Purification and Properties of a Yeast Nucleotide Pyrophosphatase*

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SUMMARY

A nucleotide pyrophosphatase (EC 3.6.1.9) has been purified from extracts of the hybrid yeast, Saccharomyces fragilis × Saccharomyces dobzhanski, to an extent where it appears homogeneous by ultracentrifugation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Multiple protein components seen on polyacrylamide and starch gel electrophoresis also exhibit enzyme activity. Ultracentrifugation, Sephadex gel filtration, and sodium dodecyl sulfate-polyacrylamide electrophoresis all gave a molecular weight of approximately 65,000 for the enzyme, the electrophoresis results indicating also that the enzyme consists of a single polypeptide chain.

Substrates for the enzyme include several sugar nucleotides (including GDP-mannose and UDP-glucose), pyridine nucleotide coenzymes, and the synthetic substrate, thymidine 5'-p-nitrophenylphosphate. Substrate competition experiments and similar inhibition of hydrolysis of different substrates by metal ion binding agents indicate that a single catalytic site is involved in the hydrolysis of the numerous substrates. No nucleotidase, endonuclease, or exonuclease activities were detectable with the purified enzyme, thus distinguishing it from other yeast and mammalian nucleotide pyrophosphatases which also exhibit nucleotidase and/or phosphodiesterase activity.

The enzyme as isolated does not require addition of any cations for maximal activity, but is strongly inhibited in the presence of metal-binding agents. This inhibition can be reversed by addition of zinc salts or other metal ions, suggesting that protein-bound metal ion is necessary to catalytic activity.

Two seemingly different reports have appeared on nucleotide pyrophosphatase activity in yeast. Takei (1, 2) has purified and characterized an enzyme from Saccharomyces oviformis which exhibited both 5'-nucleotidase activity and nucleotide pyrophosphatase activity. Cabib and Carminatti (3) reported the presence of nucleotide pyrophosphatase activity in extracts of Saccharomyces cerevisiae. Dialyzed cell extracts catalyzed a magnesium-dependent liberation of P1 from GDP-mannose. They suggested that this was due to the combined action of a nucleotide pyrophosphatase and a phosphatase which catalyzed cleavage of the resulting GMP to guanosine and P1. The fact that the enzyme preparation was active on a variety of nucleoside 5'-phosphoryl esters did not warrant a conclusion on the question of whether one or several enzymes were responsible.

The work reported here was directed toward the purification and characterization of an enzyme from the hybrid yeast Saccharomyces fragilis × Saccharomyces dobzhanski which catalyzes the hydrolysis of two commonly used synthetic substrates for phosphodiesterase activity, i.e. bis-p-nitrophenyl phosphate and deoxythymidine 5'-p-nitrophenyl phosphate, in addition to the pyrophosphate bond of several sugar nucleotides and other dinucleotides. No nucleotidase activity was demonstrable and no degradation of a variety of oligonucleotides and polynucleotides occurs. Evidence is presented which indicates that one enzyme is responsible for the hydrolysis of the various substrates.

EXPERIMENTAL PROCEDURE

Materials

The following chemicals and enzymes were obtained from the sources indicated: bis-p-nitrophenyl phosphate, p-nitrophenylphosphate, NAD+, NADH, NMN, C6C, C6A, G6A, ApG, glucose-6-P dehydrogenase, phosphoglucomutase, and alcohol dehydrogenase from Sigma; UDG-glucose, nitrophenyl-pdT, and ApC from Calbiochem; GDP-glucose, GDP-glucose, ADP-glucose, and NADP+ from P-L Biochemicals; dTp-nitrophosphoryl from Raylo Chemicals, Ltd.; oligo- and polynucleotides from Miles Laboratories; GDP-[p-32]mannose from New England Nuclear; phenylmethylsulfonyl fluoride from Cyclo Chemical. Thymidine oligonucleotides were a generous gift from Dr. J. G. Moffatt, and TDP-glucose and GDP-[p-32]glucose were a gift of...
Dr. R. G. Hansen. Hydroxylapatite was prepared as described by Jenkins and Robinson (4). DEAE-cellulose was purchased from Bio-Rad and washed with 0.5 M NaOH and 0.5 M HCl before equilibration with the appropriate buffer.

**Methods**

**Enzyme Assays—**Assay mixtures in which nitrophenyl-p-dT, dT'-nitrophenyl, or bis-p-nitrophenyl phosphate were used as substrates contained 2 to 8 mM substrate, 100 mM Tris-chloride, pH 8.5, and suitably diluted enzyme in a final volume of 1.0 ml. Divalent cations were included in the assay mixture where indicated. The liberation of p-nitrophenol was followed at 400 nm continuously at 30°C in a thermostated cell with a 1-cm light path using a Beckman DB-C spectrophotometer with recorder. For qualitative assays, a lower concentration (0.1 mM) of substrate was used. A molar absorption coefficient of 18,000 for p-nitrophenol at 400 nm and pH 8.5 was used for calculating enzyme units. A unit of activity is defined as the number of micromoles of p-nitrophenol produced per min.

A chromatographic assay was used to follow hydrolysis of GDP-mannose. The reaction mixture consisted of 2.1 mM GDP-[14C]mannose (2.1 x 10^6 cpm per μmole), 100 mM Tris-chloride, pH 8.5, and enzyme in a final volume of 0.25 ml. Aliquots of 50 μl were removed at zero and subsequent times. The reaction was stopped by addition of 25 μl of 0.1 M EDTA in 0.1 M sodium phosphate at pH 6.7. The mixture was then spotted on Schleicher and Schuell 589 Green Ribbon C paper and developed with ethanol-1.0 M ammonium acetate, pH 5.0 (7:3, v/v), for 15 hours. This solvent gave excellent separation of GDP mannose, mannose 1-P, and mannose. Radioactivity was detected with a radiochromatogram scanner. A more quantitative determination was made by counting 1-cm strips of the chromatam immerssed in scintillation fluid (4.0 g of 2,5-diphenyloxazole and 50 mg of 1,4-bis[2-(5-phenyloxazoyl)]benzene made up to 1 liter with toluene) with a liquid scintillation counter.

The production of glucose-1-P from glucose-containing sugar nucleotides was measured spectrophotometrically at 340 nm with phosphoglucomutase and glucose-6-P dehydrogenase. The initial reaction mixture contained 2 mM glucose-containing sugar nucleotide, 100 mM Tris-chloride, pH 8.5, and 250 μl of enzyme in a final volume of 0.25 ml. The mixture was incubated at 30°C and 50-μl aliquots were removed at zero and subsequent times and added to 1.0 ml of the assay mixture for phosphoglucomutase (5) which also contained 2 mM NADP+ and excess phosphoglucomutase and glucose-6-P dehydrogenase.

NAD+ and NADH pyrophosphatase activities were measured in a similar reaction mixture as for sugar nucleotides. Aliquots were removed and analyzed for remaining substrates, NAD+, and NADH, with alcohol dehydrogenase and ethanol or acetaldehyde (6). The assay for NADP+ pyrophosphatase utilized excess glucose-6-P and glucose-6-P dehydrogenase for the measurement of residual substrate (7). The reliability of the enzyme assays involving pyridine nucleotide oxidation or reduction was verified by adding known quantities of the substrates (glucose-1-P, NAD+, NADH, or NADP+) to the appropriate assay mixture and observing nearly stoichiometric (usually 95%) recovery in the form of oxidized or reduced pyridine nucleotide.

The assay mixture for alkaline phosphatase contained 0.9 ml of 1 mM p-nitrophenyl phosphate in 0.5 M Tris-chloride, pH 8.5, and 0.1 ml of diluted enzyme solution. The reaction was followed continuously at 400 nm.

Nucleotidase activity was assayed by measuring the release of P3 as described by Hanson and Fairley (8), and by paper chromatography of assay mixtures with the solvent ethanol-methyl ethyl ketone-0.5 M morpholinium tetrabromide, pH 8.6, in 0.01 M EDTA (7:2:3 v/v). The assays for 5'-nucleotidase activity of 5'-AMP were carried out at pH values of 6.7, and 8.5.

The hydrolysis of dinucleoside monophosphates containing adenosine was followed by the adenosine deaminase-coupled spectrophotometric assay of Ipatov and Felicioli (9). Hydrolysis of dinucleoside monophosphates and of oligo- and polynucleotides was also measured chromatographically. The paper chromatograms were developed with propanol-NH4OH-H2O (55:10:35 v/v) or with ethanol-1.0 M ammonium acetate, pH 7.5 (7:3 v/v), and the presence of mononucleotides was detected with an ultraviolet lamp. Hydrolysis of polynucleotides was also followed by the increase in absorbance at 260 nm of nucleotides and oligonucleotides soluble in cold 2.5% perchloric acid containing 0.25% uranyl acetate.

**Gel Electrophoresis—**Starch gels and buffers were prepared as described by Ferguson and Wallace (10). After electrophoresis, the gel was sliced and one half was stained for protein with 0.01% nigrosin. The other half was overlayed with a solution of 0.25 M Tris-chloride, pH 8.5, and 5 mM nitrophenyl-pdT in warm 1% agar. Release of p-nitrophenol was noted by the appearance of a bright yellow band.

Polyacrylamide disc gel electrophoresis was performed in 7% gels at 4°C in Tris-glycine buffer, pH 9.5 (11). All gels were polymerized by using riboflavin as catalyst. Electrophoresis was performed in a Canalco electrophoresis unit at a current of 5 ma per gel. After electrophoresis, the gels were treated in two different ways. One method consisted of staining one gel for enzyme activity by immersing the whole gel in a 1% agar solution containing 2 mM nitrophenyl-pdT. Another duplicate gel was stained for protein by immersing it in a solution of Amido black for 2 hours and destaining in the Canalco continuous destaining apparatus. The other method consisted of slicing the whole gel from the top to the bottom in 0.25-cm sections, followed by cutting each section into two equal pieces. One piece was stained for enzyme activity by adding it to 1 ml of 4 mM nitrophenyl-pdT for 3 hours at 30°C after which the absorbance of the solution was read at 400 nm. The other piece was homogenized in 0.2 ml of 100 mM Tris-chloride, pH 8.5, for 10 hours. After centrifugation, the protein content of the supernatant fluid was determined by the method of Lowry et al. (12).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis, including the preparation of 10% gels and protein solutions and staining and destaining, was carried out as described by Weber and Osborn (13). The gels were calibrated with trypsin, glyceraldehyde phosphate dehydrogenase (rabbit muscle), aldolase (rabbit muscle), ovalbumin, glutaminate dehydrogenase (bovine liver), and serum albumin (bovine).

**Centrifugation—**Sedimentation equilibrium studies were performed by the high speed meniscus depletion method of Yphantis (14) using the Spinco model E ultracentrifuge equipped with a photoelectric scanner. Double sector cells were used, containing sample and buffer in the two chambers. Sedimentation velocity experiments were monitored with either sehirken optics or a photoelectric scanner at 200 nm. All protein samples were...
**Table I**

**Purification of nucleotide pyrophosphatase**

The total activities and specific activities shown in columns 4 and 5 are for 2 mM nitrophenyl-pdT. The ratio of specific activities of GDP mannose to nitrophenyl pdT is shown in the last column.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Volume (ml)</th>
<th>Protein (mg)</th>
<th>Total Activity (units)</th>
<th>Specific Activity (units X 100/mg)</th>
<th>Ratio of Specific Activities</th>
<th>GDP- Mannose/ Nitrophenyl pdT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract</td>
<td>340</td>
<td>5806</td>
<td>10.5</td>
<td>1.82</td>
<td>2.37</td>
<td></td>
</tr>
<tr>
<td>Heat</td>
<td>390</td>
<td>2400</td>
<td>6.9</td>
<td>2.62</td>
<td>2.89</td>
<td></td>
</tr>
<tr>
<td>pH 4.5</td>
<td>3080</td>
<td>6.2</td>
<td>2.00</td>
<td>3.06</td>
<td></td>
<td></td>
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<tr>
<td>Hydroxylapatite</td>
<td>90</td>
<td>159</td>
<td>3.2</td>
<td>19.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DEAE-cellulose, pH 9.4</td>
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<td>59</td>
<td>2.6</td>
<td>44.1</td>
<td>2.41</td>
<td></td>
</tr>
<tr>
<td>DEAE-cellulose, pH 7.6</td>
<td>2</td>
<td>2.5</td>
<td>0.9</td>
<td>387</td>
<td>2.70</td>
<td></td>
</tr>
<tr>
<td>Sephadex G-200...</td>
<td>1</td>
<td>1.3</td>
<td>0.8</td>
<td>640</td>
<td>2.72</td>
<td></td>
</tr>
</tbody>
</table>

Results

**Preparation of Enzyme**

The major difficulty encountered in the purification of pyrophosphatase is the removal of alkaline phosphatase. The heat step and pH 4.5 step in the purification procedure eliminate a large percentage of the phosphatase, even though a substantial increase in specific activity of the pyrophosphatase is not obtained. Furthermore, the latter two steps were found to be necessary prior to the hydroxylapatite step in order to obtain reproducible and complete removal of the alkaline phosphatase by hydroxylapatite chromatography. The purification procedure as described is highly reproducible.

**Homogeneity and Molecular Weight of Enzyme**

A summary of the purification procedure is given in Table I. Usually, a 300-400-fold purification is obtained with an 8 to 10% recovery of enzyme units. The last column in Table I also shows that the ratio of specific activities using the substrates GDP-mannose and nitrophenyl-pdT are essentially constant throughout purification.

**Analytical Ultracentrifugation**—A single, apparently symmetrical peak was observed with schlieren optics at a protein concentration of 5 mg per ml. At a protein concentration of 0.15 mg/ml, the enzyme eluted with 400 ml of 0.08 M sodium phosphate, pH 6.85, by stirring for 10 min. Following centrifugation, the supernatant fluid was concentrated to 100 ml by vacuum dialysis.

Since some contaminating alkaline phosphatase remained, the enzyme solution was applied to a column (2.5 cm x 20 cm) containing 20 g of hydroxylapatite in 1 mM sodium phosphate at pH 6.85. The column was eluted with 100 ml of 0.05 M sodium phosphate, pH 6.85, and then with 90 ml of 0.08 M sodium phosphate, pH 6.85. The latter eluent, containing the enzyme, was entirely free of alkaline phosphatase.

**DEAE-cellulose, pH 9.4, Step**—The enzyme solution was concentrated to 10 ml by vacuum dialysis and dialyzed against 3 liters of 5 mM Tris-chloride, pH 9.4, containing 0.1 M NaCl. This solution was applied to a column (1.5 cm x 50 cm) of DEAE-cellulose equilibrated with the same buffer. Elution was carried out with a linear gradient of 1 liter of the equilibration buffer and 1 liter of 5 mM Tris-chloride, pH 9.4, containing 0.35 M NaCl. Fractions containing enzymatic activity against nitrophenyl-pdT were combined and concentrated to 26 ml by vacuum dialysis.

**DEAE-cellulose, pH 7.6, Step**—The preceding solution was dialyzed against 3 liters of 0.05 M Tris-chloride, pH 7.6, and concentrated to 10 ml. The solution was applied to a column (1.5 cm x 30 cm) of DEAE-cellulose equilibrated with the same buffer. A linear gradient of 500 ml of the equilibration buffer and 500 ml of 0.05 M Tris-chloride, pH 7.6, containing 0.3 M NaCl was used for elution. The fractions containing enzyme activity were pooled and concentrated to 2 ml.

**Sephadex G-200, A chromatographic column (1 x 100 cm)** was filled with Sephadex G-200 which had been equilibrated with 0.05 M Tris-chloride, pH 7.6, containing 0.1 M KCl. The 2.2 ml enzyme solution from the previous step was applied and eluted with the same buffer at a flow rate of 5 ml per hour. Fractions containing enzyme activity were pooled and concentrated by vacuum dialysis to 1 ml. The enzyme at this stage of purity was entirely free of alkaline phosphatase.
O.D.₅₅₀ per ml, an observed sedimentation coefficient of 3.85 was calculated from data obtained with a photoelectric scanner. At the same protein concentration, sedimentation equilibrium runs (20 hours at 22,000 rpm) also indicated lack of heterogeneity of the enzyme preparation in that a straight line was obtained from a plot of log concentration (O.D.₅₅₀) versus the square of the distance from the center of rotation. Although the partial specific volume has not been determined, an assumed average value of 0.735 ml/g results in a calculated molecular weight of 64,500.

Gel Filtration—The purified enzyme emerged in a single, symmetrical peak from a Sephadex G-200 column. After calibration of the column with standard proteins of known molecular weight, a plot of elution volume versus molecular weight of the standard proteins gave a molecular weight of 60,000 for the nucleotide pyrophosphatase.

Gel Electrophoresis—Analysis of the enzyme by polyacrylamide gel electrophoresis at pH 9.5 revealed four distinct protein bands. However, when assayed for enzyme activity with nitrophenyl-pdT using either the whole gel or gel sliced as described in “Methods,” all four protein bands were found to be active. Slicing the gel into 0.25-cm sections followed by splitting each section into two pieces allowed a more quantitative determination of protein and enzyme activity. As shown in Fig. 1, only those sections which contained protein exhibited enzyme activity. Furthermore, the specific activity of the enzyme was nearly constant through the region of the four protein bands.

Starch gel electrophoresis revealed a broad protein band of about 0.6 cm width. The entire band exhibited enzyme activity when overlaid with an agar solution of nitrophenyl-pdT.

Electrophoresis of the reduced, denatured enzyme (40 μg) on SDS-polyacrylamide gels resulted in the detection of only one protein band, again indicative of a homogeneous protein. A plot of log molecular weight versus mobility for several standard proteins gave a molecular weight of 65,000 for the nucleotide pyrophosphatase. This evidence suggests that the protein consists of a single polypeptide chain.

Since limited proteolysis of the nucleotide pyrophosphatase during the isolation procedure may account for the multiple components seen on gel electrophoresis of the native enzyme, the isolation procedure was carried through with all of the working solutions containing 5 × 10⁻³ M phenylmethylsulfonyl fluoride, a protease (esterase) inhibitor previously shown to be effective in isolation of yeast enzymes (17, 18). No major differences in fractionation characteristics were noted, and multiple, enzymatically active protein bands were still observed on gel electrophoresis. Although it is still possible that these multiple forms are due to proteolysis, the nearly constant specific activities of the polyacrylamide gel components (Fig. 1) would argue against any major alteration in catalytic activity, and the single protein band observed on SDS gel electrophoresis would argue against any major alteration in size.

Properties of Enzyme

Substrates—After the final purification step, no hydrolysis of the following compounds by the enzyme was detectable: poly(A); poly(C); poly(U); yeast-soluble and transfer RNA; Up(Up)₃Up; Ap(Up)₃Up; m'T(m'T₃)m'T; m'T₃(m'T₃)m'T; ApC; CpA; ApG; GpA; CpA; ApA; FpG; 2'(3')-AMP; cyclic adenosine 2',3'-monophosphate; 5'-AMP; 5'-GMP; ADP; ATP; p-nitrophenyl phosphate.

The nucleotide pyrophosphatase exhibited activity against all sugar nucleotides and all coenzymes tested which are diesters of pyrophosphoric acid (Table II). The rates of hydrolysis of the sugar nucleotides tested are dependent on both the sugar and nucleoside moieties, GDP-mannose being hydrolyzed the fastest. A difference in rate of hydrolysis is also noted for the pyridine nucleotide coenzymes. The Michaelis constant, Kₘ, is 0.17 nM for NAD⁺ as compared with 1.0 nM for GDP-mannose and 3.3 nM for UDP-glucose. However, the rates of hydrolysis are much greater for the sugar nucleotides than for NAD⁺.

In addition, substrates which are phosphodiester containing...
Effect of zinc chloride concentration on EDTA-inhibited nucleotide pyrophosphatase. Enzyme activity was measured as described in "Methods" immediately after the addition of 2 mM EDTA and ZnCl₂ as indicated. Enzyme activity is expressed as a percentage of a control incubation which contained neither EDTA nor metal. △—△, nitrophenyl-pdT; ○—○, GDP-mannose.

Product Identification—The products have been conclusively shown to be due to the action of a nucleotide pyrophosphatase. Hydrolysis of GDP-mannose yields equimolar quantities of GMP and mannose-1-P. The GMP was identified by chromatography with the solvents ethanol-1.0 M ammonium acetate, pH 7.5 (7:3 v/v), and 0.5 M LiCl on polyethyleneimine impregnated Whatman No. 1 paper. The fact that no GTP or GDP was found and that no PP₁ was present in the incubation mixture rules out the participation of GDP-mannose pyrophosphorylase (19). Similar evidence from other sugar nucleotides also rules out the participation of any of the other sugar nucleotide pyrophosphorylases which have been reported to be present in yeast (20). The lack of a requirement for P₁ in the absence of any nucleoside diphosphate production similarly rules out the participation of the sugar nucleotide pyrophosphatase (21). The pH optimum for the enzyme with UDP-glucose, GDP-mannose, or nitrophenyl-pdT as substrates was pH 8.6 to 8.7 when tested at relatively high substrate concentrations (8 mM) in either Tris-chloride or glycine-KOH buffers. Activity was half-maximal at pH 8.2.

Activators and Inhibitors—Monovalent cations such as sodium and potassium had no effect on the enzyme activity below a concentration of 1 M. Although the enzyme is not inactive in the absence of any additions of divalent cations, it is inhibited completely by chelating agents such as 2 mM EDTA and O-phenanthroline. Partial or total reactivation is achievable with several different divalent cations. The percentage of reactivation with 4 mM metal ions was: zinc, 80%; cobalt, manganese, and copper, 55%; calcium, iron, magnesium, and nickel, 45%. The reactivation of the EDTA-inhibited enzyme with different concentrations of zinc is shown in Fig. 1. A zinc concentration of 1 to 2 mM above the concentration of EDTA is optimal; higher levels of zinc result in inhibition. Fig. 2 also shows that the
enzyme-catalyzed hydrolysis of two substrates, nitrophenyl-pdT and GDP-mannose, is maximally reactivated at approximately the same zinc concentration.

The enzyme is also inhibited by various reducing agents. Dithiothreitol and L-cysteine (4 mM) inhibit completely, and higher concentrations (16 mM) of mercaptoethanol inhibit activity by 90%. Ascorbic acid (16 mM) is without effect. These compounds probably do not exert this effect as reducing agents, but rather by virtue of their metal-binding properties, since the inhibition can be overcome by addition of excess zinc.

Evidence for Single Enzyme Catalyzing Hydrolysis of Different Substrates —The numerous substrates hydrolyzed by the isolated enzyme and the previous reports of different nucleotide pyrophosphatasos in yeast (1, 2, 23) led us to examine whether a single protein was responsible for the observed catalysis. The following experiments, in addition to the previously described studies on homogeneity of the protein, indicated that a single enzyme was involved.

Coincidence of Enzyme Activity during Purification.—The activity against nitrophenyl-pdT, GDP-mannose, NAD+, and bis-p-nitrophenyl phosphate coincided on Sephadex G-200 and on two DEAE-cellulose columns. Also, the ratio of specific activities against GDP-mannose and nitrophenyl-pdT remained essentially constant throughout purification (Table I).

Kinetics.—The hydrolysis of nitrophenyl-pdT was inhibited by GDP-mannose, UDP-glucose, and NAD+ in a competitive manner as expected if the same enzyme is involved. Furthermore, the measured total velocities of hydrolysis of GDP mannose and nitrophenyl-pdT when presented together were not additive as compared with each substrate alone but agreed quite closely with total velocities calculated from the equation
\[ v = \frac{V_0}{K_s + \frac{V_0}{(a)K_a + (b)K_b}} \]
for a reaction in which mutual competition of two substrates for a single enzyme occurs (24) (Fig. 3).

Inhibitors.—As already documented, activity against both GDP-mannose and nitrophenyl-pdT is inhibited by EDTA and restored to the EDTA-inhibited enzyme by addition of metal ions.

Discussion

The nucleotide pyrophosphatase activity in dialyzed yeast extracts as previously reported by Cabib and Carminatti (3) is probably the same enzyme as reported here. The relative rates of hydrolysis of GDP-mannose, UDP-glucose, and NAD+ (Table II) decrease in that order as previously observed (3). The reported magnesium requirement may have been due to the partial inactivation of the enzyme by the EDTA present in their buffers. EDTA inhibition and reactivation by zinc and other metals suggests that the enzyme, as isolated, contains essential metal ions. These results are in agreement with the demonstration by Corder and Lowry (25) of a zinc requirement for mammalian nucleotide pyrophosphatase.

Several properties of the S. oviformis nucleotide pyrophosphatase (1, 2) differ from those reported here. In particular, we observe no 5'-nucleotidase activity. Since it was not reported whether the S. oviformis enzyme catalyzes hydrolysis of sugar nucleotides, it is possible that the enzyme reported here is basically a sugar pyrophosphatase, whereas the S. oviformis enzyme is a NAD+ pyrophosphatase.

The hydrolysis of nitrophenyl-pdT and bis-p-nitrophenyl phosphate, substrates commonly used for detecting and assaying phosphodiesterases (26–28), points out again (29, 30) the relative nonspecific nature of these compounds and the possible danger in using them as specific diesterase substrates.

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REFERENCES

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