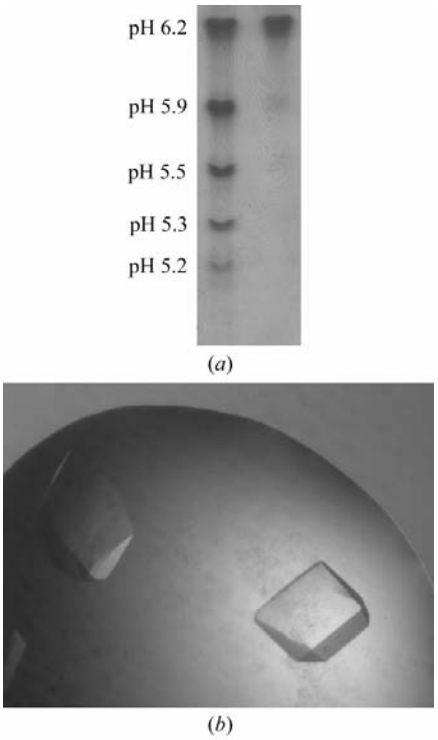

Figure 1
(*a*) Analytical isoelectric focusing showing ABL. Left lane, after the affinity chromatography step: the five isoforms are visible. Right lane, after the preparative isolectric focusing step: this separated the most basic form, which was crystallized. (*b*) Crystals of ABL grown by the hanging-drop method. The size of the largest crystal is approximately 0.3 × 0.3 × 0.1 mm.

2. Crystallization

ABL was purified from *A. bisporus* fruiting bodies by affinity chromatography in a column of human erythrocytic stroma incorporated into a polyacrylamide gel (Betail *et al.*, 1975) as described elsewhere (Irazoqui *et al.*, 1992). Polyacrylamide gel electrophoresis of the protein thus purified in the presence of sodium dodecyl sulfate showed a single band with an apparent molecular weight of 16 000 Da, but isoelectric focusing in the pH range 4.0–6.5 revealed, in our hands, the existence of (at least) five main different isoforms as shown in Fig. 1(*a*) (see also Sueyoshi *et al.*, 1985). Since this mixture could not be crystallized, the isoforms were separated by preparative isoelectric focusing. The three most abundant forms were used for initial screening of crystallization conditions with the hanging-drop method at 277 and 293 K using Hampton Research Screens, mixing 1 µl of the protein solution and the same volume of precipitating solution and equilibrating against a 0.5 ml reservoir. Diffraction-quality crystals could only be obtained with the most basic of the isoforms. The final optimized conditions for crystal growth were 4 *M* sodium formate, 0.02 *M* Tris–HCl pH 8.0 and a protein concentration of 30 mg ml^{-1}. The crystals can be grown by both the vapour-diffusion and microdialysis methods to about 0.5 mm in the largest dimension in about a week (see Fig. 1*b*). Other conditions were found that yielded smaller or more poorly diffracting crystals.

3. X-ray analysis

The data were collected at room temperature from crystals mounted in glass capillaries. The detector was a Rigaku R-AXIS II imaging plate mounted on a Rigaku RU-300 rotating-anode X-ray generator. The source was operated at 50 kV and 100 mA using a focal spot size of 0.3 × 3 mm. Monochromatic Cu $K\alpha$ radiation was obtained using Yale mirrors. The data were processed using the program *MOSFLM* (Leslie, 1990), initially in the space group *C*222 and, after a careful examination of the output thus produced, in the correct space group.

Table 1
X-ray data-collection statistics.

Values in parentheses refer to the highest resolution shell, 2.3–2.2 Å.

Space group	$C222_1$
Unit-cell parameters	
a (Å)	93.06
b (Å)	98.16
c (Å)	76.38
Observed reflections	95470
Independent reflections	18119
Resolution limit (Å)	30.0–2.2
$R_{\rm sym}$ (%)	5.0 (12.9)
$\langle I/\sigma(I)\rangle$	13.4 (5.4)
Completeness (%)	99.9 (100)

The space group of the ABL crystals is $C222_1$. Assumption of the presence of eight tetramers (MW = 64 000 Da) in the unit cell yields a Matthews coefficient $V_{\rm M}$ (Matthews, 1968) of 1.36 Å^3 Da^{-1}, whereas the assumption of eight dimers in the unit cell (MW = 32 000 Da) yields a more reasonable value of 2.73 Å^3 Da^{-1}. Therefore, the estimated solvent content for these crystals with one dimer in the asymmetric unit is 55%, which is within the range of values typically found for protein crystals. A consequence of the presence of four tetramers in the unit cell is that they must possess a crystallographic twofold axis of symmetry. The statistics of a complete data set collected to a resolution of 2.2 Å and processed with the program *MOSFLM* and the crystallographic suite *CCP*4 (Collaborative Computational Project, Number 4, 1994) are shown in Table 1.

We have also soaked one crystal in mother liquor containing a high concentration of the T-antigen (purchased from Calbiochem) and have found that the crystal remains intact, that it diffracts to approximately the same resolution as the native crystals and that it appears to be totally isomorphous. Since the lectin does not show any sequence homology to any protein of known three-dimensional structure, we have undertaken solution of the phase problem using the classical MIR method. Several isomorphous heavy-atom derivatives have been prepared and are in the process of being evaluated.

Determination of the three-dimensional structure of this remarkable protein will undoubtedly yield new information on the folds that can bind the T-antigen and further studies on co-crystals are likely to reveal details of the protein–ligand interactions.

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