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One-step partial purification of *Solanum tuberosum* tuber lectin using magnetic chitosan particles

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Abstract

A one-step procedure for a partial purification of *Solanum tuberosum* tuber lectin has been developed. Lectin from tuber extract or from potato wastewater was adsorbed to magnetic chitosan particles and eluted with low pH buffer. The specific activity of separated lectin increased 13 times during the purification process and the recovery was 50%.

Introduction

Lectins are sugar binding proteins or glycoproteins of non-immune origin which agglutinate cells and/or precipitate glycoconjugates. They are widely distributed in nature being found mainly in seeds but also in other parts of certain plants, and in many other organisms from bacteria to mammals. Lectins are widely used experimentally in carbohydrate biochemistry and cell biology, e.g., for the separation of various biologically active compounds and cells.

A number of adsorbents for affinity chromatography separation of plant lectins have been developed, usually those using immobilised specific polysaccharides or oligosaccharides. In the case of potato tuber lectin, which exhibits *N*-acetylglucosamine specificity, immobilized *N*,*N*′,*N*″-triacetylchitotriose (Dessai & Allen 1979), *N*-acetylglucosamine (Wierzba-Arabska & Morawiecka 1987) and fetuin (Owens & Northcote 1980) can serve as examples. Affinity chromatography is a very efficient purification procedure but it requires the application of pre-purified samples to eliminate column clogging and increase of back-pressure.

An alternative technique that eliminates the need for prior particle removal is the adsorption of the protein of interest to magnetic affinity adsorbents. Magnetic separation techniques enable separation of target biologically active compounds and cells from a variety of materials including raw extracts, cultivation media, blood, body fluids, environmental samples, etc. (Šafařík & Šafaříková 1997, 1999). Magnetic affinity adsorbents have been used for the separation of various proteins (enzymes, antibodies, antigens, receptors, histidine-tagged proteins), nucleic acids (DNA, RNA, oligonucleotides), low-molecular weight biologically active compounds (drugs) and xenobiotics (carcinogens, water soluble dyes, heavy metals ions, radionuclides) (Šafařík & Šafaříková 1997).

Up to now there is no information available about the application of magnetic separation procedure for the isolation of lectins. In this work a rapid procedure for one-step partial purification of potato tuber lectin is described. Magnetic derivatives of chitosan, acetylated chitosan and chitin have been prepared and tested for rapid separation of lectin from crude extract. It was shown that magnetic chitosan is the adsorbent of choice.

Materials and methods

Materials and equipment

Chitosan (medium molecular weight) was from Fluka, Switzerland while chitin and Bradford reagent were from Sigma, USA. Magnetite [iron(II,III) oxide, declared particle size < 5 μm] was from Aldrich, USA. Glutaraldehyde (25% solution, w/v) was from Serva, Germany. Acetic anhydride and common chemicals were obtained from Lachema, Czech Republic. Mixed citrated human blood was obtained from the haematology laboratory of a local hospital. Erythrocytes were isolated by centrifugation and washed several times with 0.15 M NaCl. Potato tubers were obtained locally, potato wastewater from a potato starch factory in Chýnov, Czech Republic. Magnetic separators MPC-1 and MPC-6 were from Dynal, Norway. Flat magnetic separator (Šafaříková *et al.* 1996) was from SVUM, Praha, Czech Republic. Fast protein liquid chromatography apparatus (FPLC) and MONO S chromatography column were from Pharmacia, Sweden. HP 1040 A diode-array detector was from Hewlett-Packard, USA. Ultrafiltration was performed with a Millipore device (USA), using immersible CX-10 single ultrafiltration units.

Preparation of magnetic affinity adsorbents

Magnetic cross-linked chitosan was prepared in the following way. Chitosan (1 g) was dissolved in 20 ml 5% (v/v) acetic acid. After its dissolution (approx. 3–4 h) 2 g magnetite powder were added and the viscous suspension was mixed thoroughly. Then 30–40 ml 1.5 M NaOH were added to convert the suspension into the gel form. The black gel was cut into small pieces and after overnight standing at ambient temperature the black chitosan gel was homogenised in a mixer to obtain fine particles. Magnetic chitosan particles were thoroughly washed with water to remove sodium hydroxide. To 130 ml of the suspension 15 ml of 1 M phosphate buffer, pH 7.4 and 5 ml of 25% (w/v) glutaraldehyde were added. The suspension was incubated at ambient temperature under mixing for 18 h. Cross-linked magnetic chitosan was thoroughly washed with water. The free aldehyde groups were blocked with ethanolamine (2 ml ethanolamine added to 130 ml suspension; incubation 18 h) and then the magnetic particles were washed with water again and stored at 4 °C.

Magnetic acetylated chitosan was prepared from water washed fine chitosan particles prepared as described above. The magnetic particles (prepared from 1 g of chitosan) were washed with 70% (v/v) methanol. The particles were then suspended in 150–200 ml of 70% (v/v) methanol and 5 ml acetic anhydride were added to convert chitosan to acetylated chitosan. The suspension was stirred at room temperature for 18 to 20 h. The magnetic *N*-acetylated chitosan particles were thoroughly washed with water and stored at 4 °C (Šafařík & Šafaříková 1993).

Magnetic chitin was prepared similarly as described previously (Šafařík 1991). Shortly, chitin (5 g) was suspended in 200 ml of cold (5 °C) HCl. The suspension was held at 5 °C under occasional mixing for 44 h. The supernatant, 100 ml, containing dissolved chitin was poured into a smooth water suspension (1000 ml) containing fine magnetite particles (3 g) under intensive stirring. The magnetic particles were thoroughly washed with water and stored at 4 °C.

Isolation of potato lectin

Potato tubers (100 g) were homogenised with 100 ml of 0.15 M NaCl in a mixer. Then 5 ml of the crude filtered extract were mixed with 1 ml of suspension of magnetic chitosan particles (the particles settled volume after 24 h sedimentation at 1 g was 0.5 ml). The mixture was incubated at ambient temperature for 1 h. Magnetic adsorbent was separated from the mixture using a magnetic separator and washed three times with water. The lectin was desorbed with 2 ml 0.1 M glycine/HCl buffer, pH 2.2. After 15 min magnetic particles were separated and the pH of the eluate was adjusted by the addition of 3 ml 0.1 M phosphate buffer, pH 8.8. Alternatively lectin was isolated from potato wastewater using the same procedure.

Analytical procedures

The determination of hemagglutination activity (HA) was performed in 96-well U-shaped microtitration plates by serial two-fold dilution of 50 μl samples in 50 μl of 0.15 M NaCl after which 50 μl of 2% (v/v) suspension of native erythrocytes in 0.15 M NaCl was added. The titre of HA was evaluated after 1 h incubation at 20 °C and expressed as the reciprocal of the last sample dilution causing visible agglutination. The amount of lectin in the last test well with positive hemagglutination was defined as 1 HA unit (1 HAU).

To evaluate the purity of isolated potato tuber lectin fast protein liquid chromatography using a MONO S column was performed. Acetate buffer (0.05 M, pH 4.6) was used as a mobile phase A, while the

Table 1. Adsorption of potato tuber lectin on magnetic adsorbents and subsequent elution with low pH buffer. Procedure described in Materials and methods was used. 1 HAU was defined as the amount of lectin in the last test well with positive hemagglutination.

Sample	Original activity of the crude extract (HAU)	Remaining activity of the extract after adsorption step (HAU)	Activity of lectins after elution with glycin/HCl buffer, pH 2.2 (HAU)
Crude potato tuber extract	2560	–	–
Magnetic chitin	–	10	5
Magnetic acetylated chitosan	–	10	5
Magnetic chitosan	–	10	1280

Table 2. Purification of *Solanum tuberosum* tuber lectin with magnetic chitosan. 1 HAU was defined as the amount of lectin in the last test well with positive hemagglutination.

Step	Volume (ml)	Lectin activity (HAU ml^{-1})	Total lectin activity (HAU)	Protein (mg ml^{-1})	Total protein (mg)	Lectin specific activity (HAU mg^{-1})	Purification factor	Yield (%)
Crude extract	5	512	2560	4.50	22.50	113	1	100
Low pH buffer eluate (after neutralisation)	5	256	1280	0.18	0.89	1438	12.6	50

same buffer containing sodium sulphate (1 M) as a mobile phase B. The flow rate was 1 ml min^{-1}. The distribution of proteins in the effluent was monitored at 280 nm using a HP 1040 A diode-array detector. In some cases samples to be analyzed were concentrated using ultrafiltration with immersible CX-10 single ultrafiltration units.

Protein concentrations were determined with the Bradford (1976) method using bovine serum albumin as a standard.

Results

Potato tuber lectin is a hydroxyproline-rich glycoprotein with *N*-acetylglucosamine specificity. In order to be able to perform magnetic separation of the lectin we prepared three types of adsorbents. Magnetic derivative of chitin and acetylated chitosan (with high concentration of *N*-acetylglucosamine residues) and magnetic chitosan (with low concentration of *N*-acetylglucosamine residues) were used. Preliminary experiments with these magnetic adsorbents indicated that all of them can efficiently adsorb lectin from the crude extract of potato tubers. Elution of adsorbed lectin with low pH buffers was possible only from magnetic chitosan (see Table 1).

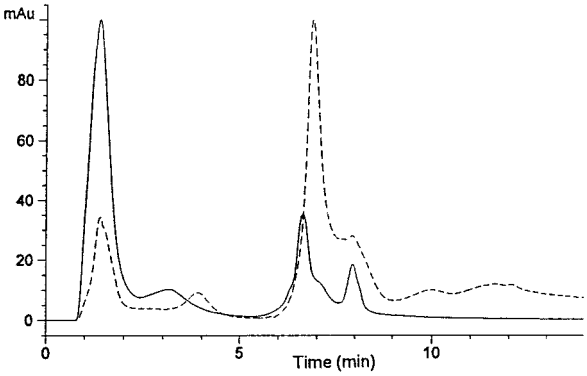

Fig. 1. Cation-exchange chromatography of crude potato tuber extract (—) and purified lectin (– – – –). Experimental details are given in Materials and methods. Normalised chromatograms are presented.

In the next step magnetic chitosan particles were used for the one-step isolation of potato tuber lectin from the crude extract. The lectin was eluted with low pH buffer and the degree of purification was checked by chromatography. Figure 1 shows the cation exchange chromatography of crude potato tuber extract

and the isolated lectin. It can be clearly seen that this one-step purification procedure led to the removal of substantial amount of the accompanying compounds and that potato lectin (peak eluting at ca. 7 min) is substantially purified. Totally 50% of lectin activity was recovered and the specific activity of the purified lectin was ca 13 times higher in comparison with the crude extract (see Table 2).

Potato tuber lectin can be found in the wastewater originating in the course of production of potato starch. Also this material was successfully used for the isolation of lectin with magnetic chitosan.

Discussion

Many methods are available for the isolation and purification of plant lectins. Affinity chromatography enables rapid one-step preconcentration or isolation of target lectins from filtrated or centrifuged samples. This procedure, however, is not suitable for the isolation of the target compounds from crude extracts containing suspended solid particles. In this case magnetic affinity adsorbents represent a promising alternative (Šafařík & Šafaříková 1997).

Our attempt was to develop a simple magnetic batch affinity adsorption process. As the potato tuber lectin is known to have *N*-acetylglucosamine specificity, we tested magnetic derivatives of chitin and related polysaccharides, namely chitosan and acetylated chitosan as adsorbents. All the adsorbents adsorbed the lectin equally well. The differences were found in the elution step. The cheapest and simplest way to elute adsorbed target compounds is the change of pH or ionic strength of the elution buffer. Having in mind the compatibility with the possible following isolation steps (e.g., ion-exchange chromatography), we tested elution with low pH buffers. This elution system enabled efficient elution of 50% of lectin activity from magnetic chitosan particles. This material is thus a very promising adsorbent for the isolation of potato tuber lectins not only from the laboratory prepared potato extracts, but also from wastewaters available from the potato starch industry. Efficient large-scale separation of lectin from this waste material is thus possible, also due to the fact that the preparation of this magnetic adsorbent is very simple and the sorbent is very cheap. Of course, other biologically active compounds with affinity similar to that of lectin can be adsorbed on the magnetic affinity matrix. These components can be separated in the next purification step.

Magnetic derivatives of chitin and chitosan thus represent useful affinity adsorbents which up to now have been used for the isolation of hen egg white lysozyme (Šafařík 1991, Šafařík & Šafaříková 1993) and the lysozyme from the gut of the soft tick *Ornithodoros moubata* (Kopáček *et al.* 1999). Alternatively, magnetic chitin and chitosan particles have also been used for the immobilization of enzymes (Bendikene *et al.* 1995) and low-molecular-weight affinity ligands (Šafařík 1995) and for the separation of microbial cells (Honda *et al*. 1998).

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