Inhibition of Bacteriophage-Q β Ribonucleic Acid Polymerase by Guanosine Triphosphate Analogues

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(Received 8 February 1979)

Sixteen compounds related to GTP were evaluated as inhibitors of bacteriophage-Q β poly(C)-dependent poly(G) polymerase. Non-phosphorylated compounds, including guanine, guanosine and deoxyguanosine, were inactive. Phosphorylated compounds gave significant inhibition at millimolar concentrations. For nucleotides the feature important for inhibition was the 5'-phosphate chain. Four triphosphates, XTP, ITP, 7-methyl-GTP and 2'-O-methyl-GTP, gave 50% inhibition of both the poly(C)- and poly(U₂C)-dependent reactions at concentrations from 0.1 to 5 mM. XTP was 10-fold more potent an inhibitor of the reaction with poly(U₂C) as template. None of these four compounds was able to substitute for GTP as substrate to a significant extent. The most active compound, 2'-O-methyl-GTP, was a competitive inhibitor ($K_i = 0.4$ mM) of GTP in the poly(C)-dependent reaction.

Infection of *Escherichia coli* by the bacteriophage Q β results in the appearance of a new nucleotide polymerase which selectively replicates viral RNA (Haruna *et al.*, 1963). Bacteriophage-Q β replicase (RNA-dependent RNA polymerase) consists of four non-identical subunits, only one of which is coded for by viral RNA (Landers *et al.*, 1974). The other three host-derived subunits normally play a role in protein synthesis: they are elongation factors EF-Tu and EF-Ts (Blumenthal *et al.*, 1972) and a 30S-ribosomal-subunit protein (Wahba *et al.*, 1974; Inouye *et al.*, 1974). This system has been well studied from the standpoint of template selection and regulation; for review see Weissmann (1974).

Several results suggest a special role for GTP in the bacteriophage-Q β replicase system. Thus GTP is the only ribonucleotide with which normal RNA synthesis is initiated (Hori *et al.*, 1967; Spiegelman *et al.*, 1968; Banerjee *et al.*, 1969). Hori (1974) found that there are two binding sites for GTP on bacteriophage-Q β replicase: one site is shared with the other three nucleoside triphosphates and the other is a unique GTP-binding site where binding is inhibited by the thiol-blocking reagent, *p*-chloromercuribenzoate. Brooks & Andersen (1978) provided kinetic evidence for two GTP-binding sites and showed that this substrate is able to form relatively stable complexes with the enzyme. Such GTP-enzyme complexes modulated inhibition by ortho- and pyro-phosphate. Guanosine 3'-diphosphate 5'-diphosphate (ppGpp) inhibited initiation of synthesis, but not chain elongation, by bacteriophage-Q β replicase with poly(C) as template (Landers *et al.*, 1974). Silverman (1973) found that GTP

was necessary to form a salt-resistant complex between bacteriophage-Q β replicase and its template, either RNA or poly(C).

The present work was undertaken to study further the role of GTP analogues as inhibitors of bacteriophage-Q β replicase. Specifically, the questions asked were: (1) what features of a GTP analogue influence its ability to inhibit the enzyme, and (2) what is the nature of the inhibition by active compounds? Two reactions of bacteriophage-Q β replicase with the synthetic templates poly(C) and poly(U₂C) were chosen, since they are well characterized (Hori, 1973; Mitsunari & Hori, 1973; Brooks & Andersen, 1978) and do not require extra protein factors (Franze de Fernandez *et al.*, 1972).

Materials and Methods

Enzyme preparation

Escherichia coli Q13 was grown in 15-litre fermenter batches and infected with a replicase-over-producing mutant (am 12; Horiuchi & Matsushashi, 1970) of bacteriophage Q β as described by Kamen (1972) and Brooks & Andersen (1978). Poly(C)-dependent poly(G) polymerase was purified by the method of Eoyang & August (1971) with the omission of hydroxyapatite chromatography. The final polymerase showed four bands on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. The specific activity in the poly(C)-dependent assay with Mn²⁺ at 32°C was over 3000 units/mg of protein; one unit is the incorporation of 1 nmol of GMP in 20 min. Other details of the preparation and storage

of the enzyme were given by Brooks & Andersen (1978).

Enzyme assay

Both poly(C) and poly(U₂C) were used as templates to direct incorporation of GMP and of GMP and AMP into acid-insoluble products. Standard reactions with poly(C) as template were run for 20 min at 32°C in final volumes of 0.1 ml, containing 0.1 M-Tris/HCl (pH 7.6 at 22°C), 4 mM-2-mercaptoethanol, 0.4 mM-[8-¹⁴C]GTP (approx. 1 Ci/mol), 0.5 mg of bovine serum albumin/ml, 1 mM-phosphoenolpyruvate, 18.2 µg of pyruvate kinase/ml, 2 A₂₇₀ units of poly(C)/ml, 10 mM-MnCl₂ and 18–30 µg of bacteriophage-Qβ RNA polymerase/ml. Final (NH₄)₂SO₄ concentration (from the enzyme storage buffer) was 25 mM. With poly(U₂C) as template (150 µg/ml), 1 mM-ATP and 1 mM-MnCl₂ were used. Reactions with labelled ATP used [2,8-³H]ATP with a specific radioactivity of approx. 10 Ci/mol.

Reactions with [8-¹⁴C]GTP as the labelled substrate were stopped by pipetting portions on to 2.5 cm-diam. Whatman 3MM filters as described by Brooks & Andersen (1978). Reactions containing ³H-labelled substrate were stopped by the addition of 1 ml of cold 10% (w/v) trichloroacetic acid/4 mM-sodium pyrophosphate. The tubes were set on ice for at least 10 min before the precipitate was collected on 0.45 µm Millipore filters which had been soaked previously in 5% trichloroacetic acid/2 mM-sodium pyrophosphate. Filters were washed with 3 × 6 ml of the same solution. Both Millipore and Whatman discs were dried and counted for radioactivity in a toluene-based scintillation fluid (Brooks & Andersen, 1978).

Compounds were tested as inhibitors of bacteriophage-Qβ replicase by preincubating them with enzyme for 60 min at 0°C in 0.05 ml. A mixture of the remaining components in 0.05 ml was added to start the reaction.

Replication-fidelity, substrate-capability and substrate-protection experiments

The effect of selected inhibitors on the fidelity of replication by bacteriophage-Qβ RNA polymerase was assessed with poly(U₂C) as template in reactions containing both [8-¹⁴C]GTP and [2,8-³H]ATP. The ratio of AMP to GMP incorporation was taken as a measure of the fidelity of replication. To determine if a GTP analogue could serve as a substrate, the poly(U₂C) reaction was run with [³H]ATP alone. The ability of an analogue to promote AMP incorporation in the absence of GTP indicates that it can serve as a substrate (Kapuler & Spiegelman, 1970). To assess whether GTP could protect bacterio-

phage-Qβ polymerase from inhibition by 2'-O-methyl-GTP in the poly(C)-dependent assay, the enzyme was preincubated for 5 min at 0°C with various concentrations of GTP before addition of the inhibitor and the rest of the reaction mixture. Reactions were then run for 5 min at 32°C.

Calculations

Radioactivity from samples containing both ³H and ¹⁴C was analysed by the discriminator-ratio method (Okita *et al.*, 1967). I₅₀ values, i.e. compound concentrations giving 50% inhibition of GMP or AMP incorporation, were determined from linear regression of percentage inhibition on log(concentration). Confidence limits (95%) were calculated for single determinations as described by Snedecor & Cochran (1967). Linear regression lines were fitted to the data of the double-reciprocal plot (Fig. 2) and K_i values were calculated from the equations of the lines.

Reagents

[2,8-³H]ATP (tetrasodium salt) (25.3 Ci/mmol) was obtained from New England Nuclear (Boston, MA, U.S.A.). Compounds tested as inhibitors (Table 1) were obtained commercially: guanine, guanosine and deoxyguanosine from Nutritional Biochemicals (Cleveland, OH, U.S.A.), N²-acetyl-guanine (2-acetamido-6-hydroxypurine hemihydrate) and 8-bromoguanosine 2',3',5'-triacetate from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.), disodium GMP, disodium dGMP, sodium GDP, tetrasodium guanosine 5'-[β,γ-methylene]triphosphate, disodium 2'-O-methyl-GTP, and disodium 7-methyl-GTP from P-L Biochemicals (Milwaukee, WI, U.S.A.), dilithium 8-azaguanosine 5'-phosphate and the 4-morpholine-*NN'*-dicyclohexylcarboxamide salt of guanosine 5'-monophosphomorpholidate from Sigma Chemical Co. (St. Louis, MO, U.S.A.), trisodium 8-bromo-GTP from Terra-Marine Bio-research (La Jolla, CA, U.S.A.), and sodium XTP and trisodium ITP from United States Biochemical Corp. (Cleveland, OH, U.S.A.). Structures of the uncommon compounds are shown in Fig. 1. All other reagents were the same as reported by Brooks & Andersen (1978).

Results

Inhibition survey

In a preliminary survey, GTP-related compounds were assayed as inhibitors of the poly(C)-directed reaction at concentrations up to 20 mM. Non-phosphorylated analogues, including the natural base and nucleoside, were inactive at 0.1 or 0.2 mM

Fig. 1. Structures of selected compounds evaluated as inhibitors of bacteriophage-Q β RNA polymerase (a) *N*²-Acetylguanosine, (b) 8-bromoguanosine 2',3',5'-triacetate, (c) 8-aza-GMP, (d) guanosine 5'-monophosphomorpholidate, (e) 2'-O-methyl-GTP and (f) 7-methyl-GTP.

(Table 1). Insolubility precluded testing at higher concentrations.

Inhibition was found with mono- and diphosphorylated compounds. At 20mM, GDP gave 98% inhibition, whereas GMP and dGMP were practically inactive (Table 1). Guanosine 5'-monophosphomorpholidate is an active compound with a bulky hydrophobic substituent. Although its phosphate area is blocked by a bulky group, guanosine 5'-monophosphomorpholidate inhibited by 93% at 20mM (Table 1). 8-Aza-GMP stimulated

GMP incorporation by 42% at 10mM (Table 1). Stimulation from 19 to 40% was observed between 0.5 and 5mM, but no concentration-response relationship was evident (results not shown).

Triphosphate analogues of GTP gave significant inhibition in the millimolar concentration range (Table 1). Five of the six analogues in this group have unmodified phosphate ester chains, whereas guanosine 5'-[β,γ -methylene]triphosphate has a stabilized interphosphate linkage. In separate experiments it was determined that the counterions of the tested

Table 1. *Inhibition of bacteriophage-Q β poly(C)-dependent polymerase by GTP-related compounds*

Inhibition of GMP incorporation was determined in reactions run as described in the Materials and Methods section. Results from multiple determinations are given as means $\pm$ s.e.m. with the numbers of experiments in parentheses.

Compound	Concentration (mM)	Inhibition (%)
Non-phosphorylated		
Guanine	0.1	5
Guanosine	0.2	5
Deoxyguanosine	0.2	-8
N ² -Acetylguanosine	0.2	-20
8-Bromoguanosine	0.2	-12
2',3',5'-triacetate		
Mono- and di-phosphates		
GMP	20	15
dGMP	20	4
8-Aza-GMP	10	-42
Guanosine 5'-mono-phosphomorpholidate	20	93*
GDP	20	98
Triphosphates		
8-Bromo-GTP	0.8	85 $\pm$ 0.5 (2)
Guanosine 5'-[β , γ -methylene]-triphosphate	1.0	45
2'-O-Methyl-GTP	0.5	89 $\pm$ 0.1 (3)
7-Methyl-GTP	0.5	22 $\pm$ 1.0 (3)
XTP	3.5	34 $\pm$ 0.8 (2)
ITP	3.5	70 $\pm$ 0.5 (3)

* Reaction contained 4 mM-MnCl₂ instead of the usual 10 mM.

compounds, namely Na⁺, Li⁺ and 4-morpholine-NN'-dicyclohexylcarboxamide, did not account for the observed inhibition or stimulation (results not shown).

All the triphosphates and the active diphosphates were reassayed to determine I₅₀ values. 2'-O-Methyl-GTP was the most active, with an I₅₀ value of 95 μ M (Table 2).

Effects on replication fidelity

Since the poly(U₂C)-dependent reaction of bacteriophage-Q β replicase promotes incorporation of both AMP and GMP (Hori *et al.*, 1967), it is possible to determine an inhibitor's effect on replication fidelity

Table 2. *I₅₀ values for inhibitors of bacteriophage-Q β poly(C)-dependent RNA polymerase*

Values in parentheses are 95% confidence limits on the I₅₀ value. Missing confidence limits indicate that no calculation was possible because of lack of sufficient activity or scatter of the data.

Compound	I ₅₀ value (mM)
2'-O-Methyl-GTP	0.095 (0.07-0.13)
GDP	0.2
8-Bromo-GTP	0.39 (0.23-0.64)
Guanosine 5'-[β , γ -methylene]-triphosphate	1.2 (0.72-2.0)
7-Methyl-GTP	1.6 (0.87-2.8)
ITP	1.7 (0.79-3.0)
XTP	4.4 (1.4-13)
Guanosine 5'-monophosphomorpholidate	9.5

Table 3. *Effect of selected nucleotides on the ratio of AMP incorporated into product by bacteriophage-Q β poly(U₂C)-dependent polymerase*

Reactions (0.1 ml) were run for 20 min as described in the Materials and Methods section with both [2,8-³H]ATP and [8-¹⁴C]GTP. Protein concentration was 29.5 μ g/ml. Mean values of duplicate determinations were used in the calculations. Control reactions incorporated 1594 $\pm$ 19 (s.e.m., *n* = 4) pmol of AMP and 1723 $\pm$ 23 pmol of GMP in 20 min.

Compound	Concentration (mM)	Relative incorporation		Molar ratio AMP/GMP
		AMP	GMP	
None		1.00	1.00	0.925
2'-O-Methyl-GTP	0.75	0.407	0.362	1.04
	0.25	0.660	0.603	1.01
	0.075	0.881	0.815	1.00
7-Methyl-GTP	12.7	0.500	0.301	1.52
	2.54	0.785	0.643	1.13
	1.27	0.903	0.806	1.04
ITP	8.80	0.157	0.077	1.88
	4.84	0.211	0.115	1.69
	1.76	0.684	0.534	1.28
XTP	50.0	0	0	—
	11.4	0.017	0.020	0.80
	3.75	0.228	0.216	0.98

by measuring the molar ratio of AMP to GMP in product synthesized with and without the inhibitor. Reactions were run with both [2,8- 3 H]ATP and [8- 14 C]GTP. In the absence of any inhibitor the AMP/GMP ratio was 0.925 (Table 3). Neither XTP nor 2'-O-methyl-GTP affected the AMP/GMP ratio at concentrations giving less than 80% inhibition (Table 3). On the other hand, both ITP and 7-methyl-GTP markedly increased the ratio at concentrations giving 30–70% inhibition (Table 3). No obvious structural feature accounts for this division among these four compounds. The effect on replication fidelity increased with increasing concentration, and there is no evidence that this effect could be dissociated from inhibition of overall incorporation.

I_{50} values calculated from the results of the above experiment with poly(U_2C) as template are shown in Table 4. Values for 2'-O-methyl-GTP, ITP and 7-methyl-GTP were comparable with those found in the poly(C)-directed reaction (Table 2), whereas XTP was a 10-fold more potent inhibitor of the poly(U_2C)-dependent reaction (I_{50} = 0.3 mm; Table 4) than of the poly(C)-dependent system (I_{50} = 4.4 mm; Table 2).

Substrate capability of selected inhibitors

The poly(U_2C)-dependent reaction was used to test the ability of XTP, ITP, 2'-O-methyl-GTP and 7-methyl-GTP to serve as substrates in place of GTP. In the absence of GTP, 12 pmol of AMP was incorporated into acid-insoluble product (Table 5). An identical reaction run with 1 mm-GTP incorporated 812 pmol of AMP. The ability of a compound to substitute for GTP as a substrate in this reaction is indicated by incorporation of labelled AMP in the absence of GTP (Kapuler & Spiegelman, 1970). Only 7-methyl-GTP (1 mm) showed any activity as a substrate by this measure and gave 12% of the GTP-supported incorporation. XTP, ITP and 2'-O-methyl-GTP were completely inactive as substrates at a concentration of 1 mm (Table 5). Thus the effects

on replication fidelity seen with ITP and 7-methyl-GTP are not explained trivially by their substituting for GTP. The product formed in poly(U_2C)-dependent reactions inhibited by ITP or 7-methyl-GTP contained only AMP and GMP in an abnormal ratio.

Substrate protection

GTP was able to protect bacteriophage-Q β replicase from inhibition by 2'-O-methyl-GTP, the most potent inhibitor reported here. As GTP concentration increased from 0.51 to 2.01 mm, inhibition of the poly(C)-dependent reaction by 0.1 mm-2'-O-methyl-GTP decreased from 47 to 18% (Table 6). Similar decreases were seen at lower

Table 5. *Substrate capabilities of selected GTP analogues in the bacteriophage-Q β poly(U_2C)-dependent polymerase reaction*

Reactions (0.1 ml) were run for 20 min with [2,8- 3 H]-ATP as described in the Materials and Methods section. Protein concentration was 29.5 μ g/ml. Concentration of GTP and analogues was 1 mm. Incorporation is reported as the mean of two (GTP) or three (the rest) determinations, $\pm$ s.e.m. Percentage incorporation is based on the reaction run with GTP taken as 100%. The AMP incorporation with no addition (12 pmol) was subtracted from GTP- and analogue-supported incorporation before calculation of percentages.

Compound	AMP incorporation	
	(pmol)	(%)
None	12 $\pm$ 6.3	0
GTP	812 $\pm$ 34	100
2'-O-Methyl-GTP	6.9 $\pm$ 1.5	0
7-Methyl-GTP	104 $\pm$ 7.5	12
ITP	13 $\pm$ 8.4	0
XTP	0 $\pm$ 0	0

Table 4. *I_{50} values for XTP, ITP, 2'-O-methyl-GTP and 7-methyl-GTP for inhibition of the bacteriophage-Q β poly(U_2C)-dependent polymerase reaction*

I_{50} values were calculated as described in the Materials and Methods section from the data of Table 3. Confidence limits (95%) are given in parentheses.

Compound	I_{50} value (mm)	
	AMP incorporation	GMP incorporation
2'-O-Methyl-GTP	0.20 (0.14–0.30)	0.16 (0.11–0.22)
XTP	0.36 (0.25–0.48)	0.30 (0.09–0.59)
ITP	1.1 (0.39–2.4)	0.71 (0.20–1.6)
7-Methyl-GTP	5.0 (2.4–13)	2.0 (1.4–3.0)

Table 6. *Protection by GTP of bacteriophage-Q β poly(C)-dependent polymerase from inhibition by 2'-O-methyl-GTP*

GTP and enzyme were preincubated on ice for 5 min before the addition of the compound. Reaction mixtures were then completed and incubated at 32°C for 5 min. Protein concentration was 18 μ g/ml. Apparent K_m and K_i values were determined from the double-reciprocal plot of these data.

2'-O-Methyl-GTP concentration (mm)	Inhibition (%) at GTP concentration (mm)			Appar-ent K_m (mm)	Appar-ent K_i (mm)
	2.01	1.20	0.51		
0.1	18.4	31.1	47.3	0.56	0.041
0.075	14.4	20.9	34.4	0.71	0.042
0.05	7.4	15.5	25.5	1.10	0.030

inhibitor concentrations. The double-reciprocal plot of these data (Fig. 2) suggests competitive inhibition and a K_i of 0.4 mM. These results do not distinguish which of the two GTP-binding sites on bacteriophage-Q β replicase is involved in competitive binding of 2'-O-methyl-GTP.

Discussion

Work on the interaction of smaller molecules with bacteriophage-Q β replicase has uncovered a variety of inhibitors, both nucleotides (Kapuler & Spiegelman, 1970) and non-nucleotides (Haruna *et al.*, 1970; Nakamura *et al.*, 1971; Blumenthal & Landers, 1973). The focus of studies reported here has been on GTP-related compounds because of the special role of GTP described above. In addition, Brooks & Andersen (1978) found that GTP could change the sensitivity of bacteriophage-Q β replicase to inhibition by P_i and PP_i , giving protection at low concentrations and increasing inhibition at high concentrations. Thus the role of GTP analogues in inhibition seemed worth investigating.

None of the 16 analogues studied here was a very potent inhibitor of bacteriophage-Q β RNA polymerase. The most active compound, 2'-O-methyl-

GTP, had an I_{50} value of 0.095 mM. This may be compared with the reported K_m values for GTP of 0.016 mM in the poly(C)-dependent reaction and of 0.2 mM in the replicase reaction (Mitsunari & Hori, 1973).

The most evident feature required for inhibition of bacteriophage-Q β RNA polymerase by these compounds is phosphorylation. Without at least one phosphate moiety in the 5'-position the compound was inactive. Increased phosphorylation seemed to correlate with increased inhibition (Table 1). Retention of the hydrogen-bonding capability of GTP seemed of lesser importance in explaining inhibitory potency. Of the eight compounds whose I_{50} values were determined, two (XTP and ITP) lacked the hydrogen-bonding face of GTP and six retained it.

In comparing I_{50} values for XTP, ITP, 2'-O-methyl-GTP and 7-methyl-GTP derived from the poly(U_2C)-dependent reaction with those from the poly(C)-directed assay, it was noted that only the potency of XTP was a function of template. XTP was 10-fold more inhibitory when the heteropolymer was template (Tables 2 and 4). The differential effect of XTP is analogous to the previous finding that sensitivity of the enzyme to inhibition by P_i and PP_i varied with template (Brooks & Andersen, 1978). In general the poly(U_2C)-directed reaction was more sensitive to P_i and PP_i than the reaction with poly(C) as template. The exception was inhibition by P_i when Mn^{2+} was the cofactor: the I_{50} value for the poly(C) reaction was 150 times lower than that for the poly(U_2C) system.

The mechanism of inhibition by these GTP analogues is not uniform. Of the four compounds tested for an effect on replication fidelity in the poly(U_2C)-dependent reaction, two increased the AMP/GMP molar ratio in the product and two did not (Table 3). None of the four nucleoside triphosphates was an effective substrate, however (Table 4).

In a study of the *E. coli* DNA-dependent RNA polymerase (transcriptase), Asano *et al.* (1971) found that ITP could substitute for GTP as a substrate in a reaction with calf thymus DNA as template. When 0.32 mM-ITP replaced GTP, 26% of normal CMP incorporation and 35% of normal AMP incorporation was observed. In contrast, ITP could not replace GTP as substrate in the bacteriophage-Q β system (Table 4).

In a study of ten nucleotide analogues, Kapuler & Spiegelman (1970) compared the ability of *E. coli* transcriptase and bacteriophage-Q β replicase to use such analogues as substrates. The host enzyme was more tolerant of UTP, CTP and ATP analogues, and gave initial reaction velocities that were from 30 to 138% of normal. Bacteriophage-Q β replicase was more stringent in its requirements and gave initial velocities from 1 to 28% of normal with such analogues. 6-Methyl-CTP was a poor substrate for

Fig. 2. Double-reciprocal plot for inhibition of bacteriophage-Q β poly(C)-dependent polymerase by 2'-O-methyl-GTP

Poly(G) synthesis was measured after 5 min at 32°C in reactions with 0.51, 1.21 or 2.01 mM-GTP and (○) 0, (□) 0.05, (△) 0.75 or (●) 0.1 mM-2'-O-methyl-GTP. Bars indicate s.e.m. for three determinations.

both enzymes. Kapuler & Spiegelman (1970) found that two GTP analogues modified in the 8-position of the purine ring, 8-bromo- and 8-oxo-GTP, were not used by either enzyme as replacements for GTP. Both compounds inhibited transcriptase when added to reaction mixtures containing GTP. 8-Oxo-GTP inhibited replicase to a lesser extent than it did the host enzyme, and 8-bromo-GTP was inactive even at an analogue/GTP molar ratio of 10. In the present work 8-bromo-GTP was found to be an inhibitor even at a ratio of 0.5. The difference is probably due to the use of different metals (Mn²⁺ instead of Mg²⁺) and templates [poly(C) instead of bacteriophage-Q β RNA] in the two studies.

Results similar to those in the bacteriophage-Q β system were obtained by Kapuler *et al.* (1969), who compared the ability of mengovirus RNA-dependent RNA polymerase and *E. coli* transcriptase to use nucleotide analogues as substrates. Both enzymes accepted 5-substituted pyrimidine nucleoside triphosphates and 7-deaza-adenosine (tubercidin) triphosphate. Although only analogues with unperturbed hydrogen-bonding capabilities functioned as substrates, this alone was not a sufficient condition, since neither 8-bromo-GTP nor 8-oxo-GTP served as substrates.

In summary, selected GTP-related compounds were found to be relatively weak inhibitors of bacteriophage-Q β RNA polymerase. Activity depended primarily on phosphorylation at the 5'-position. Lack of a uniform inhibition mechanism was indicated by the ability of only two of four tested triphosphates to alter product base composition.

I thank Ms. Donna Ball, Mr. William Searfoss, Ms. Maureen Appler and Ms. Esther Shearer for able assistance in various phases of this work. Dr. Jon A. Andersen kindly reviewed the manuscript.

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