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Membrane-bound and soluble polyphosphatases of mitochondria of *Saccharomyces cerevisiae*: identification and comparative characterization

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Abstract

Isolated mitochondria of *Saccharomyces cerevisiae* possess polyphosphatases insensitive to a number of inhibitors of ATPase and pyrophosphatase of the same organelles and differing from the last two by neutral pH optima and molecular masses. After subfractionation of mitochondria, the polyphosphatase activity is distributed among the membrane and soluble preparations. The membrane-bound and soluble polyphosphatase activities are represented by different enzymes distinguished by molecular masses, substrate specificity, K_m values, and relation to mono- and divalent cations. The membrane-bound polyphosphatases have molecular masses of 120 and 76 kDa, and the soluble one of about 36 kDa. All three enzymes appear to have a monomeric structure. The soluble polyphosphatase activity is stimulated by divalent cations in contrast to the membrane-bound one which is inhibited by the same cations, including Mg^{2+}. Monovalent cations do not actually change the activity of the soluble enzyme, but stimulate it in the membrane preparation. Specific activities for the hydrolysis of polyphosphates with average chain lengths of 9–188 phosphate residues increase under increasing degree of substrate polymerization in the membrane preparation and are actually unchanged in the soluble one. The affinity of the soluble enzyme to polyphosphates is 5–10 times higher than that of the membrane-bound polyphosphatases.

Keywords: Exopolyphosphatase; Tripolyphosphatase; Pyrophosphatase; Mitochondrion; (Yeast)

1. Introduction

Eucaryotic microorganisms, in particular yeast, possess polyphosphate pools localized in different cell compartments [1]. However, the enzymes carrying out the synthesis and degradation of these biopolymers are still little-studied. We have demonstrated polyphosphatase activities of cell envelope [2,3], cytosol free from cell organelles [4], vacuoles [5], and nuclei [6] of *Saccharomyces cerevisiae*. The cell-envelope and cytosol polyphosphatases were purified to homogeneity [3,7]. Comparison of polyphosphatase activities by a number of biochemical properties suggests that each of the compartments in question possesses its own polyphosphatase.

The comparative study of the enzymes of polyphosphate metabolism is of interest both in the context of understanding of the role of polyphosphate in

Abbreviations: Cat^+- and Cat^{2+}, mono- and divalent cations; Con A, concanavalin A; DCCD, *N*,*N*′-dicyclohexylcarbodiimide; PMSF, phenylmethylsulfonyl fluoride; PPase, pyrophosphatase

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various cell compartments and the evolutionary origin of cell organelles. In this connection, the study of the mitochondrial polyphosphatase and its comparison with the known polyphosphatases of eubacteria [8,9] might be very productive, since the evolutionary origin of mitochondria from ancient eubacteria is now generally recognized [10].

The present work demonstrates that the mitochondria of *S. cerevisiae* possess membrane-bound and soluble forms of the polyphosphatase activity which are now being compared.

2. Materials and methods

2.1. Chemicals

All chemicals used were of the analytical grade. Polyphosphates with the average chain lengths of 9, 15, and 188 (Monsanto, USA) or 45 (Sigma, USA) were separated from P_i and PP_i by gel filtration on Sephadex G-10 (Pharmacia, Sweden) as described [2]. Tripolyphosphate (Sigma), ATP (Reanal, Hungary), and PP_i (Koch-Light, UK) were used without further purification. Sephacryl S-200 was purchased from Pharmacia/LKB, and Con A from Serva (Germany).

2.2. Cultivation, isolation of spheroplasts and mitochondria

Cultivation of the yeast *S. cerevisiae* VKM-1173 (IBFM-366) and isolation of spheroplasts were performed as described earlier [6].

To isolate mitochondria, spheroplasts were shocked osmotically in 20 mM Tris-HCl, pH 6.8, containing 1 mM EDTA, 4 mM $MgSO_4$, 1 mM PMSF, 0.2% bovine albumin, and 0.05% Con A. Spheroplasts were incubated in this osmotic lysis buffer with gentle agitation for 10 min at 25°C followed by a quick addition of an equal volume of cooled 1 M sorbitol in the same buffer containing no Con A. All the following manipulations were carried out at 0°C according to the method described for isolation of mitochondria [11] with the addition of 1 mM PMSF to all the solutions.

Mitochondria were purified in a Percoll gradient in 10 mM Tris-HCl, pH 6.8, with 0.6 M sorbitol and 1 mM PMSF. The mitochondria were suspended in 1–2 ml of buffer and layered on top of discontinuous Percoll gradient (10 ml 40% and 30 ml 20% Percoll). The gradient was centrifuged for 30 min at $105\,000 \times g$. The band at the interphase was collected, diluted with more than 20 buffer volumes, and centrifuged for 5 min at $1400 \times g$. Mitochondria from the supernatant were harvested for 20 min at $17\,000 \times g$, washed again with buffer, and pelleted for 10 min at same speed. Mitochondria were suspended in 20 mM Tris-HCl, pH 6.8, containing 0.6 M sorbitol, 1 mM EDTA, 4 mM $MgSO_4$, and 1 mM PMSF (buffer A).

2.3. Subfractionation of mitochondria

Subfractionation was performed by sonication of freshly prepared mitochondria for 3×20 s at the maximal output using a MSE ultrasonic disintegrator. The suspension was centrifuged for 10 min at $12\,000 \times g$ to remove unbroken mitochondria. The membrane fraction of mitochondria was pelleted by centrifugation for 60 min at $130\,000 \times g$. The pellet was washed with buffer A containing 0.1 M NaCl and once again sedimented under the same conditions. After the first high-speed centrifugation, the supernatant was a soluble preparation of mitochondria which included both intermembrane space and matrix.

2.4. Solubilization of the membrane preparation of mitochondria and gel filtration

Proteins of the mitochondrial membrane preparation were solubilized with Triton X-100 in 20 mM Tris-HCl, pH 7.2, containing 20% glycerol, 5 mM dithiothreitol, 4 mM $MgSO_4$, and 0.5 mM PMSF. The solution for solubilization was added to the membrane sediment to obtain the ratio detergent/protein 5:1 (w/w). The suspension was treated with Teflon pistil from time to time for 20 min at 0–4°C and centrifuged for 60 min at $200\,000 \times g$. The pellet was treated once more with Triton X-100 in the solution for solubilization. The concentration of Triton X-100 was doubled. Then the suspension was centrifuged under the same conditions, and supernatants were combined.

Proteins of the soluble preparation and membrane preparation after solubilization with Triton X-100

were chromatographed using a column (1.6×90 cm) with Sephacryl S-200 equilibrated with 20 mM Tris-HCl, pH 7.2, containing 0.1% Triton X-100, 1 mM dithiothreitol, 0.1 M NaCl, and 4 mM $MgSO_4$, and then eluted with the same buffer at a rate of 15 ml/h. Fraction volume was 3 ml.

2.5. Enzyme assays

Polyphosphatase (exopolyphosphatase, polyphosphate phosphohydrolase, EC 3.6.1.11), tripolyphosphatase (EC 3.6.1.25), and pyrophosphatase (EC 3.6.1.1) activities were determined by the rate of P_i formation at 30°C for 30–60 min in 1 ml of reaction mixture. The incubation mixtures for determination of the phosphohydrolase and succinate dehydrogenase (EC 1.3.99.1) activities were described in [12]. To study the phosphohydrolase activities in the native mitochondria, 0.1% Triton X-100 was added to the incubation mixture. The resulting P_i was determined with ascorbic acid and a solution containing ammonium molybdate and SDS [13].

To estimate the effect of polyclonal antiserum against the purified cell-envelope polyphosphatase on mitochondrial polyphosphatase activity, the reaction was started by adding the substrate after incubation of mitochondria or preparations obtained as a result of subfractionation with immunoserum for 5 min at room temperature. Polyphosphate with an average chain length of 15 was essentially used in the work. The use of other polyphosphates was indicated specially.

To study the effect of Cat^+ and Cat^{2+} on the polyphosphatase activities of isolated mitochondria and soluble and membrane preparations obtained from them, they were first dialyzed for 5 h at 4°C against 10 mM Tris-HCl, pH 7.2, containing 20% glycerol. The buffer was changed four times during the procedure. The enzyme activities were determined as described above using sulfates in the case of divalent cations, and chlorides, with monovalent ones.

2.6. Other methods

Protein concentration was assayed by the modified Lowry method [14] using bovine serum albumin as the standard. Electrophoretic analysis under denaturating conditions was performed in 7.5% PAG according to Laemmli [15]. Immunoblotting was carried out as described earlier [16]. pH-dependence of the phosphohydrolase activities was determined using 100 mM Tris-acetate, pH 5.5–9.0. Polyclonal antiserum against the purified cell-envelope polyphosphatase of *S. cerevisiae* was prepared from rabbit as described earlier [16]. The rate of O_2 uptake by mitochondria was estimated as earlier in a buffer containing 0.6 M sorbitol, 0.36 mM EDTA, 10 mM KH_2PO_4, 10 mM Tris-HCl, pH 6.5, and 10 mM KCl, and measured by a LP-7 Polarograph (Laboratorni Pristroje, Czechia) in a closed thermostatted cell using a Clark-type platinum electrode [11]. The differential spectrum of the mitochondrial preparation was measured using a Shimadzu UV-180A spectrophotometer (Japan). The isolated mitochondria oxidized with $K_4[Fe(CN)_6]$ were placed in the reference cell, and the experimental cell was filled with the same quantity of the preparation reduced with dithionite.

3. Results

3.1. Characterization of isolated mitochondria

The isolated mitochondria had the following characteristics. When oxidizing NADH, O_2 consumption was 0.22 μmol O_2/min per mg protein. This process was sensitive to KCN. The succinate dehydrogenase activity was 0.1 and 0.3 μmol/min per mg protein before and after purification in the Percoll gradient, respectively, and this was in good agreement with the literature data [17].

The mitochondrial absorption spectrum had maxima at 549, 561, and 605 nm corresponding to cytochromes *c*, *b*, and aa_3, and this confirms the data obtained for the yeast mitochondria [11,13].

In preliminary experiments, the isolated mitochondria contained ATPase inhibited by vanadate more than 2-fold, indicating the presence of plasma membranes in the preparation. The plant lectin Con A was used to bind superficial glycoproteins of the yeast plasma membranes [18]. When treated with Con A just before spheroplast lysis, the plasma membranes produced large fragments which were easily sedimented at a low-speed centrifugation. This allowed us to obtain a relatively pure preparation of

Table 1
Effect of 0.1% Triton X-100 on some phosphohydrolase activities of isolated mitochondria of *S. cerevisiae*

Phosphohydrolase	Specific activity (mU/mg protein)	
	Without Triton X-100	With Triton X-100
Polyphosphatase	35	80
Tripolyphosphatase	10	120
ATPase	805	1225
PPase	90	165
Alkaline phosphatase	20	40

mitochondria free from the plasma, vacuolar, and nuclear membranes.

The polyphosphatase activity of the isolated mitochondria was fairly low (Table 1). Triton X-100 (0.1%) increased the activity more than 2-fold (Table 1). This might be explained by a facilitated access of the substrate to the enzyme. ATPase, PPase, and alkaline phosphatase activities also increased in the presence of 0.1% Triton X-100. So, all the activities of the isolated mitochondria were thereafter measured with 0.1% Triton X-100.

The effect of some inhibitors on phosphohydrolase activities of the isolated mitochondria was studied to exclude the possibility of polyphosphate hydrolysis by other mitochondrial phosphohydrolases (Table 2). Heparin, an effective inhibitor of polyphosphatase activities of cytosol, vacuoles, and nuclei [4–6] of the yeast, completely suppressed the polyphosphatase activity of the isolated mitochondria. This acidic mucopolysaccharide appears to be a substrate analog and does not act on the mitochondrial ATPase and PPase (Table 2).

The mitochondrial polyphosphatase activity was almost insensitive to the action of NaF, which completely suppressed the PPase activity (Table 2). It was also insensitive to such known inhibitors of the mitochondrial ATPase as azide, oligomycin, DCCD, and orthovanadate, an inhibitor of plasma membrane ATPase (Table 2). The inhibitor of vacuolar ATPase, KNO_3, decreased the polyphosphatase activity of mitochondria by $\sim$40%. This effect, however, was due to the action of K^+ but not NO_3^-.

When used purified mitochondrial ATPase and PPase, no polyphosphatase activity was observed with polyphosphate as a substrate.

The tripolyphosphatase activity of the isolated mitochondria of *S. cerevisiae* was about 3-fold lower than the polyphosphatase one (Table 1). However, it increased 12-fold on addition of 0.1% Triton X-100 (Table 1). We suggested that the tripolyphosphatase activity might be localized inside mitochondria, and Triton X-100 facilitated the access of tripolyphosphate to the enzyme. This prompted us to dis-

Table 2
Effect of some reagents on the phosphohydrolase activities of isolated mitochondria of *S. cerevisiae*

Reagent	Concentration	Activity (%)			
		Polyphosphatase	Tripolyphosphatase	ATPase	PPase
No addition		100	100	100	100
NaN_3	5 mM	99	105	29	98
Oligomycin	10 μg/ml	99	105	22	100
Heparin	2 μg/ml	0	100	–	–
	20 μg/ml	0	75	130	113
NaF	1 mM	90	88	109	0
Vanadate	0.1 mM	98	90	98	105
DCCD	0.1 mM	103	120	31	100
KNO_3	50 mM	63	120	118	108

Specific activities of polyphosphatase, tripolyphosphatase, ATPase, and PPase taken as 100% were 95, 110, 1300, and 200 mU/mg protein, respectively.

integrate the isolated mitochondria and to study separately the membrane and soluble fractions obtained.

3.2. Subfractionation of isolated mitochondria, solubilization and gel filtration of mitochondrial proteins

Membrane and soluble preparations were obtained by ultrasonic treatment of the isolated mitochondria of *S. cerevisiae* followed by membrane sedimentation. Both preparations contained approximately equal proportions of the protein and polyphosphatase activities (Table 3). The soluble preparation contained more than 80% of the tripolyphosphatase and PPase activities of mitochondria (Table 3).

The membrane preparation contained the polyphosphatase activity which was difficult to extract from the membranes. We failed to find the appropriate conditions to solubilize it. The membrane-bound polyphosphatase activity was not solubilized with deoxycholate; Zwittergent TM-314 extracted only about 15% of this activity, and it was inactivated during storage at −20°C. We succeeded in solubilizing approximately 40% of the polyphosphatase activity with Triton X-100. The tripolyphosphatase and PPase activities were solubilized with Triton X-100 by 70 and 90%, respectively.

Membrane proteins after solubilization with Triton X-100 (∼5 mg) and soluble proteins (∼4 mg) were chromatographed using the column with Sephacryl S-200 (Fig. 1). The polyphosphatase activity was partially separated from the tripolyphosphatase and PPase activities through gel filtration. The proteins with the polyphosphatase activity were revealed in the membranes. Their molecular masses were of 120 and 76 kDa. The molecular mass of the soluble enzyme was 36 kDa.

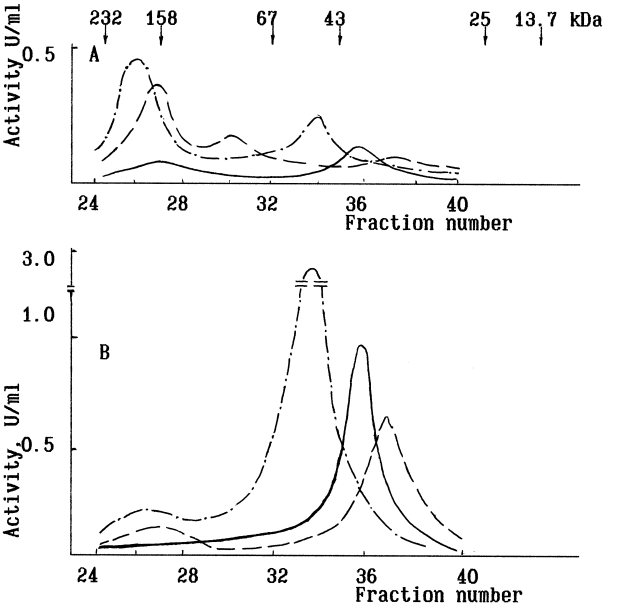

Fig. 1. Gel filtration of proteins of the mitochondrial preparations using Sephacryl S-200 column. Membrane preparation solubilized with Triton X-100 (A) and soluble preparation (B). - - -, polyphosphatase; —, tripolyphosphatase; - · -, PPase. Ribonuclease A (13.7 kDa), chymotrypsinogen A (25 kDa), ovalbumin (43 kDa), bovine serum albumin (67 kDa), aldolase (158 kDa), and catalase (232 kDa) were used as protein standards for calibration of the column. Their positions are shown by arrows.

The tripolyphosphatase activity was revealed mainly in the soluble preparation and corresponded to a protein with a molecular mass of about 44 kDa (Fig. 1). The only known eucaryotic tripolyphosphatase from mitochondria of *Neurospora crassa* had two identical subunits of about 40 kDa each [19].

The PPase activity of the membrane preparation was associated with a protein of about 185 kDa, and that of the soluble preparation, with a protein of about 58 kDa. These data coincide very closely

Table 3
Phosphohydrolase activities of isolated mitochondria of *S. cerevisiae* and of preparations obtained under their subfractionation

Preparations	Protein (mg)	Activity (mU)		
		Polyphosphatase	Tripolyphosphatase	PPase
Mitochondria	16	1200	1825	3775
Membrane preparation	9.25	785	305	620
Soluble preparation	6.75	575	1430	2565
Membrane fraction after solubilization with Triton X-100	5.5	310	215	560
Sediment	3.8	450	120	70

with those obtained under PPase purification from bovine heart mitochondria [20].

3.3. Immunoassay of mitochondrial polyphosphatases

Antibodies against the purified cell-envelope polyphosphatase of *S. cerevisiae* appear to have rather high specificity relative to all the yeast polyphosphatases. These antibodies inhibited polyphosphatase activities of the cell envelope and cytosol and did not affect those of isolated vacuoles and nuclei [5,6]. Nevertheless, these antibodies were able to react with all the yeast polyphosphatases in the Western blot [16]. Yeast polyphosphatases appear to have certain similar sites in their structures, which are not exactly related to the activity.

In experiments with mitochondria, the antibodies did not affect the polyphosphatase activity in the membrane preparation and inhibited the soluble polyphosphatase by approximately 30%. Consequently, the soluble polyphosphatase of mitochondria differed by its immune properties from those of the cytosol and cell envelope notwithstanding that they all had similar molecular masses.

The polyphosphatases revealed both in the membrane and soluble preparations of the yeast mitochondria appear to have a monomeric structure, since PAGE under dissociating conditions followed by immunoblotting showed that antibodies against the purified cell-envelope polyphosphatase reacted with polypeptides of about 115, 78, and 37 kDa (Fig. 2). The two membrane polyphosphatases may differ in the content of carbohydrate or lipid moieties.

A good agreement of molecular masses determined by these two methods suggests that antibodies against the cell-envelope polyphosphatase were able to react with the mitochondrial polyphosphatases.

3.4. Effect of pH on mitochondrial phosphohydrolases

The membrane-bound and soluble polyphosphatase activities of mitochondria were optimal at the neutral pH with a less pronounced pH optimum of the soluble enzyme (Fig. 3). The mitochondrial polyphosphatase activities were similar in their pH optima both to polyphosphatases from other compartments of the same yeast strain of *S. cerevisiae* [3–6]

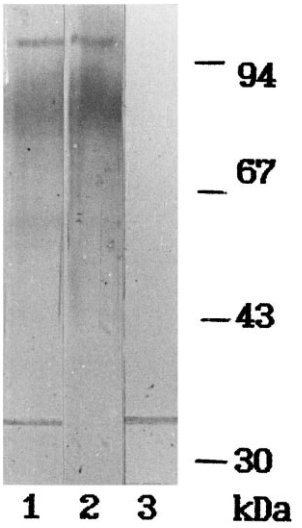

Fig. 2. Immunoblot of mitochondrial proteins with antibodies against the cell-envelope polyphosphatase. Molecular mass standards used are shown to the right: phosphorylase (94 kDa), bovine serum albumin (67 kDa), and ovalbumin (43 kDa). In addition, thyroglobulin (330 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20.1 kDa), and α-lactalbumin (14.4 kDa) were run in parallel. Lane 1, preparation of isolated mitochondria; lane 2, preparation of mitochondrial membranes; lane 3, preparation of soluble mitochondrial fraction.

and to most of the known microbial polyphosphatases [21–23].

The tripolyphosphatase activity of the membrane preparation had no well-defined pH optimum, and in the soluble preparation it was shifted to the alkaline region (Fig. 3).

PPases of the membrane and soluble preparations were most active in the alkaline region, pH 7.5–9.0 (Fig. 3). The shift of pH optimum of the soluble tripolyphosphatase activity to the alkaline region may be due to the fact that PP_i formed by hydrolysis of the substrate by tripolyphosphatase was hydrolyzed by PPase, the activity of which was 6-fold higher in the soluble preparation as compared with the membrane one. This is especially probable as NaF, the PPase inhibitor, strongly suppressed the tripolyphosphatase activity in the soluble preparation of mitochondria: about 60% as compared with 20% for the polyphosphatase activity.

3.5. Substrate specificity and affinity to polyphosphates

The polyphosphatase activity in the soluble prep-

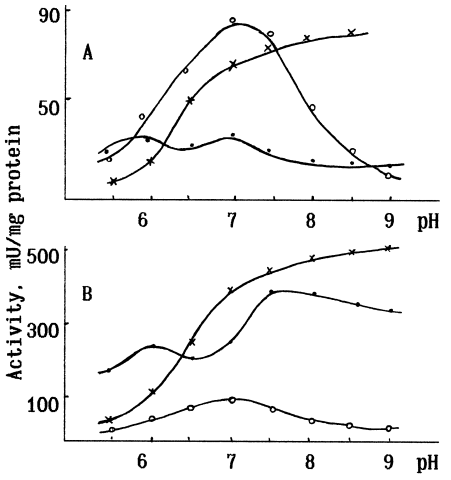

Fig. 3. Effect of pH on the phosphohydrolase activities of membrane-bound (A) and soluble (B) preparations of mitochondria of *S. cerevisiae*. ○, polyphosphatase; ●, tripolyphosphatase, ×, PPase.

aration of mitochondria was nearly the same when using polyphosphates with the average chain lengths of 9, 15, 45, and 188 phosphate residues (Table 4). In this respect, the polyphosphatase activity of the soluble preparation of mitochondria was similar to those of cytosol and nuclei, which were almost unchanged with the increase of substrate polymerization from 9 to 208 phosphate residues [4,6]. The polyphosphatase activity in the membrane preparation of mitochondria was enhanced with the increase of polymerization degree (Table 4), which was characteristic of vacuolar polyphosphatases revealed in the same yeast strain [5].

The apparent K_m values of the membrane-bound polyphosphatase activity in the presence of 2.5 mM Mg^{2+} were 25 and 1 μM on hydrolysis of polyphosphates with the chain length of 15 and 188 phosphate residues, respectively. The values obtained and the shape of dependences of substrate concentrations differed little from those for the isolated mitochondria.

Table 4
Polyphosphatase activities (mU/mg protein) of soluble and membrane preparations of mitochondria with polyphosphates having different polymerization degrees

Preparation	Polyphosphates with average chain length equal to:				
	3	9	15	45	188
Soluble preparation	210	65	80	60	75
Membrane preparation	40	45	85	100	110

The soluble polyphosphatase of mitochondria had the highest affinity to polyphosphates among the reported yeast polyphosphatases. The apparent K_m values were 4 and 0.06 μM on hydrolysis of polyphosphates with the average chain length of 15 and 188 phosphate residues, respectively. When working with the preparations of isolated mitochondria, this higher affinity to the substrate was concealed by the low affinity of the membrane-bound polyphosphatase activity.

3.6. Effect of mono- and divalent cations

In the presence of 50 mM Tris-HCl and 2.5 mM Mg^{2+}, monovalent cations did not actually affect the soluble polyphosphatase activity of mitochondria while stimulating the polyphosphatase activity in the membrane preparation (Table 5). NH_4^+ was the best stimulator.

Most polyphosphatase activities from various microorganisms were stimulated by addition of K^+ or NH_4^+ in the presence of $Tris^+$ [8,9,23,24]. However,

Table 5
Effect of monovalent cations on the polyphosphatase activities of membrane and soluble preparations of isolated mitochondria of *S. cerevisiae*

Cation concentration (mM)	Activity (%)		
	Na^+	K^+	NH_4^+
10	100 (115)	110 (120)	115 (115)
50	130 (105)	115 (110)	140 (130)
100	160 (100)	120 (100)	160 (115)
200	140 (95)	135 (95)	190 (110)

Activities of soluble preparation are given in brackets. Activities in the absence of cations taken as 100% were 85 mU/mg protein in both preparations.

Table 6
Effect of divalent cations on the polyphosphatase activities of membrane and soluble preparations of isolated mitochondria of *S. cerevisiae*

Cation concentration (mM)	Activity (%)			
	Mg^{2+}	Co^{2+}	Zn^{2+}	Mn^{2+}
0.005	– (–)	80 (125)	80 (120)	– (–)
0.01	105 (160)	75 (380)	80 (370)	90 (210)
0.1	95 (410)	70 (380)	60 (250)	50 (340)
1.0	70 (450)	10 (195)	10 (70)	45 (255)
2.5	70 (370)	– (–)	– (–)	– (–)

Activities of the soluble preparation are given in brackets. Activities in the absence of divalent cations taken as 100% were 31 and 46 mU/mg protein for the membrane and soluble preparations, respectively.

there is an example of suppression of the polyphosphatase activity by K^+ in the homogenate of *Endomyces magnusii* [25].

The polyphosphatase activities in the membrane and soluble preparations of mitochondria were 31 and 46 mU/mg protein, respectively, in the absence of Cat^{2+} (Table 6). Addition of Cat^{2+} to the soluble preparation resulted in the increase of this activity, which depended on the nature and concentration of Cat^{2+} (Table 6). Based on the degree of stimulation of the soluble enzyme, the tested Cat^{2+} were arranged in the following order: $Co^{2+} > Mg^{2+} > Zn^{2+} > Mn^{2+}$. This correlated with the order for both isolated nuclei [6] and mitochondria of *S. cerevisiae*.

Addition of Cat^{2+} to the membrane preparation of mitochondria resulted in inhibition of the polyphosphatase activity, which also depended on the nature and concentration of Cat^{2+} (Table 6). Based on the degree of inhibition of the membrane-bound polyphosphatase activity, the tested Cat^{2+} were arranged in the following order: $Co^{2+} = Zn^{2+} > Mn^{2+} > Mg^{2+}$.

Similar to the soluble polyphosphatase of mitochondria, the polyphosphatases of yeast compartments studied previously (cell envelope, cytosol, vacuole, and nucleus) were stimulated by Cat^{2+} [3–6]. The only reported example of Cat^{2+}-inhibited polyphosphatase was represented by the enzyme purified from the cell homogenate of *Corynebacterium xerosis* [21]. Cat^{2+}-independent polyphosphatase activities were described in some other microorganisms [26].

4. Discussion

The properties of the soluble and membrane-bound polyphosphatase activities of mitochondria of *S. cerevisiae* are summed up in Table 7. It is evident that these activities are represented by different enzymes distinguished by molecular masses, substrate specificity, K_m values, and relation to Cat^+

Table 7
Characterization of polyphosphatase activities in membrane and soluble preparations of mitochondria of *S. cerevisiae*

Properties	Membrane preparation	Soluble preparation
Molecular masses	120 and 76 kDa	36 kDa
Substrate specificity	Activity increases on substrate polymerization degree increasing	Activity does not actually depend on substrate polymerization degree
K_m	25 μm ($PolyP_{15}$) 1 μM ($PolyP_{188}$)	4.00 μM ($PolyP_{15}$) 0.06 μM ($PolyP_{188}$)
Effect of Cat^{2+}	Inhibition	Activation
Effect of Cat^+	Activation	No action
Effect of antibodies against purified cell-envelope polyphosphatase	No inhibition	Inhibition by 30%

$PolyP_{15}$ and $PolyP_{188}$, polyphosphates with average chain lengths of 15 and 188, correspondingly.

and Cat^{2+}. Some properties of the soluble polyphosphatase of mitochondria, such as a small molecular mass and activation by Cat^{2+}, bring it close together with polyphosphatases of the cell envelope and cytosol of the same yeast strain. Yet, a weak inhibition by antibodies against the purified cell-envelope polyphosphatase, an absence of stimulation by EDTA (not illustrated), and a high affinity to polyphosphates distinguish it from the enzymes mentioned. The most interesting property of the soluble polyphosphatase of mitochondria is its high affinity to polyphosphates with the long chain (10-fold higher than those for the other known yeast polyphosphatases).

The comparison of the soluble polyphosphatase of mitochondria and that of bacteria makes it possible to find the features of both similarity and distinction. While the polyphosphatase of *A. johnsonii* has a monomeric structure with the molecular mass of 55 ± 15 kDa [9], the enzyme from *Escherichia coli* is a dimer with the subunit molecular mass of 58 kDa [8]. Among similar features of the soluble mitochondrial enzyme and bacterial polyphosphatases are their stimulation by Cat^{2+} and high affinity to polyphosphates.

While the soluble polyphosphatase of mitochondria has a certain similarity to other known polyphosphatases, the membrane-bound polyphosphatase activity is unique. First, the polyphosphatases tightly bound to the membranes are still unknown. Second, there is only one example of polyphosphatase, which not only required Cat^{2+} for polyphosphate hydrolysis, but even was inhibited by these ions [21]. The proteins of mitochondrial membranes possessing polyphosphatase activities appear to be highly hydrophobic and have high molecular masses.

At present, polyphosphate–polyhydroxybutyrate complexes involved in the regulation of membrane penetration have been detected in bacterial and eucaryotic membranes [27,28]. Their content in the membranes depends on the cell state. We suggest that polyphosphatases localized in the membrane were able not only to degrade, but to synthesize polyphosphates entering these complexes. Detection of the membrane-bound polyphosphatase activity in mitochondria prompt us to search for similar activities in other cell membranes, since the information about the polyphosphatase activities in membranes is rather scant.

Thus, the analysis of polyphosphatases of the yeast mitochondria demonstrates that these enzymes are highly diversified and difficult to classify. The only key for classification might be their purification and sequencing.

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