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mRNA-Decapping Enzyme from *Saccharomyces cerevisiae*: Purification and Unique Specificity for Long RNA Chains

AUDREY STEVENS

Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831

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An enzyme that hydrolyzes one PP_i bond of the cap structure of mRNA, yielding m^7GDP and 5′-p RNA was purified from *Saccharomyces cerevisiae* to a stage suitable for characterization. The specificity of the enzyme was studied, using both yeast mRNA and synthetic RNAs labeled in the cap structure. A synthetic capped RNA (540 nucleotides) was not reduced in size, while as much as 80% was decapped. Yeast mRNA treated with high concentrations of RNase A, nuclease P1, or micrococcal nuclease was inactive as a substrate. The use of synthetic capped RNAs of different sizes (50 to 540 nucleotides) as substrates showed that the larger RNA can be a better substrate by as much as 10-fold. GpppG-RNA was hydrolyzed at a rate similar to that at which m^7GpppG-RNA was hydrolyzed. The PP_i bonds of an RNA containing a 5′-triphosphate end group were not hydrolyzed.

A 5′-terminal cap, m^7G(5′)ppp(5′)N, is a feature of almost all eucaryotic mRNAs. Capping occurs shortly after the synthesis of the RNA chain is started in the nucleus, and caps serve to stabilize the mRNAs in both the nucleus (5) and cytoplasm (4). The presence of the cap structure is important in the process of translation (16). It follows then that an enzyme or enzymes that remove the cap structure could cause severe inactivation of mRNA, as well as render it more susceptible to degradation.

Nucleotide pyrophosphatase activities from tobacco (17) and potato (2, 7) that cleave the mRNA cap structure to yield m^7GMP have been described. Both enzymes are nonspecific pyrophosphatases and cleave intact capped mRNA. An enzyme from HeLa cells hydrolyzes m^7GpppN and m^7G-capped oligonucleotides up to 10 nucleotides long but not capped mRNA (12, 13). Again, m^7GMP is the product.

By using ^{3}H-cap-labeled mRNA of *Saccharomyces cerevisiae* as a substrate, a decapping enzyme yielding [^{3}H]m^7GDP was detected in enzyme fractions derived from *S. cerevisiae* (20). The enzyme appeared to be a specific pyrophosphatase since m^7GpppA and UDP-glucose were not hydrolyzed. The enzyme has now been purified free of most RNase activity and found to have a unique specificity for long RNA chains. The purification and specificity experiments are described here.

MATERIALS AND METHODS

Purification of the decapping enzyme. *S. cerevisiae* S288C was grown and stored as previously described (21). The purification steps used were similar to those described previously, including the use of the hydroxylapatite (Sigma Chemical Co.) column (20). The protease inhibitors leupeptin (1 μg/ml) and antipain (0.5 μg/ml) were used in the buffers in addition to α-tosyl fluoride (0.2 mM). For further purification (from 100 g of *S. cerevisiae*), the active fractions from the hydroxylapatite column were concentrated by $(NH_4)_2SO_4$ precipitation (0 to 70%) and dissolved in 2 ml of buffer A (20 mM Tris hydrochloride buffer [pH 7.7], 10% glycerol, 0.5 mM dithiothreitol) containing 50 mM NH_4Cl and the protease inhibitors described above. The enzyme fraction was then dialyzed for 5 h against the same buffer. The dialyzed enzyme fraction was chromatographed on a heparin-agarose (Sigma) column (0.9 by 3 cm) equilibrated with buffer A containing 25 mM $(NH_4)_2SO_4$. The column was eluted with a linear gradient (52 ml) of 25 to 400 mM $(NH_4)_2SO_4$ in buffer A, and fractions (1.3 ml) were collected and assayed. The active fractions were stored at −30°C.

Assay of decapping enzyme. The conversion of [^{3}H]m^7Gppp[^{14}C]RNA of *S. cerevisiae* to [^{3}H]m^7G in the presence of both the decapping enzyme and alkaline phosphatase was used as an assay for the decapping enzyme. One nanomole of labeled RNA (as nucleotides) was incubated in a reaction mixture (50 μl) containing 50 mM Tris hydrochloride buffer (pH 8.0), 1 mM $MgCl_2$, 50 mM NH_4Cl, and decapping enzyme (5 to 50 U) for 15 min at 30°C. The reaction mixture was then heated for 3 min at 80°C, followed by the addition of 5 μg of *Escherichia coli* alkaline phosphatase (Sigma). After a 30-min incubation at 37°C, marker m^7G was added, and the reaction mixture was applied to Whatman 3MM filter paper. High-voltage paper electrophoresis was carried out at 2,000 V for 75 min using pyridine-acetate buffer (pH 3.5). The m^7G spot was eluted with water (0.5 ml), and the radioactivity was counted. A reaction mixture lacking enzyme served as a blank. One unit of activity is the amount of enzyme required to convert 1% of the ^{3}H cap label to [^{3}H]m^7G. The decapping activity was linear with enzyme concentration and time under the conditions described here.

Preparation of substrates. The synthetic [^{3}H]methyl-capped RNAs were prepared with SP6 polymerase (Promega Biotec) in the presence of GpppG (Pharmacia, Inc.), followed by methylation with vaccinia virus guanylyltransferase (Bethesda Research Laboratories, Inc.). Fragments of the cDNAs of p33 (9) (*Pst*I→*Hin*dIII at 800 base pairs) and pcTAT-3 (15) (*Eco*RI→*Pst*I at 540 base pairs) which had been cloned into the corresponding linker sites in the transcription vectors pSP64 and pSP65, respectively (14), were obtained from K. Isham and K.-L. Lee. The two DNAs were linearized with restriction enzymes so RNAs of various chain lengths would be obtained in transcription reactions with SP6 polymerase. The restriction enzymes used with the pcTAT-3 DNA fragment were *Pst*I, *Hin*dIII, and *Rsa*I, yielding RNAs with chain lengths of 540, 100, and 50 nucleotides. The p33 DNA fragment was cleaved with *Fnu*4HI, *Hin*fI, and *Rsa*I to yield RNAs with 68, 172, and 342 nucleotides. The SP6 polymerase reaction mixtures (50 μl) were similar to those described by Konarska et al. (8).

Each contained 3 μg of DNA, 40 mM Tris hydrochloride buffer (pH 7.5), 6 mM $MgCl_2$, 10 mM dithiothreitol, 0.4 mM ATP, UTP, and CTP, 0.04 mM GTP, 0.5 mM GpppG, 40 U of RNase inhibitor (RNasin) (Promega Biotech), 60 μCi of [α-^{32}P]UTP (ICN Biomedicals Inc.), and 15 U of SP6 polymerase. After incubation for 1 h, the mixture was extracted with phenol, and the aqueous phase was precipitated with ethanol (75%) and dialyzed for 18 h against 10 mM Tris hydrochloride buffer (pH 7.5). The dialyzed sample was precipitated with ethanol (75%) in the presence of 1 M ammonium acetate. To the ethanol-washed pellet, reagents for the guanylyltransferase recation were added. The reaction mixture (50 μl) contained 100 mM Tris hydrochloride buffer (pH 7.7), 1 mM $MgCl_2$, 2.5 mM dithiothreitol, 40 U of RNasin, 5 μCi of *S*-adenosyl[*methyl*-^{3}H]methionine (73 Ci/mmol; Amersham Corp.), and 3.5 U of guanylyltransferase. After 1 h, the reaction mixture was extracted with phenol and RNA was precipitated from the aqueous phase with ethanol (75%).

[^{3}H]m^{7}Gppp[^{14}C]RNA was prepared as previously described (20) from a 1-liter culture of *S. cerevisiae* A364A, mutant *ts*368. For labeling, 5 mCi of [*methyl*-^{3}H]methionine (ICN) were used.

G[^{32}P]ppp[^{3}H]RNA of 540 nucleotides was prepared by using the pcTAT-3 DNA fragment described above. First, [^{3}H]RNA with a triphosphate end group was prepared by using reaction mixtures similar to those described for making the labeled synthetic RNAs, except that the GTP concentration was 0.4 mM, GpppG was omitted, and [^{3}H]ATP (25×10^6 cpm; ICN) was included. The product was isolated by phenol extraction and dialysis as described for the synthetic RNAs. One microgram was then capped in a reaction mixture (50 μl) containing 50 mM Tris hydrochloride buffer (pH 7.7), 2 mM $MgCl_2$, 1 mM dithiothreitol, [α-^{32}P]GTP (36×10^6 cpm; ICN), 20 U of RNasin, and 2.5 U of guanylyltransferase. After 1 h at 37°C, the RNA was isolated by Sephadex G-75 column chromatography, using a 7-ml column with 10 mM Tris hydrochloride buffer (pH 7.5) as the eluting buffer.

m^{7}GpppG[^{3}H]RNA and GpppG[^{3}H]RNA of 540 nucleotides were prepared by using the pcTAT-3 DNA fragment described above. Reaction mixtures similar to those described for making the labeled synthetic RNAs were used, and m^{7}GpppG (Pharmacia) or GpppG at 0.5 mM was added to make the RNAs containing them. [^{3}H]ATP (25×10^6 cpm; ICN) was used to label the RNA.

[^{3}H]RNA with a triphosphate end group containing γ-^{32}P label was prepared with *E. coli* RNA polymerase, using bacteriophage T4 DNA as a template. The reaction mixture (50 μl) contained 10 μg of phage T4 DNA, 20 mM Tris hydrochloride buffer (pH 7.7), 5 mM $MgCl_2$, 4 mM dithiothreitol, 50 mM KCl, 20 μM ATP (10^6 cpm of ^{3}H label and 45×10^6 cpm of γ-^{32}P label), 200 μM UTP, CTP, and GTP, and 2 U of *E. coli* RNA polymerase (Pharmacia). After 30 min at 37°C, the reaction mixture was extracted with phenol and the RNA was precipitated from the aqueous phase with ethanol.

Characterization of substrates. The amount of the synthetic RNA substrates which terminated in GpppG was determined before methylation by treatment of the preparations with a fraction of the 5′→3′ exoribonuclease of *S. cerevisiae* (23). This enzyme hydrolyzes capped RNA very poorly, so the fraction of the RNA rendered acid soluble was taken as the amount that was not capped. All of the synthetic RNAs showed values of 50 to 65% for the extent of capping. These values are in agreement with those reported by Contreras et al. (3) and Ahlquist and Janda (1) for incorporation of GpppG when it was added to polymerase reaction mixtures.

The extent of methylation of the capped RNA substrates by guanylyltransferase was calculated from the amounts of ^{3}H and ^{32}P label in the final products. The amount of label in the methyl group of the cap structure of each RNA substrate was determined by treating the RNA with nucleotide pyrophosphatase (Sigma) and *E. coli* alkaline phosphatase, followed by assay of the label in m^{7}G as described above for assay of the decapping enzyme. In all instances, the extent of methylation was 90 to 100%, as originally reported by Monroy et al. (11) in their characterization of guanylyltransferase from vaccinia virus. The extent of methylation provided additional evidence that the level of GpppG incorporation was 50 to 65%.

The extent to which the substrate G[^{32}P]ppp[^{3}H]RNA was capped was determined from the ^{32}P and ^{3}H label in the final product.

RNase treatment of capped RNAs and assay of products. RNA samples (yeast mRNA and a synthetic RNA with a chain length of 342 nucleotides) were pretreated with different RNases. The RNase A reaction mixtures (10 μl) contained 10 mM Tris hydrochloride buffer (pH 7.5), 1 nmol of yeast mRNA or 0.18 pmol (as a capped RNA chain) of synthetic RNA, and different amounts of RNase A. The nuclease P1 reaction mixture (10 μl) contained 1 nmol of yeast mRNA, 25 mM sodium acetate buffer (pH 5.4), and enzyme. The micrococcal nuclease reaction mixture (6 μl) contained 1 nmol of yeast mRNA, 1.5 mM $CaCl_2$, 10 mM Tris hydrochloride buffer (pH 7.5), and enzyme. The reaction mixtures were incubated for 30 min at 37°C, followed by the addition of decapping reaction reagents. Control reaction mixtures were set up in the same manner, but after the RNase A or micrococcal nuclease treatment, they received a second addition of labeled RNA after the addition of 40 U of RNasin to the RNase A mixtures and 25 nmol of EGTA [ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid] to the micrococcal nuclease mixture.

Other materials. Nuclease P1, RNase A, and micrococcal nuclease were obtained from Pharmacia, Worthington Diagnostics, and Sigma, respectively. Restriction enzymes were from New England BioLabs, Inc., and were used as described by the manufacturer. The protease inhibitors, Sephacryl S-200, and standard proteins were from Sigma, and T4 polynucleotide kinase was from Pharmacia.

RESULTS AND DISCUSSION

Purification of the decapping enzyme free of most RNase activity. The purification scheme used for the decapping enzyme was similar to that described previously (20), except that heparin-agarose chromatography was included as a final step in the procedure and additional protease inhibitors were used in the buffer solutions. The previously described steps, including the use of the hydroxylapatite column, are similar to those described for purifying a 5′→3′ exoribonuclease of *S. cerevisiae* (21). The results of the hydroxylapatite chromatography of the decapping and exoribonuclease activities are shown in Fig. 1A. The decapping enzyme eluted with a small peak of poly(A)-hydrolyzing activity, which was found to be a 5′→3′ exoribonuclease similar to that found in the main peak of poly(A)-hydrolyzing activity shown in Fig. 1A. The two activities (decapping and exoribonuclease) could be separated by heparin-agarose chromatography (Fig. 1B). The heparin-agarose chromatography gave a threefold further purification of the decapping enzyme, with 70% recov-

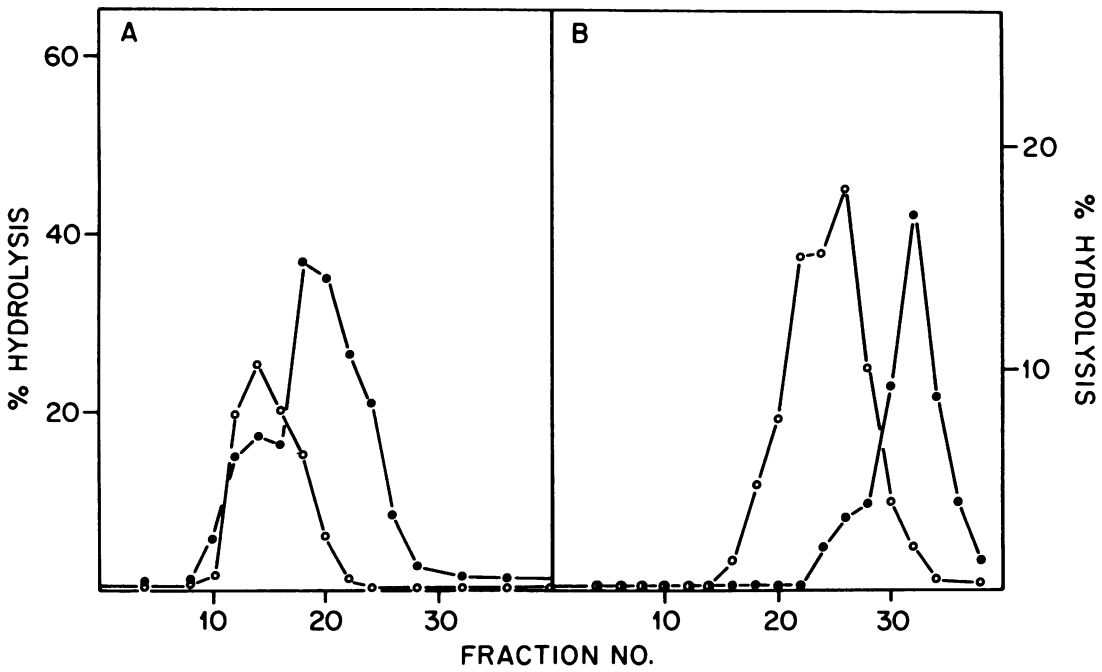

FIG. 1. Hydroxylapatite and heparin-agarose chromatography of the decapping enzyme. (A) Hydroxylapatite chromatography was carried out as described previously (21) with a DEAE-cellulose fraction after ammonium sulfate precipitation and dialysis. An 80-ml linear gradient of 50 to 500 mM potassium phosphate in buffer A was used for the elution, and 2-ml fractions were collected. Samples (1 and 2 μl, respectively) of the fractions were analyzed for percent poly(A)-hydrolyzing activity, using the assay for the 5′→3′ exoribonuclease (21), and for percent decapping activity, as described in the text. (B) Heparin-agarose chromatography was carried out as described in the text, and 1- and 2-μl samples of the fractions were assayed for poly(A)-hydrolyzing and decapping activity, respectively. Percent hydrolysis per microliter of enzyme is shown. Symbols: ●, 5′→3′ exoribonuclease activity; ○, decapping activity.

ery. The active fractions from the heparin-agarose column contained about 15 U of decapping activity per μl, and they were used in the assays described below. The removal of most of the exoribonuclease activity allowed a study of whether intact mRNA is decapped, as described below.

Properties and apparent molecular weight of the decapping enzyme. The optimum pH for activity of the decapping enzyme was 7.5 to 8.5. Fifty percent of the maximal activity was found at pH 7.0, and 60% was found at pH 9.5. A divalent cation is required for activity since EDTA inhibited the reaction completely. Mg^{2+} stimulated maximally at 1 mM. Mn^{2+} stimulated 85% as well at 1 mM. Salt (NH_4Cl) was not required for the reaction and was inhibitory at higher concentrations (37% at 100 mM and 100% at 200 mM). The activity of the heparin-agarose enzyme fractions was increased about 1.6-fold by the addition of RNasin. The temperature optimum of the enzyme was 30°C. The K_m for yeast mRNA (as nucleotides) was 12.5 μM.

To determine the apparent molecular weight of the decapping enzyme, it was subjected to molecular-sieve chromatography to determine the Stokes radius and to gradient centrifugation to determine the sedimentation coefficient. The enzyme was chromatographed on a Sephacryl S-200 column which was standardized with proteins of known molecular weight (ovalbumin, bovine serum albumin, aldolase, and *E. coli* alkaline phosphatase). A Stokes radius of 3.8 nm was determined from the elution volume as described by Siegel and Monty (18). A sedimentation coefficient of 5.0S for the enzyme was determined by the procedure of Martin and Ames (10), using the same proteins as markers. By using the Svedberg equation (18) with the values for Stokes radius and sedimentation coefficient, an apparent molecular weight of 79,000 was calculated. (A partial specific volume of 0.73 was assumed.) Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the heparin-agarose enzyme fractions showed about 10 distinct bands, suggesting that the enzyme was not more than about 10% pure. It was not possible to obtain a more highly purified preparation because of losses encountered in further purification steps.

Hydrolysis of intact capped mRNA. That long RNA chains are substrates for the decapping enzyme and that the RNA is not degraded before or after decapping were determined by gel electrophoresis of reaction mixtures containing capped ^{32}P-labeled RNA. The extent of decapping was also determined. The results are shown in Fig. 2. The size of the [^{32}P]RNA was the same in a reaction mixture incubated for 15 min without enzyme (lane 1) as in reaction mixtures incubated for 5 min (lane 2) or 15 min (lane 3) with a high concentration of enzyme. Counting of the radioactivity in the RNA bands showed less than 10% degradation. Determinations of the extent of decapping in the reaction mixtures showed 65 and 81% for the 5- and 15-min reaction mixtures, respectively.

Effect of RNase treatment of substrate RNA. The effects of treating the capped RNA substrates with different concentrations of pancreatic RNase A and high concentrations of nuclease P1 and micrococcal nuclease on their activity as substrates are shown in Table 1. Treatment of both yeast mRNA and a synthetic capped RNA with increasing concentrations of RNase A led to a gradual decrease in the rate at which they were decapped. At the highest concentrations of RNase A used, the RNAs were rendered inactive as substrates. Nuclease P1, which hydrolyzes yeast mRNA to yield m^7GpppA and m^7GpppG (19), completely destroyed its activity as a substrate for the decapping enzyme. It was previously shown that m^7GpppA was not cleaved (20).

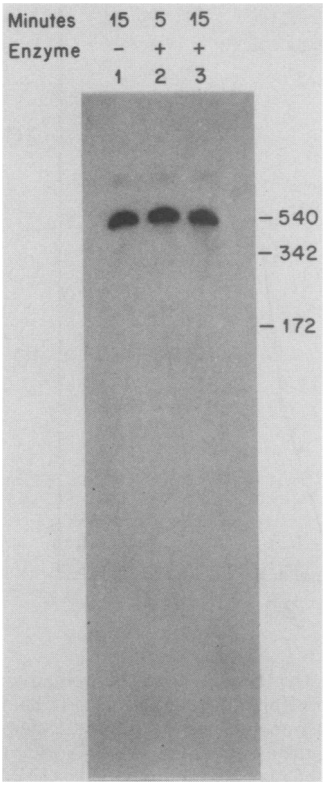

FIG. 2. Analyses of possible degradation of a capped RNA substrate by the decapping enzyme. A synthetic [^{3}H]methyl-capped [^{32}P]RNA (0.5 pmol, as a capped RNA chain) with a chain length of 540 nucleotides (10,000 cpm of ^{3}H label and 17,700 cpm of ^{32}P label) was used as the substrate with a fraction of the decapping enzyme from heparin-agarose chromatography. The reaction mixtures were as described in the text but were doubled in volume to 0.1 ml, and each contained 40 U of RNasin. Reaction mixture 1 (lane 1) was incubated for 15 min at 30°C in the absence of enzyme. Reaction mixtures 2 and 3 (lanes 2 and 3, respectively) were incubated with 75 U of enzyme at 30°C for 5 and 15 min, respectively. After the incubation period, 50 μl of each was processed for the determination of percent decapping. The remaining 50 μl of each reaction mixture was extracted with phenol, and the aqueous phase was precipitated with ethanol. The pellets were dissolved in urea-containing buffer and electrophoresed in a 5% polyacrylamide–8 M urea gel as described by Inoue et al. (6). The gel was exposed to X-ray film for 8 h at −80°C. The numbers on the right are chain lengths in nucleotides.

Micrococcal nuclease also inactivated yeast mRNA. When the enzyme itself was preincubated with micrococcal nuclease, its decapping activity was not affected (data not shown). That the products of RNase A and micrococcal nuclease action did not inhibit hydrolysis by the decapping enzyme is shown by the results for the control reaction mixtures (Table 1). The results shown in Table 1 suggest that long RNA chains are the best substrates for the decapping reaction.

Effect of size of synthetic capped RNAs on the decapping rate. To examine more closely the effect of RNA chain length on the rate of decapping, synthetic capped RNAs of different chain lengths were made (see Materials and Methods). Three RNAs were made by using each of two DNA fragments cloned into SP6 polymerase transcription vectors and shortened by restriction enzyme digestion. The rates of decapping of RNAs of different lengths when 0.25 pmol of each was used as a substrate are shown in Fig. 3. The results obtained with RNAs with chain lengths of 540, 100, and 50 nucleotides, produced with one of the cloned fragments, show that RNA with a chain length of 100 was decapped at 50% of the rate of RNA with a chain length of 540 and that the rate of decapping dropped to less than 10% with an RNA with a chain length of 50. The second group of RNAs, with chain lengths of 342, 172, and 68 nucleotides, also showed a significant decrease in decapping rate with decrease in chain length (Fig. 3). Mixing experiments showed that the smaller

TABLE 1. Effects of nuclease treatment of capped RNA substrates on rate of decapping[a]

Substrate	RNase and concn used to treat substrate	Relative decapping activity (%)
Yeast mRNA	None	100
	RNase A (ng/ml)	
	1.5	46
	7.5	<3
	7.5 (control)	89
	Nuclease P1, 50 μg/ml	<3
	Micrococcal nuclease (μg/ml)	
	4	<3
	4 (control)	87
Synthetic RNA	None	100
	RNase A (ng/ml)	
	0.45	84
	2.35	29
	4.5	<3
	9.0	<3
	4.5 (control)	93

[a] The RNase treatments and decapping assays were done as described in the text.

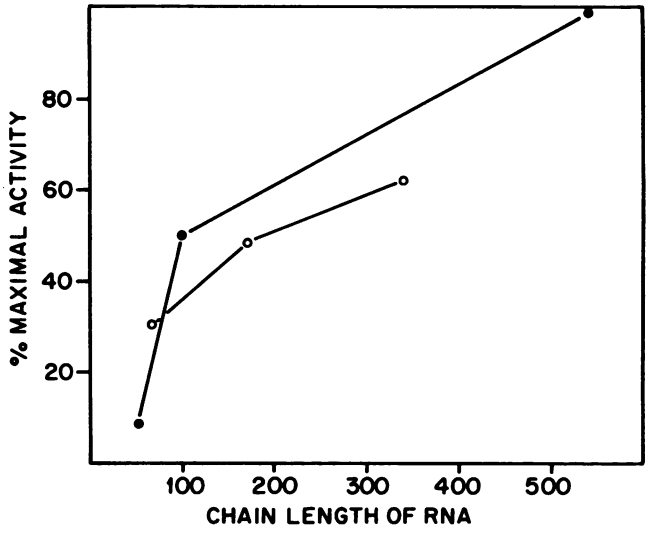

FIG. 3. Effect of chain length of synthetic capped RNA molecules on rate of decapping. Synthetic [^{3}H]methyl-capped [^{32}P]RNAs (0.25 pmol, as capped RNA chains) with chain lengths of 50, 100, and 540 nucleotides (synthesized with the pcTAT-3 DNA fragments (●) and 68, 172, and 342 nucleotides (synthesized with the p33 DNA fragments) (○) were tested as substrates in the decapping reaction, using reaction mixtures as described in the text with a 5-min incubation time and 50 U of enzyme. RNasin (40 U) was included in each reaction mixture. The rate of hydrolysis of the synthetic RNA with a chain length of 540 nucleotides was used as the maximal activity (100%).

RNAs did not contain inhibitory material. K_m values determined for the synthetic RNA substrates (as capped RNA chains) were as follows: RNA with a chain length of 540 nucleotides (RNA 540), 15 nM; RNA 100, 38 nM; RNA 50, 40 nM; RNA 172, 25 nM; RNA 68, 30 nM.

Other characteristics of the decapping reaction. That m^7GDP is cleaved from the capped RNA substrates by the decapping enzyme was shown in a previous report (20). To determine that the hydrolysis leaves the RNA undegraded further at the 5′ end, a capped synthetic RNA of 342 nucleotides terminating with 5′-pG was hydrolyzed. The structure of the synthetic RNA was such that the next G from the 5′-pG terminus was nucleotide 9. The nucleotide at the 5′ end of the decapped product was isolated by paper electrophoresis after phosphorylation of the 5′ end with T4 polynucleotide kinase and digestion of the RNA with nuclease P1. An electrophoretic analysis of the change in 5′ nucleotide content of the RNA upon hydrolysis with the decapping enzyme is shown in Fig. 4A. [^{32}P]pG was the 5′-terminal nucleotide found, and its labeling depended upon alkaline phosphatase treatment of the RNA before phosphorylation, thus showing that the product RNA terminated with pG.

Of interest was whether the specificity of the enzyme could be extended to the 7-methyl group of the cap structure. A synthetic RNA with a chain length of 540 nucleotides with the structure G[^{32}P]ppp[^{3}H]RNA was prepared, and its hydrolysis was compared with that of [^{3}H]m^7Gppp [^{32}P]RNA. The ^{32}P-labeled RNA lacking the 7-methyl group was hydrolyzed, yielding GDP (Fig. 4B). [^{3}H]m^7GpppRNA was also hydrolyzed, and m^7GDP was the product (Fig. 4B). The extent of capping of the G[^{32}P]ppp[^{3}H]RNA was considerably lower than that of the [^{3}H]m^7Gppp[^{32}P]RNA (6.6 compared with about 60%) and its extent of hydrolysis was about 50% of that of the methyl-containing RNA. To compare better the rates of hydrolysis of methylated and nonmethylated RNA species, m^7GpppG[^{3}H]RNA and GpppG[^{3}H]RNA, prepared so that the extents of capping were similar, were used as substrates. A coupled assay was used to determine the activity of the two RNAs. The assay involved measurement of the conversion of labeled RNA to acid-soluble material in the presence of both the decapping enzyme and a fraction of the 5′→3′ exoribonuclease of *S. cerevisiae* (23). The latter enzyme hydrolyzes capped RNA very poorly; therefore, the acid solubilization of the two capped RNAs would depend on prior decapping. m^7GpppG-RNA and GpppG-RNA were hydrolyzed at almost the same rate.

The hydrolysis of the PP_i bonds of the 5′-triphosphate end group of an RNA ([^{32}P]pppRNA) synthesized with *E. coli* RNA polymerase, using T4 DNA as a template, was measured by electrophoretic analysis of label released as P_i or PP_i. No hydrolysis was detected (data not shown).

The decapping enzyme described here uniquely prefers long capped RNA chains and thus appears to be highly specific for a PP_i bond of the RNA cap structure. In crude

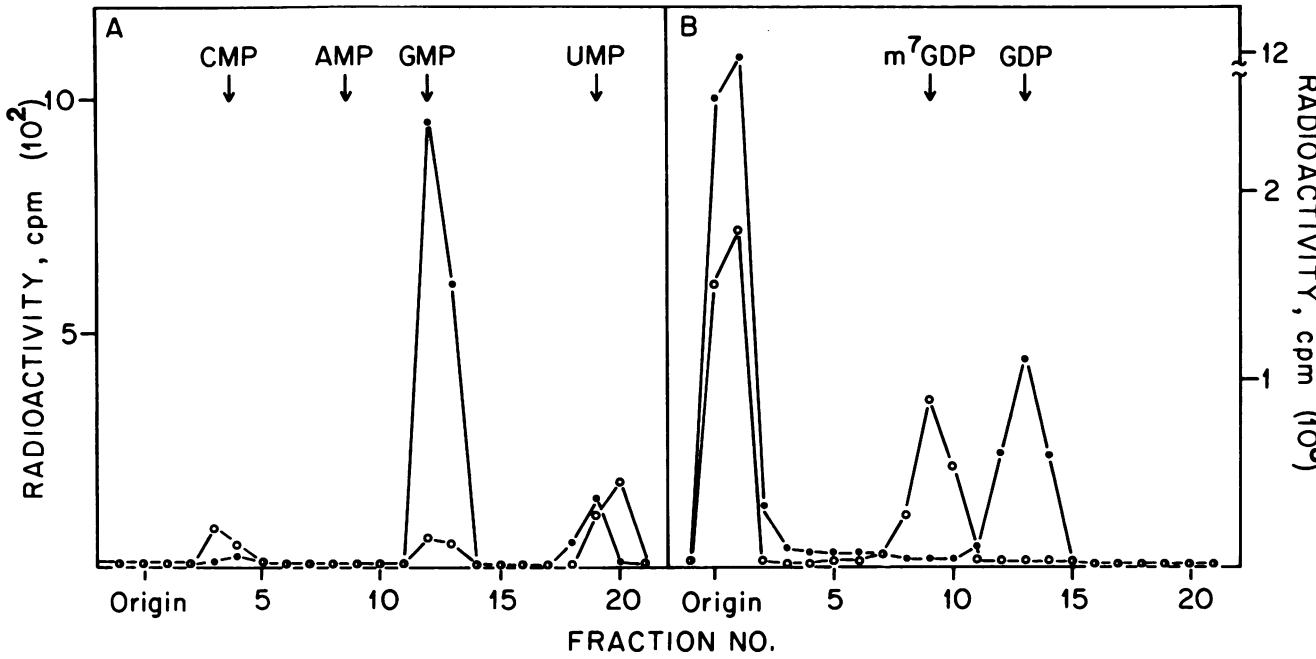

FIG. 4. Identification of the products of decapping of [^{3}H]m^7GpppRNA and G[^{32}P]pppRNA. (A) Analyses of the 5′-terminal nucleotide of the decapped RNA product. [^{3}H]m^7Gppp[^{32}P]RNA (0.2 pmol, as a capped RNA chain) with a chain length of 342 nucleotides was incubated in the absence and presence of 50 U of the decapping enzyme as described in the text. The reaction mixtures were then treated with 2 μg of *E. coli* alkaline phosphatase for 30 min at 37°C. After phenol extraction, the RNA products were phosphorylated with T4 polynucleotide kinase by using [γ-^{32}P]ATP and hydrolyzed with nuclease P1, and the products were examined by paper electrophoresis, as previously described (22). Electrophoresis was carried out for 75 min. The difference in the label content of 5′ nucleotides between the reaction mixture with the decapping enzyme and that without the enzyme is shown (●). The same experiment was also carried out without the alkaline phosphatase treatment (○). (B) Analysis of the cap products with RNAs with methylated and nonmethylated cap structures. G[^{32}P]ppp[^{3}H]RNA (0.0033 pmol, as a capped RNA chain) and [^{3}H]m^7Gppp[^{32}P]RNA (0.1 pmol, as a capped RNA chain), each with a chain length of 540 nucleotides, were each incubated with 30 U of the decapping enzyme, as described in the text. The products from the cap structures were examined by paper electrophoresis as previously described (20). Electrophoresis was carried out for 50 min. Symbols: ●, G[^{32}P]pppRNA; ○, [^{3}H]m^7GpppRNA.

extracts and partially purified enzyme fractions in which RNase activity is high, such an enzyme may have only low activity. The enzyme described here was detected by chance in a partially purified fraction of a 5′→3′ exoribonuclease. Nuss et al. (13) reported that they failed to find in HeLa cells a decapping activity that acted on long capped RNA chains. We are currently examining extracts and enzyme fractions of higher eucaryotic cells for decapping activity in the presence of RNasin.

That the decapping enzyme purifies closely with a 5′→3′ exoribonuclease could be of considerable interest, as was discussed previously (20). The two enzymes may be involved together in the turnover or degradation of at least some mRNA species. It may be of interest to compare the decapping rates of several yeast mRNAs that have different turnover rates. It is possible that the secondary or tertiary structure or both of the RNA chains may affect the rate of decapping.

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