THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 250, No. 24, Issue of December 25, pp. 9330-9335, 1975
Printed in U.S.A.

Modification of RNA by mRNA Guanylyltransferase and mRNA(guanine-7-)methyltransferase from Vaccinia Virions

(Received for publication, August 15, 1975)

SCOTT A. MARTIN AND BERNARD MOSS

From the Laboratory of Biology of Viruses, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20014

A purified enzyme system isolated from vaccinia virus cores has been shown to modify the 5′ termini of viral mRNA and synthetic poly(A) and poly(G) to form the structures m^{7}G(5′)pppA- and m^{7}G(5′)pppG-. The enzyme system has both guanylyltransferase and methyltransferase activities. The GTP:mRNA guanylyltransferase activity incorporates GMP into the 5′ terminus via a 5′-5′ triphosphate bond. The properties of this reaction are: (*a*) of the four nucleoside triphosphates only GTP is a donor, (*b*) mRNA with two phosphates at the 5′ terminus is an acceptor while RNA with a single 5′-terminal phosphate is not, (*c*) Mg^{2+} is required, (*d*) the pH optimum is 7.8, (*e*) PP_i is a strong inhibitor, and (*f*) the reverse reaction, namely the formation of GTP from PP_i and RNA containing the 5′-terminal structure G(5′)pppN-, readily occurs. The *S*-adenosylmethionine:mRNA(guanine-7-)methyltransferase activity catalyzes the methylation of the 5′-terminal guanosine. This reaction exhibits the following characteristics: (*a*) mRNA with the 5′-terminal sequences G(5′)pppA- and G(5′)pppG- are acceptors, (*b*) only position 7 of the terminal guanosine is methylated; internal or conventional 5′-terminal guanosine residues are not methylated, (*c*) the reaction is not dependent upon GTP or divalent cations, (*d*) optimal activity is observed in a broad pH range around neutrality, (*e*) the reaction is inhibited by *S*-adenosylhomocysteine. Both the guanylyltransferase and methyltransferase reactions exhibit bisubstrate kinetics and proceed via a sequential mechanism. The reactions may be summarized:

$$\text{ppN-} + \text{GTP} \rightleftarrows \text{G(5′)pppN-} + PP_i \quad (1)$$

$$\text{G(5′)pppN-} + \textit{S}\text{-AdoMet} \rightarrow \text{m}^7\text{G(5′)pppN-} + \textit{S}\text{-AdoHcy} \quad (2)$$

Vaccinia, a large DNA virus that replicates within the cytoplasm of infected cells, contains enzymes for the synthesis of mRNA. In the accompanying paper (1), we described the purification from vaccinia virus of two inseparable activities which modify the 5′ terminus of mRNA. One activity is a guanylyltransferase which incorporates GMP into the 5′ terminus of mRNA. The second activity, a guanine-7-methyltransferase, incorporates a methyl group into position 7 of the 5′-terminal guanosine residue in the mRNA. Both activities appear to be components of an enzyme system which has a molecular weight of 127,000 and which contains two polypeptides whose molecular weights are 95,000 and 31,400. In this paper we analyze the *in vitro* modification of viral mRNA and synthetic polyribonucleotides by this enzyme system.

EXPERIMENTAL PROCEDURES

Enzymes and Assays—mRNA guanylyltransferase and mRNA(guanine-7-)methyltransferase was purified from vaccinia virus and assayed as described in the preceding paper (1). In experiments in which guanylyltransferase-independent methyltransferase activity was to be measured, GTP and Mg^{2+} were omitted from the reaction mixture described previously.

Preparation of Synthetic mRNA as Suitable Acceptor for Guanylyltransferase—The chemical addition of a phosphate group to the 5′ terminus of synthetic polyribonucleotides was accomplished by the following procedure based on the studies of Rapaport and Zamecnik (2), Kozarich *et al.* (3), Hoard and Ott (4), and Cramer *et al.* (5, 6). Mono(tri-*n*-butylammonium) phosphate was prepared from orthophosphoric acid and excess tributylamine and was made anhydrous by repeated addition and evaporation of pyridine followed by dimethylformamide. The phosphate content of the final preparation was determined by the assay of Fiske and SubbaRow (7) after digestion of the material with 5 N H_2SO_4 and 30% H_2O_2. To the anhydrous tributylammonium phosphate equivalent to 10 mmol of P_i was added 50 mmol of 1,1′-carbonyldiimidazole in anhydrous dimethylformamide in a total volume of 50 ml. The formation of diimidazolylphosphonate in this mixture was allowed to proceed for 24 hours at room temperature in a desiccator, after which 1 ml of anhydrous methanol was added to decompose any remaining 1,1′-carbonyldiimidazole. Commercial polyadenylic acid (100 mg) was dissolved in 100 ml of H_2O and was precipitated as the cetyltrimethylammonium salt by the procedure of Sibatani (8). The precipitate was collected by centrifugation, washed twice with H_2O, lyophilized, and dissolved in 50 ml of anhydrous dimethylformamide. To this solution was added the diimidazolylphosphonate prepared as described above, and the mixture was reacted 24 hours at room temperature in a desiccator, after which the RNA was recovered as the sodium salt by dialysis against 0.1 M sodium acetate followed by precipitation from 0.1 M sodium acetate/70% ethanol overnight at −20°.

A similar procedure on a smaller scale was followed for adding a second phosphate group to the 5′ terminus of poly(G).

Preparation of mRNA with Blocked 5′-Terminal Structure, G(5′)pppN-—In a 15-ml mixture containing 50 mM Tris-HCl (pH 7.8),

1 mM dithiothreitol, and 2.5 mM each GTP and $MgCl_2$, were added 7 mg of poly(A) acceptor prepared as described above and 30 units of guanylyltransferase from vaccinia virus. After incubation for 1 hour at 37° the RNA was phenol-extracted three times in the presence of sodium dodecyl sulfate, and precipitated from ethanol overnight at −20°. The precipitated RNA was dissolved in H_2O and further purified by passage through a column (55 × 0.9 cm) of Sephadex G-50.

For experiments utilizing mRNAs whose blocked 5′ termini were labeled with [^{3}H]guanosine, the acceptor mRNA (either vaccinia mRNA, $pp(A)_n$, or $pp(G)_n$) was incubated with guanylyltransferase (5 units/ml) under conditions for the guanylyltransferase assay described in the preceding paper (1). Following the incubation the RNA was purified by phenol extraction, ethanol precipitation, and Sephadex gel filtration as described above.

Paper Electrophoresis—In experiments in which reaction products were analyzed by paper electrophoresis, mixtures were applied in 2-cm strips onto Whatman No. 3MM paper. Electrophoresis was performed at 30 volts/cm for 3 hours in 50 mM sodium citrate, pH 3.5. Following electrophoresis locations of nucleotides were determined by examination under ultraviolet light. The position of migration of marker $^{32}P_i$ and locations of labeled components of the reaction mixtures were identified by cutting the paper into 1-cm strips, immersing them in toluene-based scintillation fluid containing 10% H_2O and 33% Triton X-100, and counting the samples with a liquid scintillation counter.

Source of Materials—In vitro vaccinia mRNA was prepared as described in the preceding paper (1). Nucleotides and synthetic polyribonucleotides were purchased from P-L Biochemicals. *S*-Adenosylmethionine was from Boehringer-Mannheim. *S*-Adenosylhomocysteine was obtained from Sigma. Radioisotopes were the products of New England Nuclear. Tributylamine was from Eastman Organic Chemicals, and 1,1′-carbonyldiimidazole was purchased from Aldrich Chemical Co. Reagent grade pyridine, dimethylformamide, and methanol were distilled prior to use and stored in a desiccator.

RESULTS

Structural Requirements of mRNA to Act as Acceptor for Guanylyl- and Methyltransferase—All experiments described in this report were performed with enzyme purified as described in the preceding paper (1). In previous studies with crude vaccinia core extracts (9) we showed that radioactivity from [α-^{32}P]- but not [β,γ-^{32}P]GTP is incorporated into vaccinia mRNA or synthetic poly(A). In the course of these experiments we found that most lots of commercial poly(A) were not acceptors for GMP incorporation. Since the 5′ terminus of poly(A) synthesized by polynucleotide phosphorylase usually contains a single phosphate (10), this led us to consider that two or more phosphates at the 5′ terminus might be required. Using the method described under "Experimental Procedures," we added a second phosphate to the 5′ terminus of synthetic poly(A) and tested its acceptor activity in the guanylyltransferase reaction. Table I shows the results. Synthetic poly(A) with a single phosphate at the 5′ terminus was ineffective as an acceptor, whereas poly(A) which has a second phosphate added to its 5′ terminus was quite effective. Indeed poly(A) containing two phosphates at the 5′ terminus was as efficient an acceptor for the incorporation of GMP by guanylyltransferase as vaccinia mRNA synthesized *in vitro*. Similar studies also showed that two phosphates were required for poly(G) to act as an acceptor. Table I also illustrates that a divalent cation is needed for GMP incorporation, but *S*-adenosylmethionine is not required. Additional experiments showed that of the four ribonucleoside triphosphates only GTP could serve as a donor in this reaction.

TABLE I

Substrate requirements of mRNA guanylyl- and mRNA (guanine-7-)methyltransferase

Polynucleotide	Mg^{2+}	GTP	GMP incorporated	Methyl incorporated
			fmoles	fmoles
$p(A)_n$	−	−		0
$p(A)_n$	+	−		0
$p(A)_n$	−	+	8	0.7
$p(A)_n$	+	+	10	0.8
$pp(A)_n$	−	−		0
$pp(A)_n$	+	−		0.3
$pp(A)_n$	−	+	2	0.6
$pp(A)_n$	+	+	154	690
$G(5')ppp(A)_n$	−	−		333
$G(5')ppp(A)_n$	+	−		316
$G(5')ppp(A)_n$	−	+		331
$G(5')ppp(A)_n$	+	+		770

As shown in Table I, poly(A) with a single 5′-terminal phosphate was not an acceptor of methyl groups under any circumstances; however, poly(A) containing two phosphates at the 5′ terminus was an acceptor of methyl groups in the presence of GTP and Mg^{2+}, conditions under which GMP was also incorporated. That methyltransferase is not dependent upon the presence of GTP and Mg^{2+} was shown by first synthesizing poly(A) with blocked 5′ termini whose structure is $G(5')ppp(A)_n$. This molecule acted as an acceptor for methyltransferase in the absence of either GTP or Mg^{2+} (Table I). The increased incorporation of methyl groups observed with $G(5')ppp(A)_n$ acceptor in the presence of GTP and Mg^{2+} is most likely a consequence of residual unblocked $pp(A)_n$ species in the enzymatically synthesized preparation of $G(5')ppp(A)_n$, although we cannot rule out the possibility that GTP and Mg^{2+} may exert an additional allosteric effect. Unmethylated vaccinia mRNA synthesized *in vitro* contains the blocked 5′-terminal sequences G(5′)pppG- and G(5′)pppA- as well as molecules with unblocked 5′ termini, explaining our previous finding that GTP stimulates methyltransferase activity but is not required absolutely with vaccinia mRNA (9).

Stimulation of Guanylyltransferase by S-Adenosylmethionine—As illustrated in Table I, *S*-adenosylmethionine is not required for guanylyltransferase activity. The incorporation of GMP into RNA is stimulated, however, by addition of the methyl donor at concentrations as low as 1 μM (Table II).

Effect of Divalent Cations on Guanylyl- and Methyltransferase Activities—The effect of divalent cations on guanylyltransferase with $pp(A)_n$ acceptor and on methyltransferase with $G(5')ppp(A)_n$ acceptor was examined and the results are shown in Table III. The incorporation of GMP into RNA is entirely dependent upon the presence of a divalent cation. Of those tested, Mg^{2+} is by far the most effective. With Mn^{2+} instead of Mg^{2+} in the reaction mixture only 11% of the incorporation of GMP is observed. By contrast, methyltransfer-

TABLE II

Effect of S-adenosylmethionine on incorporation of GMP

S-AdoMet Concentration	GMP Incorporated*
μM	
0 (control)	1.00
0.1	1.08
0.5	1.03
1	1.16
5	1.26
10	1.51
50	1.72
100	1.92
1000	2.28

*Expressed as relative to control in which 1.5 pmoles were incorporated.

TABLE III

Effect of divalent cations on mRNA guanylyl- and mRNA (guanine-7-)methyltransferase reactions

Cation	GMP Incorporated	Methyl Incorporated
None (control)	0.008	1.00
Mg^{2+}	1.00	0.67
Mn^{2+}	0.11	0.14
Ca^{2+}	0.021	0.46
Zn^{2+}	0.039	0.0017
Cu^{2+}	0.014	0.012

Divalent cations were present in place of Mg^{2+} in standard guanylyl- and methyltransferase assays at final concentrations of 2.5 mM. 2.5 mM EDTA was present in the control mixture. Numbers are expressed as relative to the optimal condition in which either 0.49 pmole GMP or 0.60 pmole methyl groups were incorporated.

ase activity exhibits no requirement for divalent cations, and at concentrations of 2.5 mM, Mn^{2+}, Cu^{2+}, and Zn^{2+} are inhibitory.

Effect of pH on Guanylyl- and Methyltransferase Activities—Vaccinia mRNA and the specific synthetic substrates, $pp(A)_n$ and $G(5')ppp(A)_n$, were used to determine the effect of pH on the guanylyl- and methyltransferase activities. With several buffer systems, optimal guanylyltransferase activity was observed at pH 7.8. The methyltransferase exhibited optimal activity in a broad range around neutral pH. For the routine assays of both activities, a Tris-HCl buffer, pH 7.8, was used.

Effects of Time, Enzyme Concentration, and Substrate Concentration on Guanylyl- and Methyltransferase Reactions—Using either vaccinia mRNA or synthetic $pp(A)_n$ as acceptors in the standard guanylyltransferase reaction, incorporation of GMP was linear for 20 min and continued to increase up to 1 hour at a slower rate. Similarly, the incorporation of methyl groups into either vaccinia mRNA or synthetic $G(5')ppp(A)_n$ was observed to be linear during the first 20 min with continued incorporation up to 1 hour. The simultaneous incorporation of GMP and methyl groups using [α-^{32}P]GTP and *S*-adenosyl[*methyl*-^{3}H]methionine with synthetic $pp(A)_n$ acceptor was measured, and the results are shown in Fig. 1. There was no lag phase for the incorporation of methyl groups suggesting that the formation of m^7G[1] occurs rapidly. Not all GMP which was incorporated under these conditions was converted to its 7-methyl derivative since as shown in Fig. 1 only one methyl group was incorporated for every 4 to 5 residues of GMP, assuming the values given for the specific activities of the radioisotopes.

Since routine enzyme assays were incubated for 30 min the effect of enzyme concentration in both guanylyl- and methyltransferase reactions was determined. Within the range of enzyme concentrations used for all experiments, a linear relationship was observed to exist between enzyme concentration and incorporation of either GMP or methyl groups during 30-min incubations (data not shown).

The rate of incorporation of GMP with the acceptor $pp(A)_n$ and the rate of incorporation of methyl groups with the acceptor $G(5')ppp(A)_n$ were examined under conditions of variable concentrations of the reactant species. The reaction mixtures were incubated with enzyme for 5 min which is shown

[1] The abbreviations used are: PEI-cellulose, polyethyleneimine-substituted cellulose; *S*-AdoMet, *S*-adenosylmethionine; *S*-AdoHcy, *S*-adenosylhomocysteine; m^7G, 7-methylguanosine; N^m, 2′-*O*-methylribonucleoside; A^m, 2′-*O*-methyladenosine; G^m, 2′-*O*-methylguanosine.

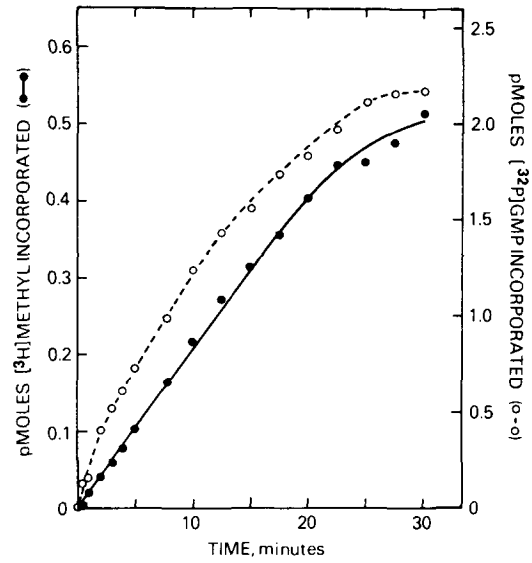

FIG. 1. Time course of incorporation of GMP and methyl groups into RNA. A 2-ml reaction mixture contained 50 mM Tris-HCl (pH 7.8), 1 mM dithiothreitol, 2.5 mM $MgCl_2$, 2.7 μM [α-^{32}P]GTP (290 mCi/mmol), 4 μM *S*-adenosyl[*methyl*-^{3}H]methionine (12.6 Ci/mmol), 200 μg of $pp(A)_n$, and purified enzyme equivalent to 15 units of guanylyltransferase and 40 units of methyltransferase. Following addition of enzyme, 100 μl of the reaction mixture were removed at various times during the incubation and were placed immediately into cold 10% trichloroacetic acid. The precipitated RNA was collected onto nitrocellulose filters, and the incorporation of radioisotopes was determined by liquid scintillation spectrometry.

in Fig. 1 to be well within the linear region of the initial reaction velocity. Plots of velocity *versus* substrate concentrations or double-reciprocal plots of the data were linear for both the guanylyl- and methyltransferase reactions. Lineweaver-Burk plots are shown in Figs. 2 and 3 for the guanylyl- and methyltransferase reactions, respectively. In both figures the series of lines, each of which represents a fixed concentration of RNA, were observed to intersect to the left of the vertical axis and below the horizontal axis. This pattern of intersection is consistent with a sequential mechanism (11) for both guanylyl- and methyltransferase reactions and indicates that in both reactions the substrates bind to the enzyme prior to release of the products.

Inhibition of Guanylyltransferase by Pyrophosphate—The effect of PP_i, a putative product of the guanylyltransferase reaction, on the incorporation of GMP into RNA was determined, and the results are shown in Fig. 4. Using GTP at a concentration of 9 μM, inhibition of the reaction was observed at concentrations of PP_i as low as 1 μM, a 50% inhibition occurred at a PP_i concentration of 4 μM, and at concentrations of PP_i greater than 50 μM, there was greater than 90% inhibition of the reaction. There was no effect on guanylyltransferase when P_i was added to the reaction mixtures instead of PP_i and at the same concentrations. It is also shown in Fig. 4 that considerably higher concentrations of PP_i are required for inhibition of the guanylyltransferase reaction in the presence of *S*-adenosylmethionine. A possible explanation of this will be presented under "Discussion."

Inhibition of Methyltransferase by S-Adenosylhomocysteine—Similarly, a putative product of the methyltransferase reaction, *S*-adenosylhomocysteine, was found to inhibit the incorporation of methyl groups into RNA. With 0.5 μM *S*-adenosylmethionine in the reaction mixture, a 30% inhibition was obtained with 1 μM *S*-adenosylhomocysteine, and greater

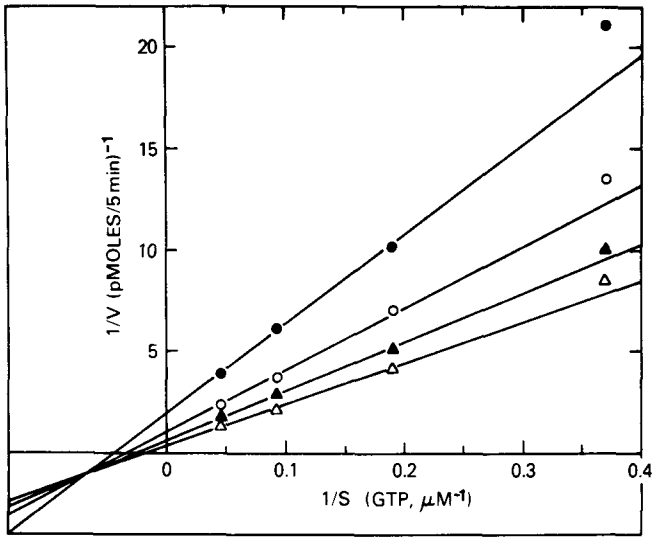

FIG. 2. Effect of substrate concentrations on the guanylyltransferase reaction. Standard 100-μl guanylyltransferase reaction mixtures containing different concentrations of GTP and pp(A)$_n$ and 0.2 unit of the enzyme were incubated for 5 min. The reactions were terminated by the addition of 1 ml of 10% trichloroacetic acid, and the incorporation of [^{3}H]GMP was determined as described in the legend to Fig. 1 and from the specific activity of the isotope in each sample. The quantity of pp(A)$_n$ in each set of samples is indicated: ●, 10 μg; ○, 20 μg; ▲, 30 μg; △, 40 μg.

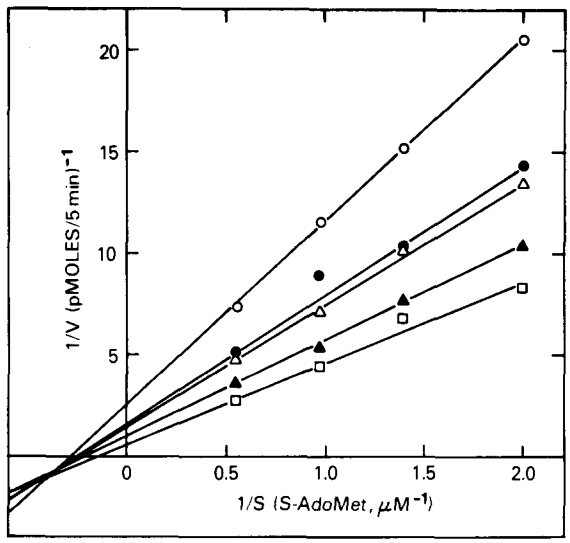

FIG. 3. Effect of substrate concentrations on the methyltransferase reaction. Methyltransferase reaction mixtures contained 50 mM Tris-HCl (pH 7.8), 1 mM dithiothreitol, different concentrations of *S*-adenosylmethionine and G(5')ppp(A)$_n$, and 0.3 unit of methyltransferase. After incubation for 5 min, the samples were treated and analyzed as described in the legend to Fig. 2. The quantity of G(5')ppp(A)$_n$ in each set of samples is indicated: ○, 5 μg; ●, 10 μg; △, 15 μg; ▲, 25 μg; □, 40 μg.

than 99% inhibition was obtained in the presence of 1 mM *S*-adenosylhomocysteine (Table IV).

Reversal of Guanylyltransferase Reaction—The products of the guanylyltransferase reaction are PP$_i$ and RNA whose 5' termini are blocked and have the structure G(5')pppN-. Using the purified guanylyltransferase, vaccinia mRNA with blocked 5' termini containing [^{3}H]guanosine was synthesized as described under "Experimental Procedures." This mRNA was purified and was then used to show the reversal of the

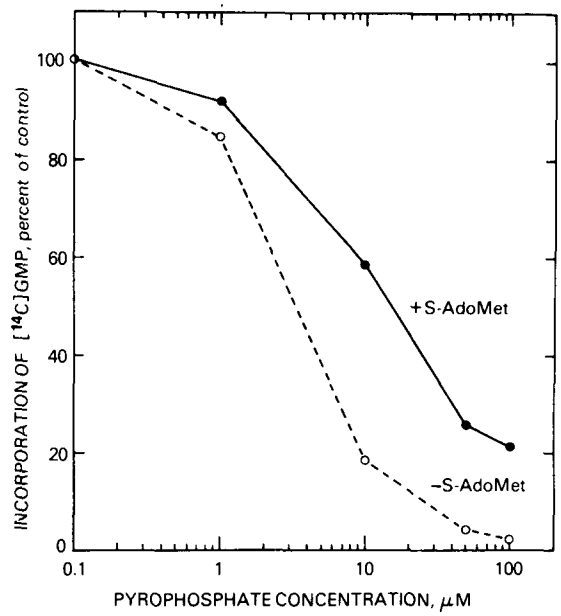

FIG. 4. Inhibition of guanylyltransferase activity by PP$_i$. The 100-μl reaction mixtures containing 50 mM Tris-HCl (pH 7.8), 1 mM dithiothreitol, 2.5 mM $MgCl_2$, 9 μM [^{14}C]GTP (545 mCi/mmol), and 0.2 unit of guanylyltransferase were incubated with different concentrations of PP$_i$ either in the presence or absence of 1 mM *S*-adenosylmethionine. The reactions were terminated by the addition of cold 10% trichloroacetic acid, and the incorporation of GMP was determined as described in the legend to Fig. 1. In the absence of PP$_i$ (control) the incorporation of GMP was stimulated 2.5-fold by the addition of 1 mM *S*-adenosylmethionine.

guanylyltransferase reaction as shown in Fig. 5. Controls in which the mRNA was incubated with enzyme alone (*Panel A*) or with ^{32}PP$_i$ alone (*Panel B*) illustrate that the majority of the [^{3}H]guanosine associated with the mRNA remains at the origin during electrophoresis. Incubation of the mRNA with both the guanylyltransferase and ^{32}PP$_i$ (*Panel C*) results in a decrease in the [^{3}H]guanosine associated with mRNA at the origin and the appearance of a new peak of material which contains both [^{3}H]guanosine and ^{32}P and which co-migrates with authentic GTP. In other experiments in which the reaction products were separated by thin layer chromatography on PEI-cellulose (12), we demonstrated the generation of [^{3}H]GTP from the vaccinia mRNA used in the above experiments as well as from [^{3}H]G(5')ppp(A)$_n$ after incubation with guanylyltransferase and PP$_i$.

DISCUSSION

An enzyme system purified from vaccinia virus cores possesses two activities which can catalyze post-transcriptional modification of the 5' terminus of RNA. Vaccinia mRNA synthesized by viral cores *in vitro* and poly(A) or poly(G) with 5'-terminal diphosphates are suitable acceptors for the guanylyltransferase. The reaction is specific for GTP, since no activity is observed with ATP, CTP, or UTP. The reaction requires a divalent cation; enzyme activity is 10-fold greater in the presence of Mg^{2+} than in the presence of Mn^{2+}. Only the guanosine residue and the α-phosphate of GTP are transferred suggesting the reaction is as follows:

$$\text{ppN-} + \text{GTP} \rightleftarrows \text{G(5')pppN-} + \text{PP}_i \quad (1)$$

The reversible nature of this reaction has been demonstrated.

A second activity of the purified enzyme system, a

TABLE IV

Effect of S-adenosylhomocysteine on incorporation of methyl groups into mRNA

S-AdoHcy Concentration	Methyl Incorporated*
μM	
0.0 (control)	1.000
0.1	0.970
1.0	0.720
10.0	0.200
100.0	0.046
1000.0	0.003

*Expressed as relative to control in which 0.6 pmoles were incorporated.

mRNA(guanine-7-)methyltransferase, can transfer a methyl group from *S*-adenosylmethionine to position 7 of the guanosine residue which has been added to the 5′ terminus of the RNA by the guanylyltransferase. No methylation of conventional 5′-terminal or internal guanosine residues has been detected indicating the specificity of the enzyme. The methyltransferase reaction which requires neither GTP nor Mg^{2+} and which is inhibited by *S*-adenosylhomocysteine may be written:

$$G(5')pppN\text{-} + S\text{-AdoMet} \rightarrow m^7G(5')pppN\text{-} + S\text{-AdoHcy} \quad (2)$$

A third activity, also solubilized from vaccinia virus cores (9) but not yet purified, can catalyze the ribose methylation of the penultimate nucleotide in the 5′-terminal structure as follows:

$$m^7G(5')pppN\text{-} + S\text{-AdoMet} \rightarrow m^7G(5')pppN^m\text{-} + S\text{-AdoHcy} \quad (3)$$

The structure $m^7G(5')pppN^m$- represents the fully modified 5′ terminus of mRNA synthesized by vaccinia virus cores in the presence of *S*-adenosylmethionine. When mRNA is synthesized in the presence of nonsaturating concentrations of *S*-adenosylmethionine, minor amounts of partially modified termini of the type $m^7G(5')pppN$- are detected which suggests that these partially modified termini are intermediates, and that Reaction 3 is the final modification step. When vaccinia virus cores are incubated with the nucleoside triphosphates in the complete absence of *S*-adenosylmethionine, only a small amount of the synthesized mRNA contains the blocked terminal structure G(5′)pppN-.[2] The absence of completely blocked 5′-terminal sequences may be explained by the inhibitory effect of PP_i on the guanylyltransferase reaction. The enzyme is inhibited by more than 90% at the concentrations of PP_i produced from the nucleoside triphosphates during RNA synthesis. A related explanation would be that the terminal structures G(5′)pppN- are actually formed, but the enzymatic reaction subsequently is driven in the reverse direction by the accumulation of PP_i. Much higher concentrations of PP_i are required to inhibit the guanylyltransferase in the presence of *S*-adenosylmethionine suggesting that following methylation of the guanosine residue, reversal of the guanylyltransferase reaction occurs less readily, if at all. This would account for the high proportion of the blocked, methylated termini of the RNA synthesized by viral cores in the presence of *S*-adenosylmethionine (13).

Although the sequence of *in vitro* Reactions 1 to 3 above provides a suitable mechanism for the post-transcriptional modification of mRNA synthesized by viral cores, we have not excluded a role for the guanylyltransferase in the initiation of mRNA synthesis. We are attempting presently to determine whether suitable conditions can be obtained for the condensation of ribonucleoside diphosphates with either GTP or m^7GTP or for the methylation of GTP or G(5′)pppG. A role for *S*-adenosylmethionine in the initiation of mRNA synthesis by cytoplasmic polyhedrosis virus has been suggested (14).

[2] Unpublished observations.

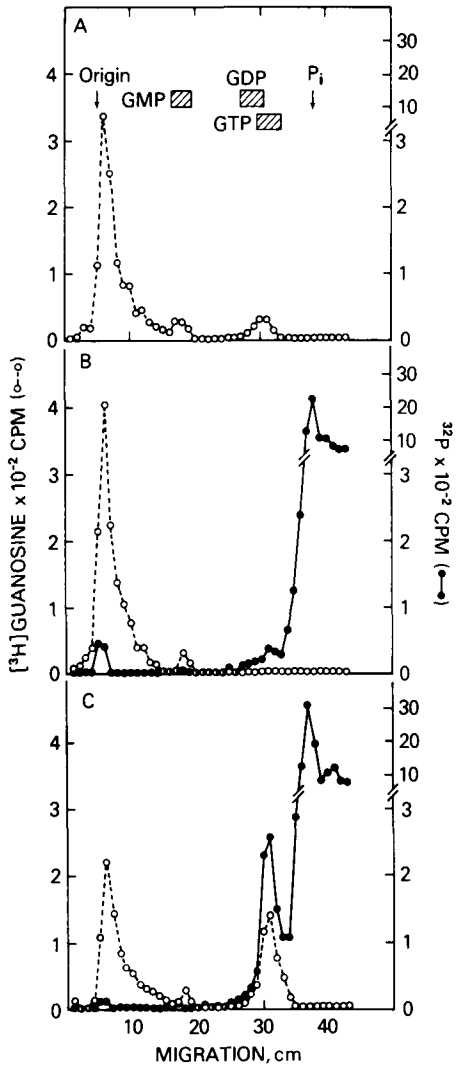

FIG. 5. Reversal of the guanylyltransferase reaction by PP_i. Each 100-μl reaction mixture contained 50 mM Tris-HCl (pH 7.8), 1 mM dithiothreitol, 2.5 mM $MgCl_2$, and 10 μg of vaccinia mRNA with [^{3}H]guanosine-blocked 5′ termini. The reaction mixtures were incubated with 0.3 unit of guanylyltransferase in *Panel A*, with 0.45 μM $^{32}PP_i$ (1.7 Ci/mmol) in *Panel B*, or with both guanylyltransferase and $^{32}PP_i$ in *Panel C*. After 30 min the reactions were terminated by the addition of EDTA, and the samples were applied to Whatman No. 3MM paper and analyzed by paper electrophoresis as described under "Experimental Procedures." The positions of migration of authentic nucleotides and P_i are indicated. Electrophoresis was from *left* to *right*.

The guanylyltransferase reaction described here is similar in certain respects to the formation of coenzymes such as NAD which have 5′-5′ diphosphate bonds or to the formation of diguanosine 5′-triphosphates and 5′-tetraphosphates by *Artemia* embryos (15). Like the vaccinia mRNA guanylyltransferase reaction, the synthesis of both NAD (16, 17) and diguanosine tetraphosphate (18, 19) involves pyrophosphorolysis of a nucleoside triphosphate. Both reactions are dependent upon Mg^{2+} and are reversed in the presence of PP_i.

Although mRNAs from a wide variety of viruses and cells contain 5′-terminal sequences of the type $m^7G(5')pppN^m$-

(discussed in the accompanying paper (1)), vaccinia virus is the only system from which the enzymes responsible for this modification have been isolated. The vaccinia enzymes appear to exhibit little sequence specificity since poly(A) and poly(G) may be suitable acceptors, and the vaccinia enzymes may be used for modifying the 5′ termini of other synthetic or natural mRNAs. Such modified mRNAs may prove useful for investigating the role of 7-methylguanosine in protein synthesis (20, 21).

REFERENCES

1. Martin, S. A., Paoletti, E., and Moss, B. (1975) *J. Biol. Chem.* **250,** 9322-9329
2. Rapaport, E., and Zamecnik, P. C. (1975) *Proc. Natl. Acad. Sci. U. S. A.* **72,** 314-317
3. Kozarich, J. W., Chinault, A. C., and Hecht, S. M. (1973) *Biochemistry* **12,** 4458-4463
4. Hoard, D. E., and Ott, D. G. (1965) *J. Am. Chem. Soc.* **87,** 1785-1788
5. Cramer, F., Schaller, H., and Staab, H. A. (1961) *Chem. Ber.* **94,** 1612-1621
6. Cramer, F., Schaller, H., and Staab, H. A. (1961) *Chem. Ber.* **94,** 1621-1633
7. Fiske, C. H., and SubbaRow, Y. (1925) *J. Biol. Chem.* **66,** 375-400
8. Sibatani, A. (1970) *Anal. Biochem.* **33,** 279-285
9. Ensinger, M. J., Martin, S. A., Paoletti, E., and Moss, B. (1975) *Proc. Natl. Acad. Sci. U. S. A.* **72,** 2525-2529
10. Harvey, R. A., and Grunberg-Manago, M. (1966) *Biochem. Biophys. Res. Commun.* **23,** 448-452
11. Cleland, W. W. (1970) in *The Enzymes* (Boyer, P. D., ed) 3rd Ed, Vol. II, pp. 7-10, Academic Press, New York
12. Randerath, K., and Randerath, E. (1967) *Methods. Enzymol.* **12A,** 340-341
13. Wei, C. M., and Moss, B. (1975) *Proc. Natl. Acad. Sci. U. S. A.* **72,** 318-322
14. Furuichi, Y. (1974) *Nucl. Acid Res.* **1,** 809-822
15. Warner, A. H., and McClean, D. K. (1968) *Dev. Biol.* **18,** 278-293
16. Kornberg, A. (1950) *J. Biol. Chem.* **182,** 779-793
17. Preiss, J., and Handler, P. (1958) *J. Biol. Chem.* **233,** 493-500
18. Warner, A. H., Beers, P. C., and Huang, F. L. (1974) *Can. J. Biochem.* **52,** 231-240
19. Warner, A. H., and Huang, F. L. (1974) *Can. J. Biochem.* **52,** 241-251
20. Both, G. W., Banerjee, A. K., and Shatkin, A. J. (1975) *Proc. Natl. Acad. Sci. U. S. A.* **72,** 1189-1193
21. Muthukrishnan, S., Both, G. W., Furuichi, Y., and Shatkin, A. J. (1975) *Nature* **255,** 33-37