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ACID PHOSPHATASE OF POTATO TUBERS (SOLANUM TUBEROSUM L.).
PURIFICATION, PROPERTIES, SUGAR AND AMINO ACID
COMPOSITION*

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1. Acid phosphatase (AcPase) from potato tubers was purified by tannic acid
fractionation, DEAE-cellulose chromatography, filtration on Bio-Gel P-150 and
affinity chromatography on Con A-Sepharose. The enzyme was purified 260-fold
and was electrophoretically homogeneous; its mol. mass is about 69,000.

2. The carbohydrate component accounts for 16.6% of the total enzyme weight
and includes mannose (5.6%), rhamnose (3.4%), glucose (2.5%), galactose (1.5%)
and glucosamine (3.6%). In the amino acid composition aspartic acid, glutamic
acid, serine and glycine account for 37.7% of total amino acid residues.

3. Optimum pH is at 5.0-5.3. The enzyme activity was reduced by half after
30 min incubation at 60°C, and was fully abolished after 2 h incubation at 70°C.
The enzyme is a nonspecific phosphomonoesterase; aromatic phosphomonoesters
and inorganic pyrophosphate can serve as substrates. Apparent $K_m$ values were
1.25 mm and 40 mm for $p$-nitrophenylphosphate and inorganic pyrophosphate,
respectively. The enzyme is inhibited by MoO$_4^{2-}$, Zn$^{2+}$, Hg$^{2+}$ and urea. Inhibition
caused by urea was reversible at urea concentration below 9 M.

Acid phosphatase (AcPase, EC 3.1.3.2) from potato tubers is a nonspecific
phosphomonoesterase (Schmidt & Laskowski, 1961). Its mechanism of action and
kinetics were investigated by several authors (Jørgensen, 1959; Alvarez, 1962; Hsu
et al., 1966). Previously we have shown that in crude extracts from potato tubers
(Kubicz & Morawiecka, 1971) as well as in partially purified AcPase (Kubicz et al.,
1974) the enzyme appears in multiple molecular forms of similar molecular mass,
but differing in isoelectric point.

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[321]
Recently Kruzel (1977) has reported on the glycoprotein nature of the enzyme. The present paper includes the purification procedure based on ion-exchange chromatography, gel filtration and the affinity of sugar component to Concanavalin A-Sepharose. General characteristics of the purified enzyme is given.

MATERIAL AND METHODS

Reagents: Disodium p-nitrophenylphosphate, disodium β-glycerophosphate and Amido Black 10B were purchased from Merck (Darmstadt, F.R.G.); N,N,N′,N′′-tetramethylethylenediamine (TEMED), N,N′-methylene-bis-acrylamide and acrylamide were obtained from Fluka A. G. (Buchs S. G., Switzerland); Whatman DEAE-cellulose was from W. R. Balston Ltd (Maidstone, Kent, England); Bio-Gel P-150 from Bio-Rad Lab. (Richmond, Calif., U.S.A.); sodium α-naphthyl phosphate was a product of Loba-Chemie (Vienna, Austria); Con A-Sepharose from Pharmacia Fine Chemicals A. B. (Uppsala, Sweden); Tris(hydroxymethyl)-aminomethane was from Malinckrodt Chem. Works (St. Louis, Mo., U.S.A.); α-methyl-d-mannoside, disodium salts of ATP, 5′-AMP and 5′-CMP, sodium salt of 2′,3′-AMP, sodium bis-p-nitrophenylphosphate, sodium β-naphthylphosphate, disodium phenylphosphate, p-nitrophenyl acetate and dithiothreitol were the products of Sigma Chem. Co. (St. Louis, Mo., U.S.A.); other reagents were purchased from P.O.Ch. (Gliwice, Poland).

Enzyme preparation. Acid phosphatase was isolated from potato tubers, variety “Uran”, 3 - 4 months after harvesting. Protein was determined according to Lowry et al. (1951) using albumin as a standard.

Enzymatic activity was determined in 200 mm-acetate buffer, pH 5.15, at 30°C using 3.23 mm-disodium p-nitrophenylphosphate as a substrate. Final volume of the incubation mixture was 2.0 ml. The reaction was stopped after 10 min incubation by adding 5 ml 100 mm-NaOH. The amount of liberated p-nitrophenol was determined spectrophotometrically at 410 nm. One unit of enzyme activity was defined as 1 μmol of p-nitrophenol liberated from the substrate per minute under the assay conditions. The enzyme activity with other substrates was determined under the same conditions by measuring the amount of liberated orthophosphate according to Fiske & Subbarow (1925). The reaction was stopped by the addition of 2.5 ml of 10% trichloroacetic acid.

Electrophoresis in 5% polyacrylamide gels at pH 9.5 was performed according to Smith et al. (1968), in 7.5% polyacrylamide gels at pH 8.3 according to Davis (1964) or at pH 4.5 after Reisfeld et al. (1962). From 10 to 50 μg protein was applied to the gel and was detected by staining with Amido Black 10B in 7% acetic acid; excess of the dye was removed by electrophoresis. The phosphatase activity was visualized on the gel with α-naphthylphosphate and Fast Blue B. The gels were stained for sugars with basic fuchsin following the method of Zacharius et al. (1969).

Analytical. Identification of sugars as alditol derivatives was performed by gas-liquid chromatography using Varian 2100 Chromatograph and 3% ECNSS-M Gas-chrom-G (Varian, Zug, Switzerland), with inositol as a standard according
to Sawarderker et al. (1965). For amino acid analysis, samples containing 1 mg protein were hydrolysed in vacuum-sealed tubes with 5.7 M-HCl at 110°C for 20 and 40 h. HCl was removed from the hydrolysates by drying under vacuum at 40°C. The cysteine content was determined in the hydrolysate previously oxidized with periodic acid. The analysis was performed with the automatic amino acid analyser AAA 881 (Microtechna-Prague, Czechoslovakia).

**Molecular mass determination.** Molecular mass of the enzyme was determined by thin-layer gel filtration on Sephadex G-100 in 100 mM-acetate buffer, pH 5.15, with the following standards: chymotrypsinogen (24 700), ovalbumin (44 600), bovine serum albumin (69 000) and haptoglobin (89 800).

**RESULTS AND DISCUSSION**

**Purification of acid phosphatase from potato tubers (Solanum tuberosum L)**

The enzyme was purified about 260-fold to electrophoretic homogeneity in five consecutive steps with 30% yield (Table 1). All operations were carried out at 4 - 8°C.

**Table 1**

**Purification of acid phosphatase from potato tubers**

The data are mean values of 8 preparations.

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Protein (mg)</th>
<th>Specific-activity (U/mg protein)</th>
<th>Total activity (units)</th>
<th>Purification factor</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 1. Crude extract</td>
<td>1500</td>
<td>1.2</td>
<td>1800</td>
<td>1.0</td>
<td>100</td>
</tr>
<tr>
<td>Step 2. Tannic acid</td>
<td>100</td>
<td>11.2</td>
<td>1120</td>
<td>9.3</td>
<td>62.2</td>
</tr>
<tr>
<td>fractionation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Step 3. DEAE-cellulose</td>
<td>28.3</td>
<td>24.2</td>
<td>684.7</td>
<td>20.2</td>
<td>38.0</td>
</tr>
<tr>
<td>Step 4. Bio-Gel P-150</td>
<td>9.8</td>
<td>64.6</td>
<td>633.1</td>
<td>53.8</td>
<td>35.2</td>
</tr>
<tr>
<td>Step 5. Con A-Sepharose</td>
<td>1.9</td>
<td>310.0</td>
<td>589.0</td>
<td>258.3</td>
<td>32.7</td>
</tr>
</tbody>
</table>

**Step 1. Crude extract.** Potatoes (1 kg) were homogenized in Waring blender with 500 ml of 100 mM-Na₂S₂O₅. The homogenate was squeezed using a hand press, and the insoluble residue was removed by centrifugation at 2600 g for 20 min.

**Step 2. Tannic acid fractionation.** The crude extract was fractionated with tannic acid according to Hsu et al. (1966).

**Step 3. Chromatography on DEAE-cellulose.** The material from step 2 was dialysed against 50 mM-Tris/HCl buffer, pH 7.5, at 5°C for 48 h and applied to a DEAE-cellulose column (3.5x50 cm) equilibrated with 50 mM-Tris/HCl buffer, pH 7.5. The proteins were eluted with a linear buffer concentration gradient from 50 mM to 500 mM. Fractions of 3.5 ml were collected at a flow rate of 1 ml/min. The phos-
phatase activity was resolved into two components in this step (Fig. 1). Fractions (no. 33 to 45) from the second major peak, which accounted for 61% of the acid phosphatase applied to the column, were combined and concentrated using the Amicon Model 52 provided with PM-10 membrane. The first peak, not adsorbed on DEAE-cellulose, was rejected.

![Diagram](image)

**Fig. 1.** DEAE-cellulose column chromatography of acid phosphatase preparation from step 2. The column (3.5×50 cm) was equilibrated with 50 mM-Tris/HCl buffer, pH 7.5, and eluted with linear buffer concentration gradient from 50 mM to 500 mM. Fractions of 3.5 ml were collected at room temperature at a flow rate of 1 ml/min. ○, Absorbance at 280 nm; ●, enzyme activity.

**Step 4. Filtration on Bio-Gel P-150.** The material from step 3 was applied to a column (2.5×150 cm) of Bio-Gel P-150 equilibrated with 100 mM-Tris/HCl buffer, pH 7.5. Elution was carried out with the same buffer. Fractions of 3.5 ml were collected at a flow rate of 9 ml/h. Fractions of highest specific activity from tubes no. 33 to 50 (Fig. 2) were pooled and concentrated as in step 3.

![Diagram](image)

**Fig. 2.** Gel filtration on Bio-Gel P-150 of acid phosphatase from step 3. The concentrated enzyme solution from DEAE-cellulose peak II (fractions no. 33 - 45) was applied to a column (2.5×150 cm) of Bio-Gel P-150 equilibrated with 100 mM-Tris/HCl buffer, pH 7.5. Elution was carried out with the same buffer. Fractions of 3.5 ml were collected at a flow rate of 9 ml/h. Designations as in Fig. 1.
Fig. 3. Affinity chromatography on Con A-Sepharose of Bio-Gel P-150 peak I' (fractions no. 33 - 50). The column (1×6 cm) was equilibrated with 100 mM-acetate buffer, pH 6.0, containing 1 mM each Mg\(^{2+}\), Mn\(^{2+}\) and Ca\(^{2+}\). The enzyme was eluted from the column with 5% water solution of \(\alpha\)-methyl-d-mannoside. Designations as in Fig. 1.

**Step 5. Affinity chromatography on Con A-Sepharose.** The enzyme was specifically adsorbed on Con A-Sepharose column (1×6 cm) equilibrated with 100 mM-sodium acetate buffer, pH 6.0, containing Mg\(^{2+}\), Mn\(^{2+}\), Ca\(^{2+}\) (each of them at 1 mM final concentration) and 1 mM-NaCl. The column was washed with the same buffer until no absorbance at 280 nm was detected in the effluent. The enzyme was then eluted from the column with 5% water solution of \(\alpha\)-methyl-d-mannoside (Fig. 3) and after dialysis against water concentrated on Minicon-B15 (Amicon Corp., Lexington, Mass., U.S.A.). The preparation obtained was completely free of proteolytic activity and of the nonspecific esterase and diesterase activities assayed with \(p\)-nitrophenyl acetate and bis-\(p\)-nitrophenylphosphate, respectively. The enzyme in 100 mM-acetate buffer, pH 5.15 (2 mg/ml) could be stored at -18° to 0°C for a few months without significant loss of activity.

Fig. 4. Polyacrylamide gel electrophoresis at pH 4.5 (1) and pH 9.5 (2 and 3) of acid phosphatase. Experimental conditions are described in the text. Gels 1 and 2 were stained with Amido Black, and gel 3 is a zymogram by diazo coupling.
Polyacrylamide gel electrophoresis of the enzyme preparation at pH 4.5, 8.3 or 9.5 gave a single protein band which could be stained for sugar and enzymatic activity (Fig. 4). The glycoprotein nature of acid phosphatase (Felenbok, 1970; Shinshi & Kató, 1979; Lorenc-Kubis & Morawiecka, 1980; Fujimoto et al., 1980) demonstrated for the potato enzyme (Kruzel, 1977) prompted us to use immobilized Con A for purification of acid phosphatase. The developed simple method enabled us to obtain electrophoretically homogeneous AcPase (Fig. 4) with specific activity of 310 units/mg, and molecular mass determined by thin-layer gel filtration 69 000 ± 2 000.

Sugar and amino acid composition of the enzyme

The composition of the sugar component of the enzyme as estimated by gas-liquid chromatography was found to be as follows: mannose 5.6%, rhamnose 3.4%, glucose 2.5%, galactose 1.5%, glucosamine 3.6%. Thus, the sugar component constitutes 11 454, and the remaining polypeptide 57 500 in the total enzyme molecular mass.

Table 2

Amino acid composition of acid phosphatase from potato tubers

The purified enzyme was analysed by the use of amino acid analyser. Cysteine was determined as cysteic acid in the hydrolysate oxidized with periodic acid.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>µmol/mg protein</th>
<th>Amino acid residues/mol protein*</th>
<th>Amino acid (mole %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lys</td>
<td>0.45</td>
<td>25.87</td>
<td>5.08</td>
</tr>
<tr>
<td>His</td>
<td>0.23</td>
<td>13.22</td>
<td>2.60</td>
</tr>
<tr>
<td>Arg</td>
<td>0.34</td>
<td>19.55</td>
<td>3.84</td>
</tr>
<tr>
<td>Cys</td>
<td>6.12</td>
<td>6.90</td>
<td>-1.37</td>
</tr>
<tr>
<td>Asp</td>
<td>1.06</td>
<td>60.95</td>
<td>11.98</td>
</tr>
<tr>
<td>Thr</td>
<td>0.51</td>
<td>29.32</td>
<td>5.76</td>
</tr>
<tr>
<td>Ser</td>
<td>0.75</td>
<td>43.12</td>
<td>8.47</td>
</tr>
<tr>
<td>Glu</td>
<td>0.79</td>
<td>45.42</td>
<td>8.93</td>
</tr>
<tr>
<td>Pro</td>
<td>0.50</td>
<td>28.75</td>
<td>5.65</td>
</tr>
<tr>
<td>Gly</td>
<td>0.74</td>
<td>42.55</td>
<td>8.36</td>
</tr>
<tr>
<td>Ala</td>
<td>0.60</td>
<td>34.50</td>
<td>6.78</td>
</tr>
<tr>
<td>Val</td>
<td>0.54</td>
<td>31.05</td>
<td>6.10</td>
</tr>
<tr>
<td>Met</td>
<td>0.16</td>
<td>9.20</td>
<td>1.81</td>
</tr>
<tr>
<td>Ile</td>
<td>0.48</td>
<td>27.60</td>
<td>5.42</td>
</tr>
<tr>
<td>Leu</td>
<td>0.70</td>
<td>40.25</td>
<td>7.91</td>
</tr>
<tr>
<td>Tyr</td>
<td>0.47</td>
<td>27.02</td>
<td>5.31</td>
</tr>
<tr>
<td>Phe</td>
<td>0.41</td>
<td>23.57</td>
<td>4.63</td>
</tr>
<tr>
<td>Total residues</td>
<td></td>
<td>508.84</td>
<td></td>
</tr>
</tbody>
</table>

* The molecular mass assumed as 57 500.
The amino acid composition of acid phosphatase is given in Table 2. It is interesting to note that aspartic acid, glutamic acid, serine and glycine constitute about 38% of the total amino acids in the phosphatase molecule. Aspartic acid and serine are probably involved in the formation of glycosidic bonds between the protein and the sugar moiety as it was recently reported for acid phosphatase from *Rhodotorula glutinis* (Wątorek et al., 1980; Maley et al., 1981).

The molecular mass determined by thin-layer gel filtration (about 69 000) and calculated on the basis of sugar content (about 57 500) is in close agreement with the results obtained by amino acid analysis, but is lower than estimated by Kubicz (1973). This dissimilarity may result from the fact that we purified another form of the heterogeneous enzyme (Kubicz et al., 1974). Since Kubicz (1973) did not investigate properties of the enzyme, and other polymorphic forms of acid phosphatase from potato tubers have not been studied, it is difficult to compare these two preparations. Presumably all polymorphic forms of AcPases from potato tubers are glycoproteins (Kruzel, 1977) which differ in the content of sugar, isoelectric point and molecular mass.

**Properties of the enzyme**

The enzyme shows pH optimum at 5.0 - 5.3 in 200 mm-acetate buffer with *p*-nitrophenylphosphate as a substrate. AcPase lost 50% of its activity after 30 min incubation at 60°C and was completely inactivated after 2 h at 70°C. The enzyme catalysed the hydrolysis of aromatic phosphomonoesters and anhydrides such

**Table 3**

*Substrate specificity of acid phosphatase from potato tubers*

Assays were carried out at 30°C and pH 5.15. The enzyme activity was determined by estimation of inorganic phosphate released, or by measuring *p*-nitrophenol when *p*-nitrophenylphosphate was used as a substrate. The enzymatic activity was expressed as percentage of that for *p*-nitrophenylphosphate.

<table>
<thead>
<tr>
<th>Substrate (10 mm)</th>
<th>Relative activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>p</em>-Nitrophenylphosphate</td>
<td>100</td>
</tr>
<tr>
<td>Phenylphosphate</td>
<td>62</td>
</tr>
<tr>
<td>Bis-<em>p</em>-nitrophenylphosphate</td>
<td>0</td>
</tr>
<tr>
<td><em>α</em>-Naphthylphosphate</td>
<td>22</td>
</tr>
<tr>
<td><em>β</em>-Naphthylphosphate</td>
<td>40</td>
</tr>
<tr>
<td><em>α</em>- and <em>β</em>-Glycerophosphate</td>
<td>0</td>
</tr>
<tr>
<td>Methylumbelliferyl phosphate</td>
<td>52</td>
</tr>
<tr>
<td>5'-ATP</td>
<td>33</td>
</tr>
<tr>
<td>3'-AMP, 3,5'-AMP, ADP, CMP, ITP</td>
<td>0</td>
</tr>
<tr>
<td>G-1-P, G-6-P, F-6-P</td>
<td>0</td>
</tr>
<tr>
<td>Inorganic pyrophosphate</td>
<td>28</td>
</tr>
</tbody>
</table>
as ATP and inorganic pyrophosphate; it was not active toward aliphatic phosphate esters and nucleoside 3'- and 5'-mono- and di-phosphates (Table 3). The apparent $K_m$ values for $p$-nitrophenylphosphate and inorganic pyrophosphate were 1.2 mM and 40 mM, respectively. MoO$_4^{2-}$, Hg$^{2+}$ and Zn$^{2+}$ at 1 mM concentration decreased acid phosphatase activity to 5%, 21% and 32%, respectively, whereas Mn$^{2+}$ was slightly activatory. EDTA and $\beta$-mercaptoethanol were without effect. The enzymatic properties of this AcPase such as pH optimum, thermostability, sensitivity to bivalent cations, and lack of response to EDTA and $\beta$-mercaptoethanol are similar to the features of acid phosphatase from various sources (Uehara et al., 1974b; Wątorek et al., 1977; Shinshi & Kató, 1979; Lorenc-Kubis & Morawiecka, 1980).

Incubation of the enzyme with increasing concentrations of urea for different time intervals led to a gradual loss of the activity; but even after 36 h incubation with 9 M-urea the enzyme still exhibited 40% of its initial activity. Removal of urea from the reaction mixture after 36 h incubation reactivated the enzyme by 96%, provided the concentration of urea did not exceed 9 M. The effect of urea on the activity of the purified enzyme suggests a subunit structure or aggregation of the enzyme molecules (Dibenedetto & Cozzani, 1975). A dimeric nature of AcPase has also been claimed for the AcPase III from potato tubers (Kubicz, 1973) and from yeast (Wątorek et al., 1980; Maley et al., 1981). Further investigation based on this effect of urea would be necessary to find out whether the aggregation of the enzyme molecules may be responsible for the formation of the different molecular forms of acid phosphatase.

REFERENCES


**FOSFATAZA KWAŚNA Z ZIEMNIAKÓW (SOLANUM TUBEROSUM L.). OCZYŚCZANIE, WŁAŚCIWOŚCI, ORAZ SKŁAD CUKROWY I AMINOKWASOWY**

**Streszczenie**

1. Fosfatazę kwaśną z buli ziemniaków oczyszczono stosując frakcjonowane wytrącanie białek z wyciągu buforowego tanią, chromatografię na DEAE-cellulose, filtrację na Bio-żelu P-150 i chromatografię powinowactwa na Con A-Sepharose. Enzym oczyszczony 258-krotne jest elektroforetycznie homogenny, a jego ciężar cząsteczkowy wynosi 69 000.

2. Składnik cukrowy stanowi 16,6% całkowitej wagi enzymu i zawiera mannozę (5,6%), ramanozę (3,4%), glukozę (2,5%), galaktozę (1,5%) i 3,6% glukozaminy. W składzie aminokwasowym kwas asparaginowy, glutaminowy, seryna i glicyna stanowią 37,7% reszt aminokwasowych.

3. Enzym wykazuje optimum pH w zakresie od 5,0 - 5,3 i po 30 min inkubacji w 60°C zachowuje 50% aktywności, a po 2 godz. incubacji w temp. 70°C całkowicie ulega inaktywacji. Enzym jest niespecyficzną fosfomonoesterazą, hydrolizuje aromatyczne fosfomonoestry i nieorganiczny
pirosforan. Pozorna wartość $K_m$ wobec $p$-nitrofenylofosforanu jako substratu wynosi 1.25 mm a wobec pirofosforanu 40 mm. Inhibitorami enzymu są jony molibdenianowe cynku i rtęci. Mocznik w stężeniu 9 m wywołuje odwracalną inaktywację enzymu,