INHIBITION OF AMV DNA POLYMERASE BY POLYRIBOADENYLIC ACID CONTAINING ε-ADENOSINE RESIDUES

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Summary: Polyriboadenylic acid was treated with chloroacetaldehyde under conditions known to introduce ε-adenosine groups. The degree of modification was monitored by increase in fluorescence intensity. Modified ε-poly rA was found to be inhibitory when unprimed 70S AMV RNA was used as a substrate, suggesting direct competition with the poly rA tract of the RNA. Since ε-poly rA cannot effectively base pair with nucleic acids normally involved in cellular processes, it has the potential of being useful as an inhibitor of oncogenic viral polymerases.

RNA dependent DNA polymerases are known to be present in nucleocapsids of oncornaviruses and have been implicated in the onset of ocogenesis (1,2). Synthetic polyribonucleotides when primed with their corresponding deoxyoligonucleotides can serve as templates of these polymerases (3). By contrast, unprimed polyribonucleotides such as poly rU and poly rA (4,5,6) are not substrates of the polymerase reaction and have been shown to inhibit by competing for the template site on the enzyme (7). In addition, several single-stranded polyribonucleotides possess antiviral activity *in vitro* (4,8). Because the poly rA tract in 70S RNA plays a critical role in transcription when primed with oligo dT (9,10), we have chosen poly rA as a model compound and introduced ε-adenosine residues (10) in an effort to determine its potential use as an inhibitor.

In this paper, we present evidence that shows ε-poly rA is an effective inhibitor of AMV DNA polymerase.

MATERIALS AND METHODS:

Reagents: [^{3}H] TTP. 17.3 Ci/mmole was purchased from Schwarz/Mann. Polyadenylic acid was obtained from Miles Chemical Co. All organic reagents were purchased from Aldrich Chemical Co.

Preparation of AMV RNA: AMV was obtained from chicken plasma of infected chicks which was provided by Dr. J. Beard through contract NO1CP33291 within the Virus Cancer Program of NCI. The virus was purified according to published methods (11). AMV 70S RNA was extracted and purified by centrifugation using markers for size determination.

Purification and Assay Conditions for AMV DNA Polymerase:

AMV DNA polymerase was assayed essentially as described (15). The reaction mixture contained in a final volume of 0.1 ml: 50 mM Tris-HCl (pH 8.3), 6mM $MgCl_2$, 0.4 mM dithiothreitol, 50 mM KCl, 0.2 mM each of dATP, dGTP, dCTP, 0.125 mM [^{3}H]-TTP (80-300 cpm/pmole) and 70S RNA as designated in appropriate tables and figures. The oligo dT:70S RNA ratio used in all assays was 1:10 by weight. Reaction mixtures were incubated at 37° for 30 minutes, stopped by transfer to 4°, addition of 100 µl solution containing 2 µg/ml of bovine serum albumin 2 µg/ml of yeast RNA, and 100 mM pyrophosphate followed by 1 ml of cold 10% trichloroacetic acid. After 10 minutes at 4° the acid insoluble material was collected on Whatman GF/C filters. The filters were washed with 10% trichloroacetic acid and the radioactivity was counted. Omission of a single deoxytriphosphate markedly reduced 70S RNA directed DNA synthesis, indicating a polymerization reaction and not terminal addition of tri-

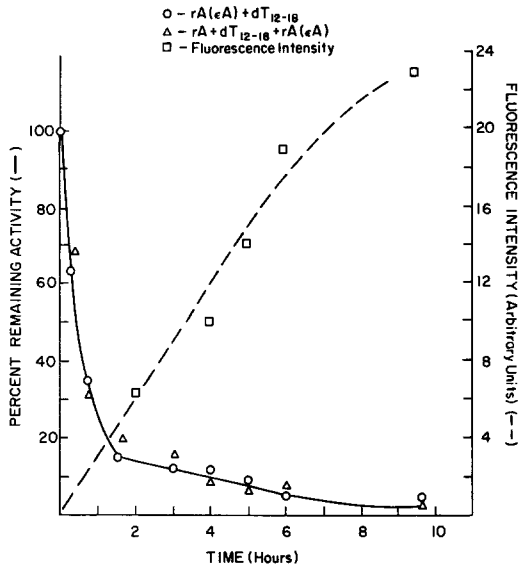

Figure 1. Correlation between the degree of poly rA modification and its effects on transcription by AMV DNA polymerase.
An aqueous solution of 0.5 mg/ml poly rA containing 50% freshly distilled chloroacetaldehyde was adjusted to pH 4.5 with 0.05M HCl, and incubated at 37^{O} for ten hours. At pH 7.0, the fluorescence of ε-poly rA exhibited excitation and emission maxima at 320 nm and 408 respectively. Aliquots were removed at time intervals. Samples were lyophilized to remove chloroacetaldehyde and effects of ε-poly rA on DNA synthesis was determined using the conditions described in Methods.

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phosphates. The product of the reaction was alkali and RNase stable but sensitive to DNase.

The purified fractions showed two distinct bands on SDS disc gel electrophoresis, representing the two non-identical subunits. Purified enzyme was used for all subsequent studies.

Chemical Modification of poly rA with Chloroacetaldehyde: Chloroacetaldehyde was prepared by hydrolysis of its dimethyl-

acetal in dilute sulfuric acid and the aldehyde was distilled over as a 2 M solution (13,14). Modification of adenine residues in poly rA was carried out at pH 4.5 (0.01-0.1M) at 37° for varied periods of time. Modified samples were lyophilized several times to remove all traces of the reagent. Fluorescence Measurements: A Hitachi Perkin Elmer MPF-2A spectrofluorometer equipped with a thermostated cell holder was used for all measurements with a 5nm band width for both excitation and emission. In addition, all measurements were done on dilute solutions to avoid correction for inner filter effects. Correction for scattering was made when necessary.

RESULTS AND DISCUSSION:

Modification of adenine residues with chloroacetaldehyde is a mild reaction which introduces ε-adenine residues in polynucleotides (14). We have adopted this method to introduce ε-adenine residues in poly rA. The characteristic fluorescence intensity was used to monitor the degree of poly rA modification over a period of ten hours (Fig. 1). At different time intervals, samples of ε-poly rA were tested as templates of the polymerase reaction by first priming with oligo dT. A rapid loss of template activity occurred within the first hour of modification, due to the inability of ε-poly rA to base pair with oligo dT. Addition of the modified polynucleotide to the oligo dT primed poly rA reaction inhibited the reaction (Table I). The action of ε-poly rA as an inhibitor of the polymerase reaction can be explained on the basis of competition for template site on the enzyme. We further extended our

TABLE I. Effects of ε-Poly rA on AMV DNA Polymerase Using Synthetic Substrates

Additions	[^{3}H] -TMP Incorporated (DPM)
Poly rA	88
ε-poly rA	2
Poly rA + dT_{12-18}	25,428
ε-poly rA + dT_{12-18}	1,055
Poly rA + dT_{12-18} + ε-poly rA	2,855

Assays were performed as described in Methods. When testing the effect of modification on poly rA, the concentration of the modified and unmodified polymers were equal (0.041 A_{260} units per assay). In experiments where ε-poly rA was tested as an inhibitor its concentration was three fold in excess (0.12 A_{260} units per assay) of that of the template.

studies by measuring the effects of ε-poly rA on the 70S RNA directed DNA synthesis. The results which are summarized in (Table II) indicate that ε-poly rA inhibited the unprimed 70S reaction to a greater extent than poly rA. By contrast, no such inhibition was observed in the presence of ε-poly rA in the oligo dT primed 70S RNA reaction.

In conclusion, this report introduces a novel inhibitor of AMV DNA polymerase. The fluorescent polymer cannot base pair with the corresponding oligonucleotides. For this reason it may have potential use as an antiviral compound. These studies have served as a model for limited modification of poly rA stretches of 70S AMV RNA in an attempt to determine

TABLE II. Effect of ε-Poly rA on AMV DNA Polymerase Using 70S AMV RNA as template.

Additions	$[^3H]$ -TMP Incorporated (DPM)
70S RNA	8,811
70S RNA dT_{12-18}	59,291
70S RNA + poly rA	1,949
70S RNA + dT_{12-18} + poly rA	81,713
70S RNA + ε-poly rA	646
70S RNA + dT_{12-18} + ε-poly rA	61,235

Assays were performed as described in materials and methods. Each assay (100 µl) contained 0.041 A_{260} units of 70S RNA. Concentration of other nucleic acids added was the same in all experiments (0.24 A_{260} units). Oligo dT_{12-18} was added to 70S RNA prior to other components.

its biological significance. Such studies have been completed and will be published elsewhere.

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