PIOTR MASŁOWSKI, HALINA MASŁOWSKA and STANISŁAW KOWALCZYK

SUBCELLULAR DISTRIBUTION AND PROPERTIES OF ALKALINE INORGANIC PYROPHOSPHATASE OF MAIZE LEAVES

Institute of Biology, Department of Biochemistry, M. Kopernik University
ul. Gagarina 11; 87-100 Toruń, Poland

1. Studies on the distribution of alkaline inorganic pyrophosphatase (pyrophosphate phosphohydrolase, EC 3.6.1.1) in the subcellular fractions of maize leaves showed that the enzyme is present in cytoplasm, chloroplasts and mitochondria. The activity observed in nuclei and microsomes may result from contamination with the mitochondrial fraction.

2. Alkaline pyrophosphatases from three subcellular fractions were purified by fractionation with (NH₄)₂SO₄, followed by ion-exchange and gel-filtration chromatography, and by isoelectric focusing. Highly purified enzyme preparations, with specific activities ranging from 55 to 188 μmoles/min/mg protein, were obtained.

3. All the enzymes exhibited the maximum activity at pH 8.5 and the Mg²⁺/PP⁺ ratio of 5. They differed in electrophoretic mobility, pI, and susceptibility to urea and thermal denaturation. This indicates that they represent isoenzymes compartmentalized in particular subcellular fractions.

Inorganic pyrophosphate is not only formed from nucleoside triphosphates as a by-product in several biosynthetic reactions but it is also involved in the energy-linked reactions in biological systems (Baltscheffsky et al., 1966; Keister & Minton, 1971; Mansurova et al., 1975): it is formed during oxidative phosphorylation in Acetobacter suboxidans and yeasts (Klungsöyr, 1959) and in the course of photophosphorylation in Rhodospirillum rubrum (Baltscheffsky & van Stedingk, 1966). Hydrolysis of pyrophosphate to orthophosphate catalysed by inorganic pyrophosphatase is known to be an important process in cell metabolism. Alkaline inorganic Mg²⁺-dependent pyrophosphatase (EC 3.6.1.1) is widespread in animals. It has also been found in the leaves of various plants (Naganna et al., 1955; Bucke, 1970; Bennett et al., 1973; Klemme & Jacobi, 1974; Masłowski & Masłowska, 1976) and

1 Abbreviations used: PP, pyrophosphate; PPase, alkaline inorganic pyrophosphatase.
in maize endosperm (Maslowska & Maslowski, 1975), its subcellular distribution is, however, still uncertain. Heber (1960) and Simmons & Butler (1969) have concluded from the results obtained with chloroplasts isolated in non-aqueous media, that the total PPase activity is restricted to chloroplasts. However, Bennett et al. (1973) have demonstrated the presence of two alkaline PPase isoenzymes in maize: one in young etiolated seedlings and another in the seedlings grown in light and treated with cycloheximide. Both isoenzymes were isolated and purified from soluble fraction of homogenate of maize leaves grown under normal conditions (Maslowska & Maslowska, 1976).

The present results give evidence for the occurrence of three alkaline inorganic pyrophosphatases compartmentalized in subcellular fractions of maize leaves. The enzymes have been extracted and purified, and some of their physico-chemical properties have been determined. It is believed that this experimental approach may facilitate understanding of the physiological role of the enzyme.

MATERIALS AND METHODS

Preparation of subcellular fractions of maize leaves

Leaves of 10-day-old maize plants (Zea Mays, var. “Wir”) were homogenized in a Macro-Waring blender (4 x 20 sec) in the medium containing in a final volume of 2.5 ml: 0.5 M-sucrose, 0.05 M-Tris-HCl buffer, pH 7.5, 1 mM-EDTA, 0.1% of albumin and 0.05% of cysteine per 1 g of tissue.

Nuclei. The homogenate was squeezed through 4 layers of cheese-cloth and then centrifuged for 15 min at 200 g. The pellet was resuspended in 40 ml of 10% sucrose and centrifuged again at 200 g for 10 min. The crude nuclear fraction was purified according to Rho & Chipehase (1962).

Chloroplasts. The supernatant obtained after removal of nuclei and cell debris was centrifuged at 1000 g for 10 min. The chloroplast pellet was washed twice with 0.5 M-sucrose in 50 mM-Tris-HCl, pH 7.5, and then purified in discontinuous sucrose density gradient according to Lockshin et al. (1971).

Mitochondria. The postchloroplastic supernatant was centrifuged at 10,000 g for 15 min. The crude mitochondrial precipitate was further purified according to Ikuma & Bonner (1967).

Microsomes. The postmitochondrial supernatant was centrifuged at 14,000 g for 15 min. The precipitate was discarded and the supernatant was recentrifuged at 100,000 g for 60 min. The pellet was resuspended in 0.4 M-sucrose in 50 mM-Tris-HCl buffer, pH 7.2, and centrifuged at 80,000 g for 60 min.

Cytoplasm. The solution obtained after the removal of microsomes at 100,000 g was regarded as the cytoplasmic fraction.

Extraction of alkaline inorganic pyrophosphatase from subcellular fractions

Preparations of the subcellular fractions of maize leaves were suspended (10 mg protein/ml) in 25 mM-Tris-HCl buffer, pH 7.5, containing 5 mM-MgCl₂ and 2 mM-EDTA. The suspensions were stored at 4°C for 24 h and were then centrifuged.
at 20,000 g for 20 min. The pellets were resuspended in the same medium, and the enzymes were released by three times repeated freezing (in solid CO₂ - alcohol) and thawing, followed each time by centrifugation at 20,000 g for 20 min. In the combined supernatants, 70 - 80% of the PPase was recovered.

**Enzyme assays**

*Inorganic pyrophosphatases.* Alkaline PPase activity was determined in the reaction mixture containing in a final volume of 5 ml: 1 mM-Na₄P₂O₇, 5 mM-MgCl₂, 40 mM-Tris-HCl buffer, pH 8.5, and 0.5 ml of enzyme (suitably diluted so that hydrolysis of pyrophosphate did not exceed 80%). Following incubation at 37°C for 15 min, 2.5 ml of ice-cold 10% (w/v) trichloroacetic acid was added and the released P, was determined according to Fiske & Subbarow (1925).

The procedure for determination of acid pyrophosphatase activity was identical with the method used for alkaline pyrophosphatase except that 50 mM-Tris-acetate buffer, pH 5, was used and MgCl₂ was omitted.

*Acid phosphatase* (EC 3.1.3.2) was determined in the incubation mixture containing in a final volume of 0.5 ml: 50 mM-Tris-acetate, 4 mM- p-nitrophenylphosphate, pH 5, and 0.5 ml of the appropriately diluted enzyme solution. The reaction was carried out for 15 min at 37°C and terminated by the addition of 4 ml of 0.5 mM-NaOH; after centrifugation, absorbance was measured at 400 nm.

*Succinate : cytochrome c oxidoreductase* was determined spectrophotometrically as described by Douce *et al.* (1973).

**Analytical**

*Protein* was assayed by the method of Lowry *et al.* (1951) with bovine serum albumin as a standard, or by measuring the absorbance at 280 nm.

*Effect of pH.* The optimum pH was determined in the assay mixture containing 1 mM-Na₄P₂O₇, 5 mM-MgCl₂ and 40 mM buffer: Tris-acetate in the pH range 4.0 - 6.0, Tris-maleate at pH 6.0 - 7.0, and Tris-HCl at pH 7.2 - 9.0. For determination of the effect of pH on the stability of the PPases, the enzyme (0.09 unit) was incubated at 25°C for 30 min in the following buffers: 0.1 mM-glycine (pH 3 - 3.5), 0.1 mM-acetate (pH 4 - 6.5), 0.1 mM-Tris-HCl (pH 7 - 9), then the enzyme activity was measured at pH 8.5 as described above.

*Isoelectric focusing.* The partly purified enzyme preparations were dialysed against 1% glycine for 24 h and separated in the apparatus constructed in this laboratory according to Doi & Ohtsuru (1974). Isoelectric focusing was carried out between pH 4 and 6, at 1000 V. After 24 h, fractions of 0.5 ml were collected and pH, PPase activity and protein content were determined in the samples dialysed against the reaction buffer (40 mM-Tris-HCl, 5 mM-MgCl₂, pH 8.5). For kinetic studies, fractions with the highest activity were combined and dialysed against the same buffer for 72 h to remove ampholine.
Polyacrylamide-gel electrophoresis. This was run in 7% acrylamide gels at pH 8.3, according to Davis (1964). The enzyme preparations containing 0.04 - 0.08 units or 0.1 - 0.2 mg protein were electrophoresed for 60 min at 0.8 mA per gel. The gels were stained for protein with Amido Black and for the enzyme with triethylenamine, according to Tono & Kornberg (1967).

RESULTS

Subcellular distribution of alkaline pyrophosphatase

To check homogeneity of the subcellular fractions obtained from maize leaves by the adopted procedure, the activity of Mg2+-inhibited acid pyrophosphatase (pH 5), a cytoplasmic enzyme (Naganna et al., 1955) and cytochrome c oxidoreductase, a marker of the plant mitochondrial membranes (Mannella & Bonner, 1975) were assayed. Neither mitochondria nor chloroplasts exhibited any acid pyrophosphatase activity. Traces of this activity (below 0.05 unit/mg protein) were found in the nuclear and microsomal fractions. The extent of contamination of the subcellular fractions with mitochondrial membranes, as judged from the cytochrome c oxidoreductase activity, is illustrated in Table 1. The cytoplasmic fraction was practically uncontaminated. The slight contamination of chloroplasts is of no significance due to the high PPase activity in this fraction. However, taking into account the PPase : cytochrome c oxidoreductase ratio one can presume that the alkaline PPase activity found in the microsomal and nuclear fractions is due to contamination with mitochondrial membranes.

Table 1
Subcellular distribution of alkaline inorganic pyrophosphatase (PPase) and succinate : cytochrome c oxidoreductase in maize leaves

The activity is expressed in units (μmoles/min/mg protein).

<table>
<thead>
<tr>
<th>Fraction</th>
<th>PPase</th>
<th>Cytochrome c oxidoreductase</th>
<th>PPase/cytochrome c oxidoreductase ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenate</td>
<td>1.62</td>
<td>0.41</td>
<td>3.95</td>
</tr>
<tr>
<td>Nuclei</td>
<td>0.02</td>
<td>0.20</td>
<td>0.10</td>
</tr>
<tr>
<td>Chloroplasts</td>
<td>4.25</td>
<td>0.52</td>
<td>8.17</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>1.10</td>
<td>12.20</td>
<td>0.09</td>
</tr>
<tr>
<td>Microsomes</td>
<td>0.04</td>
<td>0.30</td>
<td>0.13</td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>9.20</td>
<td>0.05</td>
<td>184.00</td>
</tr>
</tbody>
</table>

As shown in Table 1, specific activity of alkaline PPase was the highest in the cytoplasmic fraction; in chloroplasts it was four times higher than in mitochondria, but it was only a half of the cytoplasmic PPase activity.
Purification of alkaline pyrophosphatase from subcellular fractions

The enzyme extracted from chloroplasts and mitochondria, and the cytoplasmic enzyme were precipitated with ammonium sulphate at 0.4 - 0.85 saturation and centrifuged at 10 000 g for 20 min. The pellets were dissolved in a small volume of 0.1 M-Tris-HCl buffer, pH 7.2, containing 5 mM-MgCl₂, dialysed against the same buffer and applied to a DEAE-Sephadex A-50 column (3 × 25 cm) previously equilibrated against the same buffer. Proteins were eluted with linear (0 - 1 M) NaCl gradient at a rate of 1 ml/min. Fractions of 5 ml were collected, and those with the highest activity were combined and concentrated to a small volume in a Visking tube at 2°C. As can be seen from the elution diagrams (Fig. 1A), all the enzymatic

Fig. 1. Column chromatography of alkaline inorganic pyrophosphatase from cytoplasm (a), chlorophyll plastids (b) and mitochondria (c) of maize leaves, on DEAE-Sephadex A-50 (A) and Sephadex G-100 (B). Alkaline pyrophosphatase and acid phosphatase were assayed as described in Methods, anh expressed as μmoles/min/ml or μmoles/15 min/ml, respectively. For experimental details of the chromatographic procedure see text.
preparations obtained at this purification stage, especially the cytoplasmic enzyme, were heavily contaminated with acid phosphatase. For complete removal of the acid phosphatase, the concentrated enzymatic preparations were dialysed against 0.1 M-Tris-HCl buffer, pH 7.2, containing 5 mM-MgCl₂, and rechromatographed on a Sephadex G-100 column (2 x 32 cm). Fractions of 4 ml were collected at a flow rate of 0.5 ml/min (Fig. 1B). The results of purification are summarized in Table 2.

**Table 2**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total protein (mg)</th>
<th>Total activity (units)</th>
<th>Recovery (%)</th>
<th>Specific activity (units/mg protein)</th>
<th>Purification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenate</td>
<td>6272.0</td>
<td>7531.0</td>
<td>100</td>
<td>1.2</td>
<td>1</td>
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<tr>
<td>a. chloroplasts</td>
<td>612.0</td>
<td>2631.6</td>
<td>100</td>
<td>4.3</td>
<td>3.6</td>
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<tr>
<td>b. mitochondria</td>
<td>97.5</td>
<td>204.6</td>
<td>100</td>
<td>2.0</td>
<td>1.7</td>
</tr>
<tr>
<td>Crude extract</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>a. chloroplasts</td>
<td>397.4</td>
<td>2106.3</td>
<td>80</td>
<td>5.3</td>
<td>4.4</td>
</tr>
<tr>
<td>b. mitochondria</td>
<td>70.6</td>
<td>169.5</td>
<td>82</td>
<td>2.4</td>
<td>2.0</td>
</tr>
<tr>
<td>c. cytoplasm</td>
<td>565.0</td>
<td>4800.0</td>
<td>60</td>
<td>8.5</td>
<td>7.0</td>
</tr>
<tr>
<td>Ppt. at 0.4 - 0.85 (NH₄)₂SO₄ sat.</td>
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<tr>
<td>a. chloroplasts</td>
<td>136.3</td>
<td>1186.5</td>
<td>45</td>
<td>8.7</td>
<td>7.3</td>
</tr>
<tr>
<td>b. mitochondria</td>
<td>29.5</td>
<td>129.8</td>
<td>63</td>
<td>4.4</td>
<td>3.7</td>
</tr>
<tr>
<td>c. cytoplasm</td>
<td>430.0</td>
<td>4600.0</td>
<td>61</td>
<td>10.7</td>
<td>8.9</td>
</tr>
<tr>
<td>DEAE-Sephadex A-50</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. chloroplasts</td>
<td>7.4</td>
<td>1050.6</td>
<td>39</td>
<td>142.0</td>
<td>118.3</td>
</tr>
<tr>
<td>b. mitochondria</td>
<td>2.5</td>
<td>95.5</td>
<td>46</td>
<td>38.2</td>
<td>31.8</td>
</tr>
<tr>
<td>c. cytoplasm</td>
<td>35.5</td>
<td>2800.5</td>
<td>37</td>
<td>78.9</td>
<td>65.7</td>
</tr>
<tr>
<td>Sephadex G-100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. chloroplasts</td>
<td>3.2</td>
<td>526.3</td>
<td>20</td>
<td>164.4</td>
<td>137.0</td>
</tr>
<tr>
<td>b. mitochondria</td>
<td>1.2</td>
<td>66.2</td>
<td>32</td>
<td>55.1</td>
<td>46.7</td>
</tr>
<tr>
<td>c. cytoplasm</td>
<td>10.1</td>
<td>1800.0</td>
<td>24</td>
<td>188.0</td>
<td>150.0</td>
</tr>
</tbody>
</table>

**Properties of purified alkaline pyrophosphatase**

Optimum pH. The purified PPases of all the subcellular fractions exhibited the maximum activity at pH 8.5 - 9, similarly as it was previously shown by Bennett et al. (1973) and Masłowski & Masłowska (1976) for the two PPase isoenzymes isolated from the total homogenate of maize leaves.

Effect of pH on enzyme stability. Mitochondrial, chloroplastic and cytoplasmic PPases (0.06 - 0.09 unit/ml) were incubated at 25°C for 30 min in the buffers of
different pH values, then the enzymatic activity was measured at pH 8.5 as described
in Methods. The enzymes retained full activity on incubation at pH from 6.5 to
7.5. At lower pH, mitochondrial and chloroplastic PPases lost about 55% of their
initial activity, and cytoplasmic PPase — about 40%.

**Effect of magnesium concentration.** At 1 mM PPi and varied Mg²⁺ concentra-
tions, the PPases exhibited the maximum activity at the Mg²⁺/PPi ratio between
5 and 10. These results are consistent with those of Simmons & Butler (1969),
Bucke (1970), Bennett et al. (1973) and Maslowski & Masłowska (1976).

**Michaelis constants.** *Kₘ* values determined from the Lineweaver-Burk plots at
pH 8.5 and Mg²⁺/PPi ratio = 5, were as follows: 5 × 10⁻⁶ M for mitochondrial
PPase, 3.3 × 10⁻⁶ M for chloroplastic PPase, and 9.9 × 10⁻⁶ M for cytoplasmic
PPase.

**Substrate specificity.** None of the following nucleotides and phosphate esters:
ATP, AMP, NAD⁺, NADP⁺, β-glycerophosphate, p-nitrophenylphosphate, at
1 mM concentration, were hydrolysed by alkaline PPase on incubation in 0.1 M
-Tris-HCl buffer, pH 8.5, containing 5 mM-MgCl₂. ADP was hydrolysed to a negli-
gible extent (about 5%), thus proving high specificity of the enzyme.

**Molecular weight.** Cytoplasmic, chloroplastic and mitochondrial PPases and
marker proteins (ovalbumin, mol. wt. 45 000; bovine serum albumin, 68 000; and
myoglobin, 17 800, all from Serva Feinbiochemica, Heidelberg, G.F.R.) were sepa-
rated on Sephadex G-100 column (2.5 × 50 cm) according to Whitaker (1963).
They were eluted with 0.1 M-Tris-HCl buffer, pH 7.5 and collected in 2 ml fractions.
The molecular weight of mitochondrial and chloroplastic PPases was 32 000 ± 1500,
and of cytoplasmic PPase — 27 000 ± 1000 (Fig. 2).

**Isoelectric focusing.** As shown in Fig. 3, the purified enzymatic preparations
of alkaline PPases from cytoplasm, mitochondria and chloroplasts were eluted as
single fractions with different isoelectric points. pI was 5 for cytoplasmic PPase,
4.85 for chloroplastic PPase, and 4.9 for the mitochondrial enzyme. Although
purification by isoelectric focusing gave homogeneous fractions; their specific activ-
ities were reduced, especially in the case of chloroplastic and mitochondrial PPases
due to inactivation of the enzyme under the experimental conditions applied.

**Gel electrophoresis.** The results obtained by polyacrylamide-gel electrophoresis
are in agreement with those obtained by isoelectric focusing. The three purified
enzymatic proteins from subcellular fractions moved towards anode as single bands
with different electrophoretic mobility. Cytoplasmic PPase migrated most rapidly
(Rₑ 0.60), mitochondrial PPase slower (Rₑ 0.50), and chloroplastic PPase at the
slowest rate (Rₑ 0.40). Three distinct bands were obtained when the mixture of the
three protein fractions was separated by gel electrophoresis. This indicates the
occurrence in maize leaves of different enzymatic proteins with alkaline pyrophos-
phatase activity.

**Urea inactivation.** The purified alkaline PPases from subcellular fractions (0.24
unit) were incubated at 20°C for 60 min in 40 mM-Tris-HCl buffer, pH 8.5, con-
taining 5 mM-MgCl₂ and urea at concentration ranging from 0.2 to 3 M. The enzy-
matic activity was measured in buffer solution without urea as described in Methods. At 3 m urea concentration, the cytoplasmic, mitochondrial and chloroplastic PPases lost about 60%, 80% and 95% of their activity, respectively.

Fig. 2. Determination of molecular weight of alkaline pyrophosphatase. Cytoplasmic, chloroplastic and mitochondrial PPases and marker proteins were applied to a Sephadex G-100 column, and eluted as described in Methods. 1, Bovine serum albumin (BSA); 2, ovalbumin; 3, mitochondrial PPase; 4, chloroplastic PPase; 5, cytoplasmic PPase; 6, myoglobin.

Thermal denaturation of alkaline pyrophosphatases. Samples of the enzyme solutions (0.5 ml, 0.24 unit) were incubated at 40 - 90°C for 15 min, then cooled in ice, and their activity was determined as described in Methods. The results obtained indicate that cytoplasmic PPase was most sensitive to thermal denaturation since it lost about 50% of its activity at 50°C, whereas chloroplastic and mitochondrial PPases lost half of their activity at 65° and 60°C, respectively.

$R_f$, pH, molecular weight and $K_m$ values of the alkaline PPases from subcellular fractions of maize leaves, are summarized in Table 3.

**Table 3**

Molecular weight, $R_f$, pH and $K_m$ values of alkaline inorganic pyrophosphatase of subcellular fractions of maize leaves

<table>
<thead>
<tr>
<th>Fraction</th>
<th>$R_f$</th>
<th>pH</th>
<th>Molecular weight</th>
<th>$K_m$ (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytoplasm</td>
<td>0.60</td>
<td>5.00</td>
<td>27 000±1000</td>
<td>$9.9 \times 10^{-6}$</td>
</tr>
<tr>
<td>Chloroplasts</td>
<td>0.40</td>
<td>4.85</td>
<td>32 000±1500</td>
<td>$3.3 \times 10^{-6}$</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>0.50</td>
<td>4.90</td>
<td>32 000±1500</td>
<td>$5.1 \times 10^{-6}$</td>
</tr>
</tbody>
</table>
Fig. 3. Isoelectric focusing of alkaline inorganic pyrophosphatase from cytoplasm (a), chloroplasts (b) and mitochondria (c) of maize leaves. The experiments were performed on a 50 ml isoelectric focusing column under conditions described in Methods.

DISCUSSION

General properties of the purified alkaline inorganic pyrophosphatases from cytoplasm, mitochondria and chloroplasts of maize leaves, resemble those reported for isoenzymes from the extracts of young etiolated maize seedlings and from seedlings treated with cycloheximide (Bennett et al., 1973), and the two isoenzymes from soluble fraction of maize leaves, partially purified by Maslowski & Maslowska (1976).

The purified enzymes from the subcellular fractions were homogeneous on gel electrophoresis and isoelectric focusing. Their specific activity (μmoles P_i/mg protein/min) were 188.0, 164.4 and 55.1, respectively, for the cytoplasmic, chloroplastic and mitochondrial PPase. The three enzymes showed a similar pH optimum, Mg^{2+} requirement, substrate specificity and molecular weight. Differences in the electrophoretic mobility, K_m values, isoelectric point and re-
sistance to thermal denaturation and urea of the enzymes isolated from cytoplasm, mitochondria and chloroplasts indicate the occurrence in maize leaves of three distinct isoenzymatic forms of alkaline inorganic pyrophosphatase compartmentized in the subcellular fractions.

REFERENCES


ROZMIESZCZENIE WE FRAKCIJACH PODKOMÓRKOWYCH I WŁAŚCIWOŚCI ALKALICZNE NIEORGANICZNEJ PIROFOSFATAZY LIŚCI KUKURYDZY

Streszczenie

1. Badania nad rozmieszczeniem alkalicznej nieorganicznej pirofosfatazy w oczyszczonych frakcjach podkomórkowych wykazały, że w liściach kukurydzy enzym ten występuje w cytoplazmie, chloroplastach i mitochondriach. Aktywność enzymu stwierdzona w jądrach i mikrosomach może być wynikiem zanieczyszczenia frakcją mitochondrialną.

2. Subkomórkowe pirofosfatazy oczyszczono drogą frakcjonowania szarczanem amonu, chromatografią jonowymnenną, sześcienną molekularnego i elektrofugowania. Uzyskano wysoko oczyszczone preparaty enzymatyczne o aktywności właściwej 55 - 188 umole/min/mg białka.

3. Badane enzymy przejawiały maksymalną aktywność w pH 8,5 przy stosunku Mg²⁺/PPᵢ = 5. Różniły się natomiast Kᵣₐ, ruchliwością elektroforyetyczną, pI, oraz wrażliwością na mocznik i na denaturację termiczną. Wyniki te wskazują, że są to izoenzymy występujące w poszczególnych frakcjach podkomórkowych.

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