Isolation and Properties of Acid Phosphatases of Sweet Potato Roots

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Acid phosphatase of sweet potato root tissue was found to consist of five components by diethylaminoethyl-cellulose column chromatography, and each component was isolated by the rechromatography. They were not separated by Sephadex G-200 gel filtration. All components hydrolyzed various phosphate compounds including phosphomonoester- and pyrophosphate-linkages. Their optimum pH values were in the range of pH 5 to 6. However, there were observed some differences in optimum pH, Michaelis constant for various substrates and relative maximum velocity among the components.

Acid phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.2), the enzyme catalyzing the hydrolysis of phosphate compounds in acid medium, has been found in various organisms and supposed to play an important role in the phosphate metabolism in the tissues. The studies on phosphatase of higher plants have been focused on apyrase and phytase, although acid phosphomonoesterase activity was detected in various higher plants.

A potent acid phosphomonoesterase activity has been found in sweet potato root tissue. Watanabe and his coworkers reported that purified acid phosphatase of sweet potato root tissue catalyzed the hydrolysis of ATP, PPI, β-GP* and glucose 1-phosphate and that the enzyme resembled apyrase of potato tubers. On the other hand, a report from this laboratory showed that acid phosphomonoesterase of sweet potato roots was divided into two fractions by DEAE-cellulose column chromatography. This paper deals with the isolation of several components of acid phosphatase of sweet potato root tissue and with their properties.

MATERIALS AND METHODS

Materials. Sweet potato roots (variety Norin 1) harvested at Kariya Farm, Aichi in October were stored at 10°C until used.

ATP, ADP and AMP were products of the Sigma Chemical Company or C. F. Boehringer and Soehne Company. DEAE-cellulose and Sephadex were obtained from the Brown Chemical Company and Pharmacia Company, respectively. Sodium isoascorbate was a gift from Fujisawa Pharmaceutical Company.

Extraction of Enzyme from Sweet Potato Root Tissue. Two hundred g of chopped sweet potato root tissue was homogenized with 200 ml of 0.05 M Tris buffer (pH 8.0) containing 0.5% sodium isoascorbate in a Waring blender. The homogenate was squeezed through a double layer of cheese cloth and centrifuged for 10 minutes at 10,000 x g. The resultant supernatant was passed through a column (4.0 x 20 cm) of Sephadex G-25 (medium) to remove polyphenolic

* The abbreviations used are: DEAE-cellulose, diethylaminoethylcellulose; β-GP, β-glycerophosphate.
3) S. Watanabe, E. Ito, G. Takeuchi and T. Kondo, ibid., 74, 361 (1953).
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compounds. The column had been equilibrated with 0.05 M Tris buffer (pH 8.0). To the eluted protein fraction was added solid ammonium sulfate to 90% saturation and the precipitate was suspended in 10 ml of 0.01 M Tris buffer (pH 8.0). The suspension was then dialyzed against 0.01 M Tris buffer (pH 8.0) for 1 day with several changes. All the above procedures were carried out at 4°C.

Fractionation of Enzyme by DEAE-Cellulose Column Chromatography. The enzyme solution (10~20 ml) was applied to a DEAE-cellulose column (3.0 x 25 cm) which had been equilibrated with 0.01 M Tris buffer (pH 8.0), and the column was washed with 10 ml of the buffer. Then, the enzyme was eluted from the column by conducting a gradual increase of sodium chloride concentration in the eluting buffer. Unless otherwise indicated, the flow rate was adjusted to 0.1 ml per minute and each 10 ml of the effluent was collected. The procedures were carried out at approximately 4°C.

Fractionation of Enzyme by Sephadex G-200 Gel Filtration. The enzyme solution (2.5 ml) was applied to a Sephadex G-200 column (1.7 x 21 cm) which was pre-equilibrated with 0.01 M Tris buffer (pH 8.0). Then, the column was washed with 50~60 ml of the buffer. The flow rate was 0.43 ml per minute and the volume of each eluted fraction was 5 ml. The procedures were done at 4°C.

Assay Methods of Enzyme Activity. Acid phosphomonoesterase (orthophosphoric monoester phosphohydrolase, 3.1.3.2) activity was assayed by determining Pi liberated from β-GP during incubation.5) The reaction mixture was composed of 50 μmoles of acetate buffer (pH 5.3), 33 μmoles of β-GP and the enzyme in a final volume of 1.0 ml. After the incubation for 10 or 30 minutes at 30°C, 1.0 ml of 5% perchloric acid was added and the precipitate was removed by filtration. Then, the amounts of liberated Pi in the filtrate were determined by the method of Allen.6) The control was run without the enzyme.

Inorganic pyrophosphatase (pyrophosphate phosphohydrolase, 3.6.1.1) activity was assayed by the same method as described above except for 2.5 μmoles of PPI instead of β-GP in the reaction mixture.

The assay of the enzymatic activity hydrolyzing ADT, ADP or AMP was based on the method described by Kielley.7) The reaction mixture was composed of 50 μmoles of histidine buffer (pH 7.5), 5 μmoles of the substrate and the enzyme in a final volume of 1.0 ml. The incubation and the determination of liberated Pi were done as described above. The control was run without the enzyme.

RESULTS

Fractionation of Crude Enzyme Extract by DEAE-Cellulose Column Chromatography

Fig. 1 shows the eluting patterns of acid phosphomonoesterase, ATP-hydrolyzing and ADP-hydrolyzing activities from a DEAE-cellulose column. The crude enzyme extract from sweet potato root tissue was added to the

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column and a non-linear gradient elution was conducted with 1,000 ml of 0.01 M Tris buffer (pH 8.0) in the mixing vessel and 500 ml of 0.7 M sodium chloride in the buffer in the reservoir vessel, as indicated in Fig. 1. Three fractions of acid phosphomonoesterase were detected. Fraction III, which was eluted at last from the column, was the major fraction and other fractions, Fractions I and II, were minor.

In cases of Fractions I and II, the eluting profile of acid phosphomonoesterase activity was found to coincide with that of the activity hydrolyzing ATP or ADP. Some differences were observed in the eluting profiles among three enzymatic activities in case of Fraction III. At least two fractions were found regarding the enzyme catalyzing the hydrolysis of ADP in Fraction III. The ratio of acid phosphomonoesterase activity to the activity hydrolyzing ATP seemed to be different among the three fractions.

An attempt was made to divide Fraction III into subcomponents. Fig. 2 shows the eluting patterns of phosphatase activities from DEAE-cellulose column, when the crude enzyme extract was fractionated with the chromatography by conducting a linear gradient elution with sodium chloride in concentrations from 0.1 to 0.3 M. Fraction III was divided into three subcomponents, two of which, Fraction III-A and III-B, possessed all three enzymatic activities. Fraction III-C, which was eluted at last from the column, seemed to hydrolyze ADP at a higher rate as compared with other substrates.

Rechromatography of Each Fraction with DEAE-Cellulose Column

It has been attempted to purify each fraction by rechromatography with DEAE-cellulose column. Each fraction was collected separately and solid ammonium sulfate was added to the fraction to 90% saturation. The precipitate was collected by centrifugation and suspended in 0.01 M Tris buffer (pH 8.0). The suspension was dialyzed against the buffer for 1 day with several changes. Then, the enzyme solution was subjected to fractionation with DEAE-cellulose column.

Fig. 3 shows the rechromatogram on acid phosphomonoesterase of each fraction. Fraction I, II or III-A was found to be eluted as a single peak without any other fraction. Regarding Fraction I or II, inorganic pyrophosphatase activity was also investigated. The eluting profile of inorganic pyrophosphatase activity was observed to the coincident with that of acid phosphomonoesterase activity.

However, Fraction III-C was eluted as a peak with a shoulder which suggests the presence of an additional fraction. The eluting profile of acid phosphomonoesterase activity was observed to coincide with that of ADP-hydrolyzing activity, in case of Fraction III-C.

Fractionation of Fraction III with Sephadex G-200 Column

The protein in Fraction III was collected by adding solid ammonium sulfate to 90%
saturation and by centrifuging the suspension. After suspending the precipitate in 0.01 M Tris buffer (pH 8.0) and dialyzing the suspension against the buffer, the enzyme solution was fractionated by Sephadex G-200 gel filtration.

It was found that Fraction III-A, -B and -C could not be separated each other, as shown in Fig. 4. Both activities hydrolyzing β-GP and ADP were distributed in a great number of fractions and the Kd value estimated roughly was approximately 0.5.

**Reaction Rate as Function of pH**

Fig. 5 shows the reaction rates of acid phosphomonoesterase activity and ATP-
ADP-hydrolyzing activities of each fraction as a function of pH. Some differences were observed in the optimum pH among three enzymatic activities in both cases of Fraction I and III-C. However, no significant difference was observed in the dependency of activities on pH among the substrates in both cases of Fraction II and III-B. There were two optimum pHs, pH 5 and 7, in Fraction III-A in all cases of three substrates. This suggests that the fraction may constitute of two sub-components.

In Fraction III-C, ADP-hydrolyzing activity at pH 7.5 was higher than ATP-hydrolyzing activity, but at pH 5.0 the former was lower than the latter.

**Effects of Magnesium and Calcium ions on Enzymatic Activity**

Table I shows the effects of magnesium and calcium ions on enzymatic activities. The activities were as follows:

- **Fraction I** and **II**: Acetate and histidine buffers were used for pHs at 4~6 and at 6~8, respectively.
- **Other fractions**: Acetate buffer for pH 4~5.5, citrate buffer for pH 5.5~6.5 and histidine buffer for pH 6.5~8.

**FIG. 4.** Fractionation of Fraction III by Sephadex G-200 Gel Filtration.

- ○—○: acid phosphomonoesterase activity.
- △—△: ADP-hydrolyzing activity.
- The activities were as for Fig. 3.

**FIG. 5.** Reaction Rates as a Function of pH.

The activities were shown as μmoles Pi liberated per 30 minutes. In Fraction I and II, acetate and histidine buffers were used for pHs at 4~6 and at 6~8, respectively. In other fractions, acetate buffer was used for pH 4~5.5, citrate buffer for pH 5.5~6.5 and histidine buffer for pH 6.5~8.
TABLE I. EFFECTS OF MAGNESIUM AND CALCIUM IONS ON ATP- AND ADP-HYDROLYZING ACTIVITIES

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Ion added*</th>
<th>Activity (Pi liberated in μmoles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>None</td>
<td>0.205</td>
</tr>
<tr>
<td></td>
<td>Mg²⁺</td>
<td>0.110</td>
</tr>
<tr>
<td></td>
<td>Ca²⁺</td>
<td>0.023</td>
</tr>
<tr>
<td></td>
<td>Mg²⁺, Ca²⁺</td>
<td>0.028</td>
</tr>
<tr>
<td>ADP</td>
<td>None</td>
<td>0.388</td>
</tr>
<tr>
<td></td>
<td>Mg²⁺</td>
<td>0.383</td>
</tr>
<tr>
<td></td>
<td>Ca²⁺</td>
<td>0.145</td>
</tr>
<tr>
<td></td>
<td>Mg²⁺, Ca²⁺</td>
<td>0.164</td>
</tr>
</tbody>
</table>

Magnesium chloride or calcium chloride was added in a concentration of 5 \times 10^{-3} M in the reaction mixture.

calcium ions on the activities hydrolyzing ATP and ADP in Fraction II and III. Both ions inhibited the activities except for no effect of magnesium or calcium ions on ADP-hydrolyzing activity of Fraction II or Fraction III.

Michaelis Constant and Relative Maximum Velocity

Michaelis constant (K_m) and relative maximum velocity (Rel. V_m) were calculated by the equation of Lineweaver and Burk. Rel. V_m means the ratio of an activity in the presence of a large amount of substrate to ATP-hydrolyzing activity in percentage. Lineweaver-Burk plots were completely straight in all cases of the fractions and the substrates.

Table II indicates K_m and Rel. V_m values of the five fractions for β-GP, ADP, AMP and PPI as the substrates at pH 5.0. In case of Fraction I, K_m for AMP was the smallest and K_m for β-GP was the largest. In cases of Fraction II, III-B and III-C, any significant difference was not observed in K_m among the substrates. In both cases of Fraction I and II, ATP was hydrolyzed at the highest rate and AMP at the lowest rate. Fraction III-A, -B and -C hydrolyzed more rapidly β-GP than ATP, although Fraction I and II hydrolyzed more rapidly ATP than β-GP. Fraction I was rather specific for ATP.

TABLE II. MICHAELIS CONSTANT AND RELATIVE MAXIMUM VELOCITY

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Fraction</th>
<th>I</th>
<th>II</th>
<th>III-A</th>
<th>III-B</th>
<th>III-C</th>
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<tbody>
<tr>
<td>ATP</td>
<td></td>
<td>5.9</td>
<td>1.9</td>
<td>0.24</td>
<td>1.4</td>
<td>0.73</td>
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<tr>
<td>ADP</td>
<td></td>
<td>2.2</td>
<td>1.1</td>
<td>—</td>
<td>1.1</td>
<td>0.56</td>
</tr>
<tr>
<td>AMP</td>
<td></td>
<td>1.0</td>
<td>2.0</td>
<td>—</td>
<td>1.1</td>
<td>1.4</td>
</tr>
<tr>
<td>β-GP</td>
<td></td>
<td>11</td>
<td>2.0</td>
<td>0.95</td>
<td>3.2</td>
<td>1.2</td>
</tr>
<tr>
<td>PPI</td>
<td></td>
<td>1.6</td>
<td>1.3</td>
<td>—</td>
<td>3.7</td>
<td>0.54</td>
</tr>
<tr>
<td>Rel. V_m</td>
<td>ATP</td>
<td>100</td>
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<td>56</td>
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<td>27</td>
<td>—</td>
<td>56</td>
<td>58</td>
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<tr>
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<tr>
<td></td>
<td>PPI</td>
<td>29</td>
<td>69</td>
<td>—</td>
<td>165</td>
<td>81</td>
</tr>
</tbody>
</table>

K_m: Michaelis constant
Rel. V_m: ratio of maximum activity for a substrate to that for ATP as the substrate in percentage.

DISCUSSION

It is evident that there are at least five acid phosphomonoesterases in sweet potato root tissue. They were separated by DEAE-cellulose column chromatography. The eluting profiles shown in Figs. 1, 2 and 3 suggest that each of them catalyzes the hydrolysis of ATP, ADP, AMP and PPI as well as of β-GP. They showed differences not only in the behavior on DEAE-cellulose column chromatography but also in the pH dependency, the effects of magnesium and calcium ions on their activities and the K_m and Rel. V_m for various substrates.

All fractions of phosphatase in sweet potato roots were observed to catalyze the hydrolysis of various phosphate compounds. The fractions were found to hydrolyze phosphomonoester- as well as pyrophosphate-linkages. Their optimum pH was about 5~6 except for Fraction III-A. Thus, the properties of all fractions of phosphatase are considered to show close resemblance although there are some slight differences in the properties among the fractions.

A single fraction was obtained by the re-chromatography of each fraction with DEAE-cellulose column. This indicates that each fraction was not converted to other fractions.
Calcium chloride and magnesium chloride were reported to stimulate apyrase activity of potato tubers. However, phosphatases from sweet potato roots were rather inhibited by the addition of these inorganic compounds. ADP was hydrolyzed more slowly than ATP in all cases of five fractions of sweet potato enzyme, but ADP was hydrolyzed more rapidly in case of potato enzyme. It is concluded that acid phosphatase of sweet potato is different from apyrase of potato tubers in some main properties.