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# Inhibition of Influenza Virus Transcription by 2′-Deoxy-2′-Fluoroguanosine

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**The nucleoside analog 2′-deoxy-2′-fluoroguanosine (2′-fluorodGuo) is phosphorylated by cellular enzymes and reversibly inhibits influenza virus replication in chick embryo cells within the first 4 h of infection. RNA hybridization studies revealed that primary and secondary transcription of influenza virus RNA were blocked at a compound concentration of 10 μM, but no inhibition of cell protein synthesis was seen even at high compound concentrations (200 μM). In vitro, the triphosphate of 2′-fluorodGuo is a competitive inhibitor of influenza virus transcriptase activity from disrupted virus, with a $K_i$ of 1.0 μM. The cellular polymerases DNA polymerase α and RNA polymerase II were only weakly inhibited or were insusceptible to 2′-fluorodGTP. In kinetic studies with the influenza virus transcriptase, 2′-fluorodGTP, in the absence of GTP, blocked elongation of the virus RNA chain. Similarly, by using purified ribonucleoprotein complexes it was found that the addition of a single nucleotide of 2′-fluorodGTP to the virus RNA caused chain termination, which resulted in the blockage of further virus transcription. Furthermore, the specificity for influenza virus transcriptase was confirmed when the transcriptase from partially resistant virus was found to be 10-fold less susceptible to 2′-fluorodGTP ($K_i$ = 13.1 μM).**

The 2′-fluororibosides are potent inhibitors of influenza virus A and B strains in cell culture, in the mouse pneumonia model (16), and in the ferret model (6). All of the influenza virus strains examined were susceptible to 2′-deoxy-2′-fluoroguanosine (2′-fluorodGuo), but the level of inhibition varies with cell type and appears to be related to the levels of phosphorylation in these different cells. In human tracheal cells, growth of influenza virus A/England/40/83 (H3N2) was reduced by 4 $\log_{10}$ units, suggesting that 2′-fluorodGuo is phosphorylated very efficiently in the human target cell. Furthermore, evaluation in human respiratory epithelial explants showed that 2′-fluorodGuo was extremely potent against both influenza virus A and B strains (90% effective concentration, < 0.1 μg/ml [<0.35 μM]), and no toxicity was observed at 100 μg/ml (350 μM) (15). These results indicate that the compound may be particularly effective when it is delivered directly to the respiratory tract.

Deoxycytidine kinase is one enzyme which has been shown to be able to phosphorylate both pyrimidine and purine analogs to their 5′-monophosphates (17). Furthermore, 2′-fluorodGuo monophosphate is a substrate for GMP kinase ($K_m$, 53 μM, compared with a $K_m$ of 7 to 9 μM for GMP [11a]). Correlations between intracellular 2′-fluorodGuo triphosphate (2′-fluorodGTP) levels and influenza virus inhibition suggest that the compound may be targeting influenza virus RNA replication possibly by inhibiting the influenza virus polymerase complex.

The present study was undertaken to elucidate further the mechanism of inhibition of influenza virus replication by 2′-fluorodGuo. The triphosphate of 2′-fluorodGuo was synthesized for enzyme kinetic studies, including studies with partially resistant virus.

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## MATERIALS AND METHODS

**Virus.** Reassortant influenza virus strain X31 (E. D. Kilbourne, New York, N.Y.), which has the hemagglutinin and neuraminidase of A/Aichi/2/68 (H3N2) and the internal proteins of A/PR8/34 (H1N1), was used throughout these studies. High-titer virus stocks were prepared in the allantoic cavities of fertile 10- to 11-day-old hen's eggs. Virus was purified by differential centrifugation and then banding on 20 to 60% continuous sucrose gradients, and the virus was sedimented at 100,000 × $g$ for 1 h and stored at −70°C.

**Cells.** Primary chick embryo (CE) cells were prepared from 10-day-old fertile hen's eggs. Cells were plated out in growth medium (medium 199) containing 10% fetal calf serum, 0.1% bicarbonate, and antibiotics. For maintenance of the cells, medium 199 with 5% tryptose phosphate broth in place of calf serum was used.

**Compounds, chemicals, and isotopes.** 2′-FluorodGuo was synthesized as described previously (17). 2′-FluorodGTP and 2′-deoxy-2′-fluoro[2-$^{14}$C]guanosine (specific activity, 63 mCi/mmol) were synthesized chemically at Burroughs Wellcome, Research Triangle Park, N.C.

L-[$^{35}$S]methionine (specific activity, >600 Ci/mmol), [$^{3}$H]uridine (specific activity, 40 to 60 Ci/mmol), [5,6-$^{3}$H]UTP (specific activity, 40 to 60 Ci/mmol), and [α-$^{32}$P]CTP (specific activity, 800 μCi/mmol) were obtained from Amersham International.

**Enzymes.** Influenza virus RNA polymerase was prepared for enzyme kinetic studies by disrupting purified virus with 0.2% Triton N101 (13).

For primer initiation and elongation studies, the ribonucleoprotein (RNP) cores were purified as described previously (12). Sucrose gradient-purified virus was disrupted by incubation at 30°C for 10 min in RNP buffer (100 mM Tris-HCl [pH 8.0], 100 mM KCl, 5 mM $MgCl_2$, 1.5 mM dithiothreitol [DTT], 5% glycerol, 1.5% Triton N101, 1% lysolecithin). The disrupted virus was fractionated by centrifugation at 40,000 rpm on a 30 to 70% (wt/vol) linear glycerol gradient buffered in 50 mM Tris-HCl (pH 7.8)–100 mM NaCl–5 mM $MgCl_2$–1.5 mM DTT in a SW40 rotor for 5 h at 4°C. Fractions containing RNP complexes, determined by analysis by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (7.5%), were stored in 50% glycerol at −20°C.

Human H9 cell DNA polymerase α was extracted from H9 cells as described previously (18), and RNA polymerase II was prepared as a partially purified extract from rat liver nuclei (14).

**Compound time-of-addition and time-of-removal studies with X31-infected CE cells.** Cells (1.5 × $10^6$ cells per culture) were infected with influenza virus X31 at a multiplicity of infection of 10 PFU per cell for 1 h at room temperature. Cells were washed with phosphate-buffered saline (PBS), and maintenance medium with or without 2′-fluorodGuo (20 μM) was added. Compound was added to the maintenance medium or was removed, the cells were washed, and fresh medium was added at hourly intervals during virus growth (10 h) and at 16 and 24 h after infection. In these studies maintenance medium without the addition of trypsin was used to ensure only one cycle of virus replication, since the hemagglutinin

from progeny virus would be uncleaved. All cultures were frozen at 24 h to allow sufficient time for virus replication after removal of the compound. Virus yields were subsequently titrated by determination of hemagglutination activity.

**Labelling of virus-infected cells with [$^3$H]uridine or [$^{35}$S]methionine.** Cell cultures ($5 \times 10^7$ cells) were infected with X31 virus at a multiplicity of infection of 50 to 100 PFU per cell for 30 min at 20°C. Excess virus was removed by washing with PBS, and the cells were incubated at 37°C in medium containing Hanks balanced salts (pH 7.0). Cells were labelled either between 2 and 4 h after infection with [$^3$H]uridine (200 μCi per culture) or at 6 h after infection with a 15-min pulse of [$^{35}$S]methionine (40 μCi per culture). To examine primary RNA transcription, cells were incubated in medium containing 100 μg of cycloheximide per ml for 60 min before infection, and to examine primary and secondary transcription, cells were incubated in the absence of cycloheximide. Labelled RNA was extracted as described previously (5), and labelled protein cell extracts were prepared as described previously (8).

Labelled virus was prepared as described above except that the cells were incubated for 24 h in the presence of the appropriate label. Culture medium was clarified by centrifugation at 1,000 × *g* for 15 min at 4°C, and virus was sedimented at 100,000 × *g* for 1 h.

**RNA extraction and hybridization studies.** Purified virus was treated with proteinase K (1 h at 37°C) before phenol extraction of virus RNA. Virus-infected, [$^3$H]uridine-labelled cells were solubilized with 0.5% SDS in 0.01 M sodium acetate buffer (pH 5.0), and RNA extracts were prepared. The RNA extracts were mixed with an excess of virus RNA (20 μg/ml per reaction) in 0.01 M Tris-HCl (pH 7.5)–0.01 M NaCl–0.1 M EDTA and were denatured in 9 volumes of dimethyl sulfoxide (DMSO) at 45°C for 30 min. Hybridization conditions were 0.01 M Tris-HCl (pH 7.5), 0.03 M NaCl, and 0.0015 M EDTA in 63% DMSO for 20 h at 37°C. After hybridization, the samples were treated with S1 nuclease before running the double-stranded RNA products on 7.5% polyacrylamide gels (90 mM Tris-borate buffer [pH 8.3]) for 40 h at 50 V as described previously (5). The gels were treated with Enlightening (New England Nuclear), dried, and exposed to Kodak X-Omat AR5 film.

**Polyacrylamide gel electrophoresis of [$^{35}$S]methionine-labelled proteins.** Protein extracts were run on 7.5 to 15% gradient gels at 50 V for 18 h on the basis of the methods of Laemmli (8).

**Enzyme assays.** The polymerase activity of detergent-disrupted influenza virus was assayed as described previously (13) by using 20 μg of disrupted virus protein per assay in 50 mM Tris-HCl buffer (pH 7.8), 5 mM $MgCl_2$, 10 mM NaCl, 100 mM KCl, 1 mM DTT, 0.4 mM dinucleotide ApG or 25 μg of globin RNA per ml, 0.5 mM CTP, 0.5 mM GTP, 1 mM ATP, 0.045 mM UTP, and 0.005 mM [$^3$H]UTP at 1,900 dpm/pmol.

DNA polymerase α was assayed as described previously (11) by using 2.5 U of enzyme per ml in 60 mM Tris-HCl buffer (pH 7.4), 5 mM $MgCl_2$, 1 mM DDT, 0.2 mg of activated DNA per ml, 0.5 mg of bovine serum albumin per ml, 0.1 mM (each) dGTP, dCTP, and TTP, and 0.006 mM [$^3$H]dATP at 3,800 dpm/pmol. RNA polymerase II was assayed as described previously (7), with enzyme at 456 μg/ml (specific activity, 78.7 pmol/min/mg), in 50 mM Tris-HCl buffer (pH 7.9), 2 mM $MnCl_2$, 1 mM DTT, 60 mM $(NH_4)_2SO_4$, 10 mM $MgCl_2$, 200 μg of calf thymus DNA per ml, 0.5 mM (each) ATP, GTP, and CTP, 0.1 mM UTP, and 0.002 mM [$^3$H]UTP at 435 dpm/pmol.

The reactions were stopped with 10% trichloroacetic acid (TCA), precipitates were collected onto GFC filters and washed with 10% TCA, and radioactivity was determined by liquid scintillation counting.

A synthetic capped mRNA described by Chung et al. (2) and Hagen et al. (4) was used as a primer for studies on the inhibition of initiation and elongation. RNA transcripts were prepared from *Sma*I-digested pGEM-7Zf(+) DNA (Promega Corp) by in vitro transcription with bacteriophage SP6 in the presence of m$^7$GpppG. The capped 67-nucleotide transcript was run on 20% polyacrylamide–7 M urea gels and was identified by UV shadowing. Gel slices were soaked overnight at 4°C in 0.5 M ammonium acetate–1 mM EDTA–0.1% SDS. The eluate was spun through SpinX filters (Costar) and was ethanol precipitated. For primer initiation and elongation studies, 3 μg of purified RNP cores was incubated with 5 μCi of [α-$^{32}$P]CTP and 0.1 μg of the capped 67-nucleotide transcript in transcription buffer (50 mM Tris-HCl [pH 7.8], 50 mM NaCl, 5 mM $MgCl_2$, 2 mM DTT) to which nucleoside triphosphates and various concentrations of 2′-fluorodGTP were or were not added. After incubation for 1 h at 31°C, the reaction products were analyzed on 20% urea-polyacrylamide gels.

## RESULTS

**Studies to determine the time of action of 2′-fluorodGuo on the virus growth cycle.** In CE cells infected at a high multiplicity of infection (>10), X31 reached a peak titer within 7 to 8 h of infection (Fig. 1). Under these conditions 2′-fluorodGuo at a 20-μM concentration produced a 2-$\log_{10}$-unit reduction in virus growth. From the time-of-addition study, 2′-fluorodGuo was shown to act early in the infection (within the first 4 h), after which the compound was ineffective. Interestingly, the time-of-removal studies revealed that the inhibitory activity is

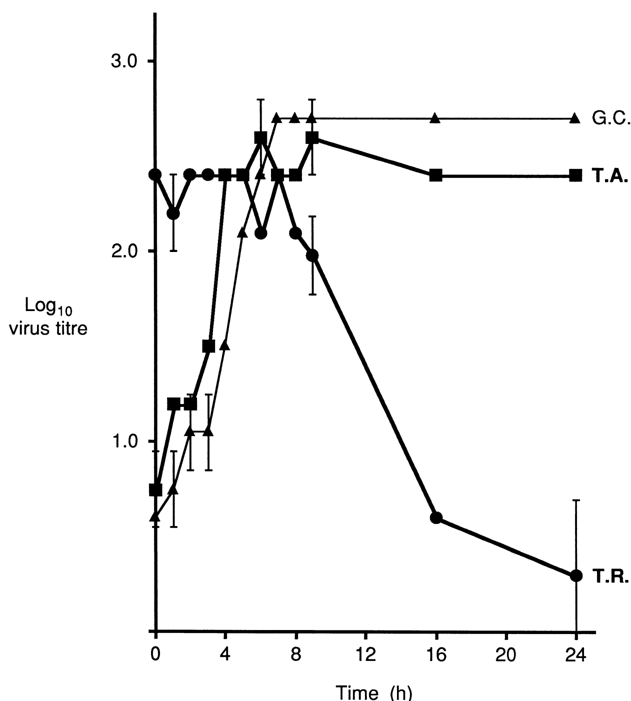


FIG. 1. Effect of time of addition (T.A.; ■) and time of removal (T.R.; ●) of 2′-fluorodGuo (20 μM) on influenza virus X31 replication in CE cells after infection with virus at 10 PFU per cell. G.C. (▲), control growth curve. Virus titers were determined by hemagglutination in duplicate at the indicated times for the growth curve and at 24 h after infection for the time-of-addition and time-of-removal studies.

completely reversible, provided that sufficient time is allowed for virus to grow after removal of the compound. The results from the time-of-addition study presented above are consistent with previous studies which suggested that the compound may act, after phosphorylation to the triphosphate form, on virus RNA synthesis. Further studies were therefore carried out to look directly at the effects of 2′-fluorodGuo on RNA and protein syntheses.

**Effect of 2′-fluorodGuo on virus RNA synthesis in CE cells.** In the presence of 2′-fluorodGuo at either 10 or 100 μM, primary and secondary transcriptions were inhibited (Fig. 2). However, the effects on secondary transcription may just be a consequence of the inhibition of primary transcription. The inhibition at 10 μM was not complete, with faint bands present in the secondary transcription tract. These results indicate that 2′-fluorodGuo inhibits virus RNA synthesis in influenza virus-infected cells and thus prevents replication of the virus.

**Effects of 2′-fluorodGuo on virus and cellular protein synthesis.** The effect of 2′-fluorodGuo on protein synthesis in infected and uninfected CE cells was determined by polyacrylamide gel electrophoresis of extracts of cultures labelled with [$^{35}$S]methionine. From the autoradiograph (Fig. 3) it may be seen that 2′-fluorodGuo from a concentration of 200 μM down to a concentration of 25 μM completely blocked virus protein synthesis and the virus-induced switch off of cellular protein synthesis. However, in uninfected cultures cellular protein synthesis even in the presence of the compound at a concentration of 200 μM appeared to be unaffected.

**Effects of 2′-fluorodGTP on virus and cell polymerases.** Initial comparative studies with influenza virus X31 RNA polymerase, human H9 cell DNA polymerase α, and rat liver RNA polymerase II were made at saturating nucleotide levels, and

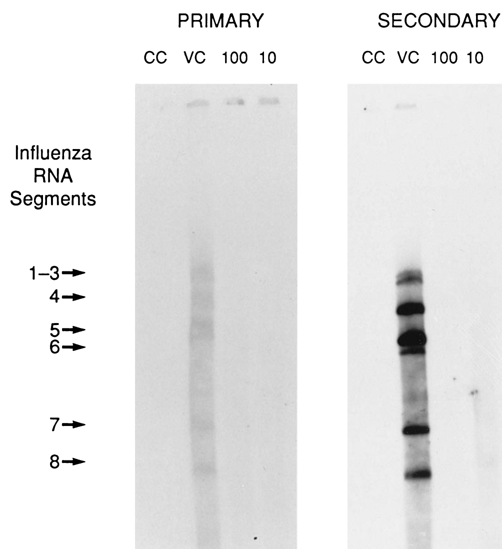


FIG. 2. Effect of 2′-fluorodGuo on primary (with cycloheximide) and secondary (without cycloheximide) transcription of virus RNA in influenza virus X31-infected CE cells. Infected [$^3$H]uridine-labelled cell extracts hybridized with virus RNA (20 μg per reaction) were run on 7.5% polyacrylamide gels as described in Materials and Methods. VC, virus control; 100, 100 μM 2′-fluorodGuo; 10, 10 μM 2′-fluorodGuo; CC, cell control.

50% inhibitory concentrations ($IC_{50}$s) were determined. For influenza virus RNA polymerase, comparisons were made with the two primers globin mRNA and ApG. Rat liver RNA polymerase II was not inhibited by 2′-fluorodGTP even at the highest concentration tested (850 μM), whereas DNA polymerase α was inhibited somewhat at high doses. In contrast, the influenza virus RNA polymerase was inhibited significantly ($IC_{50}$s, 93.2 ± 25 μM [$n$ = 11] with the dinucleotide primer ApG and 96 ± 48 μM [$n$ = 2] with the globin RNA primer). Further analysis of the inhibition of influenza virus RNA polymerase with ApG as the primer and variable nucleotide sub-

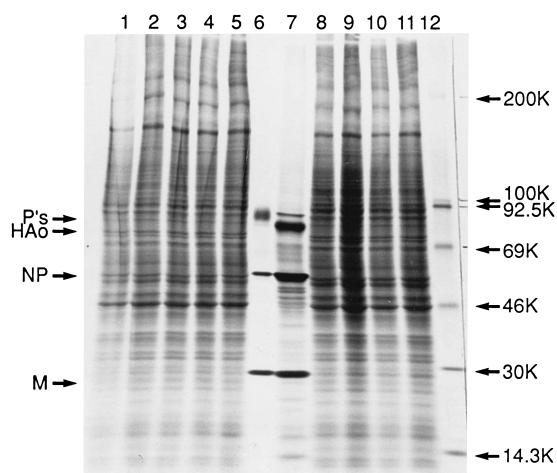


FIG. 3. Effect of 2′-fluorodGuo on polypeptide synthesis of uninfected CE cells (lanes 1 to 4 were treated with 25, 50, 100, and 200 μM 2′-fluorodGuo, respectively) and X31-infected CE cells (lanes 8 to 11 were treated with 200, 100, 50, and 25 μM 2′-fluorodGuo, respectively). Lane 5, uninfected cell control; lane 6, purified virus; lane 7, infected virus control; lane 12, $^{14}$C molecular weight markers (K indicates thousands). P's, polymerase proteins; HA, hemagglutinin; NP, nucleoprotein; M, matrix protein.

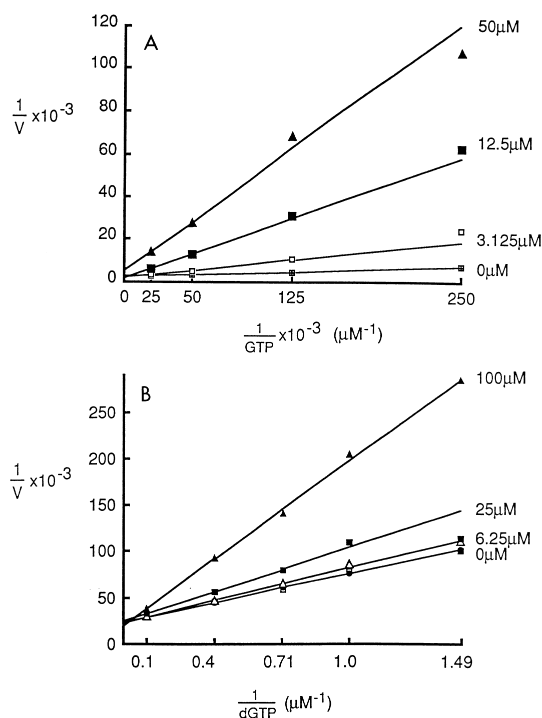


FIG. 4. Lineweaver Burke plots showing competitive inhibition of influenza virus RNA polymerase (A) and DNA polymerase α (B) by 2′-fluorodGTP. Each assay point was determined in triplicate. From these assays $K_i$ values were determined by using the computer program Grafit. The $K_i$ determined for influenza virus polymerase was 1.3 μM (standard error, ±0.2 μM), and that determined for DNA polymerase α was 48 μM (standard error, ±0.1 μM).

strate concentrations revealed that the $IC_{50}$s were dependent on GTP levels but not on the levels of ATP, CTP, or UTP. Studies with various concentrations of [$^3$H]GTP or [$^3$H]dGTP and three fixed concentrations of 2′-fluorodGTP for influenza virus RNA polymerase and DNA polymerase α, respectively, are presented as Lineweaver Burke plots in Fig. 4. From these kinetic studies 2′-fluorodGTP was found to be a competitive inhibitor of the incorporation of GMP into viral RNA, with a mean $K_i$ of 1.09 ± 0.35 μM ($n$ = 3) compared with a mean $K_m$ of 6.24 ± 1.9 μM for GTP. With DNA polymerase α, 2′-fluorodGTP also demonstrated competitive inhibition, in this case, of the incorporation of dGMP into DNA, but for DNA polymerase α the $K_i$ of 48 μM ($n$ = 2) was much higher than the $K_m$ of 0.9 μM for dGTP, indicating a much lower affinity than the natural substrate.

Further enzyme studies were designed to determine if 2′-fluorodGTP could replace GTP and be incorporated into the growing virus RNA chain. For this, the effect of 2′-fluorodGTP (100 μM) on RNA synthesis in the absence of GTP was examined (Fig. 5). Little or no RNA synthesis occurred over a 2-h period when 100 μM 2′-fluorodGTP was present. This suggests that 2′-fluorodGTP blocks RNA chain elongation by the influenza virus RNA polymerase, possibly by the addition of a single nucleotide which causes chain termination. To check that the virus RNA template was still capable of supporting RNA synthesis, an excess of GTP (500 μM) was added to the reaction mixture after 90 min. Virus RNA synthesis resumed rapidly, indicating that RNA synthesis was not blocked irreversibly.

Further kinetic studies were undertaken with influenza virus RNA polymerase from purified disrupted virus of the parent

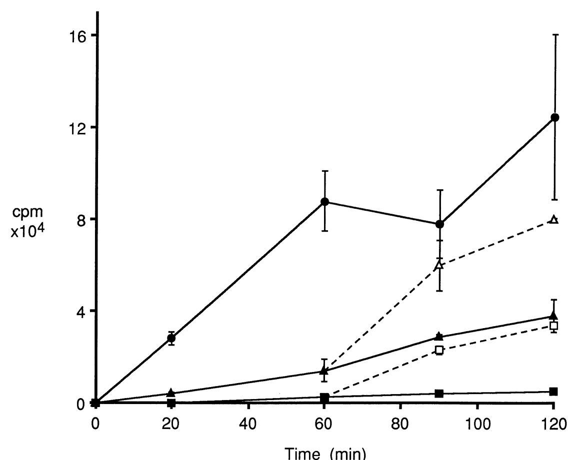


FIG. 5. Effect of 2′-fluorodGTP on influenza virus RNA polymerase activity in the absence of GTP and in the presence of GTP when GTP was added 1 h after the start of the reaction. The results presented in the figure are averages from two experiments, and each point was determined in triplicate for each assay. ●, 500 μM GTP; △, with 500 μM GTP added at 60 min; ▲, 0 μM GTP; □, with 500 μM GTP added at 60 min; ■, 0 μM GTP with 100 μM 2′-fluorodGTP.

strain (strain X31) and of a partially resistant mutant (strain X31R) (16). In direct comparisons in two assays, the parent X31 RNA polymerase gave an average $K_i$ of 1.3 μM, compared with an average $K_i$ of 13.15 μM for X31R, which is a 10-fold reduction in the susceptibility of the enzyme from resistant virus.

**Effect of 2′-fluorodGTP on purified RNPs.** More detailed studies were undertaken with purified RNPs to analyze the inhibition of initiation and the elongation of transcription primed by a synthetic capped mRNA. The products were analyzed on polyacrylamide gels (Fig. 6). Chung et al. (2) have shown that this synthetic mRNA-like substrate is cleaved after the guanosine residue, 11 nucleotides from the cap, by the endonuclease activity of the polymerase complex to produce a single 5′-capped, 11-nucleotide primer termed G11 (2, 4) which can act as a primer for influenza virus transcription. The addition of [$^{32}$P]CTP in the absence of other nucleotides resulted in a labelled, capped, 12-nucleotide product through incorporation of one [$^{32}$P]CMP residue to the 3′ end of the G11 primer (Fig. 6, lane 1). The addition of 40 μM GTP or 2′-fluorodGTP resulted in the appearance of a labelled doublet of 12 and 13 nucleotides (Fig. 6, lanes 3 and 4), indicating that during elongation 2′-fluorodGTP can be incorporated as the second nucleotide with efficiency equal to that of GTP. This doublet appeared to be of lower intensity compared with that obtained after the addition of only labelled CTP, and we have found that GTP can be incorporated as the first nucleotide during initiation with this primer. This shows that both GTP and 2′-fluorodGTP can compete with CTP at initiation but also that 2′-fluorodGuo is not a better competitor relative to GTP for initiation. Similarly, we have found that at initiation 2′-fluorodCTP does not compete with [$^{32}$P]CTP as efficiently as unlabelled CTP (data not shown). In the controls, the addition of the other three nucleotides allows elongation to proceed, producing high-molecular-weight transcription products which accumulate at the top of the gel (Fig. 6, lane 6). Elongation of the limited transcript in the presence of 2′-fluorodGTP resulted in significant inhibition of [$^{32}$P]CTP incorporation and shorter transcripts (Fig. 6, lane 5). The lower intensities of the transcription products in the presence of

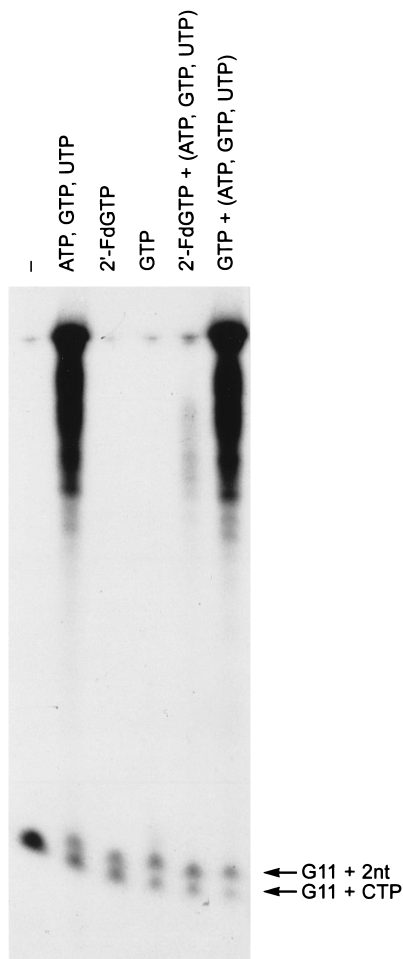


FIG. 6. Effect of 2′-fluorodGTP (2′-FdGTP) on the initiation and elongation of RNP transcription primed by a synthetic capped mRNA [capped 67-nucleotide transcript from a *Sma*I-digested pGEM-7Zf(+) DNA]. From left to right, purified RNPs incubated with capped mRNA and [α-$^{32}$P]CTP (lane 1), plus 5 μM (each) ATP, UTP, and GTP (lane 2), plus 40 μM 2′-fluorodGTP (lane 3), plus 40 μM GTP (lane 4), and plus 40 μM 2′-fluorodGTP plus 5 μM (each) ATP, UTP, and GTP (lane 5) and 40 μM GTP plus 5 μM (each) ATP, UTP, and GTP (lane 6). Transcripts not resolved at the top of the gel are more than 100 nucleotides (nt) in length.

2-fluorodGTP, together with the fact that 2′-fluorodGTP can be incorporated, would indicate that transcription is blocked after the addition of 2′-fluorodGTP. In experiments in which an excess of all nucleotides was added to reaction mixtures containing truncated transcription products, a partial reversal of the block in transcription was observed (data not shown).

## DISCUSSION

The nucleoside analog 2′-fluorodGuo has been shown to inhibit the primary transcription of influenza virus RNA in cell culture, which results in a blockage of virus replication. The compound also appears to inhibit secondary transcription, although this may be a secondary effect resulting from the inhibition of primary transcription. These findings are consistent with previous observations that intracellular 2′-fluorodGTP levels correlate with anti-influenza virus activity (16). The compound is phosphorylated efficiently by cellular enzymes, of which deoxycytidine kinase and GMP kinase are two enzymes capable of phosphorylating the compound to its mono- and

diphosphate forms, respectively. Furthermore, studies with the polymerase complex from disrupted influenza virus substantiated the fact that the triphosphate was the active component. Both 2′-fluorodGuo and 2′-fluorodGuo monophosphate had no activity against the virus enzyme complex (unpublished data). Selectivity was shown with two important cellular enzymes, DNA polymerase α and RNA polymerase II. In cellular assays a therapeutic ratio of 27 was observed with virus strain X31 in CE cells (16), which compares favorably to the therapeutic ratio of 38 seen with DNA polymerase α in the present study. In particular, RNA polymerase II was examined because it has been shown that inhibitors of this enzyme can severely disrupt the replication of influenza virus (9, 10). With 2′-fluorodGuo, virus inhibition was clearly not a consequence of inhibition of cellular RNA polymerase II, since there was no inhibition by 2′-fluorodGTP in enzyme assays. Confirmation that influenza virus transcriptase was the target of inhibition by 2′-fluorodGTP was obtained from further analysis of partially resistant virus. Interestingly, although only a 5-fold shift in the $IC_{50}$ was seen with resistant virus in cell culture, with the polymerase from resistant virus, a 10-fold shift in the $K_i$ was observed. Thus, inhibition of the transcriptase would account for the inhibition observed in cell culture.

Studies on the mechanism of inhibition of the polymerase by 2′-fluorodGTP showed that the 2′-fluoro-triphosphate was a competitive inhibitor of GMP incorporation into virus RNA. However, 2′-fluorodGTP could not replace GTP and be incorporated into the growing virus RNA chain but blocked virus RNA chain elongation. These kinetic studies suggested that 2′-fluorodGTP may be incorporated and may cause chain termination. Indeed, from the primer elongation studies it was apparent that incorporation of a single fluoronucleotide occurred. This resulted in the termination of further transcription. Interestingly, in all of the assay systems used, that is, in virus replication studies, in enzyme kinetic studies, and in primer elongation studies, the inhibition was found to be partially or totally reversible. This would suggest that, in the absence of inhibitor, terminated products are removed or are cleaved and reused as primers for the production of complete transcripts. Although cap binding and endonuclease cleavage of mRNA were not looked at directly in the present study, the presence of a labelled cleavage product on gels after treatment with 2′-fluorodGTP and the similar levels of inhibition seen with both capped RNA- and ApG-primed transcription strongly suggest that the capping mechanism was not a target for 2′-fluorodGuo. In the primer elongation experiments the presence of shorter transcripts may have arisen from the incorporation of and chain termination by 2′-fluorodGTP.

Few nucleoside analogs have been shown to inhibit influenza virus replication, in contrast to the large number of nucleoside inhibitors of herpesviruses and the human immunodeficiency virus. This may be due in part to the lack of any influenza virus-specific kinases, as is seen with herpesviruses, but probably also reflects the high degree of specificity of the influenza virus polymerase complex for ribosides, which is in contrast to the promiscuity of the human immunodeficiency virus reverse transcriptase. Ribavirin is one such nucleoside analog with activity against influenza virus which has also been shown to be a competitive inhibitor of the influenza virus polymerase complex (3). However, ribavirin appears to have a multiple mode of action, including effects on GTP pool levels by inhibition of IMP dehydrogenase and effects on the capping mechanism (1). In a further study (19), ribavirin was found to inhibit both the initiation and the elongation phases of transcription. Possibly because of this multiple mode of action, no ribavirin-resistant influenza virus has ever been isolated. Interestingly, the 2′-fluorodGuo-resistant virus did not show cross-resistance to ribavirin, indicating differences in the targets for the two inhibitors. Further studies are in progress to sequence the transcriptase gene, PB1, from resistant and parent X31 virus strains. In addition to its possible use in influenza virus chemotherapy, 2′-fluorodGuo should be a useful tool in probing the active site of the polymerase complex.

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