Short communication

Magnetic ovalbumin and egg white aggregates as affinity adsorbents for lectins separation

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Abstract

Simple and low-cost preparation of new magnetic adsorbents from ovalbumin and egg white, based on methanol precipitation and subsequent glutaraldehyde cross-linking has been developed. These adsorbents were used for preconcentration of two plant lectins from potato (\textit{Solanum tuberosum}) tubers and from wheat (\textit{Triticum} spp.) germs extracts. The adsorbed lectins were eluted with diluted hydrochloric acid. The specific activities of both lectins increased approximately 30–40 times during the preconcentration process.

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1. Introduction

Affinity chromatography techniques represent currently the most powerful tool available to the downstream processing both in term of their selectivity and recovery [1]. To isolate target lectins from different sources, specific polysaccharides or glycoproteins are usually used as affinity ligands. A great number of adsorbents with immobilized or cross-linked specific polysaccharides were described for affinity chromatography separation of plant lectins [2–5]. Also the glycoproteins ovalbumin and fetuin have already been used as affinity ligands for lectins isolation [6,7]. Column affinity chromatography, however, does not allow separation of target lectins from crude raw materials containing suspended solids.

To overcome these problems, magnetic separation procedures can be efficiently used [8]. Magnetic adsorbents have several advantages in comparison with standard chromatography adsorbents, especially due to the fact that the separation process can be performed directly in crude samples containing suspended solid material without the need of expensive equipment [9].

Ovalbumin is a monomeric phosphoglycoprotein with a molecular weight of 44.5 kDa. It is a major protein of egg white constituting about 54% of the total egg white proteins [10]. It belongs to the serpin superfamily of proteins, although unlike the majority of serpins it is unable to inhibit any protease [11]. Ovalbumin is a glycoprotein containing about three residues of N-acetylglucosamine and about five residues of mannose in its molecule [12].

This study is focused on the development of a simple and rapid procedure for the preparation of low-cost magnetic adsorbents from ovalbumin and its natural source—egg white. These new magnetic adsorbents were used for preconcentration of two plant lectins with N-acetylglucosamine specificity from potato (\textit{Solanum tuberosum}) tubers and from wheat (\textit{Triticum} spp.) germs extracts.

2. Materials and methods

2.1. Materials and equipment

Magnetsite (iron(II, III) oxide, declared particle size <5 \textmu m) was from Aldrich, USA. Glutaraldehyde (50\% solution, w/v) was from Fluka, Switzerland. Ovalbumin, methanol and common chemicals were obtained from Lachema, Czech Republic. Sample mixer and magnetic separator MPC-1 were from Dynal, Norway while flat magnetic separator was from Qiagen, USA. Mixed citrated human blood was obtained from the hematology laboratory of a local hospital. Erythrocytes were isolated by repeated centrifugation and...
careful washing with 0.15 M NaCl. Potato tubers and wheat germ were purchased from a local market.

2.2. Preparation of magnetic adsorbents

Ovalbumin (1 g) was dissolved in 10 ml of 0.1 M phosphate buffer, pH 7.0. After its dissolution (approx. 2–3 h) the solution was filtered and the obtained ovalbumin solution (8.0 ml) was mixed thoroughly with magnetite powder (60 mg). In order to convert the suspension into magnetic ovalbumin aggregates, methanol (27.5 ml) was added in ca. 3 ml aliquots rapidly, so that the suspension was well mixed. After addition of 4 ml of 50% glutaraldehyde the mixture was incubated under mixing for 1.5 h to obtain cross-linked magnetic ovalbumin aggregates. They were subsequently thoroughly washed with water and free aldehyde groups were blocked using Tris (200 mM final concentration; 1 h incubation) and then magnetic aggregates were washed with water again and stored at 4 °C. The particles settled volume after 24 h sedimentation at normal gravity was 23.2 ml.

Magnetic egg white aggregates were prepared from diluted egg white. Egg white was separated from yolk in a standard way. Subsequently egg white (total protein concentration ca. 100 mg/ml) was homogenized and filtered through a cheese-cloth. Egg white was diluted using 0.05 M sodium bicarbonate buffer, pH 9.2, at a ratio 1:2 (v/v). Diluted egg white (3.0 ml) was mixed thoroughly with 9 mg of magnetite powder. Then 7.5 ml of methanol was added in 1 ml aliquots rapidly, so that the suspension was well mixed in order to convert the suspension into the magnetic egg white aggregates. After addition of 50% glutaraldehyde (1.15 ml) the mixture was incubated under mixing for 1.5 h. The following steps were the same as described above. The particles settled volume after 24 h sedimentation at normal gravity was 16.5 ml; it corresponds to the volume of particles prepared from 1 ml of undiluted egg white.

2.3. Preparation of potato tubers and wheat germ extracts

Potato tubers (100 g) were thoroughly homogenized with 100 ml of 0.15 M NaCl in a mixer. The homogenate was filtrated and the filtrate was used as a crude extract for lectin preconcentration. Wheat germ (100 g) were mixed with 580 ml of 0.15 M NaCl for 60 min. The suspension was filtered and centrifuged at 3000 × g for 5 min; supernatant was used for preconcentration of target lectin.

2.4. Isolation of lectins

Twenty millilitres of crude filtered extracts were mixed with 1 ml (sedimented volume) of magnetic protein aggregates. The suspension was slowly mixed on a rotary mixer at room temperature for 2 h. Magnetic adsorbent was separated from the suspension using a magnetic separator and washed 10 times with water. Contaminating proteins were eluted using 3 ml of 0.3 M NaCl for 15 min. The target lectins were desorbed by repeated elution (two 1.5 ml aliquots; 30 min incubation each) with 10 mM HCl. Then magnetic aggregates were separated and the pH of each eluate was adjusted by the addition of 25 µl of 0.2 M Tris. Both eluates were poured together and analyzed.

To study the reuse of magnetic adsorbents, the lectins were eluted as described above and then the adsorbents were washed with water. The regenerated adsorbents were used for next adsorption/desorption process. The experiments were repeated three times.

2.5. Analytical procedures

Determination of hemagglutination activity (HA) was performed in 96-well microtitration plates. A serial two-fold dilution of 50 µl eluate was made in 50 µl of 0.15 M NaCl and then 50 µl of 2% (v/v) suspension of human erythrocytes in 0.15 M NaCl was added. The titer of HA was evaluated after 30 min incubation at 20 °C and expressed as the reciprocal value 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). Since the volume of lectin solution added to each well of the agglutination trays is 50 µl, a titer of 1/16, for example, would be 16 units/50 µl or 320 units/ml [13]. Specific lectin activity is defined as the activity in such units per mg of protein. Protein concentrations were determined by Bradford method with bovine serum albumin as standard.

Inhibition of lectin-induced hemagglutination by ovalbumin was studied as follows. A solution of lectin with 2 HAU (50 µl) was mixed with an equal volume of a serial two-fold dilution of the ovalbumin sample to be tested; the starting ovalbumin solution concentration was 10% (w/v). After incubation at room temperature for 30 min, 50 µl of 2% suspension of human erythrocytes were added and incubation continued for another 30 min. The concentration of ovalbumin in the final reaction mixture capable to inhibit completely 2 HAU of the lectin (minimal inhibiting concentration) was calculated [14].

Lectin purity was checked using an FPLC system (Pharmacia, Sweden) which comprised LCC-500 controller and two P500 pumps. The chromatography was carried out on a Mono S HR 5/5 column using 0.05 M acetate buffer, pH 4.6, as a mobile phase A, and the same buffer containing 1 M sodium sulphate as a mobile phase B. The flow rate was 1 ml min⁻¹. The elution profile was monitored at 280 nm with diode array detector (Agilent 1100 Series, Agilent, USA).

3. Results and discussion

Ovalbumin is an important glycoprotein both from the point of view of basic research and food technology. Due to its glycoprotein nature, immobilized ovalbumin has already been used as an affinity ligand for affinity chromatography of target lectins. However, magnetic ovalbumin derivative can bring additional advantage, namely the possibility of its selective magnetic separation from crude biological samples. That is why the simple preparation of magnetic ovalbumin aggregates using methanol precipitation and subsequent glutaraldehyde cross-linking was developed. Because ovalbumin is the main protein in egg white, magnetic cross-linked egg white aggregates were also prepared using the slightly modified procedure. Both magnetic protein aggregates exhibited similar properties both from the point of view of their stability, response to external magnetic field and affinity towards target lectins. Both types of magnetic protein aggregates differed in the compactness, ovalbumin aggregates being more compact.

Preliminary experiments were focused on the determination of appropriate ratio between magnetite powder mass and ovalbumin solution concentration and volume, amount of glutaraldehyde necessary to form stable magnetic protein aggregates, and, in case of egg white, its necessary dilution. The optimization led to the development of simple procedures for the preparation of magnetic cross-linked protein aggregates, as described in Section 2. The prepared aggregates can be simply separated using NdFeB permanent magnets or commercial magnetic separators. The ovalbumin aggregates diameters were in the range 60–110 µm (Fig. 1) while the egg white aggregates diameters ranged between 600 and 900 µm. The
aggregates were stable in water solutions containing antimicrobial compounds.

Due to the presence of N-acetylglucosamine residues in ovalbumin molecules the newly developed magnetic ovalbumin and egg white aggregates were used for magnetic separation of target lectins. Two lectins with N-acetylglucosamine specificity, namely potato tuber lectin and wheat germ lectin, were chosen. The first experiments clearly showed that both magnetic adsorbents can efficiently adsorb both lectins from the crude extracts and that the adsorbed lectins can be eluted with low pH solution (see Table 1). Also ovalbumin inhibition assay demonstrated that both lectins exhibited affinity towards ovalbumin. The lowest concentration of ovalbumin totally inhibiting 2 HAU of potato lectin was 0.1 mg/ml. Total inhibition of 2 HAU of wheat germ lectin required ovalbumin concentration 6.25 mg/ml at least.

In the next step magnetic protein aggregates were used for one-step preconcentration of lectins from crude extracts. Substantial amount of unwanted accompanying proteins was eluted with 0.3 M NaCl solution, while target lectins were eluted using two step elution with low pH solution. Tables 2 and 3 summarize the experimental data. As can be seen, 30% of lectin activity was recovered in all cases. The specific activity of the purified potato tuber lectin increased ca. 40 times after the affinity purification step (see Table 2). The specific activity of the purified wheat germ lectin was slightly lower when compared with potato tuber lectin; higher specific activity was obtained using magnetic egg white aggregates as an affinity adsorbent (see Table 3).

Ion exchange chromatography was used to check the purity of the separated lectins. Fig. 2 shows the cation exchange chromatography of the crude potato extract and of isolated lectin. It can be clearly seen that substantially purified product was obtained. Large amount of the accompanying proteins (present especially in the first major peak) were almost completely eliminated during the isolation process. The lectin activity was observed in all four main peaks in the purified preparation; most probably this can be explained by the isoelectric point heterogeneity of potato lectin [15].

Both magnetic adsorbents were repeatedly used for the isolation of lectins from crude extracts. Magnetic ovalbumin could be used at least three times without the change of the separation process. On the contrary, magnetic egg white exhibited substantial decrease of lectin recovery during the second repetition.

### Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Original activity of the crude extract (HAU)</th>
<th>Remaining activity of the extract after adsorption step (HAU)</th>
<th>Activity of lectins after elution with 10 mM HCl (HAU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potato tuber lectin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude potato tubers extract</td>
<td>102400</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Magnetic ovalbumin</td>
<td>–</td>
<td>25600</td>
<td>30720</td>
</tr>
<tr>
<td>Magnetic egg white</td>
<td>–</td>
<td>25600</td>
<td>30720</td>
</tr>
<tr>
<td>Wheat germ lectin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude wheat germ extract</td>
<td>3200</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Magnetic ovalbumin</td>
<td>–</td>
<td>400</td>
<td>960</td>
</tr>
<tr>
<td>Magnetic egg white</td>
<td>–</td>
<td>1600</td>
<td>960</td>
</tr>
</tbody>
</table>

### Table 2

<table>
<thead>
<tr>
<th>Step</th>
<th>Volume (ml)</th>
<th>Lectin activity (HAU/ml)</th>
<th>Total lectin activity (HAU)</th>
<th>Protein (mg/ml)</th>
<th>Total protein (mg)</th>
<th>Lectin specific activity (HAU/mg)</th>
<th>Purification factor</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude potato tubers extract</td>
<td>20</td>
<td>5120</td>
<td>102400</td>
<td>1.182</td>
<td>23.64</td>
<td>4332</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>Eluate after neutralization</td>
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<td>30720</td>
<td>0.056</td>
<td>0.168</td>
<td>182857</td>
<td>42.2</td>
<td>30</td>
</tr>
<tr>
<td>Crude wheat germ extract</td>
<td>20</td>
<td>160</td>
<td>3200</td>
<td>1.552</td>
<td>31.04</td>
<td>103</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>Eluate after neutralization</td>
<td>3</td>
<td>320</td>
<td>960</td>
<td>0.113</td>
<td>0.339</td>
<td>2832</td>
<td>27.5</td>
<td>30</td>
</tr>
</tbody>
</table>
Preconcentration of target lectins with magnetic egg white aggregates

Table 3

<table>
<thead>
<tr>
<th>Step</th>
<th>Volume (ml)</th>
<th>Lecitin activity (HAU/ml)</th>
<th>Total lectinit activity (HAU)</th>
<th>Protein (mg/ml)</th>
<th>Total protein (mg)</th>
<th>Lecitin specific activity (HAU/mg)</th>
<th>Purification factor</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude potato tubers extract</td>
<td>20</td>
<td>5120</td>
<td>102400</td>
<td>1.182</td>
<td>23.64</td>
<td>4332</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>Eluate after neutralization</td>
<td>3</td>
<td>102400</td>
<td>307200</td>
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<td>0.186</td>
<td>165161</td>
<td>38.1</td>
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<tr>
<td>Crude wheat germ extract</td>
<td>20</td>
<td>160</td>
<td>3200</td>
<td>1.552</td>
<td>31.04</td>
<td>103</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>Eluate after neutralization</td>
<td>3</td>
<td>960</td>
<td>1824</td>
<td>0.087</td>
<td>0.261</td>
<td>3678</td>
<td>35.7</td>
<td>30</td>
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</table>

Table 4

<table>
<thead>
<tr>
<th>Capacity</th>
<th>Potato lectin</th>
<th>Wheat germ lectin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovalbumin (HAU/ml)</td>
<td>30720</td>
<td>960</td>
</tr>
<tr>
<td>Ovalbumin (HAU/mg)</td>
<td>890.9</td>
<td>27.8</td>
</tr>
<tr>
<td>Egg white (HAU/ml)</td>
<td>30720</td>
<td>960</td>
</tr>
<tr>
<td>Egg white (HAU/mg)</td>
<td>5068.8</td>
<td>158.4</td>
</tr>
</tbody>
</table>

The capacity of magnetic adsorbents was expressed as the amount of HAU eluted from 1 ml of sedimented adsorbent; corresponding values were calculated and expressed as HAU per 1 mg of protein. The values are given in Table 4. Both ovalbumin and egg white magnetic aggregates can be successfully used for lectins separation. From the point of view of mechanical properties and possible reuse ovalbumin derivative is preferable, even if the capacity calculated per mg of protein is lower than in case of egg white aggregates; most probably higher compactness of ovalbumin aggregates is responsible for masking part of oligosaccharide chains.

4. Conclusion

The time-consuming and costly purification procedures have inspired a search for suitable low-cost adsorbents. The main aim of our work was to find a cheap and easy to get magnetic adsorbents enabling to separate target biologically active compounds from crude samples by means of magnetic separation procedures. Magnetic cross-linked proteins aggregates, especially those prepared from inexpensive and readily available individual proteins or their mixtures represent a new type of magnetic affinity adsorbents which can be successfully used for this purpose. In case of glycoproteins aggregates the saccharide moiety can be employed for target lectins separation. There is only a very limited number of papers describing magnetic affinity separation of lectins. Cheap and easy-to-prepare magnetic affinity adsorbents can be very useful for lectins separation from various biological sources. The described procedure has a potential to overcome the drawbacks of multistep purification methods.

Further possible applications of these and other adsorbents prepared from low-cost proteins are currently under study. A more detailed search will be performed to find other interesting biologically active compounds which could be separated using the described procedure.

Acknowledgements

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