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A tuber lectin from *Arisaema helleborifolium* Schott with anti-insect activity against melon fruit fly, *Bactrocera cucurbitae* (Coquillett) and anti-cancer effect on human cancer cell lines

Manpreet kaur [a], Kuljinder Singh [b], Pushpinder J. Rup [b], A.K. Saxena [c], Rizwan H. Khan [d], Mohd. Tashfeen Ashraf [d], Sukhdev Singh Kamboj [a], Jatinder Singh [a,*]

[a] *Department of Molecular Biology and Biochemistry, Guru Nanak Dev University, Amritsar 143 005, India*
[b] *Department of Zoology, Guru Nanak Dev University, Amritsar 143 005, India*
[c] *Department of Pharmacology, Regional Research Laboratory, Jammu 180 001, India*
[d] *Interdisciplinary Biotechnology Unit, Aligarh Muslim University, Aligarh 202 002, India*

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Abstract

An anti-insect and anti-cancer lectin has been isolated from *Arisaema helleborifolium* Schott by affinity chromatography using asialofetuin-linked amino activated silica beads. The bound *A. helleborifolium* lectin (AHL) was eluted with 100 mM glycine–HCl buffer, pH 2.5. It gave a single band on SDS–PAGE, pH 8.3, and PAGE, pH 4.5. However, multiple bands were obtained in PAGE at pH 8.3 and isoelectric focusing. The lectin was a homotetramer having subunit molecular mass 13.4 kDa while its native molecular mass was 52 kDa. It was a glycoprotein with 3.40% carbohydrate and was stable up to 60 °C for 30 min. It showed anti-insect activity towards second instar larvae of *Bactrocera cucurbitae* (Coquillett) with LC_{50} value of 16.4 μg/ml. Larvae fed on artificial diet containing sub-lethal dose of AHL showed a significant decrease in acid phosphatase and alkaline phosphatase activity while esterase activity markedly increased as compared to larvae fed on diet without lectin. AHL was also found to inhibit in vitro proliferation of some well established human cancer cell lines viz HOP-62 (95%), HCT-15 (92%), HEP-2 (66%), HT-29 (68%), PC-3 (39.4%), and A-549 (20.7%).

Keywords: *N*-Acetyl-D-lactosamine; Anti-insect; Araceae; *Arisaema*; *Bactrocera*; Cancer cell lines; Esterase; Lectin; Monocot; Phosphatase

Lectins, sugar-binding, and cell agglutinating proteins of non-immune origin, have elicited much attention in view of their unique ability to bind reversibly to specific carbohydrate ligands without any chemical alteration [1]. Owing to the fine specificity, lectins have been employed for various applications in biomedical sciences including cancer research. A variety of alternations in carbohydrate structure have been observed in cancer cells. These may involve increased sialylation, increased branching of complex carbohydrates, or occasionally emergence of some novel structures [2]. Lectins can serve as an excellent probe to study these altered glycosylation patterns. A few lectins including those from *Helix pomatia* [3] and *Agaricus bisporus* [4] are being investigated for their use in cancer research and therapy.

The specific carbohydrate-binding activity of lectins also plays an important role in plant interactions with other organisms [5]. There is increasing evidence that plant lectins can act as molecular defense against insects and other herbivores [6]. The anti-insect activity of many plant lectins has been documented in feeding assays in vitro and in studies with transgenic plants [6,7]. This can be economically of great potential in pest management because lectins being primary metabolic product, their genes are good candidates to confer insect resistance in transgenic crops. Therefore, the purification and characterization of lectins from new sources may reveal genes with the potential for genetic improvement of crops.

* Corresponding author. Fax: +91 183 2258819, 2258820.
E-mail address: jst2002@rediffmail.com (J. Singh).

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During the last decade, lectins with interesting properties have been isolated and characterized from various monocot families [8–10]. Interestingly, most of the lectins from these families belong to a single monocot mannose-binding lectin superfamily as revealed by their molecular structure, sequence homologies, and exclusive specificity for mannose. A few studies have, however, revealed the occurrence of some monocot lectins having specificity for complex glycoproteins and not for mannose [8–10]. The present study also reports the isolation and characterization with respect to various biochemical, biophysical, and biological properties, of a non-mannose binding monocot araceous lectin from wild Himalayan cobra lily, *A. helleborifolium* Schott. The anti-insect activity of AHL was assayed by taking melon fruit fly, *Bactrocera cucurbitae* (Coquillett) as a model insect. This fly is an important pest of cucurbits in tropical countries, besides affecting >80 host plants belonging to various families [11]. In this endeavor, the effect of lectin on its biology (i.e., developmental period, percentage pupation, and percentage emergence) were investigated. In addition, the effect of this lectin was also assessed on the activity of hydrolytic enzymes involved in digestion, growth and development. The in vitro anti-tumor potential of AHL was also investigated on some human cancer cell lines.

Materials and methods

Chemicals and reagents

Fetal calf serum from Sera Lab (GB) and RPMI 1640 from Gibco-BRL, New York, USA, were procured and stored at 4 °C. All sugars/derivatives, glycoproteins, bovine serum albumin, Freund's complete adjuvant, adriamycin, 5-fluorouracil, and paclitaxel were obtained from Sigma Chemical, St. Louis, USA. Standard molecular weight markers, gel filtration markers and ampholine of p*I* range 3.0–9.5 were from Amersham Pharmacia, New Jersey, USA. Amino activated silica beads used were from Clifmar, UK. Biogel P-200 for gel filtration chromatography was purchased from Bio-Rad USA. All other chemicals were of analytical grade.

Tumor cell lines

The various human cancer cell lines employed i.e., HOP-62 (lung), A-549 (lung), HCT-15 (colon), HT-29 (colon), HEP-2 (liver), PC-3 (prostate), SNB-78 (CNS), SiHa (cervix), and MCF (breast), were procured from National Center for Cell Science (NCCS), Pune. These cell lines were maintained in RPMI 1640 medium with 10% FCS, 10 U/ml penicillin and 100 μg/ml streptomycin at 37 °C, in humidified atmosphere (90% air and 10% CO_2) in CO_2 incubator (Heraeus, Heracell).

Plant material

Tubers of *A. helleborifolium* Schott were collected from Shimla during the month of August, 2004.

Isolation and purification of AHL

Lectin from *A. helleborifolium* tubers was extracted overnight at 4 °C with 10 mM phosphate-buffered saline (PBS),[1] pH 7.2 (1:5 w/v). After centrifugation at 20,000*g* for 30 min, the supernatant was chromatographed on asialofetuin-linked amino activated silica beads (1000 Å; pore size, 100 μ; diameter) as described earlier [8]. The bound lectin was eluted with 100 mM glycine–HCl buffer, pH 2.5. The fractions were neutralized immediately with 2 M Tris–HCl buffer, pH 8.3. The protein rich fractions were dialyzed against PBS and stored at 4 °C for further analysis.

Lectin-activity assay and sugar inhibition of lectin

To 30 μl of serial twofold dilution of the lectin in microtitre plate, an equal volume of 2% (v/v) suspension of rabbit erythrocytes (3.5×10^8 cells/ml) was added [12]. Agglutination was assessed after 1 h at 37 °C when the RBCs in the control well had fully sedimented. Agglutination activity was expressed as the reciprocal of the highest dilution that gave a positive result and was reckoned as one hemagglutination unit. To find the carbohydrate specificity of AHL, sugar inhibition was performed in a manner analogous to the hemagglutination test [12]. For this purpose, a series of 39 sugars/derivatives were used, which included four pentoses: D-arabinose, L-arabinose, D-ribose, and D-xylose; 20 hexoses or their derivatives: D-fructose, D-galactose, D-glucose, D-mannose, L-sorbose, L-fucose, L-rhamnose, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, *N*-acetyl-D-mannosamine, sialic acid, methyl-α- and methyl-β-D-glucopyranosides, methyl-α-D-mannopyranoside, methyl-α- and methyl-β-D-galactopyranosides, β-phenyl-D-glucopyranoside, *N*,*N*′,*N*″-triacetylchitotriose, adonitol, and *myo*-inositol; seven disaccharides: β-gentiobiose, D-lactose, D-maltose, D-melibiose, D-trehalose, T-disaccharide, and *N*-acetyl-D-lactosamine; two trisaccharides: D-melizitose and D-raffinose; three polysaccharides: chitin, glycogen, and inulin. Three glycoproteins, i.e., fetuin, asialofetuin, and porcine mucin were also used. Sugars or their derivatives were tested at a concentration of 100 mM while polysaccharides and glycoproteins at a concentration of 4 mg/ml. Each lectin was used at twice the lowest concentration causing agglutination of rabbit RBCs as determined through double dilution technique. The minimum concentration of the sugar in the final mixture that completely inhibited the lectin-induced hemagglutination was taken as minimal inhibitory sugar concentration (MIC).

To ascertain the biological specificity of AHL, the hemagglutination activity was tested against both native as well

[1] *Abbreviations used:* PBS, phosphate-buffered saline; MISC, minimal inhibitory sugar concentration; PAGE, polyacrylamide gel electrophoresis; TCA, trichloroacetic acid; SRB, sulforhodamine B; LacNAc, *N*-acetyl-D-lactosamine; MIC, minimum inhibitory sugar concentration; MEAPC, minimal erythrocyte agglutinating protein concentration; AcPs, acid phosphatases; AkP, alkaline phosphatase.