The Effect of “Cap” Analogs on Reovirus mRNA Binding to Wheat Germ Ribosomes

EVIDENCE FOR ENHANCEMENT OF RIBOSOMAL BINDING VIA A PREFERRED CAP CONFORMATION

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A variety of compounds related to the 5'-terminal “cap” (m'GpppN) of eukaryotic mRNA's were chemically synthesized and tested as inhibitors of reovirus mRNA binding to wheat germ ribosomes. Under our conditions of mRNA binding to ribosomes, 7-methyl-, 7-ethyl-, and 7-benzyl-GDP, but not GDP, decreased stable initiation complex formation by 70 to 80% at a concentration of 0.1 mM. The presence of the positive charge on the imidazole of the 7-substituted compounds by treatment with alkali destroyed their inhibitory activity. Similarly, reduction to 8-hydro-m'GDP reversibly decreased the activity of m'GDP. The results were consistent with the hypothesis that the positive charge resulting from 7-alkylation provides an active cap conformer for binding via interaction with phosphates on the positively charged imidazole moiety. In accord with this suggestion, 7-carboxymethyl GDP and 7,8-dimethyl GDP were found to be less inhibitory than m'GDP. A 2-amino group was also important since m'GDP was less effective than 0.1 mM m'XDP did not inhibit ribosome binding. Other poor inhibitors were 6-Cl-m'GDP and 1,7-dimethyl GDP but m'GDI and m'Gpp had essentially the same activity as m'GDP.

The 5'-terminal “cap” structure, m'GpppN, that is present in most eukaryotic mRNA's facilitates their function in protein synthesis at the level of initiation (1). The presence of the N'-methyl group on the terminal guanosine promotes the formation of stable initiation complexes between mRNA and ribosomal 40 S subunits (2, 3). Chemical or enzymatic removal of m'G from capped mRNA reduces ribosome binding (4, 5) and unmethylated mRNA's are less effective in directing protein synthesis than their capped counterparts (6). In the case of reovirus mRNA, binding of wheat germ 40 S ribosomal subunits occurs at or near the 5'-end, resulting in protection against RNase digestion of a fragment that includes the cap and is capable of efficiently rebinding to ribosomes (7). Furthermore, proteins that associate with caps, apparently selectively, have been detected in protein-synthesizing extracts prepared from Artemia salina (8), rabbit reticulocytes (9, 10), and wheat germ (10). These various observations indicate that the 5'-end of eukaryotic mRNA has a positive influence on the interaction between ribosomes and mRNA and that the cap may participate in formation of the initiation complex.

Additional support derives from the observation that cap analogs such as m'Gpp, m'Gppp, m'Gpppp, and m'GpppN effectively inhibit translation by limiting mRNA binding to ribosomes (11-13). The presence, position, and number of phosphates on m'G all affect inhibitory capacity since m'G and m'Gpp are inactive and m'Gppp is considerably more inhibitory than m'Gpp. If it has been suggested on the basis of nuclear magnetic resonance measurements that the nucleoside diphosphate is a better inhibitor because it can assume a conformationally preferred structure as a result of an electrostatic interaction between the positively charged imidazole moiety and a negatively charged phosphate group (14); presumably, the mRNA caps would have the same conformation during initiation of protein synthesis. Consistent with this suggestion was the observation (3) that the binding of poly(A,U) to ribosomes was increased by 5'-terminal addition of m'GpppN but not by an alkali-modified cap that lacked a positive charge.

Our interest in the molecular mechanism by which the caps promote initiation of translation has prompted the preparation of several cap analogs to facilitate a study of the structural features which contribute to mRNA cap function. Using reovirus mRNA and wheat germ ribosomes, we have verified the importance of the positive charge on the imidazole moiety of m'G and of the nature and arrangement of substituents on its pyrimidine ring.

EXPERIMENTAL PROCEDURES

Materials

Guanosine, 1-methylguanosine, N'-methylguanosine, 6-chloroguanosine, guanosine 5'-diphosphate, adeninosine 5'-diphosphate,
Effect of "Cap" Analogs on Reovirus mRNA Binding to Wheat Germ Ribosomes

7-Ethylguanosine 5'-Diphosphate (e7GDP; 2) - Guanosine 5'-diphosphate (12.7 mg; 24.9 pmol) was stirred in 1 ml of dimethyl sulfoxide that had been acidified with hydrochloric acid (1 drop of 1 M HCl/10 ml of dimethylsulfoxide) and treated with 195 mg (1.25 mmol) of ethyl iodide. The mixture was stirred at room temperature for 1.5 h and then adjusted to pH 10 with 10 M NaOH (1 liter total volume; 7-ml fractions) at a flow rate of 210 ml/h. The appropriate fractions (25 to 61) were pooled and desalted. Concentration under reduced pressure gave 56 A\textsubscript{260} units (60 pmol; 24%) of 7-ethylguanosine 5'-diphosphate (2), having the expected UV spectrum.

Synthesis of Cap Analogs

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Table I

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ultraviolet (pH 7)</th>
<th>Fluorescence (pH 7)</th>
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<tr>
<td></td>
<td>λ_{max} (nm)</td>
<td>λ_{min} (nm)</td>
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<tr>
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<td>m'MDP (8)</td>
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<tr>
<td>m'dGDP (9)</td>
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<td>228</td>
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</tbody>
</table>

7-Methylinosine 5'-Diphosphate (m'dGDP; 8) - Inosine 5'-monophosphate (53 mg; 152 pmol) was dissolved in 0.6 ml of dimethylsulfoxide that had been acidified with hydrochloric acid (1 drop of 1 M HCl/10 ml of dimethyl sulfoxide). This solution was treated with 50 μl of methyl iodide and then maintained at room temperature for 2 h. After treatment with 5 ml of ethanol, the suspension was centrifuged and the isolated solid was dissolved in 0.6 ml of dimethylsulfoxide. This solution was treated with 50 μl of methyl iodide and then maintained at room temperature for 3 days. Methanol (1 ml) was added to the reaction mixture, which was then concentrated to dryness. The residue was dissolved in 10 ml of water and applied to a DEAE-cellulose column (10 × 0.5 cm); elution was with ammonium bicarbonate gradient (0 to 0.25 M; 1 liter total volume; 4-ml fractions) at a rate of 225 ml/h. Fractions 34 to 40 were pooled, desalted, and concentrated to give 7-methylinosine 5'-monophosphate as a white solid, yield 231 A₂₆₀ units (19%).

7-Methyl-2'-deoxyguanosine 5'-Diphosphate (m'dGDP; 11) - Deoxyguanosine 5'-diphosphate (110 A₂₆₀ units; 9.2 pmol) was dissolved in 200 μl of water and 1 ml of dimethyl sulfoxide, then treated with 300 μl (1.2 mmol) of methyl iodide and stirred at room temperature for 6 h. The reaction mixture was then concentrated to dryness. The precipitate was isolated and purified as indicated above for compound 10, yield 39 A₂₆₀ units (53%) of 7-methyl-2'-deoxyguanosine 5'-diphosphate. The product was characterized by its uv and fluorescence spectra and by its chromatographic behavior on polyethyleneimine tlc (1 M LiCl), Rᶠ 0.49.

P'-(7-Methylguanosine-5')₅-(inosine-5')₃-diphosphate (m'GppI; 12) - 7-Methylguanosine 5'-monophosphate (150 A₂₆₀ units; 16 μmol) and tri-n-butylammonium phosphate (55 pmol) in 500 μl of dimethylformamide was added with vigorous mixing and the reaction mixture was maintained overnight at 4°C. The resulting precipitate was isolated by centrifugation, dissolved in 25 ml of water, and applied to a DEAE-cellulose column (25 × 2.0 cm; HCO₃⁻ form). The column was washed with 25 ml of water and then with a linear gradient of ammonium bicarbonate (0 to 0.25 M; 1 liter total volume; 4-ml fractions) at a rate of 225 ml/h. Fractions 89 to 97 were pooled, desalted, and concentrated to afford compound 12 as a colorless glass, yield 90 A₂₆₀ units (51%); tlc (polyethyleneimine; 1 M LiCl), Rᶠ 0.40.

7-Methylxanthosine 5'-Diphosphate (m'XDP; 10) - A solution of 175 A₂₆₀ units (19.6 pmol) of xanthosine 5'-diphosphate in 1 ml of dimethyl sulfoxide (acidified with 1 drop of 1 M HCl/10 ml of dimethyl sulfoxide) was treated with 100 μl (400 μmol) of methyl iodide for 6 h. The reaction mixture was diluted with 20 ml of ethanol and maintained overnight at 4°C. The precipitate was isolated by centrifugation, dissolved in 25 ml of water, and applied to a DEAE-cellulose column (25 × 2.0 cm; HCO₃⁻ form). The column was washed with 25 ml of water and then with a linear gradient of ammonium bicarbonate (0 to 0.25 M; 1 liter total volume; 7-ml fractions) at a rate of 225 ml/h. Fractions 84 to 97 were pooled, desalted, and concentrated to afford compound 10 as a colorless glass, yield 80 A₂₆₀ units (52%); tlc (polyethyleneimine; 1 M LiCl), Rᶠ 0.45.

7-Methyl-2'-deoxyguanosine 5'-Diphosphate (m'dGDP; 11) - Deoxyguanosine 5'-diphosphate (110 A₂₆₀ units; 9.2 pmol) was dissolved in 200 μl of water and 1 ml of dimethyl sulfoxide, then treated with 300 μl (1.2 mmol) of methyl iodide and stirred at room temperature for 3 h. The product was isolated and purified as indicated above for compound 10, yield 39 A₂₆₀ units (53%) of 7-methyl-2'-deoxyguanosine 5'-diphosphate. The product was characterized by its uv and fluorescence spectra and by its chromatographic behavior on polyethyleneimine tlc (1 M LiCl), Rᶠ 0.56.

P'-(7-Methylguanosine-5')₅-(inosine-5')₃-diphosphate (m'GppI; 12) - 7-Methylguanosine 5'-monophosphate (150 A₂₆₀ units; 16 μmol) and tri-n-butylammonium phosphate (55 pmol) in 500 μl of dimethylformamide was added with vigorous mixing and the reaction mixture was maintained overnight at 4°C. The resulting precipitate was isolated by centrifugation, dissolved in 25 ml of water, and applied to a DEAE-cellulose column (25 × 2.0 cm; HCO₃⁻ form). The column was washed with 25 ml of water and then with a linear gradient of ammonium bicarbonate (0 to 0.25 M; 1 liter total volume; 4-ml fractions) at a rate of 225 ml/h. Fractions 89 to 97 were pooled, desalted, and concentrated to afford compound 12 as a colorless glass, yield 90 A₂₆₀ units (51%); tlc (polyethyleneimine; 1 M LiCl), Rᶠ 0.40.
was converted to the anhydrous tri-n-butylammonium salt and treated with 30 mg (185 μmol) of 1,1-carboxynitromidazine in 1 ml of dimethylformamide. The reaction mixture was maintained at room temperature for 2 days and treated with 1 μl (24 μmol) of methanol for 30 min. The reaction mixture was then treated with the anhydrous tri-n-butylammonium salt of inosine 5'-monophosphate (180 A₂₆ₐ units; 15 μmol) in 0.5 ml of dimethylformamide and maintained at room temperature for an additional 3 days. The reaction mixture was concentrated and the residue was dissolved in 25 ml of water and applied to a DEAE-cellulose column (25 x 2 cm; HCO₃⁻ form). The column was washed with 25 ml of water and then with a linear gradient of ammonium bicarbonate (0 to 0.2 M; 1 liter total volume; 9-ml fractions) at a flow rate of 200 ml/h. Fractions 70 to 73 were pooled, desalted, and concentrated to afford 36 A₂₆ₐ units (16%) of the dinucleoside diphosphate.

**Alkali-induced Imidazole Ring Opening of 7-Alkylguanosine 5'-Diphosphates**

7-Methylguanosine 5'-diphosphate (2.5 μmol) was dissolved in 0.25 ml of degassed Tris buffer (10 mM, pH 7.0) and treated with 13.5 mg (250 μmol) of potassium borohydride. The resulting 8-hydro-7,9-disubstituted guanosine 5'-diphosphate treated under these conditions yielded uv-absorbing material migrating faster than GDP and intermediate between GMP and UMP. There was little or no residual m'GDP as judged by fluorescence detection. Under milder conditions (1 to 2.5 M NH₄OH) some fluorescent material remained; this material could be converted to m'G by treatment with alkaline phosphatase (10 mM Tris buffer, pH 8, 36 μg/ml, 30 min, 37°C).

**Reduction of m'GDP and Reoxidation of 8-Hydro-m'-GDP**

8-Hydro-m'GDP (0.1 mM) gave a fluorescence reading of 1.2 at excitation 290 nm, emission 390 nm) as described and purified to homogeneity by DEAE-cellulose chromatography; the purity of each sample was verified by tlc on polyethyleneimine and by its quantitative ultraviolet and fluorescence spectra. As shown in Fig. 1, 0.1 mM m'GDP markedly decreased the binding of reovirus ³H-methylated mRNA to wheat germ ribosomes. The inhibitory effect was not restricted to the methylated nucleotide since other N'-substituted guanosine 5'-diphosphates, including the 7-ethyl and 7-benzyl derivatives, were also inhibitory (Fig. 1). Thus, the presence of a bulkier 7-alkyl group did not alter the inhibitory capacity of the cap analog. Guanosine 5'-diphosphate had no effect at 0.1 mM concentration, but at higher concentrations it also reduced binding, possibly by competition with GTP during formation of the ternary complex, GTP-ëif2-methionyl-tRNA <sub>i</sub>Met (22).

[**Fig. 1.** Effect of 7-alkylated guanosine 5'-diphosphates on the binding of ³H-methylated mRNA to wheat germ ribosomes. (³H)Methyl-labeled mRNA was incubated in wheat germ S₉₀ extract, and the radioactivity bound to 80 S ribosomes in the presence of the indicated compounds was determined by density gradient centrifugation as described under “Experimental Procedures.” GDP (□-□), GDP (×-×), m'-GDP (△), GDP (●), GDP (●), and the respective ring-opened derivatives of m'-GDP (△), e'-GDP (●), and benzyl-GDP (■).](http://www.jbc.org/)
hydro-\textsuperscript{m'GDP} (13) conversion is that the transformation can be reversed slowly by reaction of the reduced species with \( \text{H}_2\text{O}_2 \) or \( \text{O}_2 \) or more rapidly by treatment with oxidants. In fact, when the \( 8\text{-hydro}-\text{m'GDP} \) was reoxidized to \text{m'GDP} with peroxide, its ability to inhibit reovirus mRNA binding to wheat germ ribosomes was almost completely recovered (Fig. 2B). Specifically, after reoxidation, binding was again decreased to 24% as compared to 77% in the control incubation mixture that contained all the components of the reduction and oxidation mixtures except \text{m'GDP}. These results provide further support for the hypothesis that the positive charge of \text{m'GDP} and its derivatives, and presumably also that of 5'-terminal \text{m'G} in mRNA caps, has an important facilitating effect on binding to ribosomes during initiation of protein synthesis.

As discussed below, the lack of inhibitory activity of \text{m'GDP} after hydride addition was thought to be due to disruption of the electrostatic interaction between the positively charged imidazole moiety and phosphate oxygen anion, with concomitant loss of preference for a single conformational isomer. Similar, albeit less dramatic, loss of inhibitory activity was also observed for \text{cm'GDP} and \text{m'GDP} (Fig. 1), both of which have substituents that might be expected to alter the presumed inhibitory conformation.

\text{m'GDP} Derivatives Modified in Pyrimidine Moiety – In addition to an N'-substituent, the 2-amino group of \text{m'GDP} also appears to be essential for maximum inhibition. Its formal removal to form 7-methylinosine 5'-diphosphate (m\text{TDP}) decreased inhibitory activity severalfold (Fig. 3). Furthermore, formal replacement of the amino group by a keto functionality, i.e. affording m\text{XDP}, eliminated most of the capacity of the diphosphate to interfere with \text{mRNA} binding to ribosomes. Other poor inhibitors of ribosomal binding were 6-Cl-\text{m'GDP} (9), in which the O\text{''} atom was replaced by chlorine and m\text{''GDP}. By contrast, the analog having a methyl substituent on the 2-amino group (m\text{m'GDP}) had the same activity as \text{m'GDP} (Fig. 3).

Alteration of Other Sites Had No Effect on Inhibition of mRNA Binding by "Cap" Analogs – As shown in Fig. 4, the 2'-deoxy analog of \text{m'GDP} (II) was nearly as inhibitory to ribosomal binding of reovirus mRNA as \text{m'GDP} itself, suggesting that the cis-diol moiety is not critical to mRNA binding. Also prepared for testing was dinucleoside diphosphate m\text{Gpp1} (12), which differs from the normal mRNA cap both in the number of phosphate groups between the nucleosides and by the replacement of G by \( \text{C} \) by 1. Compound 12 was a good inhibitor of mRNA binding in the \textit{in vitro} assay, although not as good as \text{m'GDP}.

\textit{Discussion}

Previous reports from several laboratories have shown that 5'-nucleotides of 7-methylguanosine, which resemble the 5'-terminal m\text{GppN} cap structure of most eukaryotic cellular and viral mRNA's, inhibit initiation of translation, presumably by competing for component(s) required for stable binding of mRNA to ribosomal subunits. In an effort to determine which parts of the 5'-cap structure influence mRNA function, \text{m'GDP} and other cap-related compounds were tested for their effect on the binding to wheat germ ribosomes of \( [\text{3H}] \) methyl-labeled reovirus mRNA. Conditions were optimized such that >50% and usually 70% to 80% of the input mRNA containing \( [\text{3H}] \) methyl-7-m\text{GppGm-C} . . . became associated with 80 S ribosomes and the inhibitory effects of several 7-substituted GDP's were compared. The results indicated that the nature of the 7-substituent was relatively unimportant (since, \textit{e.g.} \text{m'GDP}, \text{m'GDP}, and \text{m'GDP} were all equally as inhibitory)
but that the associated positive charge on the imidazole moiety was necessary for activity. The latter conclusion was based on several observations. First, treatment of m'GDP, eGDP, and nGDP with ammonium hydroxide afforded opening of the imidazole ring (19, 23) and concomitant loss of inhibitory activity (Fig. 1). Second, the (reversible) reduction of m'GDP to 8-hydro-m'GDP which removes the positive charge from the imidazole moiety without affecting the 7-substituent largely eliminated inhibitory activity; activity was fully restored upon reoxidation of 8-hydro-m'GDP to m'GDP (Fig. 2). 

While the ability of m'GDP to effect inhibition of mRNA binding clearly depended on the presence of a positive charge on the imidazole ring, there was no obvious correlation between expected "charge density" on the imidazole rings in the tested compounds (23) and the extent of inhibition of mRNA binding effected by those species. Since all of the 7-alkylated nucleosides with good inhibitory activity also have 5'-phosphate groups, it seemed reasonable to suggest that the function of the positively charged imidazole moiety might be in maintenance of the cap analogs in a single, conformationally preferred form involving electrostatic interaction between the phosphate and imidazole moieties. A required electrostatic interaction would be consistent with the observed lack of inhibitory activity of 8-hydro-m'GDP and of other cap analogs after ring opening of the imidazole moiety; this type of interaction was also suggested by the differences in chemical reactivity of m'G and the corresponding ribonucleoside phosphates. Hendler et al. (15) e.g. found that the stability of the imidazole ring to hydrolysis at pH 8.9 was increased in the presence of the phosphate groups. During the preparation of the m'GDP analogs used in this study, we also noted a remarkable difference in reactivity for 7-alkylated guanosine 5'-monophosphates, as compared with the respective unalkylated species. For example, under conditions that resulted in the conversion of GMP → GDP in 87% yield (25), attempted activation of the phosphate group in m'GMP with 1,1'-carbon- 
yldiimidazole, followed by treatment with inorganic phosphate, gave none of the desired product. Even under much more vigorous conditions, involving treatment with carbon- 
yldiimidazole at 50° for 48 h and subsequent treatment with inorganic phosphate for an additional 48 h, the yield of m'GDP was only 10%. Similarly, while reaction of inosine 5'-monophosphate with the phosphorimidazolidate of phosphoric acid gave the respective 5'-diphosphate in 75% yield (25), analogous treatment of m'GMP and m'IMP gave none of the desired diphosphates. The nucleophilic character of the phosphate oxygens is apparently greatly diminished by interaction with the positively charged imidazole moiety. An additional line of evidence suggesting the possible conformational rigidity of the cap analogs due to an "electrostatic interaction between the positively charged N-7 and the negatively charged phosphate groups" has been reported recently by Hickey et al. (14), who studied the conformation of several cap analogs by nmr spectroscopy. 

On the basis of the accumulated evidence, it seemed reasonable to suggest that the function of the positively charged imidazole moiety might be in maintenance of the cap analogs in a single, conformationally preferred form. To test this hypothesis, we prepared 7-carboxymethylguanosine 5'-diphosphate (4) in the belief that through electrostatic interaction with the positively charged imidazole moiety the carboxyl group might disrupt the preferred conformation normally associated with 7-substituted guanosines. In fact, cmGDP was substantially less active than compounds 1 to 3, consistent with the interpretation that the carboxyl group did interfere with an otherwise preferred conformational arrangement. Also prepared for testing as an inhibitor of mRNA binding was m'GMP (5). As shown in Fig. 1, the additional 8-substituent also diminished the biochemical activity of m'GDP, possibly by steric or electronic destabilization of a requisite conformational isomer.

Another part of m'GDP that is functionally important is the 2-amino group. Its removal diminished activity, while formal replacement by a keto group essentially eliminated any inhibitory effect (Fig. 3). The requirement for a 2-amino group is consistent with the results of Hickey et al. (14) who observed that m'Fp was a relatively weak inhibitor of the translation of tobacco mosaic virus RNA, HeLa cell RNA, and globin mRNA in wheat germ extract. Similarly, the m'GpppN-pyrophosphatase activity recently purified from HeLa cells is inhibited by m'G but not by m'(26).

Loss of inhibitory activity was also observed when m'GDP was additionally substituted on N-1 (m'GDP) or when O6 was replaced by Cl (6-Cl-m'GDP) (Fig. 3). These results can be explained in terms of the ability of m'GDP (and presumably of the m'G moiety in the mRNA cap) to utilize the N-1 and N6 atoms for hydrogen bonding which may serve to stabilize interaction with a cellular component(s) (such as an initiation factor) or perhaps even further stabilize the putative inhibitory conformation of m'Gpp . . . itself.

In contrast to the strong dependence of inhibitory activity on the structural features discussed above, formal replacement of the ribose moiety of m'GDP by 2'-deoxyribose did not significantly affect its ability to inhibit mRNA binding (Fig. 4). This suggests that the 2',3'-Cix-diol in the 5'-m7G moiety of capped mRNA is not essential for function. Previous experiments with reovirus and vesicular stomatitis virus mRNA's indicated that binding to wheat germ or rabbit reticuloocyte ribosomes was decreased but not eliminated by periodate oxidation of the C6-diol to the dicarbonyl (26, 27). In another study, however, translation of liver cell mRNA in wheat germ protein synthesizing extract was abolished by periodate oxidation (28).

The present findings obtained with m'GDP altered at different positions were interpreted on the assumption that m'GDP acts as a cap analog, although we have not tested the effect on ribosome binding of modifying the m'G in caps of viral mRNA (see "Note Added in Proof"). Attempts to obtain mRNA with
5'-terminal 8-hydro-m7G under a variety of experimental conditions were unsuccessful because of rapid reoxidation. It should be noted that translating extracts prepared from different types of cells including wheat germ, mouse ascites, and rabbit reticulocytes vary in the extent of their dependence on capped mRNA function and in their sensitivity to cap analogs (20, 27, 29). These variations are partly due to the in vitro conditions employed for study, especially differences in K+ concentration (30). They may also reflect variations in the stability of initiation factors and other cellular components required for protein synthesis. Among them may be proteins that interact with 5'-termini of mRNA during initiation (8-10). For example, mRNA translation in reticulocyte lysates would be relatively resistant to inhibition by m7GDP if they contained high levels of protein factors that associated with caps to mediate stable binding of mRNA to ribosomes. The same collection of eukaryotic initiation factors is needed for translation of capped (globin) and uncapped (encephalomyocarditis virus) RNA (31). However, some of the factors, including EIF-3, consist of multiple high molecular weight components and it is possible that different subunits of a complex factor interact with 5'-ends of capped and uncapped mRNA. In this situation, cap analogs such as m7GDP could indirectly inhibit the function of uncapped mRNA by binding to one subunit of an initiation factor and affecting the activity of another subunit by changing the overall structure of the complex. As a result, cap analogs might inhibit translation of uncapped mRNA, as observed recently for vesicular stomatitis virus mRNA (32) and satellite tobacco necrosis virus RNA. It will be of interest for elucidating the function of caps to determine whether initiation factor(s) interact with the 5'-ends of eukaryotic mRNA's.

Acknowledgment—We thank Mr. Andrew Buchman for assisting with the syntheses of the cap analogs.

Note Added in Proof—Recent studies by Y. Furuichi and A. J. Shatkin indicate that ethylated reovirus mRNA's containing either 5'-e7GpppGe . . . or 5'-e7GpppG . . . bound to wheat germ ribosomes to the same extent as methylated mRNA and were translated equally as well.

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The effect of "cap" analogs on reovirus mRNA binding to wheat germ ribosomes. Evidence for enhancement of ribosomal binding via a preferred cap conformation.

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