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Isolation and Characterization of a Dinucleoside Triphosphatase from *Saccharomyces cerevisiae*

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An enzyme able to cleave dinucleoside triphosphates has been purified 3,750-fold from *Saccharomyces cerevisiae*. Contrary to the enzymes previously shown to catabolize Ap_4A in yeast, this enzyme is a hydrolase rather than a phosphorylase. The dinucleoside triphosphatase molecular ratio estimated by gel filtration is 55,000. Dinucleoside triphosphatase activity is strongly stimulated by the presence of divalent cations. Mn^{2+} displays the strongest stimulating effect, followed by Mg^{2+}, Co^{2+}, Cd^{2+}, and Ca^{2+}. The K_m value for Ap_3A is 5.4 μM (50 mM Tris-HCl [pH 7.8], 5 mM $MgCl_2$, and 0.1 mM EDTA; 37°C). Dinucleoside polyphosphates are substrates of this enzyme, provided that they contain more than two phosphates and that at least one of the two bases is a purine (Ap_3A, Ap_3G, Ap_3C, Gp_3G, Gp_3C, m^7Gp_3A, m^7Gp_3G, Ap_4A, Ap_4G, Ap_4C, Ap_4U, Gp_4G, and Ap_5A are substrates; AMP, ADP, ATP, Ap_2A, and Cp_4U are not). Among the products, a nucleoside monophosphate is always formed. The specificity of cleavage of methylated dinucleoside triphosphates and the molecular weight of dinucleoside triphosphatase indicate that this enzyme is different from the mRNA decapping enzyme previously characterized (A. Stevens, Mol. Cell. Biol. 8:2005–2010, 1988).

Various bis(5′-nucleosidyl) polyphosphates, e.g., Ap_4N (N = A, C, G, or U) (13, 40), Ap_3N (5, 9, 12, 19, 25, 27, 28), Bp_4B' (B, B′ = C, G, or U) (8, 10), Gp_3G (35, 39), and Ap_5A (32), occur in living cells. These nucleotides are believed to have a physiological role because their intracellular concentrations may depend on environmental factors such as temperature or the presence of oxidants.

In exponentially growing *Saccharomyces cerevisiae* cells (30°C) the four Ap_4N have concentrations of between 0.20 and 0.55 μM (8, 11), the six Bp_4B' concentrations are between 0.03 and 0.21 μM (8), and the Ap_3A concentration is 0.16 μM (28). When yeast cells are exposed to heat shock or to cadmium addition, the concentrations of these nucleotides strongly increase (8, 9, 28). For instance, 3 h after a temperature shift from 30 to 46°C, the concentrations of the various Ap_4N are multiplied by factors ranging from 170- to 600-fold (8). Upon exposure to 5 mM cadmium for 1 h (30°C), the Ap_3A concentration is increased 21-fold (28).

To elucidate the mechanisms which regulate the cellular concentrations of these nucleotides, it is important to identify the enzymes responsible for their metabolism. The participation of aminoacyl-tRNA synthetases in the in vivo biosynthesis of Ap_4N was recently established in the case of *Escherichia coli* cells (4). Enzymes capable of degrading bis(5′-nucleosidyl) polyphosphates have been characterized from several organisms. Such enzymes include specific dinucleoside tetraphosphatases (2, 7, 15, 16, 18, 21, 24, 26, 30, 31), specific dinucleoside triphosphatases (6, 17, 18, 34), and nonspecific phosphodiesterases or nucleoside pyrophosphatases (3, 18, 23, 31, 33 and references therein). In *S. cerevisiae*, two Ap_4A phosphorylases catalyzing the reaction $Ap_4A + P_i \leftrightarrow ADP + ATP$ have been characterized (14, 28, 29). Recently, these enzymes were shown to be responsible for the catabolism of Ap_4N and Bp_4B', since disruption of the *APA1* and *APA2* genes encoding the two Ap_4A phosphorylases resulted in a strong increase in the cellular concentration of these nucleotides (28). The Ap_4A phosphorylases are specific for bis(5′-nucleosidyl) tetraphosphates and do not cleave Ap_3A. In agreement with this result, cellular Ap_3A concentration is not modified by the disruption of the *APA1* and *APA2* genes (28). Therefore, it was likely that another enzyme was responsible for the Ap_3A catabolism in *S. cerevisiae*.

In this study, we report the isolation and the characterization of a new enzyme from *S. cerevisiae*, able to cleave Ap_3A. This enzyme behaves as a hydrolase and not as a phosphorylase.

MATERIALS AND METHODS

DEAE-Sephadex A50, Sephadex G200, Sephadex G75 superfine, and Mono Q were from Pharmacia, Matrex Green A was from Amicon, Chelex 100 was from BioRad, Polymin P was from Serva, Lichrosorb RP18 was from Merck, TSK 3000 was from Beckman, and aminohexyl Sepharose was from PL-Biochemicals. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis was performed with the Pharmacia Phast-gel apparatus and under the conditions recommended by the supplier.

Purification of dinucleoside triphosphatase. Dinucleoside triphosphatase was purified from 840 g (wet weight) of *S. cerevisiae* D273 (A. Tzagoloff, Columbia University, New York). Preparation of the crude extract, Polymin P precipitation, and ammonium sulfate fractionation (35 to 65%) were performed as described previously (28). The ammonium sulfate precipitate was solubilized in 5 liters of a solution containing 20 mM potassium phosphate buffer, pH 7.15, 10 mM 2-mercaptoethanol, and 0.1 mM phenylmethylsulfonyl fluoride (PMSF) and applied to a DEAE-Sephadex A50 column (16-cm diameter by 20 cm; flow rate, 816 ml/h) equilibrated in the same buffer. After a 2-liter wash, elution was performed with an 80-liter linear gradient from 20 to 200 mM potassium phosphate buffer at pH 7.15. Active fractions were pooled and precipitated with ammonium sulfate (65%). The precipitate was dialyzed against a solution containing 20

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TABLE 1. Purification of *S. cerevisiae* dinucleoside triphosphatase

Purification step	Protein (mg)	Total activity (U[a])	Sp act (U[a]/g)	Yield (%)	Relative purification
Extract	112,000[b]	22	0.20		
Supernatant after Polymin P precipitation	42,480[b]	23	0.54	105	2.7
Ammonium sulfate fraction (35–65%)	23,250[b]	21.4	0.92	97.3	4.6
DEAE-Sephadex	1,200[b,c]	10.4	8.7	47	43.5
Sephadex G75	162[c]	5.8	36	26.5	180
Matrex Green	24[c]	2.9	120	13	600
Mono Q	1.6[c]	1.2	750	5.4	3,750

[a] 1 U is defined as the amount of enzyme capable of transforming 1 μmol of Ap_3A per min (37°C; 40 mM Tris-HCl [pH 7.8], 25 μM Ap_3A, 0.1 mM EDTA, 5 mM $MgCl_2$).

[b] Protein analysis by the method of Lowry et al. (22).

[c] Protein amount determined from UV absorbancy, assuming that 1 A_{280} unit corresponded to a protein concentration of 1 mg/ml.

mM potassium phosphate buffer (pH 7.15), 10 mM 2-mercaptoethanol, and 0.1 mM PMSF and applied on a Sephadex G75 superfine column (25-cm diameter by 87 cm) equilibrated in 20 mM potassium phosphate buffer (pH 7.15)–10 mM 2-mercaptoethanol at a flow rate of 52 ml/h. Active fractions were precipitated with ammonium sulfate (65%). The precipitate was dialyzed against a solution of 10 mM potassium phosphate (pH 6.75), 1 mM EDTA, and 0.1 mM PMSF and applied to a Matrex Green column (3.2-cm diameter by 5 cm). Elution was performed with a 400-ml linear gradient from 0 to 500 mM KCl in 10 mM potassium phosphate (pH 6.75; flow rate, 10 ml/h). The active fractions were dialyzed against a solution of 10 mM potassium phosphate (pH 6.75), 0.1 mM EDTA, and 0.1 mM PMSF, and samples of 10 ml containing 2.4 mg of protein were successively applied on a Mono Q column (0.5-cm diameter by 5 cm). Elution was performed with a 96-ml linear gradient from 0 to 150 mM KCl in 10 mM potassium phosphate (pH 6.75)–0.1 mM EDTA (flow rate 0.8 ml/min). Active fractions from the 10 successive chromatographies were pooled and dialyzed against a solution of 20 mM Tris-HCl, pH 7.8, 10 μM EDTA, and 60% glycerol. The enzyme pools were stored at −20°C.

Nucleotides. Ap_4C, Ap_4G, Ap_4U, and Cp_4U were extracted from *E. coli* PAL2103D (20), as described previously (28). Other nucleotides were from Boehringer (Ap_4A, Ap_5A, ATP, ADP, and AMP), Sigma (Ap_2A, Ap_3A, m^7GMP, and m^7GDP), or Pharmacia (Ap_3G, Gp_3G, Gp_3C, Gp_4G, m^7Gp_3A, and m^7Gp_3G). Ap_3C was enzymatically synthesized by using purified *E. coli* lysyl-tRNA synthetase (26). [^{3}H]ADP (666 GBq/mmol) and [^{3}H]Ap_4A (159 GBq/mmol) were from Amersham (Amersham, United Kingdom).

[^{3}H]Ap_3A was enzymatically synthesized by using purified *E. coli* lysyl-tRNA synthetase as described previously (26), except that 0.5 mM ATP and 0.5 mM [^{3}H]ADP were used. [^{3}H]Ap_3A was purified by high-pressure liquid chromatography (HPLC) on a Lichrosorb RP18 column (0.46 by 20 cm, isocratic elution with 25 mM potassium phosphate, pH 5.3, 1.5 ml/min), and salts were removed by boronate chromatography (1).

Enzymatic assays. To monitor Ap_3A or Ap_4A degradation activities, in crude extracts and during the course of the purification of dinucleoside triphosphatase, the reaction mixture contained 40 mM Tris-HCl (pH 7.8), 5 mM $MgCl_2$, 0.1 mM EDTA, 300 U of alkaline phosphatase (Boehringer) per ml, and either 25 μM [^{3}H]Ap_3A (640 GBq/mol) or 25 μM [^{3}H]Ap_4A (640 GBq/mol). After incubation at 37°C, the reaction was quenched and the [^{3}H]adenosine formed was counted as described previously (29). Michaelis constants for Ap_3A and Ap_4A were measured under the same assay conditions.

HPLC. To follow the hydrolysis of various substrates catalyzed by dinucleoside triphosphatase, the reaction sample (105 μl) was applied to a column (0.46 by 20 cm) packed with Lichrosorb RP18. Nucleotides were isocratically eluted at a flow rate of 1.5 ml/min with either (i) 50 mM potassium phosphate (pH 6.4) containing 0.6 mM tetrabutylammonium bromide and 6% methanol (Cp_4U) or (ii) 50 mM potassium phosphate (pH 5.3) (other nucleotides minus Cp_4U). The A_{254} of the column effluent was monitored and recorded on a Hitachi D2000 integrator. The concentration of each nucleotide in the reaction sample was deduced by comparison of its chromatogram with the chromatograms of standard solutions of known concentrations.

RESULTS

Purification and molecular ratio of dinucleoside triphosphatase from *S. cerevisiae*. In order to search for the occurrence of an Ap_3A-degrading activity in *S. cerevisiae* [^{3}H]Ap_3A was enzymatically synthesized. It was used in a radioisotopic assay adapted from that previously developed to monitor Ap_4A degradation. This assay allowed us to detect Ap_3A degradation by yeast crude extract and, then, to follow the enzyme responsible for this activity during the course of its purification.

The yeast dinucleoside triphosphatase was purified 3,750-fold with a yield of 5.4% (Table 1). The purification included precipitation of nucleic acids with Polymin P, ammonium sulfate fractionation, and successive chromatographies on DEAE-Sephadex, Sephadex G75 superfine, Matrex Green, and Mono Q (Table 1). As seen on the SDS-polyacrylamide gel in Fig. 1, the enzyme preparation obtained was not homogeneous. However, a more highly purified enzyme fraction could not be obtained because of the losses of activity encountered in the further purification steps assayed (chromatography on aminohexyl Sepharose, blue dextran Sepharose, or TSK 3000 or preparative gel electrophoresis).

Molecular ratio of the dinucleoside triphosphatase was determined by Sephadex G200 chromatography (1-cm diameter by 60 cm; flow rate, 5 ml/h; 50 mM Tris-HCl buffer, pH 7.8, with 10 mM 2-mercaptoethanol). On this column, the dinucleoside triphosphatase activity migrated as a single, very symmetrical peak. Cochromatography of a 180-fold-purified sample of the hydrolase with cytochrome c from horse heart (14,000), carbonic anhydrase from bovine erythrocytes (30,000), isoleucyl-tRNA synthetase from *E. coli*

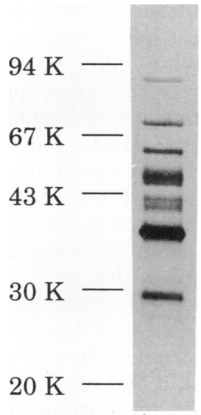

FIG. 1. SDS-polyacrylamide gel electrophoresis of the *S. cerevisiae* diadenosine triphosphatase preparation obtained after chromatography on the Mono Q column. Electrophoresis was performed on a 12.5% polyacrylamide gel. The gel was stained with the Phast-gel silver kit (Pharmacia). The following molecular weight markers were used: phosphorylase B, 94,000; bovine serum albumin, 67,000; ovalbumin, 43,000; carbonic anhydrase, 30,000; and soybean trypsin inhibitor, 20,000.

(110,000), and phenylalanyl-tRNA synthetase from *E. coli* (250,000) indicated an M_r of 55,000 ± 2,000.

Metal ion requirements. The hydrolysis of Ap_3A catalyzed by the purified *S. cerevisiae* dinucleoside triphosphatase was strongly stimulated by the presence of divalent cations. As shown in Table 2, Mn^{2+} displayed the strongest stimulating effect on the activity of the enzyme, followed by Mg^{2+}, Co^{2+}, Cd^{2+}, and Ca^{2+}. The Zn^{2+}, Ni^{2+}, and Fe^{2+} ions had weak stimulating effects, whereas Cu^{2+} had none. In the presence of 100 μM Mn^{2+}, the initial rate of Ap_3A hydrolysis was 71-fold higher than in the presence of 100 μM EDTA (Table 2).

Characterization of the enzyme reaction. The products of Ap_3A cleavage by the dinucleoside triphosphatase were identified by HPLC analysis. In either the presence or the absence of potassium phosphate (1 mM), the degradation of Ap_3A resulted in the stoichiometric appearance of AMP and ADP. The rate of Ap_3A hydrolysis was also independent of the presence or absence of phosphate. Therefore, the reaction catalyzed by this enzyme is a hydrolysis and not a phosphorolysis.

TABLE 2. Ap_3A hydrolysis in the presence of various divalent cations[a]

Buffer additive	Relative activity (%)
None	5.1
EDTA	1.4
Ca^{2+}	57
Cd^{2+}	67
Co^{2+}	67
Cu^{2+}	6.0
Fe^{2+}	17
Mg^{2+}	70
Mn^{2+}	100
Ni^{2+}	23
Zn^{2+}	19

[a] Buffers and Ap_3A were freed from contaminating divalent ions by passage through a Chelex 100 column. Incubation mixture contained 40 mM Tris-HCl (pH 7.8), 20 μM Ap_3A, 100 μg of bovine serum albumin per ml, and catalytic amounts of dinucleoside triphosphatase. EDTA or the metal ion under study, in the form of chloride, was added at a final concentration of 100 μM. The reaction (10 min, 37°C) was stopped by heating the samples for 2 min at 95°C. Products of the reaction was quantitated by HPLC analysis as described in Materials and Methods. Initial velocities of Ap_3A hydrolysis measured in the presence of metal ions are expressed as percentages of the values measured at 100 μM $MnCl_2$.

Ap_4A (20 μM) was also cleaved by diadenosine triphosphatase. However, the cleavage into AMP + ATP occurred at a rate about 8-fold lower than that observed at the same concentration of Ap_3A. Since the preparation of diadenosine triphosphatase was not homogeneous, it was possible that the observed Ap_4A hydrolysis was caused by an enzyme distinct from diadenosine triphosphatase but copurifying with it. To examine this, the K_m value for Ap_4A in the Ap_4A hydrolysis reaction was measured and compared with the K_i value exhibited by Ap_4A in the Ap_3A hydrolysis reaction. Similar values for the K_m and K_i constants are expected if the hydrolyses of Ap_4A and Ap_3A are catalyzed by a unique enzyme. The hydrolysis of Ap_4A followed typical Michaelis-Menten kinetics in the concentration range studied (0.5 to 500 μM). The corresponding K_m value was 17.4 ± 2.6 μM. Then, the inhibition by Ap_4A of the Ap_3A hydrolysis reaction was searched for by measuring apparent K_m values for Ap_3A in the presence of various Ap_4A concentrations. Since hydrolysis of Ap_4A occurred slower than that of Ap_3A, experimental conditions could be designed in which the Ap_4A concentration did not significantly vary during the assay of [^{3}H]Ap_3A hydrolysis. The results showed that Ap_4A behaved as a competitive inhibitor of Ap_3A in the Ap_3A hydrolysis reaction. The entire set of data obtained with Ap_3A concentrations ranging from 0.5 to 20 μM and Ap_4A concentrations ranging from 10 to 1000 μM was analyzed using a least-squares multiple regression method. A K_m value for Ap_3A of 5.3 ± 1.0 μM and a K_i value for Ap_4A of 18.3 ± 1.6 μM were determined.

The above-described experiment shows that diadenosine triphosphatase can bind Ap_4A, and the identity of the values of the K_i and K_m constants strongly indicates that diadenosine triphosphatase is responsible for the Ap_4A hydrolysis observed.

Substrate specificity and products of the reaction. As summarized in Table 3, dinucleoside triphosphatase catalyzed the cleavage of all bis(5′-nucleosidyl) polyphosphates assayed, provided the phosphate chain contained at least three phosphates and that one of the two bases composing the nucleotide was a purine; Ap_3N (N = A, G, or C), Ap_4N (N = A, G, C, or U), Gp_3G, Gp_3C, Gp_4G, and Ap_5A were substrates, whereas Ap_2A and Cp_4U were not. A nucleoside monophosphate was always one of the products. Thus, Ap_4A was cleaved into AMP + ATP, Ap_5A was cleaved into AMP + Ap_4, Gp_3G was cleaved into GMP + GDP. In the case of asymmetrical bis(5′-nucleosidyl) polyphosphates (Ap_3C, Ap_3G, Ap_4C, Ap_4G, Ap_4U, and Gp_3C) a purine monophosphate was systematically obtained. Ap_3G was cleaved into AMP + GDP (64%) as well as into GMP + ADP (36%), and Ap_4G was cleaved into AMP + GTP (86%) as well as into GMP + ATP (14%). Ap_3C and Gp_3C were exclusively converted into AMP + CDP and GMP + CDP, respectively.

The rates of hydrolysis of all assayed bis(5′-nucleosidyl) triphosphates were similar. Ap_4G and Gp_4G were hydrolyzed with rates 1.35- and 1.41-fold slower than that observed with Ap_3A. On the other hand, the rates of hydrolysis of Ap_4A, Ap_4C, and Ap_4U were much smaller (Table 3). This enzyme was named a dinucleoside triphosphatase rather

TABLE 3. Substrate specificity of *S. cerevisiae* dinucleoside triphosphatase[a]

Substrate	Relative rate of hydrolysis (%)[b]	Products (%)
Ap_3A	100	AMP + ADP
Ap_3G	95	AMP + GDP (64) or GMP + ADP (36)
Ap_3C	94	AMP + CDP (100)
Ap_4A	13	AMP + ATP
Ap_4G	76	AMP + GTP (86) or GMP + ATP (14)
Ap_4C	4.3	AMP + CTP (100)
Ap_4U	5.2	AMP + UTP (100)
Ap_5A	3	AMP + Ap_4
Gp_3G	74	GMP + GDP
Gp_3C	96	GMP + CDP (100)
Gp_4G	61	GMP + GTP
m^7Gp_3G	47	GMP + m^7GDP (97) or m^7GMP + GDP (3)
m^7Gp_3A	37	AMP + m^7GDP (97) or m^7GMP + ADP (3)
Ap_2A, Cp_4U	ND	
AMP, ADP, ATP	ND	

[a] Buffers and substrates were freed from contaminating divalent ions by passage through a Chelex 100 column. The incubation mixture (105 μl) contained 40 mM Tris-HCl (pH 7.8), 0.5 mM $MgCl_2$, 20 μM EDTA, 200 μg of bovine serum albumin per ml, 20 μM of the substrate under study, and catalytic amounts of Ap_3A hydrolase. After incubation at 25°C for 5 or 10 min, the reaction was stopped by freezing the samples in liquid nitrogen. Products of the reaction were analyzed by HPLC as described in Materials and Methods. Initial rates of hydrolysis are expressed (in parentheses) as percentages of the rate observed with the Ap_3A substrate.

[b] ND, not detectable.

than a dinucleoside tetraphosphatase because it hydrolyzes Ap_3A eightfold faster than Ap_4A.

The cap dinucleotides m^7Gp_3G and m^7Gp_3A were also degraded by dinucleoside triphosphatase. They were converted, almost exclusively, into GMP + m^7GDP and AMP + m^7GDP, respectively. The rates of hydrolysis of m^7Gp_3G and m^7Gp_3A were 1.6- and 2.6-fold lower than those of the corresponding nonmethylated nucleotides. AMP, ADP, and ATP were not substrates of the enzyme.

The Ap_4A degradation activity persisting in an *apa1 apa2* strain originates from dinucleoside triphosphatase. A *S. cerevisiae* strain devoid of Ap_4A phosphorylase activity was recently obtained by disruption of the *APA1* and *APA2* genes encoding Ap_4A phosphorylases I and II (28). In crude extracts of this strain, the remaining Ap_4A degradation activity is 60-fold lower than that in extracts of the parental strain (28). Since, as shown above, dinucleoside triphosphatase is capable of hydrolyzing Ap_4A, experiments were performed to determine whether dinucleoside triphosphatase was responsible for the Ap_4A degradation activity still detectable in a crude extract of the *apa1 apa2* strain.

Both Ap_4A and Ap_3A degradation activities were measured in a crude extract of *S. cerevisiae* YPALSHU (*apa1 apa2*) prepared by sonication as described previously (29). The ratio between the rates of Ap_4A and Ap_3A degradation (10%) in this extract was close to the ratio (13%) predicted from the rate values measured with the purified dinucleoside triphosphatase (Table 3). Then, after Polymin P precipitation and ammonium sulfate fractionation, the crude extract of the *apa1 apa2* strain was successively chromatographed on DEAE-Sephadex A50 and Matrex Green columns by using the same elution conditions as those used during the purification of dinucleoside triphosphatase. The two Ap_4A and Ap_3A degradation activities comigrated during each chromatography. The ratio between Ap_4A and Ap_3A degradation activities remained constant and equal to about 13%. Finally, it was verified by HPLC that the enzyme obtained after the Matrex Green chromatography cleaved Ap_4A into AMP + ATP. All these results bring evidence that the residual Ap_4A hydrolase activity in the YPALSHU (*apa1 apa2*) strain originates from the presence of the dinucleoside triphosphatase identified in this study.

Conclusion. In this report, a dinucleoside triphosphatase was isolated from *S. cerevisiae*. Like the other Ap_3A hydrolases so far evidenced from rat liver (34), rat brain (6), lupin (18), or *E. coli* (17, 26), it cleaves Ap_3A into AMP + ADP. The K_m values for Ap_3A of all these hydrolases are comparable: 5.9, 14, 7, 1.2, and 12 μM for *S. cerevisiae*, rat brain, rat liver, lupin, and *E. coli* Ap_3A hydrolases, respectively. The molecular ratios of these enzymes are also rather close, the yeast enzyme being slightly larger (55,000) than the enzymes of lupin (41,000), *E. coli* (36,000), rat brain (34,000), or rat liver (29, 800).

The *S. cerevisiae* dinucleoside triphosphatase hydrolyzes all the bis(5'-nucleosidyl) triphosphates assayed. Strikingly, it also efficiently cleaves the purine-rich Ap_4G and Gp_4G. It is noteworthy that the in vivo concentrations of the various Ap_4N species were differently modified upon disruption of the *APA1* and *APA2* genes encoding Ap_4A phosphorylases I and II. In an *apa1 apa2* mutant strain, Ap_4A, Ap_4C, and Ap_4U concentrations were 35- to 50-fold higher than in the control *APA1 APA2* strain. The Ap_4G concentration was only fivefold higher (28). In the context of the present study, it becomes likely that such a relatively small increase in Ap_4G concentration was caused by a persistent hydrolysis of this nucleotide by dinucleoside triphosphatase in vivo.

Finally, the *S. cerevisiae* dinucleoside triphosphatase also cleaves the cap analogs m^7Gp_3A and m^7Gp_3G. In yeasts, the 5'-terminal structures of capped mRNAs are composed of m^7Gp_3Ap and m^7Gp_3Gp in a relative distribution of 75% and 25% (36). An mRNA decapping enzyme which hydrolyzes one pyrophosphate bond on the cap structure of mRNA, yielding m^7GDP and 5'-p RNA (38), has been isolated from S. cerevisiae. The hydrolysis of m^7Gp_3A and m^7Gp_3G by the dinucleoside triphosphatase, as evidenced here, also results in the appearance of m^7GDP. However, dinucleoside triphosphatase is probably distinct from the already described mRNA decapping enzyme, since the latter enzyme was reported to be inactive on the m^7Gp_3G and m^7Gp_3A dinucleotides (37). Moreover, the molecular ratio found for the mRNA decapping enzyme (79,000) (38) is different from that determined here for dinucleoside triphosphatase (55,000). The possibility remains, however, that yeast dinucleoside triphosphatase may behave in vivo as an mRNA decapping enzyme.

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