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RESEARCH ARTICLE

CRYSTALLIZATION AND PRELIMINARY X-RAY DIFFRACTION STUDIES OF ABELMOSCHUS ESCULENTUS LECTIN

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ABSTRACT

The *Abelmoschus esculentus* lectin has been reported with putative anti cancer activity. The lectin of approximately 16 kDa from the seeds has been purified. After ammonium sulfate fractionation and ion exchange chromatography, the lectin precipitated out as shower of needles. The precipitate which got dissolved with optimized condition was crystallized using 20% (w/v) PEG 1000, 0.2 M lithium sulfate, and 0.1 M phosphate/citrate by hanging drop vapour diffusion method. X-ray diffraction data up to 2.99 Å have been collected under cryo condition. The crystals belong to space group P1 with unit cell parameters a=50.47 Å, b=50.74 Å, c=74.02 Å, α=74.02º, β=84.66º and γ=78.43º. The protein is found to be active from pH 5 to 12.

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INTRODUCTION

Lectins are proteins or glycoproteins of non immunoglobulin nature exhibit specific recognition of carbohydrate moieties and thereby cell agglutination[1]as well. The reversible binding to complex glycoconjugates that too by not altering the covalent structure makes this class of proteins interesting[2].The purification and characterization of lectins are a very heterogeneous. Lectins were primarily classified on the sugar binding specificity. The mode of binding and thereby the functional role varies depends on source[3]. where a particular lectin exists. Mostly, plant lectins are directed against foreign glycans i.e to interact with another organism. Many lectins are responsible for disease resistance by preventing infection. Lectins also been characterized as a response to stress, particularly salt stress.

The ladyfinger seeds have been reported with many medicinal importance and chymotrypsin inhibitors from the seeds were well characterized[4]. The ladyfinger lectin belongs to the subgroup that shows binding with fructose, lactose and mannose[3]. It also been reported with putative anti cancer activities[5]. In this study, the lectin (~16 kDa) has been purified to homogeneity and crystallized. Preliminary X-ray diffraction suggests a dimeric association which may be relevance for its biological activities.

MATERIALS AND METHODS

Purification of Abelmoschus Esculentus Lectin

The mature and dry seeds were grounded into a fine powder using an electric mill coupled with a sieve. The flour was defatted with ice cold n-hexane twice and air dried at room temperature. The resultant powder was extracted at room temperature by continuous stirring for 6 hours with a solution of 1:8 (w/v) 20 mM Tris-HCl, pH 7.4, and 10 mM NaCl. After centrifugation at 9,500g for 60 min, the insoluble fraction was discarded, and the supernatant was used to check for the lectin activity. The total protein content was quantified by UV-Spectroscopy at 280 nm. The supernatant was fractionated and precipitated with various ammonium sulfate saturation levels (0 – 30%, 30-60%, 60 – 80 % and 80 – 100%) each with 9 hours mixing at 18 °C. Pellets were collected by centrifugation at

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9,500g for 60 min at 4 °C. The active fraction (30 – 60 %) with hemagglutination was dialyzed, centrifuged to remove debris and analyzed on SDS PAGE.

The supernatant was applied onto an anion-exchange DEAE column equilibrated with buffer-A (20 mM Tris-HCl pH 7.4, 10mM NaCl). After washing extensively with the equilibration buffer, bound proteins were elutedusing salt gradient with buffer-B (20 mM Tris-HCl pH 7.4 and 800 mM NaCl). The elution was monitored at 280 and 254 nm through inbuilt UV detector on FPLC.

Hemagglutination Activity

By following classical glass slide method using 10 micro liters of fresh blood from healthy volunteer, the hemaglutination activity was confirmed by microscopic inspection. The lectin activity was found with the flow through only. When the fraction (with two major bands on 12 % SDS-PAGE) was taken out of cold room after four days for further purification, a milky white (Fig.1) precipitate was noticed. The precipitate which could not be resolubilized by buffer-A was found to be needles of crystals under polarizer microscope. SDS-PAGE confirms the crystals as lectin (by molecular weight) and subsequent analysis proves hemagglutination activity.

Crystallization of Lectin

Needles were finally got dissolved in buffer-A by adding few micro liters of 1N NaOH. Small crystals grew after 10 days from trials using vapour diffusion method in a drop consisting of 0.2 M lithium sulfate, 0.1 M phosphate/citrate buffer (pH4.2 & 6.0) and 20% PEG 1000 (from molecular dynamics JCSG-1; condition No.6). To optimize crystal growth, the trials were repeated with different protein concentrations and different volumes of protein to reservoir ratios. Diffraction quality crystals were obtained in 2 to 5 days from a drop consisting of 18 mg/ml lectin with 2:1 ratio.

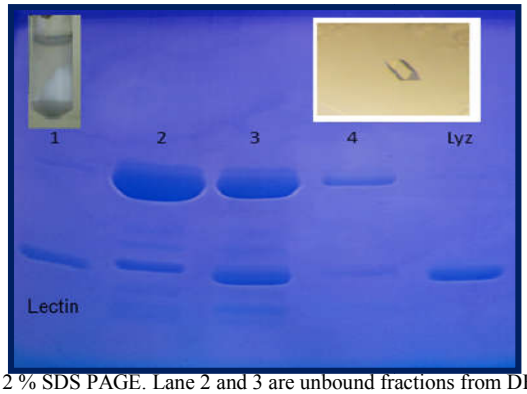

Fig. 112 % SDS PAGE. Lane 2 and 3 are unbound fractions from DEAE. Lane 1. The precipitate (shower of crystals) and Lane 4. Corresponding supernatant. Lyz-Lysozyme. (Inlets: The precipitate-needles and Diffraction quality crystal).

X-Ray Data Collection and Data Processing

The X-ray diffraction experiments were carried out on XRD1 macromolecular crystallography beamline at Elettra Synchrotron facility, Italy using a CCD detector. The crystal diffracted to a maximum resolution of 2.99 Å at 100 K (Fig. 2).

The diffraction data were collected up to 360 frames with a crystal-to-detector distance of 200 mm with 1° φ oscillation and 15 seconds exposure time. The collected data set was processed using the imosflm software suite[6] and scaled using SCALA[7] suite.

Table 1 Crystallization

Method	Vapour diffusion method
Plate type	24 well Limbro plate
Temperature (K)	293
Protein concentration	18 mg/ml
Buffer composition of protein solution	20 mM Tris. Hcl (7.4) and 10 mM NaCl
Composition of reservoir solution	0.2 M lithium sulfate, 0.1 M phosphate/citrate buffer (pH4.2 & 6.0) and 20% PEG 1000
Volume and ratio of drop	2:1
Volume of reservoir	200 µl

Table 2 Data collection and processing (Values for the outer shell are given in parentheses)

Diffraction source	XRD1 Beam line; Elettra Synchrotron
Wavelength (Å)	0.979
Temperature (K)	100
Detector	CCD
Crystal-detector distance (mm)	200
Rotation range per image (°)	1
Total rotation range (°)	360
Exposure time per image (s)	15
Space group	P1
a, *b*, *c* (Å)	a = 50.70, b = 50.66, c = 58.73
α, β, γ (°)	α = 74.02°, β = 84.66°, γ = 78.43‘
Mosaicity (°)	2.23
Resolution range (Å)	26.0 – 2.99
Total No. of reflections	23145 (3641)
No. of unique reflections	9086 (1435)
Completeness (%)	82.0 (79.9) *
Redundancy	2.5
$\langle I/\sigma(I)\rangle$	5.2 (2.2)
$R_{r.i.m.}$	12.5 (33.4)

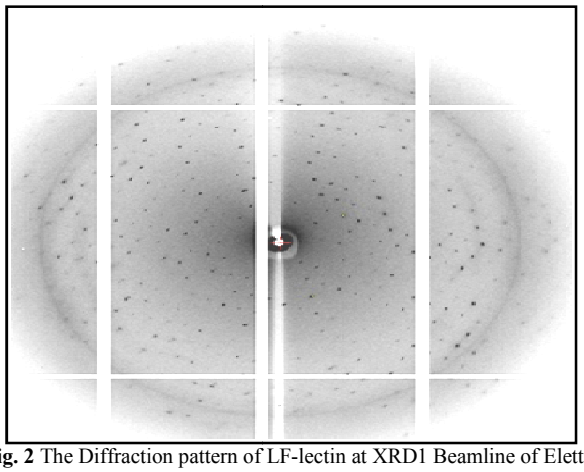

Fig. 2 The Diffraction pattern of LF-lectin at XRD1 Beamline of Elettra Synchrotron Facility

RESULTS AND DISCUSSION

A lectin of ~16 kDa has been purified, crystallized and diffraction data measurements have been carried out. The fraction of 30-60% ammonium sulphate cut exhibit lectin activity and didn’t bind with the anionic resin at pH 7.4. The flow through fraction containing two major bands on SDS PAGE retained the lectin activity and yielded precipitate when

kept at cold room for four to five days. The precipitate resists solubilization with water as well as buffers. Though unknowingly discarded twice, third time, we could find needles of crystals. The crystalline precipitate (shower of crystals) got dissolved in Tris buffer only in the presence of small aliquot of NaOH and showed the lectin activity. The crystals were washed thrice with buffer and directly dissolved in loading buffer and analyzed on 12 % SDS-PAGE which confirm ~16 kDa (Fig. 1).

The shower of needles was dissolved in buffer-A in presence of small aliquot of NaOH. Different shapes of crystals could be obtained. Ring crystals, small rectangular rods with shower of needlesat both ends are found common. Crystals both small and large sized were found to be merged and also decayed soon. Quite large crystals within a week were obtained during protein to reservoir ratio and reservoir volume optimizations. Diffracting quality crystals with rectangular prismatic shape (Fig. 1) were obtained with protein-reservoir as 2:1 and reservoir of 0.1 ml at 293K.

X-ray diffraction data (Fig. 2) were collected at Elettra synchrotron facility, Italy using a CCD detectorto a maximum resolution of 2.99 Å and processed using the 'imosflm' software suite[6]. The data statistics are given in Table 1. Analysis of the diffraction pattern indicated that this crystals exhibit triclinic space group P1. From the molecular weight (~16 kDa) and Matthews's coefficient (2.78 $Å^3Da^{-1}$), a dimer in an asymmetric unit can be assumed with a solvent content of 72 %. Lectin structure (PDB: 1OUW) from *Calystegiasepium*[8] was used as template for molecular replacement where we could get a convincing solution with a decent electron density map. Phase refinement and attempts to get high quality diffraction data are in progress. These results will be useful to correlate its structure, specificity and dimeric association with anti cancer properties.

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