INHIBITION OF DNA POLYMERASES ONCORNAVIRUSES BY MODIFIED NUCLEIC ACIDS.

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The DNA polymerase activity in virions of the RNA tumor viruses can be studied in two different ways: 1) The endogenic reaction: the disrupted virions are incubated with substrates in the absence of any added template, and synthesis of RNase

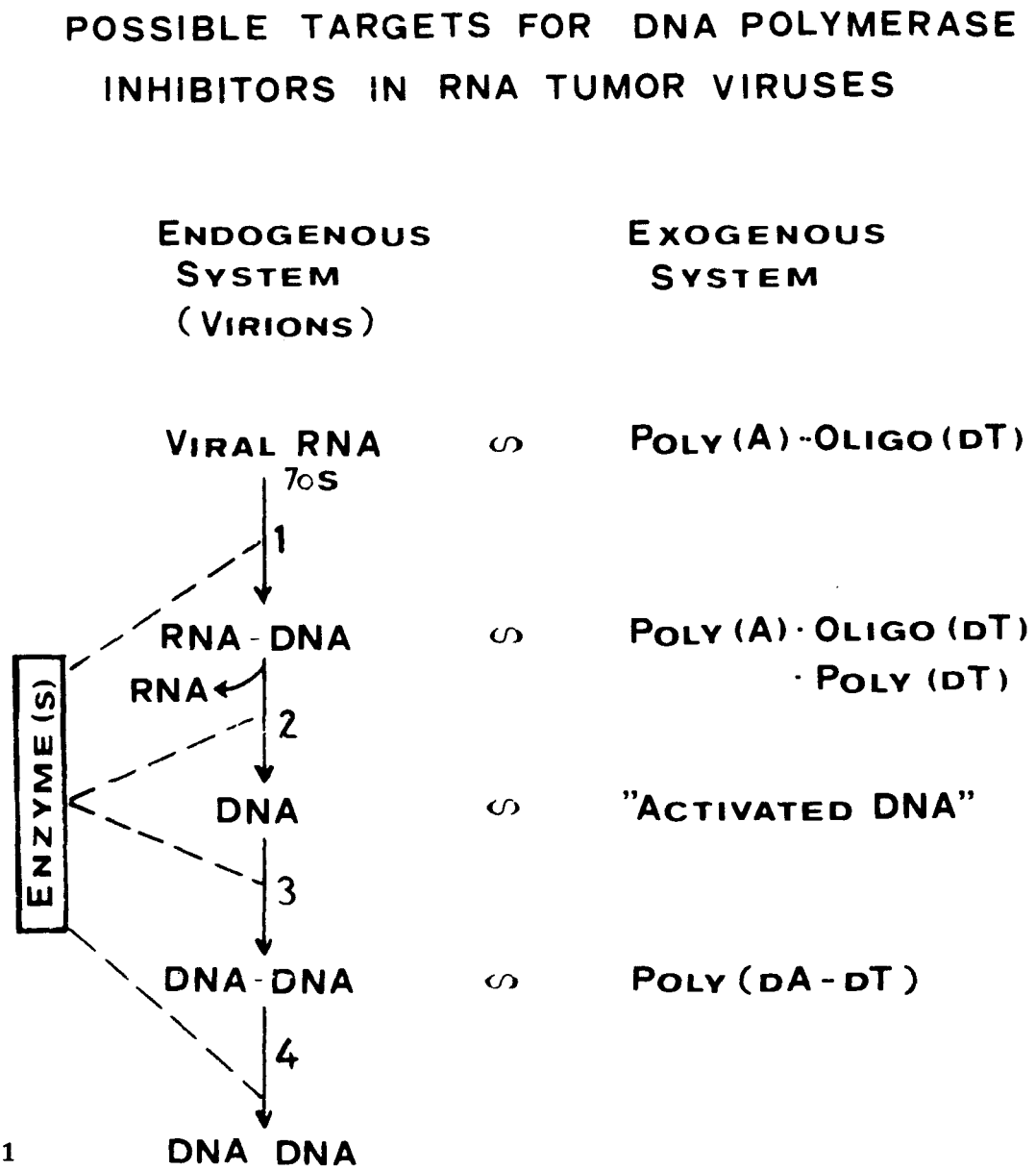

Fig. 1

sensitive DNA synthesis is measured, which indicates the transcription of endogenous viral RNA (60–70s); 2) The template-dependent reaction: this reaction can be studied by purifying the solubilized enzyme, or by using disrupted virions. The reaction can be carried out in the presence of a viral RNA, activated DNA, or synthetic polynucleotides of various composition (see Fig. 1).

A large number of compounds have been reported to inhibit the DNA polymerase activity of oncornaviruses in the absence (endogenic reaction) and in the presence of a variety of templates (see Table 1). On the basis of template specificity of the reactions which are most sensitive to these inhibitors, possible targets have been postulated in Table 1.

Most of the inhibitors reported so far act by forming complexes with the template(s), so that their specificity is not strictly for the viral enzyme, and bacterial as well as mammalian enzymes will also be inhibited by such inhibitors; the exception to this generalization is an inhibitor which specifically will bind to 70S viral RNA. An inhibitor of latter type has not been reported so far. Another way to develope specific inhibitors is to search for compounds which will specifically bind to DNA polymerases of oncornaviruses. The present report describes the inhibition of DNA polymerases of oncornaviruses by a novel template analogue, a partially thiolated (5-SH) polycytidylic acid (MPC), which acts by complexing with the enzyme(s), and shows some selectivity in its mode of action on the viral DNA polymerase.

Table 1. Reactions inhibited by various inhibitors of DNA polymerases of oncornaviruses

Inhibitor	Target Reaction (see Fig. 1)	References
Dideoxythymidine-Triphosphate (ddTTP)	1,2 und 4	SMOLER et al., 1971
Actinomycin D	2,3 and 4	GURGO et al., 1971 MÜLLER et al., 1971
Rifampicin derivatives	1-4	GURGO et al., 1971 KOTLER and BECKER, 1971 TING et al., 1972 CHANDRA et al., 1972a
Ara-CTP	1	MÜLLER et al., 1972
Distamycin derivatives	3 and 4	CHANDRA et al., 1972b
Daunomycin derivatives	3 and 4	CHANDRA et al., 1972c
Tilorone-HCl	(1), 4	CHANDRA et al., 1972d
Ethidium bromide	1,2	HIRSCHMAN, 1971 MÜLLER et al., 1971 FRIDLENDER & WEISSBACH, 1971
Anthracyclines	1	APPEL and HASKELL, 1971

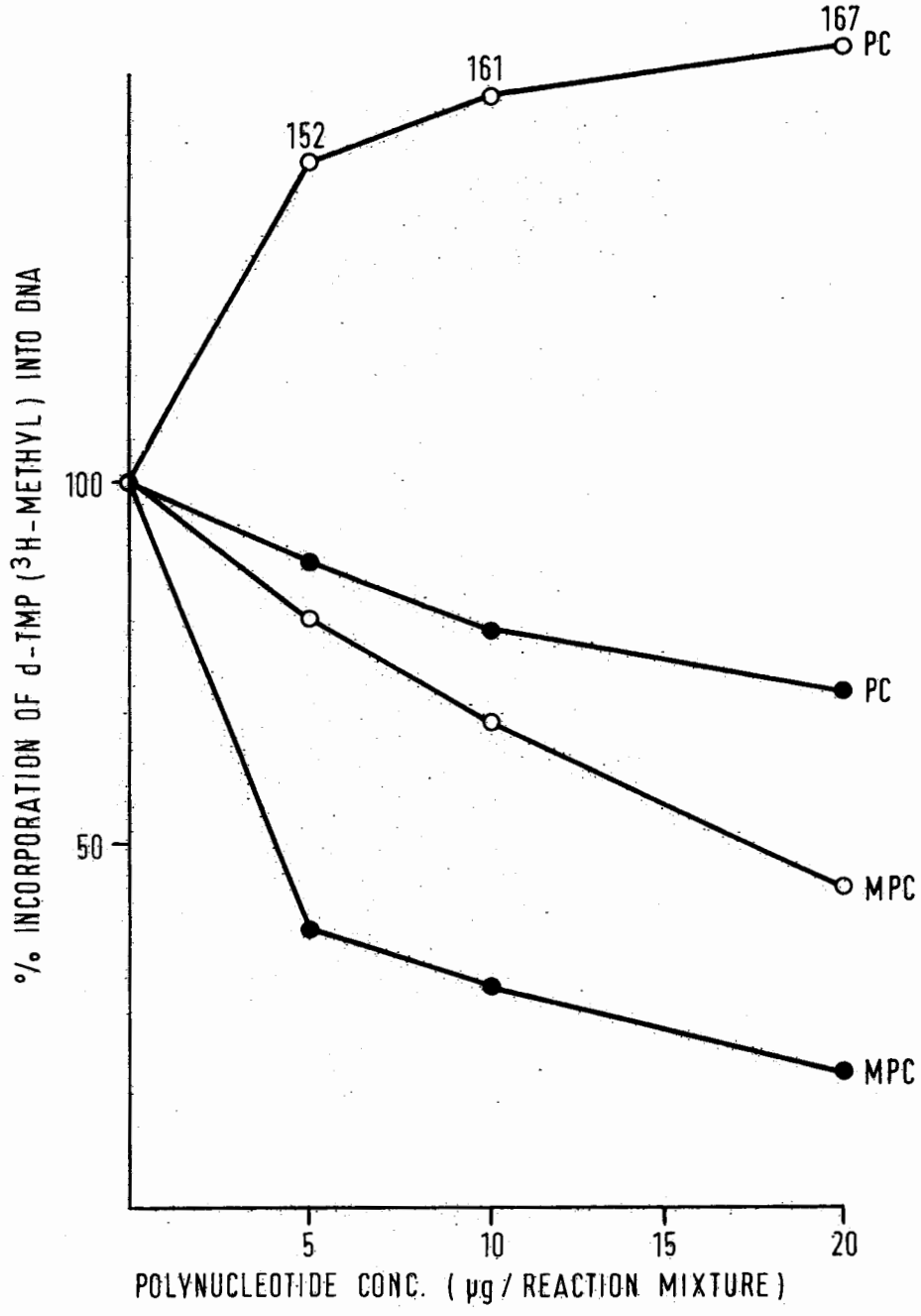

Fig. 2: Effects of thiolated polycytidylic acid (MPC) and unmodified polycytidylic acid (PC) on the DNA polymerase activities from MSV(Moloney) in the presence of poly rA. $(dT)_{14}$ (●——●) and poly (dA-dT) (○——○) as templates.

Fig. 2 shows the effects of 5-mercapto-(8.68 %)-polycytidylic acid (MPC) and unmodified polycytidylic acid (PC), respectively, on the incorporation of ^{3}H-TMP into DNA by the DNA-polymerases of the MSV(Moloney), in the presence of either poly (dA-dT) or poly rA. $(dT)_{14}$ as the template. The results obtained with the

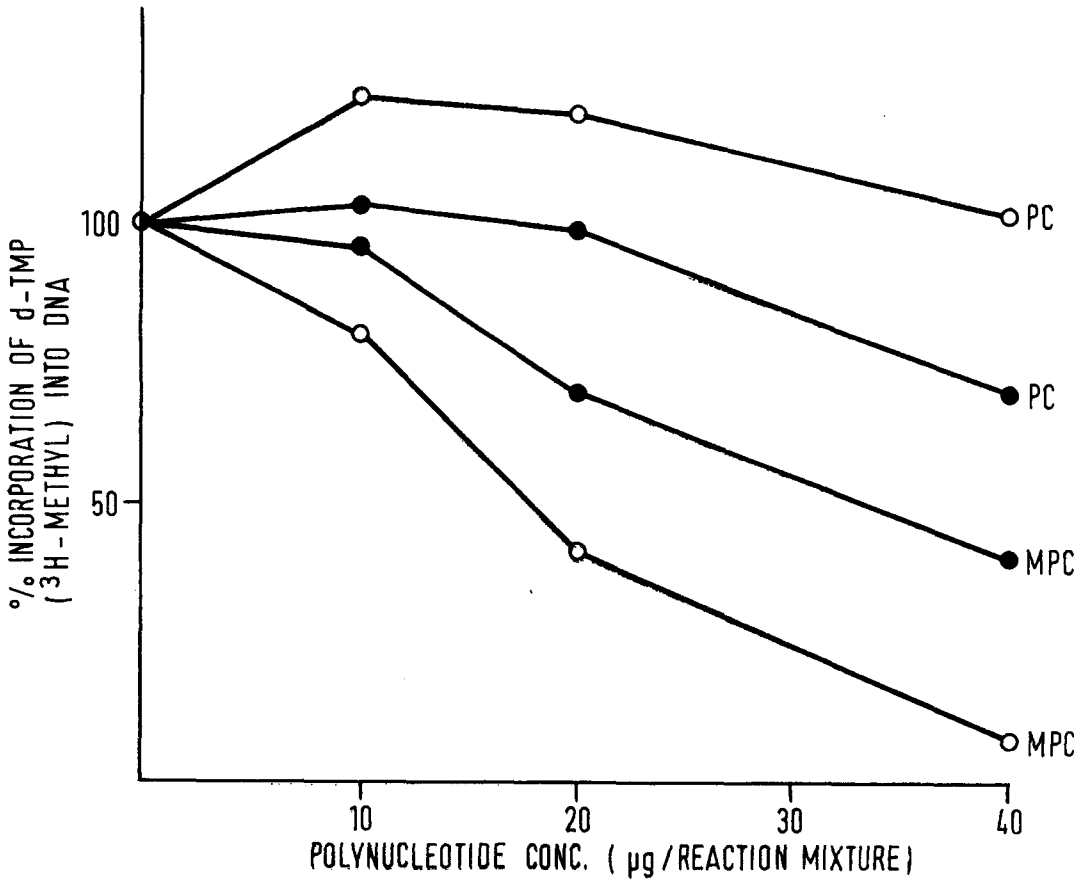

Fig. 3: Effects of thiolated polycytidylic acid (MPC) and unmodified polycytidylic acid (PC) on the DNA polymerase activities from FLV (Friend) in the presence of poly rA. $(dT)_{14}$ (●——●) and poly (dA-dT) (○——○) as templates.

same pair of modified and unmodified polycytidylic acid samples in the FLV DNA-polymerase assay, using the same pair of templates, are graphically represented in Figure 3.

It is clear from these graphs that the modified polynucleotide, MPC, significantly inhibits the DNA polymerases present in both viral extracts; furthermore, the inhibitory activity of MPC is very nearly the same in the two systems when poly (dA-dT) is used as the template (50 % inhibition at 18 µg/0.25 ml of reaction mixture), but in the presence of poly rA. $(dT)_{14}$ as the template, MPC acts as a much more potent inhibitor of ^{3}H-TMP incorporation in the MSV(M) assay system (50 % inhibition at 4 µg/reaction mix.) than in the FLV system (50 % inhibition at 35 µg/reaction mix.). In contrast, the unmodified polynucleotide, PC, stimulates DNA polymerase activity in both viral systems with poly (dA-dT) as the template, and it shows slight inhibitor activity in the presence of the poly rA. $(dT)_{14}$ template.

A time study of the effect of MPC, added at various periods after incubation of the reaction mixture showed that once the formation of the enzyme-template complex has occured, this RNA-directed polymerase is no longer sensitive to MPC.

The interpretation of the mode of MPC action on DNA polymerases of oncornaviruses needs a clarification of many questions, one may ask: 1) Is the inhibition dependent on the degree of thiolation of the polymer? ; 2) Does it inhibit DNA

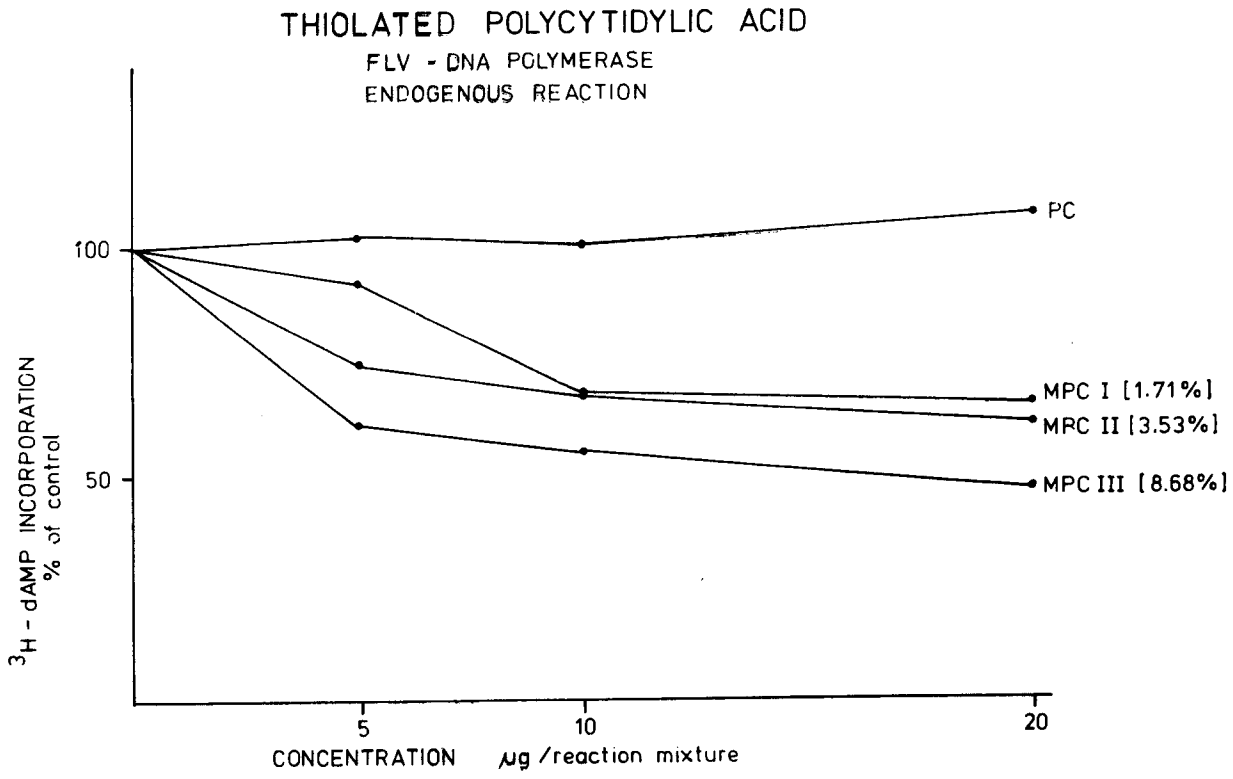

Fig. 4: Inhibition of FLV-DNA-polymerase activity in the absence of any added template by thiolated polycytidylic acids (MPC), containing 1.71 %, 3.53 % and 8.68 % of 5-SH-cytidylate units.

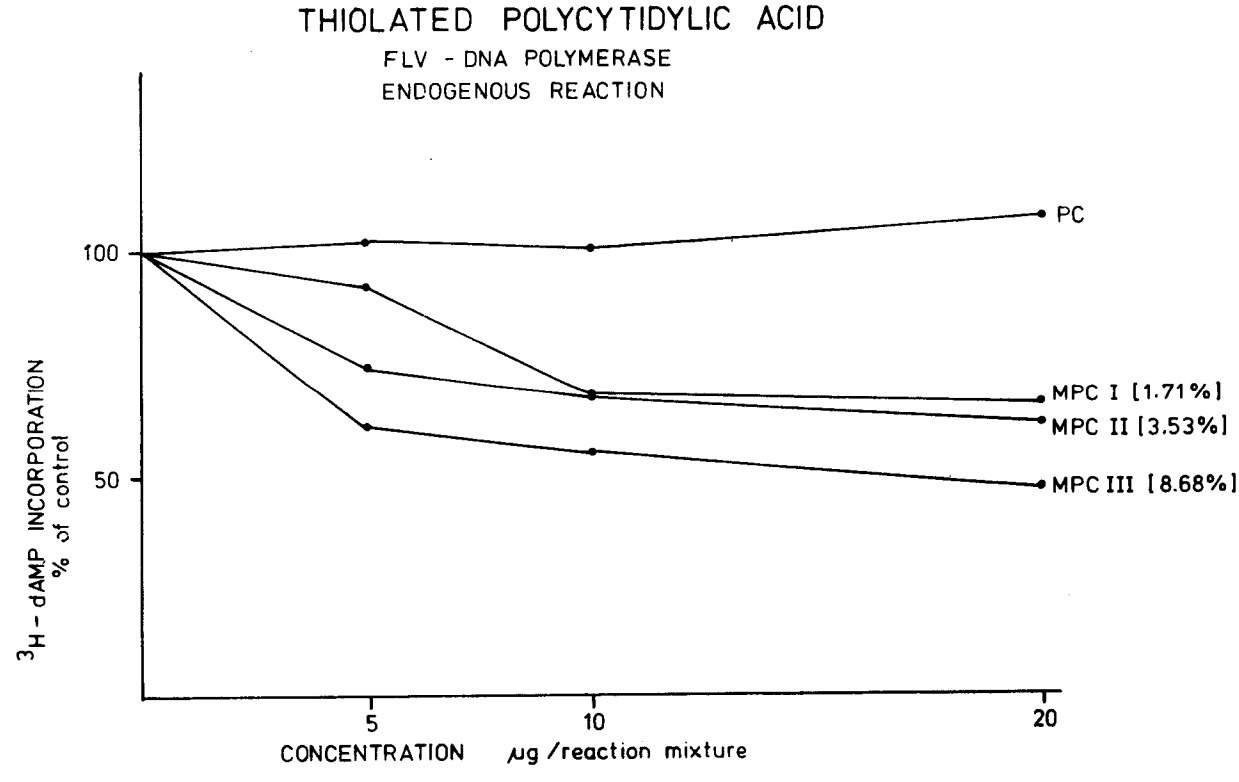

Fig. 5: Inhibition of FLV-DNA-polymerase activity in the presence of poly rA. $(dT)_{14}$ by thiolated polycytidylic acids (MPC), containing 1.71 %, 3.53 % and 8.68 % of 5-SH-cytidylate units.

polymerases from other sources and, 3) How is the affinity of this compound towards enzymes from other types of virus particles?

The study with polycytidylic acid preparations with varying degree of thiolation showed a clear dependence of inhibiting capacity on the degree of thiolation of the polymer. These studies were carried out with MPC containing 1.71 %, 3.53 % and 8.68 % of SH-cytidylate units in the polymer. The degree of thiolation was estimated by neutron activation analysis.

As follows from Fig. 4, the endogenic reaction in FLV is inhibited by all MPC preparations, and the amount of inhibition is dependent on the degree of thiolation of the polymer. Similar results were obtained in the FLV-reaction catalyzed by poly rA. $(dT)_{14}$, a template known to be specific for viral DNA polymerases.

Regarding the selectivity of MPC action we have done some experiments on the DNA-polymerase of *E. coli* K_{12} cells, isolated by the procedure of RICHARDSON (1966).

Table 2. Effect of polycytidylic acid (PC) and mercapto-polycytidylic acid (MPC) on DNA-polymerase of E. coli K_{12} in the presence of denatured DNA

Compound added+	^{3}H-dAMP Incorporation into DNA (cpm/reaction mixt.)	% of control
None	575	100
PC	608	106
MPC++–I	572	99.5
MPC –II	554	96
MPC –III	591	103

+ All compound were added at a concentration of 5 µg/reaction mixt. (0,30 ml);
++ MPC-I, MPC-II and MPC-III represent mercaptocytidylic (poly) acids with 1.71 %, 3.53 % and 8.68 % 5-SH cytidylate units respectively.

As follows from Table 2 the DNA polymerase reaction of bacterial cells is insensitive to MPC; even at higher concentrations no inhibition occured. More experiments are necessary to understand the specificity of MPC in viral systems. These studies are now being substantiated in collaboration with Dr. Robert C. Gallo.

The basic idea to initiate these studies was to find out the role of such modifications on natural viral templates, 60–70 sRNA. We, therefore, have done some model experiments with partially thiolated DNA and various types of RNA, isolated and purified from Ehrlich ascites cells.

In these experiments best results were obtained using modified soluble RNA (sRNA) in the MSV-DNA polymerase reaction. As follows from Table 3 the unmodified sRNA shows a slight inhibition of the endogenic and poly rA. $(dT)_{14}$-catalyzed reactions; however, the modified sRNA inhibits both the reactions very significantly.

Table 3. Effect of soluble RNA (sRNA) from Ehrlich ascites cells on the DNA polymerase of MSV(M)

Compound added	^{3}H-TMP incorporation into DNA (% of control)		
	-Template	+poly rA. $(dT)_{14}$	+poly (dA-dT)
Non-thiolate sRNA (μg/reaction mix.)			
10	93	98	104
20	92.5	86	100
40	92	71	102
Thiolated (1-3 %) sRNA (μg/reaction mix.)			
10	77	69	56
20	75	58	43
40	63	49	40

Conclusions

The present results indicate that modification of polynucleotides and nucleic acids may lead to the development of useful inhibitors of DNA polymerases of oncornaviruses. Model studies with partially thiolated polycytidylic acid (MPC) show that 1) MPC inhibits the DNA-polymerase activity of oncornaviruses by forming a complex with the enzyme, 2) the inhibition can be potentiated by increasing the number of 5-SH-cytidylate units in the polymer, and the inhibition is to some extent selective. The last point needs more experiments which, we hope our future studies will substantiate. The studies regarding the implication of this inhibition on biological activity of these compounds are in progress.

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