

Crystallization and Preliminary Crystallographic Studies of an Antitumour Lectin from the Edible Mushroom *Agrocybe aegerita*

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Abstract: An antitumour lectin named AAL has been purified from the fruiting body of edible mushroom *Agrocybe aegerita*. In addition to having a distinct bioactivity, AAL shows strong inhibition effects on human and mouse tumour cells. It has been shown that AAL exerts its antitumour effects *via* apoptosis-induction. AAL and AAL-lactose complex have been crystallized and their diffraction data were collected with resolution of 2.6 Å and 3.0 Å, respectively. Both crystals belong to space group P 6122 with unit cell parameters $a = 123.98 \text{ \AA}$, $b = 123.98 \text{ \AA}$, $c = 56.86 \text{ \AA}$, $\alpha = 90^\circ$, $\beta = 120^\circ$ and $\gamma = 120^\circ$, respectively.

Keywords: lectin, mushroom, antitumour, apoptosis, DNase activity, crystallization.

1. INTRODUCTION

Lectins are a group of proteins that recognize the carbohydrate moieties of cell surface proteins and have diverse physiological functions [1, 2]. They are involved in growth regulation, cell adhesion, cell migration, cell apoptosis and immune responses.

Mushrooms are famed for their nutritional and medicine values. A variety of compounds with important pharmacological properties have been isolated from mushrooms [3-5]. Among them, the study of mushroom lectins with immunomodulatory, antiproliferative, antitumour/cytotoxic and hypotensive activities has attracted growing interest [6-8].

Recently, a novel antitumour lectin, named AAL, has been purified from the edible mushroom *Agrocybe aegerita* [9]. It consists of two identical subunits of 15.8 kDa and its pI is about 3.8 as determined by isoelectrofocusing electrophoresis. In the purified native AAL, there was no carbohydrate discerned. It has shown a series of distinct bioactivities, including agglutinating human and animal erythrocytes regardless of blood types or animal species, inhibition activities to tobacco mosaic virus on *Nicotiana glutinosa*, and promotion of the differentiation of fruit body primordial from the mycelia of *Agrocybe aegerita* and *Auricularia polytricha* [9]. Most recently it was identified that AAL could strongly inhibit the growth of several human tumour cell lines like HeLa, SW480, SGC-7901, mouse

sarcoma S-180, and also the viability of S-180 tumour cells antitumor effects *via* apoptosis-inducing activities [10].

Therefore, determination of the three-dimensional structure of AAL becomes of great interest since it will provide a sound basis for explaining the structural mechanism of the significant properties of this antitumour protein. It may thus further lead to the development of a novel antitumour drug through structure-based drug design. As a first step, we report here the crystallization and preliminary crystallographic studies of the wild AAL and the complex of AAL with its carbohydrate ligand, lactose.

2. MATERIALS AND METHODS

2.1. Purification and Characterization

Crude AAL was isolated from the dry fruiting bodies of *Agrocybe aegerita*. The crude proteins were further purified by ion exchange chromatography on DEAE-Sepharose Fast Flow column, gel filtration chromatography on Sephacryl S-200 HR column and GF-250 HPLC column, respectively. The details of the purification procedures have been described previously [9].

2.2. Crystallization

AAL was dissolved in pure water at a concentration of 10 mg/ml for crystallization. Initial screening was performed using a sparse matrix method [11] with Hampton Research Crystal Screen I and II at 293 K. The hang-drop vapour-diffusion method was used. Each drop contained equal amounts (1 μ l) of protein (10 mg/ml) and reservoir solution equilibrated against 500 μ l of reservoir solution in the well. In initial screening some crystallites and large twinned crystals were appeared from a few conditions. Through optimization, the diffraction-quality crystals were grown in the following conditions: drops formed by mixing equal volumes (2 μ l) of 10 mg/ml protein in water and 0.1 M Tris

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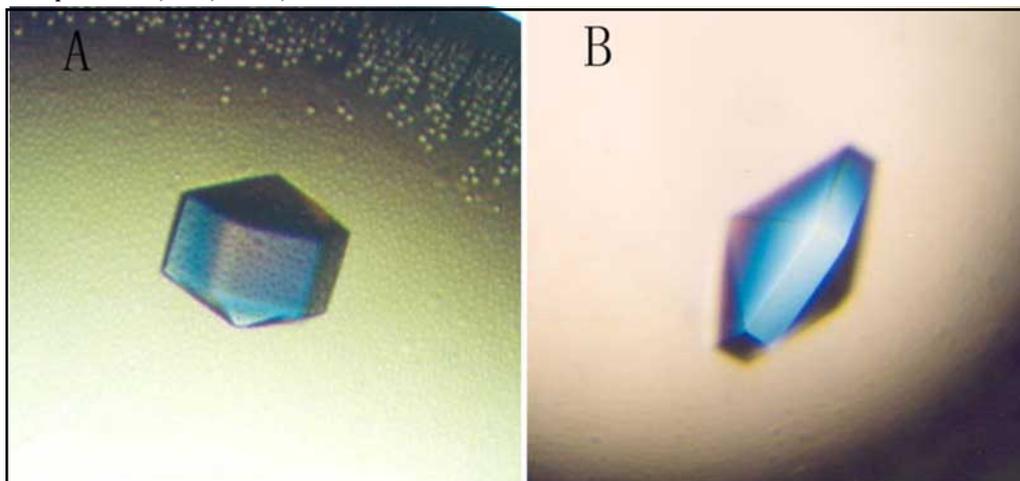


Figure 1. Crystals of AAL. (A) Hexahedron crystal of AAL ($0.3 \times 0.3 \times 0.15 \text{ mm}^3$). (B) Hexahedron crystal of AAL-lactose complex ($0.4 \times 0.4 \times 0.1 \text{ mm}^3$).

Table 1. X-ray data-collection statistics

Data set	AAL	AAL-Lac
X-ray source		Cu K
Wavelength	1.54	1.54
Temperature	85 K	85 K
Space group	P 6122	P 6122
Unit cell parameters	a = 123.98 Å	a = 123.69 Å
	b = 123.98 Å	b = 123.69 Å
	c = 56.86 Å	c = 56.64 Å
	$\alpha = 120^\circ$	$\alpha = 120^\circ$
Resolution range (Å)	50-2.6	50-3.0
	(2.69-2.60)	(3.11-3.00)
Observed reflections	60155	24943
Unique reflections	8227	5422
Completeness (%)	98.7 (93.2)	99.4 (100)
Mean I / σ (I)	15.6 (3.3)	14.3 (5.0)
R_{sym} (%) ^a	6.8 (32.6)	8.3 (32.4)

^a $R_{\text{sym}} = \frac{(|I| - \langle |I| \rangle)}{|I|}$.

^b Values in parentheses are for outer resolution shell.

hydrochloride buffer (pH 7.0) containing 6~8% (v/v) glycerol anhydrous and 1.6~1.7 M $(\text{NH}_4)_2\text{SO}_4$ were equilibrated with the same buffer solution. For the complex of AAL with lactose, crystals were obtained with the same conditions as described above by adding 10 mM lactose in the reservoir solution.

2.3. Data Collection and Crystallographic Analysis

X-ray diffraction data of the native AAL crystal were collected at the resolution 2.6 Å on a Rigaku R-Axis IV⁺⁺ image plate using Cu K radiation ($\lambda = 1.5418 \text{ Å}$) from a Rotating Anode operating at 40 kV and 20 mA with 0.1 mm cofocus incident beam diameter. Data were collected at 85K with a crystal-to-detector distance of 200 mm, $\omega = 1^\circ$ and 300 s exposure time. A total of 70 frames were collected. Paraffin oil was approved to be the best cryo-protectant after trying a lot of other general ones, like PEG, glycerol, sucrose

or so. The data of the AAL-lactose complex crystal were collected at a resolution 3.0 Å by the same condition and a total of 114 of frames were collected. All data sets were processed with the *mosflm6.2.3* program and scaled by CCP4 programs SCALA [12].

3. RESULTS AND DISCUSSION

Crystals of AAL appeared in one week and grew to their full size in three to four weeks. They are typical hexahedron with maximum dimensions of $0.3 \times 0.3 \times 0.15 \text{ mm}^3$ (Fig. 1A). In the optimization procedure we found that the growth of AAL crystals was very sensitive to buffers and pH. When changed to 0.1 M HEPES-Na (pH 7.0) or 0.1 M sodium cacodylate (pH 6.5), only very small crystals were obtained. When the pH of Tris hydrochloride was over 7.5, many small crystals with bad qualities appeared. Other essential factors were the concentration of $(\text{NH}_4)_2\text{SO}_4$ and the addition

of glycerol anhydrous. When the concentration of $(\text{NH}_4)_2\text{SO}_4$ was less than 1.5 M, no crystals could be obtained. Further, crystals became large badly twinned, losing their hexahedron shape when the concentration of glycerol was less than 3% (v/v). The addition of Mg^{2+} or Zn^{2+} and variation of the protein concentration seemed to have little effect on the crystal growth, as crystals could be obtained over a range of AAL concentrations from 5 mg/ml to 15 mg/ml. Crystals of AAL-lactose complex grew in a similar way to AAL and typical crystals suitable for X-ray diffraction experiments have maximum dimensions of $0.4 \times 0.4 \times 0.1 \text{ mm}^3$ (Fig. 1B).

The statistics of data collection and processing are shown in Table 1. Crystals of both AAL and AAL-lactose complex belong to space group P 6122. The unit cell parameters are $a = 123.98 \text{ \AA}$, $b = 123.98 \text{ \AA}$, $c = 56.86 \text{ \AA}$, $\alpha = 90^\circ$, $\beta = 120^\circ$ and $\gamma = 120^\circ$ for AAL and $a = 123.69 \text{ \AA}$, $b = 123.69 \text{ \AA}$, $c = 56.64 \text{ \AA}$, $\alpha = 90^\circ$, $\beta = 120^\circ$ for AAL and AAL-lactose complex crystals, respectively. Further analysis showed that lactose had definitely bound to AAL molecules. Therefore, the binding of the lactose has only a little effect on the crystal growth and the crystal packing of the molecules. Assuming that one molecule is present in the asymmetric unit, a value for the Matthews coefficient, V_m , [13] is estimated as $3.70 \text{ \AA}^3 \text{ Da}^{-1}$ with a corresponding solvent content of 66.8%, which is within the reasonable range found in protein crystals.

So far, no crystallographic or structural studies of apoptosis-inducing antitumour lectins from mushroom have been reported. Single isomorphous replacement with anomalous scattering (SIRAS) the structures methods has been successfully used in the determination of AAL and AAL-Lactose complex structures.

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