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PURIFICATION AND PROPERTIES OF LECTIN FROM POTATO TUBERS AND LEAVES; INTERACTION WITH ACID PHOSPHATASE FROM POTATO TUBER*

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The haemagglutination activity of lectin, (STA) was determined during vegetation of potatoes (Solanum tuberosum var. Uran). Traces of haemagglutination activity were found in young stalks and first leaves. In the next leaves the maximal activity appeared after 6 - 8 weeks of vegetation and, simultaneously with the decrease in leaves it appeared in young tubers (at the time of their differentiation from stolons), reaching the maximum in the 18th week of vegetation.

Lectins from potato tubers and leaves were extracted with acetate buffer, pH 3.6, salted out with ammonium sulphate, and purified alternatively by affinity chromatography on GlcNAc-Spheror or by adsorption on trypsinated and glutaraldehyde-fixed rabbit erythrocytes. Chromatography on Sephadex G-200 was used for preparative separation of isomeric forms. In the tubers four isolectins were found, differing in molecular mass, pl values, content of sugars and haemagglutination activity. Two of these forms were isolated in homogeneous form: a heavy dimeric form, $M_r$ 130 000 - 142 000 containing 59.6% of sugars (including 28.8% arabinose, 6.9% galactose, 16.5% glucose, 1.6% xylose and 3.1% mannose), and a light monomeric form, of $M_r$ 21 000 - 22 000, containing 20% of neutral sugars (2.5% of pentoses and 17.5% of hexoses). From leaves, a single heavy dimeric lectin of $M_r$ 136 000, containing 14.3% of neutral sugars was isolated.

The effect of tuber isolectin on the activity, pH optima and heat-stability of the potato acid phosphatase has been described.

Lectins of plant origin are either simple proteins, metallo-proteins or glycoproteins [1, 2]. They bind carbohydrates and agglutinate various types

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of cells and cellular organelles [1, 2]. Lectin from potato-tuber (STA-tb) was first discovered by Marcussen-Begun [3] and isolated by Marinkovich [4] and Morawiecka [5]. Physico-chemical characteristics of STA was given by Allen et al. [6] and, more recently, by Matsumoto et al. [7]. It has been shown that STA is an unusual glycoprotein containing over 50% of sugars, mainly arabinose, rich in hydroxyproline, and binding specifically mono- and oligomers of β-D(1,4)-N-acetylglucosamine [6, 7]. Multiplicity of STA forms differing in pl was reported by Allen et al. [6] and differing in \( M_r \) values, by Van Driessche & Kanarek [8] and Kilpatrick et al. [9]. Besides, Kilpatrick described the occurrence of specific STA form in the juice and pericarp of potato fruit [10].

Common occurrence of lectins in the storage organs and developing plant tissues led to several hypotheses on their physiological role [2, 11]. Recently, Casalongue & Lezica [12] have demonstrated that STA is associated with cell wall, and suggested that it is involved in the maintenance of the cell-to-cell interaction.

In the present work we studied the time-course of changes in the haemagglutination activity of STA during vegetative development of potato plants as well as physico-chemical properties of isolectins isolated and purified from potato tuber and leaves. The evidenced effect of STA on some catalytic properties of acid phosphatase from potato tubers suggests that this effect may also concern other enzymes.

MATERIAL

Material. Potato tubers (Solanum tuberosum var. Uran) were planted in the field in Spring (April - May), and a few plants were collected every two weeks; they were separated into roots, stalks, leaves, stolons, young tubers, and old maternal tubers. For isolation of lectins, tubers collected in Autumn and leaves collected in Summer (July - August) were used.

Erythrocytes from rabbit blood were obtained from the Medical Academy in Wroclaw.

Reagents. Concanavalin A was from Serva (F.R.G.), ampholin from LKB (Sweden). Acid phosphatase from potato tubers was prepared as described by Kruzel & Morawiecka [13], other reagents were of analytical grade, either purchased from the known producers or of Polish production.

1 Abbreviations used: STA, Solanum tuberosum agglutinin; STA-tb, lectin isolated from potato tubers; STA-ll, lectin isolated from potato leaves; ConA, lectin from Canavalia ensiformis; SDS, sodium dodecyl sulphate; PAGE, polyacrylamide gel electrophoresis; GlcNAc, N-acetylglucosamine; GalNAc, N-acetylgalactosamine; GlcNAc-Spheron, N-acetylglucosamine-Spheron; pNPP, p-nitrophenyl phosphate.
METHODS

Trypsination and glutarylation of rabbit erythrocytes. The method applied was essentially that described by Turner & Leiner [14].

The agglutination test. The haemagglutination activity was determined on microplates Microtiter U with decreasing concentrations of lectin in 10 mM phosphate buffer, pH 7, containing 150 mM NaCl. Agglutination of TGF erythrocytes was determined after 1 h at room temperature. The lowest amount of protein agglutinating TGF rabbit erythrocytes was designated as one unit of activity.

Protein determination. This was performed by the method of Lowry et al. [15].

Determination of haemagglutination activity during plant vegetation. Plant material collected successively during vegetation was homogenized with an equal volume of 100 mM acetate buffer, pH 3.6, containing 2 mM Na₂S₂O₅. The homogenates were left for 2 h at 4°C, then centrifuged at 6000 g for 30 min. In the supernatants, protein and agglutination activity with TGF erythrocytes were determined [15].

Isolation of lectin from potato tubers (STA-ub) and leaves (STA-ff). Potato tubers were washed, peeled and cut into small fragments. The fragmented tubers (1000 g) or leaves (100 g) were treated as described above in the lectin assay. After 2 h extraction, the suspensions were decanted, squeezed through four layers of gauze, and the remainder was treated again with half of the initial volume of acetate buffer. The combined extracts were centrifuged at 6000 g for 30 min at 4°C. The extracted proteins were salted out with ammonium sulphate at 0.6 saturation. After 12 h, the sediment was collected by centrifugation, dissolved in 20 mM acetic acid, dialysed for 24 h against two changes of the same solvent, and finally freeze-dried. In preparation of lectin from leaves, after salting out the dissolved sediment was desalted on Bio-Gel P5 column (5 × 60 cm) and freeze-dried. The preparations were further purified either by GlcNAc-Spheron affinity chromatography or by specific adsorption on TGF rabbit erythrocytes [16].

Affinity chromatography on GlcNAc-Spheron. Glycosylation of Spheron P-300 with N-acetyl-d-glucosamine was performed by the method of Filka et al. [17]. The partially purified lyophilized preparations of lectin (about 2 g protein) from tubers or leaves were dissolved in 10 mM phosphate buffer, pH 7.2, containing 150 mM NaCl and were subjected to affinity chromatography on GlcNAc-Spheron column (2 × 30 cm). The non-adsorbed proteins were eluted with the same buffer till complete disappearance of absorbance at 280 nm. The adsorbed proteins showing agglutination
activity were eluted with 100 mM acetic acid [8], dialysed against 20 mM acetic acid and freeze-dried.

**Adsorption on TGF rabbit erythrocytes.** The same partially purified lyophilized lectin preparations were dissolved in phosphate buffer, pH 7.2, containing 150 mM NaCl. The TGF erythrocytes centrifuged at 3000 g (1 ml) were then mixed with 4 ml of protein solution (25 mg/ml) and left for 1 h at room temperature. After centrifugation, the supernatant containing non-adsorbed proteins was discarded and erythrocytes were washed with 4-ml portions of the buffer till disappearance of absorbance at 280 nm. The packed erythrocytes were then washed with 4 - 5 portions of 100 mM acetic acid to release bound lectin proteins. The active fractions were pooled, dialysed against 20 mM acetic acid, and freeze-dried.

**Separation of isolectins on Sephadex G-200.** The lectin preparations from potato tubers purified either by chromatography on GlcNAc-Spheron or by adsorption on TGF erythrocytes were dissolved in 4 M urea (10 mg protein/ml) and kept for 24 h before being applied on Sephadex G-200 column (1 × 100 cm) equilibrated with 4 M urea. Fractions of 2 ml were collected at a flow rate of 4 ml/h, at room temperature. The active fractions were pooled, urea was removed on a Bio-Gel P4 column (5 × 30 cm), and finally the samples were freeze-dried.

**Polyacrylamide gel electrophoresis.** Electrophoresis of the purified lectin preparations was run in 15% gel at pH 2.7 according to Panyim & Chalkley [18] and in 5% gel at pH 9.5 according to Smith et al. [19] in tubes (0.5 × 10 cm). Lectin (50 μg) dissolved in 0.9 M acetic acid or Tris/borate buffer, pH 9.5 was applied on the gel. Gels run at pH 2.7 were stained for protein with Coomassie Brilliant Blue (CBB R-250) according to Fairbanks et al. [20], and at pH 9.5 according to Chrambach et al. [21]. Sugars in gels were detected with basic fuchsin [22].

**Determination of molecular mass.** The M<sub>r</sub> values of lectins were determined by electrophoresis in 9% polyacrylamide gel at pH 7.1 in the presence of 0.1% SDS [23]. For this purpose, lectins (50 μg) previously heated at 100°C for 3 min were applied on the gel together with standard proteins: phosphorylase b (M<sub>r</sub>, 94 000), bovine serum albumin (70000), ovalbumin (43 000), carbonic anhydrase (30 000), trypsin inhibitor (20 100) and α-lactoalbumin (14 400). Gels were stained for protein with 0.25% Coomassie Brilliant Blue R-250 in a mixture of 1.5% methanol and 3.2% acetic acid in water and destained with a mixture of 7.5% methanol - 50% acetic acid in water. Sugars were stained with basic fuchsin according to Zacharius et al. [22].

**Molecular masses of isolectins** (*STA*-tb-1, *STA*-tb-4 and *STA*-lf) were determined by molecular gel filtration on Bio-Gel P-150. The column
(0.8 × 100 cm) was equilibrated with 100 mM buffer, pH 5.1. The following proteins were used as standards: aldolase (M, 158 000), bovine serum albumin (68 000), ovalbumin (45 000), chymotrypsinogen A (25 000) and cytochrome c (12 500). Fractions of 2 ml were collected at a flow rate of 3 ml/h, at room temperature.

Molecular masses were additionally determined from the sedimentation constant (S_{20,w}) and the diffusion coefficient (D). A MOM type 3170b (Hungary) ultracentrifuge was used. The protein concentration gradient was measured in the Philbot-Svenson optical system at 20°C at 55 000 rev./min, against three concentrations of the lectin solution (2, 4 and 5 mg/ml). The diffusion coefficient was determined at 8 000 rev./min [24]. Partial specific volume (ν) of the protein was calculated from the amino acid and sugar composition of the STA-tb preparation and from the partial specific volumes of the amino acid and sugar residues.

Isoelectric focusing. For determination of the isoelectric point, the method of Karlsson et al. [25] was used; 5% polyacrylamide gel contained 6% ampholine (pH range 3.5-10). Isoelectric focusing was performed on a plate (260 × 125 × 2 mm) using the LKB (Sweden) Multiphor 2117 System. To determine pH gradient, the unstained gel was cut into 5-mm slices, each slice was extracted overnight with 1 ml water. The haemagglutination activity was located by a similar procedure, except that 2-mm slices were extracted with 0.1 ml portions of 100 mM phosphate-buffered saline, pH 7.

Determination of carbohydrate components of lectin. Neutral sugars were determined by the phenol-sulphuric acid method [26] with galactose as a standard, pentose by the method of Mejbaum [27] with arabinose as a standard, and hexosamines according to Ludowieg & Benmaman [28] using as standards glucosamine (at 535 nm) and galactosamine (at 525 nm).

Monosaccharides in the STA-tb preparation were identified as alditol derivatives by gas-liquid chromatography using Varian 2100 Chromatograph and 3% ECNSS-M Gaschrom-G (Varian, Zug, Switzerland) with inositol and xylose as internal standards [29].

Effect of STA-tb lectin on acid phosphatase from potato tubers. The preparation of STA-tb purified on GlcNAc-Spheron and acid phosphatase, isolated according to Kruzel & Morawiecka [13] were used. The activity of the enzyme was determined using pNPP as a substrate [13] or by the Fiske-Subbarow method [30] when other phosphate monoesters served as substrates. The enzymatic activity was expressed in micromoles of phosphorus liberated during 1 min at 30°C at pH 5.1.

To determine the effect of lectin, acid phosphatase was preincubated for 20 min at 30°C at the enzyme-lectin ratios ranging from 1:0.5 to 1:10.
The apparent $K_m$ values for acid phosphatase were determined at the two enzyme-lectin ratios (1:0.5 and 1:10) against $p$NPP concentration ranging from 0.25 to 8 mM. After 20 min preincubation at 30°C, the enzyme activity was determined and referred to control incubated without lectin.

Substrate specificity of acid phosphatase was determined at the enzyme-lectin ratios of 1:0.5 and 1:10 using (10 mM) $p$NPP and ATP. Lectin was incubated with the enzyme for 20 min at 30°C and the amount of phosphorus liberated was determined by the Fiske-Subbarow method [30].

To measure thermal stability of acid phosphatase, the enzyme was preincubated with lectin under the above-mentioned conditions, then the mixture was kept for 10 min at temperatures ranging from 20 to 80°C; and phosphatase activity was determined [13].

The pH optimum of acid phosphatase in the presence of lectin was examined with $p$NPP as a substrate at 3.45 - 9.0 pH.

RESULTS

The haemagglutination activity in potato plants during vegetation. The agglutination of rabbit TGF erythrocytes by the extracts from roots, stalks, leaves, stolons and both maternal and young tubers (if any) is presented in Fig. 1. The haemagglutination activity decreased in the maternal tuber during plant vegetation, was absent in roots, and but traces were found in young stalks. After 4 weeks the activity appeared in the first leaves, rose rapidly reaching the maximum after 6 - 8 weeks of vegetation, and then gradually declined. In young tubers formed from stolons the haemagglutination activity appeared in the 14th week of vegetation and
increased rapidly to the maximum within the 18th week. At that time the maternal tuber decayed and leaves became dry.

**Isolation and properties of lectins**

The procedure for purification of lectins from potato tubers and leaves is given in Table 1. The lectin obtained either by affinity chromatography on GlcNAc-Spheron or adsorption on TGF erythrocytes were purified about 200-fold; the yield was 27% in the case of potato leaves, and 48% for tubers. Electrophoresis of the preparations under non-denaturing conditions is given in Plate 1. At this stage of purification the leaf lectin showed on PAGE-SDS at pH 7.1 a single glycoprotein band (Plate 2) whereas the lectin from tubers was a mixture of four isolectins (STA-tb-1-4) which were resolved on Sephadex G-200 in the presence of 4 M urea (Fig. 2). Fraction STA-tb-1 agglutinates erythrocytes at the protein concentration as low as 0.01 µg, whereas fraction tb-1 and tb-4 subjected to PAGE-SDS gave a single protein band, of $M_r$ 70,500 and 22,700, respectively, whereas fractions STA-tb-2 and STA-tb-3 were still heterogeneous (Plate 3). It was found that $M_r$ of the leaf lectin was 70,500, i.e. the same as that of STA-tb-1 (Plate 1).

To check whether $M_r$ for STA-tb-1 and STA-lf are comparable the preparations were subjected to ultracentrifugation (Fig. 3) and gel filtration

### Table 1

**Purification of lectin from tubers (STA-tb) and leaves (STA-lf) of potato (Solanum tuberosum)**

For purification, 1000 g of tubers harvested in Autumn and 100 g of leaves collected after 6 weeks of vegetation, were used

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Haemagglutination activity</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>specific (units/mg protein)</td>
<td>total ($10^{-3}$ x units)</td>
</tr>
<tr>
<td>Extract with 100 mM acetate buffer, pH 3.6</td>
<td>105</td>
<td>980</td>
</tr>
<tr>
<td>Ammonium sulphate 0.6 sat., dialysis, lyophilization</td>
<td>75</td>
<td>133</td>
</tr>
<tr>
<td>Chromatography on GlcNAc-Spheron or adsorption on TGF-erythrocytes</td>
<td>115</td>
<td>730</td>
</tr>
<tr>
<td>Sephadex G-200 chromatography in the presence of 4 M Urea</td>
<td>1430</td>
<td>127</td>
</tr>
<tr>
<td>20000</td>
<td>472</td>
<td>48</td>
</tr>
<tr>
<td>20000</td>
<td>36</td>
<td>27</td>
</tr>
<tr>
<td>90000</td>
<td>272</td>
<td>90000</td>
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<tr>
<td>12000</td>
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<td>—</td>
</tr>
<tr>
<td>15000</td>
<td>130</td>
<td>—</td>
</tr>
<tr>
<td>5000</td>
<td>8</td>
<td>—</td>
</tr>
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</table>
Fig. 2. Gel filtration of tuber lectin on Sephadex G-200 in the presence of 4 M urea

on Bio-Gel P150 under non-denaturing conditions. The value of $M_r$ obtained by ultracentrifugation were similar to those obtained by molecular sieving, and were: 130 000 for STA-tb-1 and 129 000 for STA-lf.

When the results of $M_r$ determination by gel filtration, ultracentrifugation and PAGE-SDS (Plate 1, 2) are compared, it becomes evident that STA-tb-1 and STA-lf have dimeric structure while STA-tb-4, of the same $M_r$ in denaturing PAGE-SDS and non-denaturing conditions, appears to be a monomer.

Gas-liquid chromatography showed that the non-resolved tuber lectin purified by the affinity chromatography on GlcNAc-Spheron contained 28.8%
Plate 1. Electrophoresis of potato lectin at pH 2.7 in 15% polyacrylamide gel (A) and at pH 9.5 in 5% gel (B). 1. STA-tb purified on GlcNAc-Spheron; 2. STA-tb purified on TGF erythrocytes; 3. STA-If purified on GlcNAc-Spheron. P, Proteinogram; G, glycoproteinogram. For details see Methods.

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Plate 2. Electrophoresis of potato lectin in 9% polyacrylamide gel at pH 7.1 in the presence of 1% sodium dodecyl sulphate. Preparation purified on GlcNAc-Spheron: 1, STA-tb; 2, STA-lf. P, Proteinogram; G, glycoproteinogram. For details see Methods

Plate 3. Electrophoresis in 9% polyacrylamide gel at pH 7.1 in the presence of 0.1% SDS, of four tuber lectin fractions eluted from Sephadex G-200 with 4 M urea. P, Proteinogram; G, glycoproteinogram
arabinose, 1.6% xylose, 16.5% glucose, 6.9% galactose and 3.1% mannose, i.e. about 60% of neutral sugars. The subunit of tuber lectin STA-tb-1 contained about 45% of neutral sugars, including about 20% of pentoses. Other isolectin forms were generally poorer in the carbohydrate component and differed in proportions of hexoses and pentoses. Lectin from leaves contained only 14.3% of natural sugars (Table 2) as measured by gas-liquid chromatography.

Isoelectric focusing of STA-tb purified on GlcNAc-Spheron (Fig. 3) resulted in separation of four fractions of isoelectric points corresponding to pH 6.3, 7.3, 8.3 - 8.5 and 9.4. The last one corresponded to STA-tb-1 and STA-lf.

Interaction of tuber lectin with acid phosphatase. The effect of lectin on optimum pH, thermostability and substrate specificity was studied with

Table 2

<table>
<thead>
<tr>
<th>Lectin</th>
<th>$M_r$</th>
<th>Neutral sugars %</th>
<th>Hexoses %</th>
<th>Pentoses %</th>
</tr>
</thead>
<tbody>
<tr>
<td>STA-lf*</td>
<td>70 500</td>
<td>14.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>STA-tb-1b</td>
<td>70 500</td>
<td>45.2</td>
<td>25.8</td>
<td>19.4</td>
</tr>
<tr>
<td>STA-tb-4b</td>
<td>22 700</td>
<td>20.0</td>
<td>17.5</td>
<td>2.5</td>
</tr>
</tbody>
</table>

* Preparation after GlcNAc-Spheron chromatography

 STA-tb-4b Fractions from Sephadex G-200 chromatography

acid phosphatase isolated from **Solanum tuberosum** tubers [13] at different enzyme-lectin ratios. For comparison, concanavalin A was used.

The effect of STA-tb on the activity of tuber acid phosphatase depended on the enzyme:lectin ratio and was the most pronounced (39%) at the 1:10 ratio; at the lower ratio of 1:0.5 this increase was half of the maximal value (Fig. 4A). It is noteworthy that no differences in the enzyme activity were observed on interaction with ConA. It has been shown that the increase in the activity of potato acid phosphatase was not due to a higher affinity of the tuber enzyme to the substrate, pNPP; the $K_m$ value for the enzyme was 0.27 mM, and in the presence of STA-tb, at the enzyme-lectin ratios of 1:10, $K_m$ was 0.21 mM. It is of interest that the purified preparation of tuber acid phosphatase showed no activity towards ATP whereas in the presence of STA-tb ATP became the substrate of the enzyme. The enzymatic activity was 50% of that with pNPP as a substrate.
As shown in Fig. 4B the optimum time for the enzyme-STA-tb interaction (1:10, w/w) was 20 min; on longer preincubation the enzymatic activity was decreased although the agglutination of erythrocytes by STA-tb was not changed during interaction with the enzyme.

In the presence of potato tuber lectin, the pH optimum of catalytic activity of tuber acid phosphatase was shifted from pH 5.5 to pH 5.0 both at the enzyme:STA-tb ratio of 1:0.5 and 1:10 (Fig. 4C).

On 20 min incubation of tuber phosphatase at 20-80°C with lectin at 1:10 or 1:0.5 ratios, its thermostability was increased. In the absence of STA-tb at temperatures exceeding 40°C the enzyme activity decreased rapidly, whereas in the presence of lectin the enzyme was affected only at temperature above 50°C (Fig. 4D).
DISCUSSION

Physico-chemical characterization of lectin isolated from potato tubers (STA) requires application of improved methods of purification of this protein [6, 7, 8]. Allen et al. [6], Kilpatrick et al. [9], and recently Matsumoto et al. [7] described methods based either on classical procedure of protein purification or on affinity chromatography [7, 11, 12, 16].

The first steps of lectin purification used by us were those of Matsumoto et al. [7]. However, in affinity chromatography (GlcNAc)_3-Sepharose 6B was replaced by GlcNAc-Spheron, or alternatively adsorption on TGF rabbit erythrocytes was tried. The preparation obtained from tubers (STA-tb) was homogeneous on PAGE at pH 9.5 and on ultracentrifugation, similarly as reported by Allen et al. [6] and Matsumoto et al. [7]. However, on electrophoresis in 15% polyacrylamide gel at pH 2.7 this preparation appeared heterogeneous, and after Sephadex G-200 gel filtration in the presence of 4 M urea it separated into four distinct forms differing in molecular mass, subunit structure and pI value. Two of these forms, STA-tb-1 and STA-tb-4 were homogeneous but the remaining two were still heterogeneous. Isolectin STA-tb-1 showed the $M_r$ value of 130 000 - 142 000, depending on the method for molecular mass determination, and dimeric structure, with the monomer $M_r$ value of 75 000. According to other authors [6, 7, 10] the dominating form of potato tuber lectin has the $M_r$ of 100 000 and is composed of two identical subunits, of $M_r$ ranging from 50 000 to 54 000. Since the “heavy” isolectin (STA-tb-1) isolated by us is the dominating form of lectin one may conclude that it corresponds to the form described by the authors cited.

The other homogeneous isolectin was the “light” form (STA-tb-4), of $M_r$ 21 400 as described by the molecular gel filtration. On PAGE-SDS the $M_r$ of the “light” form was found to be 22 700 which suggests the lack of subunit structure. Also Kilpatrick et al. [9] demonstrated the presence in potato tuber of another “light” form of lectin, with $M_r$ of 15 000.

Isolectin isolated from potato leaves (STA-lf), which so far has not been studied, is a dimer with $M_r$ of 129 000. Both the pI and the haemagglutination activity of this form are similar to those of “heavy” isolectin from tubers. However, the content of neutral sugars in leaf lectin was lower by 30% than in STA-tb-1; this could be due to difference in glycosylation of this form. “Heavy” isolectin from tubers (STA-tb-1) contained 42.5% of neutral sugars, including 20% of pentose; in the remaining isolectins the content of the carbohydrate component was significantly lower. The sugar component of STA-tb-1 included xylose, glucose and mannose, not detected by Allen et al. [6] and Ashford et al. [31] in STA of $M_r$. 
100,000 but reported by Matsumoto et al. [7] for the dominating form of STA.

Despite the numerous studies [1, 2, 5, 6, 7, 11] on the physico-chemical properties and intracellular localization [12, 32, 33, 38] of lectins their biological functions still remain unknown.

In the present work it was demonstrated that, in the maternal tuber planted in Spring, the haemagglutination activity gradually decreased whereas the activity in the first leaves reached the maximum after 6-8 weeks and in young tubers in the 14th week of vegetation. In germinating wheat seed the amount of lectin decreased by a half after 34 days and 70% of the activity was found in stalks, whereas only 30% in roots [32].

We have demonstrated previously [34, 35] that lectins raised the activity of acid phosphatases and ribonucleases. In the present work it was demonstrated that, at the enzyme:lectin ratio of 1:10, the enzymatic activity was increased by 30%, the apparent $K_m$ value remaining unchanged. The association with the enzyme did not affect the haemagglutination activity of the lectin which suggests that interaction with the enzyme involves other domains of the STA molecule. In the presence of tuber lectin the pH optimum for the catalytic activity of acid phosphatase shifted from pH 5.5 to 5.0, its heat stability was increased, and the substrate specificity was extended as the enzyme became active with ATP. Similar effects of other lectins: concanavalin A and wheat and rye germ agglutination were observed with Mg$^{2+}$-ATPase [36] and acid phosphatase from Poa pratensis seeds [37]. The latter enzyme in the presence of ConA was more resistant to the action of proteolytic enzymes. Activation by lectin of acid phosphatases from rye germ could be reversed by GlcNAc [35, 38].

Modification of some catalytic activities and physico-chemical properties of enzymes by lectins leads to the supposition that these compounds may play a role in the metabolism of both mature cells and organisms, and during their ontogenesis.

REFERENCES


