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2'-FLUOROPOLYNUCLEOTIDE-DIRECTED REVERSE TRANSCRIPTASE REACTIONS. EFFECT OF HOMOLOGOUS POLYNUCLEOTIDES

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Several homologous polynucleotides have been tested as inhibitors on the reactions catalyzed by avian myeloblastosis virus (AMV) reverse transcriptase, in the presence of polyribonucleotides and 2'-fluorinated polynucleotides as templates. Polynucleotides differentially inhibited the reactions catalyzed by reverse transcriptase in the presence of these synthetic templates. Polyriboadenylic acid (poly(rA), poly(2'-0-methyladenylic acid) (poly(Am)), poly(2'-fluoro-2'-deoxyadenylic acid) (poly(dAfl), polyinosinic acid (poly(rI)) and polyuridylic acid poly(rU)) inhibited the polyribonucleotide-, but not the 2'-fluorinated polynucleotide-directed reverse transcriptase activity.

KEY WORDS: Reverse transcriptase, 2'-fluoropolynucleotides, templates

INTRODUCTION

Reverse transcriptase [E.C.2.7.7.7] first described in a retrovirus, can utilize synthetic polyribonucleotides and polydeoxyribonucleotides as templates in the presence of complementary deoxyribo-oligomers as primers.[1–4] Other polynucleotides has been tested as templates in the reactions catalyzed by reverse transcriptase. The O-methyl- and fluoroderivaties at the 2' position of polycytidylic acid and polyadenylic acid could be recognized as templates by the viral enzyme.[5–7] On the other hand, several other polynucleotides inhibited the reactions catalyzed by reverse transcriptase. Murine leukemia virus and AMV reverse transcriptases were inhibited by poly(rU), poly(2'-fluorouridylic acid)[8–9] poly(2'-O-ethyladenylic acid)[10] and poly(2'-O-ethylcytidylic acid).[11]

We have previously described the effects of 2'-fluoropolynucleotides as templates and inhibitors on the DNA synthesis catalyzed by DNA- and RNA-dependent DNA polymerases.[7] In contrast to poly(rA)-, the poly(dAfl)-directed reverse transcriptase activity was not affected in the presence of polycytidylic acid analogs.[7] We have also observed that the AMV reverse transcriptase reaction, catalyzed in the presence of both poly(rA) or poly(dAfl) as template, was inhibited by intercalating and

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nonintercalating drugs.[12] In the present work, we describe the effect of single stranded polynucleotides on the reaction catalyzed by AMV reverse transcriptase with polyribonucleotides and 2'-fluoro 2'-deoxypolynucleotides as templates.

MATERIALS AND METHODS

Chemicals

AMV reverse transcriptase was obtained from Boehringer Mannheim. Poly(rA), poly(rC), poly(rI), oligo $(dT)_{12-18}$, oligo$(dG)_{12-18}$, dithiothreitol, bovine serum albumin, TTP, and dGTP were purchased from Sigma Chemical Company (St, Louis, MO). Poly(Am), was obtained from P.L. Biochemicals, Inc. (Milwaukee, WIS). Poly(dAfl) and poly(dCfl) were gifts from Dr. W. Guschlbauer (C.E.N., Saclay, France). Tritium-labelled TTP and dGTP were purchased from New England Nuclear (Du Pont).

Preparation of template-primers

The annealing of the template-primer duplexes was performed as previously described.[7] Synthetic polynucleotide solutions were $2.4A_{260}$ units/ml for A-T duplexes and $4.4A_{260}$ units/ml for G-C duplexes in 10 mM Tris-HCI (pH 7.9). Ater mixing, the solutions containing A-T duplexes were heated for 15 min at 75°C, while those containing G-C duplexes were heated at 90°C and then left for 30 min at room temperature.

Enzyme assay

The incubation mixture contained the following reagents in a final volume of 0.05 ml; 50 mM Tris-HCl (pH 7.9), 2 mM dithiothreitol, 200 μg/ml bovine serum albumin, 40 mM KCL, 6 mM magnesium acetate, $0.24A_{260}$ units/ml poly(A)-oligo(dT) or $0.44A_{260}$ units/ml poly(C)-oligo(dG), 10 μM [^{3}H] TTP or [^{3}H] dGTP (900 cpm/pmol), and 1 unit reverse transcriptase. Incubation was carried out at 37°C for 30 min. The reaction was stopped by the addition of 1 ml ice-cold trichloroacetic acid solution. The precipitates were filtered through Millipore nitrocellulose membranes, washed with ice-cold 2% trichloroacetic acid solution containing 0.1 M sodium pyrophosphate, dried and counted in a PPO/POPOP/toluene scintillation mixture.

RESULTS AND DISCUSSION

We have previously reported the ability of poly(dAfl) and poly(dCft) to replace the respective polyribonucleotides or polydeoxyribonucleotides as template in the DNA synthesis reaction catalyzed by DNA- and RNA-dependent DNA polymerases.[7] In this work, we present the effect of various polynucleotides on the ribo- and on the 2'-fluoropolymers-directed reverse transcriptase activities.

Table 1 shows the effect of some polyadenylic acid analogs on the reactions catalyzed by reverse transcriptase with poly(rC) and poly(dCfl) as template. Poly(rC)- but not

TABLE 1
Effect of poly(A) analogs on DNA synthesis catalyzed by reverse transcriptase

Addition	(μg/ml)	DNA synthesis (%)	
		Poly(rC)-oligo(dG)	Poly(dCfl)-oligo(dG)
None		100 (136)	100 (45)
Poly(rA)	1	87	100
Poly(rA)	5	63	100
Poly(Am)	1	46	100
Poly(Am)	5	41	88
Poly(dAfl)	1	6	93
Poly(dAfl)	5	3	88

20 μg/ml poly(rC) or poly(dCfl), 2 μg/ml olio(dG), and 50 μM[^{3}H] dGTP (300 cpm/pmol) were incubated as described in Materials and Methods. Picomoles of radioactive precursor incorporated are given in brackets. The DNA synthesis values represent the mean of triplicate determinations (SD = ± 2%).

poly(dCfl)-directed reverse transcriptase reaction was inhibited in the presence of poly(rA), poly(Am) and poly(dAfl), with the following order of strength:

$$\text{poly(dAfl)} \gg \text{poly(Am)} > \text{poly(rA)}$$

The corresponding fluorinated analog of poly(C) was a noncompetitive inhibitor with regard to TTP and to poly(rA)-oligo(dT) in the reactions catalyzed by AMV reverse transcriptase.[7] The non inhibition of the poly (dCfl)-directed reverse transcriptase reaction with poly(A) analogs was similarly observed using poly(dAlf) as template and poly(dCfl) as inhibitor.[7] Other polynucleotides were assayed in order to verify if the presence of fluoro at the 2'-position of synthetic templates really might confer a stabilizing effect to the reaction catalyzed by reverse transcriptase. Poly(rI) inhibits the reverse transcriptase reaction in the presence of poly(rA)-oligo(dT) as template-primer, but not significantly with poly(dAfl)-oligo(dT)(Table 2). Poly(rI) was not tested in the poly(dCfl)-directed reverse transcriptase since an interaction (1:1) occurred between these polynucleotides.[13]

Figure 1 shows the effect of polyuridylic acid on the reations catalyzed by AMV reverse transcriptase in the presence of poly(A) and poly(C) analogs as templates. 10 μml poly(rU) inhibited about 35% and 50% of the reverse transcriptase activity, respectively, in the presence of poly(rA) and poly(rC) as templates. Under the same conditions, poly(dAfl)-and poly(dCfl)-directed reverse transcriptase reactions were not affected by poly(rU). Analogous results were obtained in the presence of Mn^{2+}, instead of Mg^{2+} as divalent cation (Table 3). Poly(rU) inhibited the reactions catalyzed

TABLE 2
Effect of polyinosinic acid on the reverse transcriptase activity

Addition	(μg/ml)	DNA synthesis (%)	
		Poly(rC)-oligo(dT)	Poly(dAfl)-oligo(dT)
None		100 (140)	100 (58)
Poly(rI)	5	68	88
Poly(rI)	10	64	89

10 μg/ml poly(rA) or poly(dAfl), 2 μg/ml oligo(dT), and 10 μM[^{3}H] TTP (900 cpm/pmol) were incubated as described in Materials and Methods. Picomoles of radioactive precursor incorporated are given in brackets. The DNA synthesis values represent the mean of triplicate determinations (SD = ± 2%).

by DNA polymerases of Rauscher leukemia virus, in the presence of poly d(A-T) as template,[8] and of avian myeloblastosis virus, in the presence of poly(rC)-oligo(dG).[9]

In order to minimize the dissociation of template-primer, Parnaik and Das have worked at 23°C and observed a higher affinity of AMV reverse transcriptase for template primers. Poly(rU) inhibits the poly(rA)- but not the poly(dAlf)-directed reverse transcriptase reaction working at 25°C and 30°C, suggesting that these effects are not dependent on the incubation temperature (results not shown).

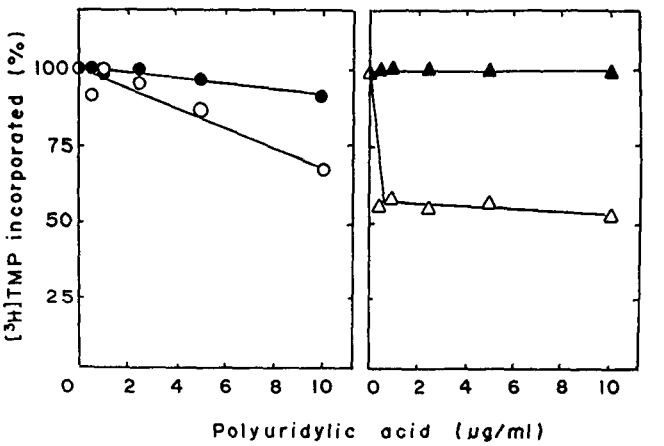

FIGURE 1 Effect of polyuridylic acid on the Mg^{2+}-dependent reverse transcriptase activity.(A) 10 μg/ml poly(rA) or poly(dAfl), 2 μg/ml oligo(dT), and 10 μM[^{3}H] TTP (900 cpm/pmol) and (B) 20 μg/ml poly(rC) or poly(dCfl), 2 μg/ml oligo(dG), and 10 μM[^{3}H]dGTP (900 cpm/pmol) were incubated as described in Materials and Methods. Results are given in percentages of radioactive precursor incorporated in poly(rA) (○), poly(dAfl) (●), poly(rC) (△) and poly(dCfl) (▲). 100% activity correspond to 137 pmol, 60 pmol, 155 pmol and 72 pmol of labelled precursor incorporated in poly(rA), poly(dAfl), poly(rC) and poly(dCfl), respectively, for 30 min. Each point represents the mean of triplicate determinations. The size of the symbols indicates the mean ±SD.

TABLE 3
Effect of polyuridylic acid on the Mn^{2+}-dependent reverse transcriptase activity

Template-primer	DNA Synthesis (%)
poly(rA)-oligo(dT)	8.3
poly(dAfl)-oligo(dT)	50.0
poly(rC)-oligo(dG)	48.8
poly(dCfl)-oligo(dG)	93.0

The assay conditions were the same as described under Figure 1, in the presence of 0.5 mM Mn^{2+} and 10 μg/ml poly(rU). 100 % activities (without polyuridylic acid) correspond to 37.8 pmol, 43 pmol, 13 pmol and 15.5 pmol of labelled precursor incorporated in poly(rA), poly(dAfl), poly(rC) and poly(dCfl), respectively, for 30 min. The DNA synthesis values represent the mean of triplicate determinations (SD = ±2%).

The results described in this paper suggest a stabilizing effect of 2'-fluoro polynucleotide-directed reverse transcriptase reaction in relation to other synthetic polynucleotides as inhibitors. A stabilizing effect on polynucleotide structure by highly electronegative 2'-substituents (N_3 and F) has been demonstrated.[13,15,16]

Other non-polynucleotide compounds seem to inhibit the reactions catalyzed by reverse transcriptase with poly(dAfl) or poly(dCfl) as template.[12]

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