Lectin purification from fruiting bodies of brown roll-rim fungus, *Paxillus involutus* (Fr.) Fr., and its application in histochemistry

ROSTYSLAV ANTONYUK¹, ALEXANDER LUTSYK¹, VOLODYMIR ANTONYUK¹,²

¹Department of Histology and Embryology, "Danylo Halytsky" Lviv National Medical University, Ukraine
²Department of Regulation of Cell Proliferation and Apoptosis, Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv

Abstract

A lectin (agglutinin) from fresh fruit bodies of the brown roll-rim fungus [*Paxillus involutus* (Fr.) Fr.] has been purified with output ≈ 60 mg/kg of raw material. Method of purification included the sedimentation of viscous polysaccharide by ethanol, removal of ethanol by dialysis, ion-exchange chromatography on DEAE-Toyopearl and affinity chromatography on Sepharose 6B column with immobilized mannose-specific *Polygonatum multiflorum* lectin. The obtained lectin preparation (abbreviated PIFA) is a glycoprotein with 6.5±1% carbohydrates, molecular mass of 64 kDa, consisting of four identical subunits. Lectin interacted only with N-acetyl-lactosamine and glycoproteins that contained Galβ1-4GlcNAc disaccharide moieties; agglutinated erythrocytes of dog, sheep and horse, but not of humans. The specificity of PIFA binding to tissue samples of the rat has been investigated. Lectin selectively reacted with gastric parietal cells, submandibular salivary gland duct cells. In the kidney, PIFA labeled epithelial cells of renal tubules, collecting ducts, nuclei of podocytes and mesangiocytes. It was also revealed selective lectin binding to Purkinje cells of cerebellum. Brush border of absorptive cells in small intestine was also strongly reactive, while goblet cells both in small and large intestine were completely negative. Considering similarities in carbohydrate specificity of *PIFA* and *Ricinus communis* agglutinin (RCA-120), histochemical reactivity of these two lectins was compared. It was similar, yet not identical: differences included absence of PIFA binding to the brush border of renal tubules, higher interaction with absorptive cells of the small intestine, lower background staining of cerebellar cortex and renal corpuscles. A conclusion was made that due to the unique carbohydrate specificity *PIFA* lectin can cover prospective position in experimental histochemistry and diagnostic histopathology comparable to PNA (Peanut agglutinin) and SNA (*Sambucus nigra* agglutinin).

Keywords: *Paxillus involutus* lectin, purification, properties, application in histochemistry.

Introduction

Lectins due to their interaction with carbohydrate moieties of cell and tissue glycoconjugates are considered sensitive and valuable tools in histochemical research [1, 2]. Age-related, physiological or pathological changes of carbohydrate determinants can be detected by specific lectins that selectively react with them [3, 4]. However, in most cases lectins demonstrate rather broad specificity of binding, reacting with a wide score of non-reducing saccharide residues [5]. Therefore, search for new lectins with high and rare specificity, i.e., selectively labeling certain morphological structures, remains an important issue.

From more than 2000 of registered lectin preparations, obtained from different sources, fungal lectins due to certain difficulties in their purification protocols give only few examples. These include *Aleuria aurantia* [6], *Marasmius oreades* [7], *Polyporus squamosus* [8] lectins, which are intensely used in histochemical investigations. Recently, in our laboratory was purified a new lectin from *Mycena pura* fungus, which exposed high affinity to alkaline phosphatase of small intestine [9].

Genus of basidiomycetes *Paxillus* in Ukraine is represented by four species. The most common among them are two – *Paxillus involutus* (Fr.) Fr. (brown roll-rim) and *Paxillus atrotomentosus* (Fr.) Fr. (velvet roll-rim). These fungi are represented almost in every country of East Europe, but *P. involutus* is more widespread than *P. atrotomentosus* species [10]. Purification of a lectin from the fruiting bodies of *P. atrotomentosus* was described in 1996 [11], while lectin from *P. involutus* remained obscure.

The aim of present investigation was to elaborate a simple method of lectin (agglutinin) purification from *P. involutus* fungus and to study its applicability in histochemical research.

Materials and Methods

Purification protocol

Fruiting bodies from *P. involutus* were gathered in August – early October in Scole district, Lviv region (Ukraine, Carpathian Mountains). Four hundred g of fresh mushrooms were knifed to small pieces (20×20×20 mm) and placed in electromixer with adding 800 mL of 1% aqueous sodium chloride solution. The mixture was homogenized and the homogenate was centrifuged at 6000–10 000 g for 15 minutes. Brown supernatant was filtered. Four hundred thirty mL of 96% ethanol was added to 860 mL of the supernatant. Viscous precipitating polysaccharide was removed by centrifugation at 500–1000 g for 10 minutes. Ethanol was removed by dialysis against 1% aqueous sodium chloride solution. Small precipitate
was removed by filtration, and then the protein fraction was precipitated by adding ammonium sulphate (600 g/L). The resulting precipitate was concentrated by centrifugation, thereafter dissolved in a small amount of water and dialyzed against 0.05 M phosphate buffer, pH 7.

After dialysis completed, solution was filtered, and its volume and agglutination titer for rabbit erythrocytes was measured. The resultant solution was applied to a column of DEAE-Toyopearl (h=5 cm, d=0.8 cm), previously equilibrated by 0.05 M phosphate buffer, pH 7. Then, the column was washed with 0.05 M, and afterwards with 0.2 M phosphate buffer solution. Fifteen mL fractions were collected during washing of the column, in which the lectin content was controlled by hemagglutination titer (Figure 1).

Figure 1 – Purification of PIFA (Paxillus involutus fungus agglutinin) by ion-exchange chromatography on DEAE-Toyopearl column: (A) Scale of protein adsorption A280 (continuous line); (B) Scale of hemagglutination activity towards rabbit erythrocytes (bulkhead on diagram).

Most of the lectin under the described conditions was eluted from the column with 0.05 M phosphate buffer solution. The lectin was fully eluted with 0.2 M phosphate buffer solution, this later fraction was slightly contaminated with a pigment. However, most of the brown pigment and ballast proteins under the above-mentioned conditions were lagged on top of the column. Regeneration of the column by removing the adsorbed brown pigment was performed by washing it with a mixture of dioxane–acetic acid–30% aqueous solution of sodium chloride 2:1:3. Fractions obtained from the DEAE-Toyopearl column were combined and precipitated by ammonium sulfate.

Further purification of lectin was performed on the affinity sorbent as follows. One mL of immobilized glyco-protein or polysaccharide gel was placed in the graduated centrifuge tube and thrice washed with 10 mL of phosphate buffered saline, pH 7.4. Thereafter that an equal volume of P. involutus extract with estimated hemagglutination titer (preferably higher then 1:32) was added to the washed sorbent. Sorbent and extract were carefully stirred for 10 minutes. After the sorbent settled on the bottom, hemagglutination titer was estimated in the supernatant. If hemagglutination titer decreased twice (due to dilution of extract), that meant absence of sorbent interaction with lectin. In case the hemagglutination titer decreases for 4, 8 or 16 times, it was proof of 50%, 75% and 87.5% of lectin coupling respectively. The sorbent was selected in experimental series, being most effective for lectin purification.

Further purification was performed by affinity chromatography on a Sepharose 6B column with immobilized lectin from Polygonatum multiflorum rhizome. To the column with affinity sorbent (total volume of 20 mL), 10 mL of purified Paxillus involutus lectin (PIFA) was applied (the lectin solution obtained in the previous stage was subjected to dialysis against 0.1 M acetate buffer, pH 6.4). After passing this solution through the column, it was washed with acetate buffer to reduce of extinction in the eluting solution at λ280 nm to a value less than 0.1. PIFA elution from the column was carried out using a 3% solution of D-mannose, which was dissolved in acetate buffer. Fractions containing protein were combined, precipitated by ammonium sulfate and after dialysis against distilled water freeze-dried.

Lectin characteristics

Molecular weight of PIFA subunits was estimated by electrophoresis in 15% PAAG (Polyacrylamide gel) in the presence of 0.1% sodium dodecyl sulphate (SDS-Na). A mixture of proteins with known molecular weight (Fermentas, Lithuania) was used as a standard (Figure 2). Total molecular weight of PIFA lectin was estimated by column chromatography on Toyopearl HW-55 gel, using as marker substances listed in Figure 3. The content of carbohydrates in PIFA molecules was estimated by DuBois et al. (1956) method [12].

Figure 2 – PAAG electrophoresis of protein molecular weight markers (1) and of purified PIFA lectin (2) in the presence of 0.1% SDS-Na.

Carbohydrate specificity of the obtained lectin was determined according to inhibition of rabbit erythrocytes hemagglutination by carbohydrates and glycoproteins. Minimal concentration of carbohydrates or glycoproteins, which completely inhibited activity of lectin (1:4 titer), was estimated using step-by-step dilution of them [13]. To estimate the carbohydrate specificity of lectin, it were used: D-glucose, D-fructose, D-galactose, sucrose, maltose, lactose (Soyuzhimreaktiv, Russia), raffinose (Fluka, Switzerland), α- and β-methyl-D-galactoside, L-rhamnose, N-acetyl-D-galactosamine and N-acetyl-D-glucosamine (Chemapol, Czech Republic), D-mannose, D-turanose, L-ribose (Bratislava Chemical Institute, Slovakia), melibiose,
α-methyl-D-mannoside, L-fucose (Koch Light, UK). To study PIFA interaction with glycoproteins and polysaccharides, were used water-soluble starch, liver pig glycogen, ovomucoid and ovalbumin (Biolar, Olaine, Latvia), gum arabic and heparin (Loba Feinchemie, Austria), alkaline phosphatase from calf intestine (Serva, Germany), purified inulin [14], yeast mannan [15] and bovine thyroglobulin [16].

**Tissue specimens and lectin histochemistry**

In order to test the applicability of new lectin preparation for histochemical research, 11 organ samples of Wistar male adult rats (4–6 months of age, weighting 150–200 g, n=7) were used. Animals were sacrificed by diethyl ether narcosis overdose with subsequent decapitation. Organs were removed immediately, fixed in 4% neutral formalin, and embedded in paraffin wax according to the standard protocol. Histological material included: submandibular salivary gland, stomach, small intestine, colon, lymph node, heart, liver, adrenal gland, kidney, cerebellar and cerebral cortex. The investigation was carried out according to the ethical criteria for the use and handling of laboratory animals established by Lviv National Medical University in accordance with the “General ethical principles on experiments with animals” of the 1st National Congress on Bioethics (Kyiv, 2001) and in compliance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (1996).

For general morphology studies, 5- to 7-µm-thick sections were stained with Hematoxylin and Eosin. For histochemical investigation, sections were labeled with P. involutus lectin, conjugated to horseradish peroxidase by method of Nakane and Kawai [17] in our modification [18]. Briefly, carbohydrate part of peroxidase was oxidized by sodium meta-periodate; thereafter, the appeared aldehydic groups of peroxidase interacted with PIFA amino groups in an alkaline medium (pH 8.4–9.8), with the formation of peptide bonds; for conjugation purposes, amounts of peroxidase and lectin were taken in equivalent quantity.

To detect carbohydrate determinants, deparaffinized sections were incubated for 20 minutes in the methanol with 0.3% hydrogen peroxide to block endogenous peroxidase; through descending ethanol adjusted to phosphate buffered saline (PBS, pH 7.4); incubated for 45 minutes at room temperature with lectin-peroxidase conjugate (dilution 10–25 mg/mL in PBS); washed in three portions of the PBS; visualization of the lectin receptor sites was carried out in a solution of 0.05% 3,3′-diaminobenzidine tetrahydrochloride (Sigma, St. Louis, MO, USA) in the presence of 0.015% hydrogen peroxide.

Control on specificity of histochemical reactions was performed as follows: (1) excluded of lectin-peroxidase conjugate from the staining protocol; (2) oxidation of the carbohydrate determinants prior to lectin-peroxidase application by 60 minutes incubation in 1% solution of meta-periodic acid (Reanal, Budapest, Hungary). In both cases, negative reactivity was detected. More detailed information concerning staining protocols of lectin histochemistry and control on a specificity of binding is described elsewhere [19].

Tissue labeling with PIFA was compared to that of Ricinus communis agglutinin (RCA-120) – lectin, which possesses similar to PIFA carbohydrate specificity. Microscopic investigation was performed using a Carl Zeiss Ng (Jena, Germany) microscope equipped with a Canon IXUS 700 digital camera (Canon, Tokyo, Japan).

**Results**

The largest portion of PIFA was obtained after washing the column as described in “Materials and Methods” section by 0.05 M phosphate buffer. Lectin was completely removed from the column with the 0.2 M phosphate buffer solution, but this fraction was slightly contaminated with pigment. Most of the brown pigment and ballast proteins were detained on the top of the column (Figure 1). After the ion-exchange chromatography, lectin was released from most of the pigment, but for its complete purification it was necessary to implement affinity chromatography, which was performed on a column with immobilized mannone specific lectin from Polygonatum multiflorum.

About 60 mg of lyophilized PIFA was obtained from 1 kg of mushrooms according to the described method. Purified lectin is a white amorphous powder, soluble in aqueous salt solutions at pH 4–9. PIFA withstand heating at 65°C for one hour, but after 15 minutes at 75°C its hemagglutinating activity decreased to 75%. Soluble lectin is well preserved in a frozen state; multiple freezing–defrosting of the solution did not cause a decrease in hemagglutination titer. PIFA did not lose hemagglutinating potency after dialysis against 1% solution of EDTA disodium salt for eight hours. This indicates that Ca2+ and Mg2+ ions are not necessary for its activity. Lectin is a glycoprotein with carbohydrate content of 6.5±1%.

Electrophoresis in 15% PAAG with 0.1% sodium dodecyl sulphate showed one band with molecular mass of ~16 kDa (Figure 2). Total molecular weight of PIFA was determined as 64 kDa by gel chromatography on a Toyopearl HW-55 column (Figure 3). This evidenced tetrameric structure of the lectin molecule, apparently consisting of four identical subunits.

![Figure 3 – Estimation of PIFA molecular weight by gel-chromatography on Toyopearl HW-55 column (39×1.5 cm, eluted by 0.1 M acetate buffer pH 6.4, supplemented with 0.5 M sodium chloride, elution rate – 0.3 mL/min.); LABA (Laburnum anagyroides bark agglutinin, M, 100 kDa); HPA (Helix pomatia agglutinin, M, 79 kDa); HSA (Human serum albumin, M, 69 kDa); GNA (Galanthus nivalis agglutinin, M, 50 kDa); PSA (Pisum sativum agglutinin, M, 48 kDa); SIT (soybean tripsin inhibitor, M, 21 kDa); Lys (egg lysozyme, M, 14.3).](image-url)
The lectin can be purified by affinity chromatography on the immobilized mannose-specific lectins, *i.e.*, *Polygonatum multiflorum*, *Leucojum vernum* and concanavalin A, as evidenced from results on PIFA interaction with the immobilized glycoproteins (Table 1).

### Table 1 – Adsorption of *Paxillus involutus* lectin to immobilized glycoproteins and affinity sorbents

<table>
<thead>
<tr>
<th>Sorbent</th>
<th>Titer of agglutination (rabbit erythrocytes)</th>
<th>% of lectin adsorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1:64</td>
<td>–</td>
</tr>
<tr>
<td>Partially hydrolyzed Sepharose</td>
<td>1:32</td>
<td>–</td>
</tr>
<tr>
<td>Bovine submaxillary mucin-agarose</td>
<td>1:32</td>
<td>–</td>
</tr>
<tr>
<td>Bovine thyroglobulin-agarose</td>
<td>1:32</td>
<td>–</td>
</tr>
<tr>
<td><em>Leucojum vernum</em> lectin-agarose</td>
<td>1:8</td>
<td>75%</td>
</tr>
<tr>
<td>Wheat germ agglutinin-agarose</td>
<td>1:32</td>
<td>–</td>
</tr>
<tr>
<td>α2-Macroglobulin-agarose</td>
<td>1:32</td>
<td>–</td>
</tr>
<tr>
<td><em>Polygonatum multiflorum</em> lectin-agarose</td>
<td>1:4</td>
<td>87%</td>
</tr>
<tr>
<td>Vicia sativa lectin-agarose</td>
<td>1:32</td>
<td>–</td>
</tr>
<tr>
<td>Concanavalin A-biogel</td>
<td>1:4</td>
<td>87%</td>
</tr>
<tr>
<td>Sorbent “Ovogel”</td>
<td>1:32</td>
<td>–</td>
</tr>
<tr>
<td>Sorbent “Ovomucin”**</td>
<td>1:32</td>
<td>–</td>
</tr>
<tr>
<td>Human blood group-specific substance B-Sepharose</td>
<td>1:32</td>
<td>–</td>
</tr>
</tbody>
</table>

“Ovogel”** and “Ovomucin”** are sorbents, effectively used for the purification of a wide variety lectins (except L-fucose specific); both sorbents are prepared using glycoproteins from chicken eggs according to method described earlier [20, 21].

PIFA eagerly agglutinates erythrocytes of dog, sheep, horse, rabbit, but not of humans and cow, even at maximal concentration of 10 mg/mL (Table 2).

### Table 2 – Minimal concentration of *Paxillus involutus* lectin, causing agglutination of erythrocytes

<table>
<thead>
<tr>
<th>No.</th>
<th>Type of erythrocytes</th>
<th>Minimal lectin concentration in [mkg/mL], causing agglutination of erythrocytes</th>
<th>Relative potency of interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Dog</td>
<td>1.22</td>
<td>128</td>
</tr>
<tr>
<td>2.</td>
<td>Sheep</td>
<td>4.9</td>
<td>32</td>
</tr>
<tr>
<td>3.</td>
<td>Horse</td>
<td>4.9</td>
<td>32</td>
</tr>
<tr>
<td>4.</td>
<td>Rabbit</td>
<td>9.7</td>
<td>16</td>
</tr>
<tr>
<td>5.</td>
<td>Guinea pig</td>
<td>18.4</td>
<td>8.4</td>
</tr>
<tr>
<td>6.</td>
<td>Goat</td>
<td>155</td>
<td>1</td>
</tr>
<tr>
<td>7.</td>
<td>Rat</td>
<td>155</td>
<td>1</td>
</tr>
<tr>
<td>8.</td>
<td>Human (O, A and B groups)</td>
<td>&gt;10 000</td>
<td>&lt;0.015</td>
</tr>
<tr>
<td>9.</td>
<td>Cow</td>
<td>&gt;10 000</td>
<td>&lt;0.015</td>
</tr>
</tbody>
</table>

*The minimal concentration of lectin that agglutinated rat erythrocytes is considered as one point in the calculation of the relative potency of interaction.*

Interation of the PIFA with carbohydrates and glycoproteins (Table 3) indicates that its binding center is complementary to the oligosaccharide structure. N-acetyl-D-lactosamine inhibited hemagglutinating activity, while lactose demonstrated no inhibitory effect. Among the tested glycoproteins, lectin preferentially interacted with fetuin and did not interact with human blood group-specific substances and mannose- or glucose biopolymers.

### Table 3 – Interaction of *Paxillus involutus* lectin with carbohydrates and glycoproteins

<table>
<thead>
<tr>
<th>No.</th>
<th>Carbohydrate or glycoprotein</th>
<th>Minimal concentration of carbohydrate or glycoprotein that inhibits hemagglutination of 4 units lectin activity [mM or %]</th>
<th>Relative inhibitor potency*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>N-acetyl-lactosamine</td>
<td>75</td>
<td>–</td>
</tr>
<tr>
<td>2.</td>
<td>Fetuin</td>
<td>0.015%</td>
<td>16</td>
</tr>
<tr>
<td>3.</td>
<td>α2-Macroglobulin</td>
<td>0.03%</td>
<td>8</td>
</tr>
<tr>
<td>4.</td>
<td>Bovine thyroglobulin</td>
<td>0.06%</td>
<td>4</td>
</tr>
<tr>
<td>5.</td>
<td>1% Bovine salivomucin</td>
<td>0.125%</td>
<td>2</td>
</tr>
<tr>
<td>6.</td>
<td>Orosomucoid</td>
<td>0.125%</td>
<td>2</td>
</tr>
<tr>
<td>7.</td>
<td>Transferrin</td>
<td>0.125%</td>
<td>2</td>
</tr>
<tr>
<td>8.</td>
<td>1% Sheep submaxillary mucin</td>
<td>0.125%</td>
<td>2</td>
</tr>
<tr>
<td>9.</td>
<td>Human immunoglobulin G</td>
<td>0.25%</td>
<td>1</td>
</tr>
<tr>
<td>10.</td>
<td>1% Bovine asialosalivomucin</td>
<td>0.25%</td>
<td>1</td>
</tr>
</tbody>
</table>

*Table does not include carbohydrates that do not possess interaction with lectin in the concentration of 100 mM: D-glucose, D-galactose, D-mannose, D-fructose, L-arabinose, D-ribose, D-xylene, α-methyl-L-rhamnose, α-methyl-D-mannopyranoside, α-methyl-D-galactopyranoside, β-methyl-D-galactopyranoside, α-methyl-D-glucopyranoside, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, lactose (Galβ1,4Glc), melibiase (Galα1,6Glc), gentiobiose (Glcβ1,6Glc), tregalose (Glcβ1,1 Glc); polysaccharides and glycoproteins in 1% concentration: yeast mannans, pig liver glycogen, starch, egg’s albumin, alkaline phosphatase from calf intestine. *Relative inhibitor potency of human immunoglobulin G was accepted as the unit of measure. PIFA demonstrated no interaction with glycoconjugates that contained terminal Man, GlcNAc or DGlc residues, disaccharides GlcNAcβ1-2Man, Manα1-3Man, but interacted with glycoproteins that exposed chains of N-acetyl-lactosamine (Galβ1-4GlcNAc). However, connection of L-fucose to N-acetyl-lactosamine completely inhibited its interaction with the lectin (oligosaccharide of LFucα1-2Galβ1-4GlcNAc structure, which is a part of group-specific substances of human blood was completely inactive). Similarly, alkaline phosphatase, whose antennas are represented by oligosaccharidic chains of Galα1-3Galβ1-4GlcNAc also did not interact. Obviously, connection not only of αLFuc, but also of αDGal to N-acetyl-lactosamine completely deprived such structure binding activity. Lectin histochemistry It was documented complete lack of PIFA binding to tissue samples of heart and adrenal gland. In the lymph node, thymus and spleen, lectin faintly labeled collagen fibers and plasma membranes of lymphocytes. In the liver, lectin receptor sites were detected within the stromal components of portal tracts, vascular membranes of hepatocytes, rest of the hepatic parenchyma being non-reactive. In the submandibular salivary gland, lectin labeling was characteristic for cells of ductal system (intercalated, striated, and interlobular ducts), with acinar cells being completely negative (Figure 4). The most intensive signal was received from perinuclear cytoplasm apparently corresponding to Golgi complex of ductular epithelium.
Lectin purification from fruiting bodies of brown roll-rim fungus, *Paxillus involutus* (Fr.) Fr., and its application...

In the kidney, PIFA label was restricted to tubular epithelium (predominantly to perinuclear and basal cytoplasm with negative nuclear images); on the contrary, nuclei of podocytes and mesangioocytes in within renal corpuscles demonstrated strong lectin reactivity (Figure 5A). Histotopography of RCA-120 receptor sites in kidney was similar, yet not identical: *i.e.*, located predominantly in the apical cytoplasm and brush border of tubular epithelium, with higher compared to PIFA background staining of renal corpuscles (Figure 5B).

In the cerebral cortex, scattered cells mainly in the pyramidal and ganglionic cell layers were lectin-positive.

In cerebellum, PIFA selectively labeled Purkinje cells, with rather faint background staining of granular layer cells and medullary fibers (Figure 6A). RCA, similarly to PIFA, labeled Purkinje cells of cerebellum, but showed higher reactivity with cells and fibers of granular and molecular layers (Figure 6B).

In the stomach, PIFA receptor sites richly decorated entire cytoplasm of parietal cells with negative image of nuclear regions. It should be noted also strong lectin binding to mucin rich surface barrier of gastric mucosa, with higher exposition in pylorus then in the body of stomach (Figure 7A). RCA compared to PIFA expressed higher affinity towards carbohydrate determinants of mucin barrier, epithelial lining of gastric pits and stromal elements of gastric mucosa (Figure 7B).

In the small intestine, it was detected PIFA labeling of absorptive cells of villi and crypts: in these cells, lectin receptor sites were concentrated mainly in the apical cytoplasm and brush border glycoconjugates. Goblet cells of both villi and crypts were PIFA negative (Figure 8, A and B). RCA expressed significantly higher reactivity towards apical cytoplasm and to the brush border of absorptive cells of villi and crypts (Figure 8, C and D).

In the colon, similarly to small intestine, PIFA and RCA labels were restricted mainly to apical cytoplasm and brush border of luminal absorptive cells, lacking in goblet cells of both surface and crypts of the mucosa (Figure 9, A–D). Similarly to other gastrointestinal organs, PIFA and RCA intensely labeled stromal elements (collagen fibers, scattered connective tissue cells) of mucosa and serosa.

With regard to background staining seen in Figures 4–8, we presume that it is caused by the exposure of residual amounts of lectin reactive carbohydrates, since areas lacking a tissue and certain cell nuclei look optically empty.

Figure 4 – Histotopography of PIFA receptor sites in rat submandibular salivary gland: strong selective reactivity of ductular epithelium (perinuclear cytoplasm, basement membrane, luminal surface), acinar cells being completely areactive (SD – Striated ducts, A – Acini, ×400).

Figure 5 – Renal structures reactivity with PIFA (A) in comparison to RCA (B): receptor sites for both lectins restricted to perinuclear cytoplasm of tubular epithelium, nuclei of podocytes and mesangioocytes; RCA binding additionally exposed in the apical cytoplasm of proximal tubules cells, background staining of renal corpuscle exceeding that with PIFA (PT – Proximal tubules, PC – Podocytes, MC – Mesangioocytes nuclei, ×400).
Figure 6 – Cerebellar cortex: selective labeling of Purkinje cells with PIFA (A); exposition of RCA receptor sites (B) – besides Purkinje cells, components of molecular and granular cell layers are stained (PCL – Purkinje cell layer, PC – Purkinje cell, ML – Molecular layer, GCL – Granular cell layer, ×400).

Figure 7 – Gastric mucosa: PIFA reactivity restricted to parietal cells and cytoplasmic glycoconjugates of surface epithelium (A); RCA demonstrate more intensive binding to luminal mucus, as well as to apical cytoplasm of surface epithelium (B) (PC – Parietal cells, SML – Surface mucosal layer, F – Gastric pits, ×400).
Figure 8 – Small intestine labeled with PIFA (A and B) in comparison to RCA (C and D), villi (A and C) and crypt (B and D) regions represented: strong reactivity of brush border and apical cytoplasm of absorptive cells with negative reaction of goblet cell glycoconjugates; RCA reactivity exceeds that of PIFA with no qualitative differences (BB – Brush border, AC – Absorptive cells, GC – Goblet cells, ×400).
Figure 9 – Large intestine: PIFA (A and B) versus RCA (C and D) reactivity in the luminal region (A and C) and within the depth of crypts (B and D); receptor sites for both lectins located predominantly in the brush border and apical cytoplasm of luminal absorptive cells with zero reactivity of goblet cells of both surface and crypt regions; no significant differences in PIFA and RCA binding (AC – Absorptive cells, BB – Brush border, GC – Goblet cells, ×400).

Discussion

The main difficulty in PIFA purification consisted in the fact that during extraction a high amount of viscous polysaccharide passed into the solution and strongly interfered all subsequent operations. It was found that the polysaccharide almost completely precipitated after adding to extract 0.5 volume of 96% ethanol, lectin retained solubility at this concentration of ethanol. However, lectin is inactivated during precipitation by two volumes of ethanol. Thereafter polysaccharide was removed by dialysis, after that lectin was concentrated by precipitation with ammonium sulfate. Our studies demonstrated that this last step is not necessary – it should be preferred immediately after dialysis against 0.05 M phosphate buffer, pH 7 to perform ion-exchange chromatography on DEAE-Toyopearl column, balanced with the same buffer.

Surprisingly, it turned out that despite the relatively good interaction with submaxillary mucin, bovine thyroglobulin, and α₂-macroglobulin of human blood serum (Tables 1 and 3), PIFA lectin did not interact with these same immobilized glycoproteins, whereas immobilized mannose-specific lectins were of appropriate quality adsorbents for PIFA (Table 1).

Interaction of PIFA lectin with Polygonatum multiflorum lectin, concanavalin A and Leucojum vernum lectin, which are pure proteins (do not containing carbohydrate moieties in their structure) apparently indicates presence of mannose-containing carbohydrate chains in the molecule of Paxillus involutus lectin. For instance, it is possible to purify some lectins (which are glycoproteins) on immobilized concanavalin A. Among them, soybean lectin, which contains 7% carbohydrates. Carbo-
and interaction with erythrocytes of immobilized fetuin. Whereas the native lectin readily interacted with fetuin, whereas the native lectin readily interacted with immobilized bovine thyroglobulin, and α₂-macroglobulin from human blood, that is quite surprising observation, considering the interaction of native substances with lectin (Table 3). Similar data was reported by Haiselova et al. [11], who purified lectin from close species P. atrotomentosus, which weakly sorbed on immobilized fetuin, whereas the native lectin readily interacted with fetuin.

Our studies evidence that carbohydrate specificity and interaction with erythrocytes of P. involutus lectin is significantly different from that of P. atrotomentosus lectin. For instance, according Haiselova et al. [11] affinity purified P. atrotomentosus lectin agglutinated human erythrocytes (non-specifically to groups ABO) at a minimum concentration of 0.13 mg/mL, while the lectin from P. involutus did not agglutinate human erythrocytes even at a concentration of 10 000 mg/mL (Table 2).

To lactosamine-specific lectins according to the literature data belong Ricinus communis (RCA-120), Datura stramonium, Erythrina crista-galli and marine sponges Geodia cydonium [23], as well as the lectin from the fungus Laetiporus sulphureus [24]. Among animal lectin specific to lactose and N-acetyl-lactosamine are galectins – lectin family, well represented in both – lower invertebrates and in mammals [25]. Recent studies revealed their involvement into multiple important biological processes, i.e. cell adhesion, regulation of cell growth, inflammation and metastasis in cancer [26]. Therefore, search for novel lactosamine-specific lectins as prospective histochemical reagent should be treated as an important issue. Since all the above-mentioned lectins, except R. communis agglutinin (RCA-120), are still expensive and hardly obtainable, for the comparative histochemical studies we have used RCA-120.

It was detected high homology in the histochemical specificity in between PIFA and RCA-120. These similarities of binding can be referred to the fact that both lectins among disaccharides preferably interact with N-acetyl-lactosamine. However, there are existing certain differences in the carbohydrate specificities of these two lectins. In particular, RCA-120 well interacts with tri-antennal structure with Man(α1-2)Man residues on terminal endings (Figure 10) [22].

Such structure of the carbohydrate chains is quite common among lectins obtained from plants and is quite possible for the PIFA lectin as well.

Earlier we detected high affinity to alkaline phosphatase of a lectin, purified from Mycena pura fungus [9]. Comparison histochemical specificities of P. involutus lectin with those of M. pura fungus [9], as well as 10 other lectins with different carbohydrate affinities [29] proved that PIFA is closely related to DGalNAc-specific lectins, namely to RCA-120. However, biochemical and histochemical studies suggest that carbohydrate specificities of these two lectins is somewhat different as reflected by high agglutination ability of RCA-120 to human erythrocytes and complete absence of the same of PIFA.

P. involutus lectin demonstrated heavy labeling of glycoconjugates in the apical cytoplasm and brush border of small and large intestine, but showed zero reactivity with goblet cell of the same location. We presume this lack of binding due to masking of lactosamine or other PIFA-reactive carbohydrates by sialic acid and fucose residues as epitopes of goblet cell glycoconjugates. Interestingly, that earlier it was reported a selective and differential labeling of goblet cells in the rat colon by means of HPA, WGA and GS-I lectins [30]. These supplementary data encompass presence of high amount of DGlcNAc residues, lack of terminal lactosamine, addition of DGalNAc and DGal determinants to glycoconjugates. Probably, that due to the unique carbohydrate specificity PIFA lectin can cover position in experimental histochemistry and diagnostic histopathology comparable to PNA (Peanut agglutinin) and SNA (Sambucus nigra agglutinin).

Conclusions

We report here a method of lectin purification from Paxillus involutus fungus. Combining of biochemical and histochemical data make it possible to assume that PIFA lectin has unique carbohydrate specificity to N-acetyl-lactosamine determinants, unshielded with α-L-fucose and α-D-galactose. Reported results on the selectivity of PIFA binding proved prospective usefulness of this lectin in histochemistry, for instance, as selective label of gastric parietal cells, submandibular gland ductual epithelium, cerebellar Purkinje cells, as well as a tool to investigate redistribution of its receptor sites under physiological and pathological conditions. We presume that due to the unique carbohydrate specificity PIFA lectin can cover position in experimental histochemistry and diagnostic histopathology comparable to PNA (Peanut agglutinin) and SNA (Sambucus nigra agglutinin).

References

Antonyuk VO, Yashchenko
Winter HC, Mostafapour K, Goldstein IJ,
Varki A, Cummings RD, Esko JD, Freeze HH, Stanley P,
Gabius HJ, Roth J,
Shifrin S, Consiglio E, Kohn LD,
Kochetkova NK (ed),
Lazurevsky GV, Terentyeva IV, Shamshurin AA,
Lutsyk MD, Panasiuk EN, Antonyuk VA, Lutsyk AD, Ladnaya LY,
DuBois M, Gilles KA, Hamilton JK, Rebers PA, Smith F,
Reviews and selected papers from the 16
In: Van Driessche E, Rougé P, Beeckmans S, Bøg-Hansen TS

Antonyuk VO, Yashchenko
Winter HC, Mostafapour K, Goldstein IJ,
Varki A, Cummings RD, Esko JD, Freeze HH, Stanley P,
Gabius HJ, Roth J,
Shifrin S, Consiglio E, Kohn LD,
Kochetkova NK (ed),
Lazurevsky GV, Terentyeva IV, Shamshurin AA,
Lutsyk MD, Panasiuk EN, Antonyuk VA, Lutsyk AD, Ladnaya LY,
DuBois M, Gilles KA, Hamilton JK, Rebers PA, Smith F,
Reviews and selected papers from the 16
In: Van Driessche E, Rougé P, Beeckmans S, Bøg-Hansen TS

Effect of the complex
A process for preparing an affinity adsorbent for the purification of lectins, author’s certificate USSR N

Barondes SH, Cooper DNW, Gitt MA, Leffler H, Galecins.

