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A VINYL POLYMER WITH PURINE RESIDUES DEFICIENT IN BASE PAIRING INHIBITS MURINE LEUKEMIA VIRUS REPLICATION

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SUMMARY

Poly(9-vinylpurine), a polynucleotide analog with purine residues unable to form complete base-pairs, inhibited replication of murine leukemia virus (MLV) without changing the growth rate of the host cells. Poly(9-vinylpurine) was also an inhibitor of *in vitro* activity of MLV DNA polymerase. The amount required for 50% inhibition depended upon the kind of template-primer that was used in the assay. But inhibition was not specific to the virus DNA polymerase, since the cellular DNA polymerases α and γ were also inhibited. Poly(9-vinylpurine) was capable of complex formation with poly(rA). However an analog, with an N,N-dimethylamino group substituted in the 6 position of the purine ring, did not form a complex with poly(rA) and did not inhibit virus replication.

INTRODUCTION

A majority of pharmacologically important nucleoside and nucleotide analogs contain residues that form complete base pairs with two or more hydrogen bonds (1). For example, we had found previously, that poly(9-vinyladenine), which forms base pair complexes with polynucleotides, inhibited replication of murine leukemia virus in cell culture without changing the growth rate of the host cells and inhibited *in vitro* replication of nucleic acid in a template specific manner (2,3). Nevertheless, there are several compounds lacking the capacity to form complete base pairs that have pronounced biological effects; such as the antibiotic puromycin containing the 6-dimethylaminopurine moiety and nebularine containing the purine moiety (1).

In the current study we prepared a new vinyl polymer, poly(9-vinylpurine), containing purine residues that can contribute only one hydrogen bond in a base pair. Unlike poly(9-vinyladenine), this compound does not form hypochronic

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complexes when mixed with poly(rU). However, we found that poly(9-vinylpurine) exerts biological effects similar to those of poly(9-vinyladenine). Thus, a vinylpolymer can exhibit the characteristic inhibitions of virus replication and DNA polymerase activity without the ability to form complete base-pairs.

MATERIALS AND METHODS

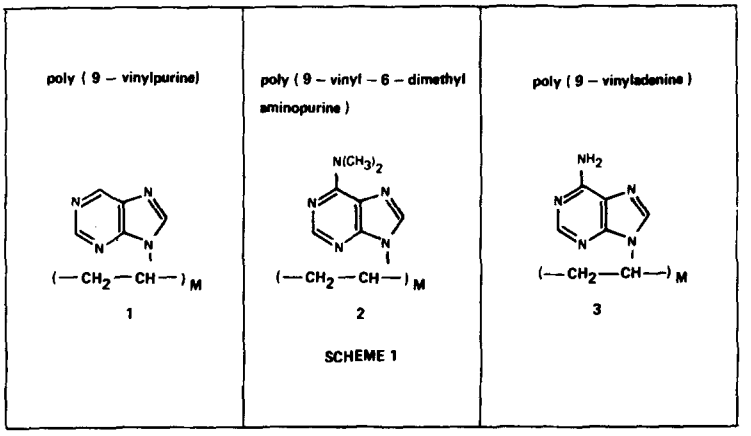
Synthesis of the monomers, 9-vinylpurine, and 6-dimethylaminopurine, has been described (4, 5). Polymers were prepared in 60-70% yield from the respective monomers by polymerization (100° for 2-12 hr) initiated with sodium persulfate and then purified by exhaustive dialysis. The molecular weights of the two vinyl polymers were estimated by electrophoresis in polyacrylamide gels in the presence of sodium dodecyl sulfate (6). Relative migration of the polymers, measured by UV absorption, was essentially identical. About 70% of both polymers had lower mobility than the protein standard of 68,000 daltons and over 95% had lower mobility than the standard of 13,700 daltons. Polymer 1 has a UV absorption maximum at 262 nm, ε4400; polymer 2 has a UV absorption maximum at 264 nm, ε9800 (10 mM sodium phosphate buffer at pH 7). Interaction of polymers with polynucleotides in solution was studied by UV absorption and the continuous variation method as described (2). For the immobilization of poly(rA), BrCN-activated Sepharose was used (7), and attachment of polymer was followed by UV absorption of the supernatant after overnight equilibration at room temperature.

The preparation of DNA polymerases alpha and gamma from mouse myeloma and DNA polymerase beta from mouse liver was as described (8). The viral DNA polymerase was the generous gift of A.K. Bandyopadhyay and was prepared as described previously (9). To obtain the data in Table II, reaction mixtures were incubated at 37° for 20 min in a final volume of 10 µl; all the mixtures contained 50 mM Tris-HCl, 20% glycerol, 1 mM dithiothreitol, and 80 µg/ml of template. Reactions with poly (dT) as template were at pH 7.7 and, in addition to the above components, contained 6 mM magnesium acetate, 40 µg/ml oligo(rA), 40 mM KCl and 100 µM [^{3}H]dATP (11,000 cpm/pmole). Reactions with poly(rA) as template were at pH 8.1 and contained 0.5 mM $MnCl_2$, 16 µg/ml oligo(dT), 100 mM KCl, and 15 µM [^{3}H]dTTP (11,000 cpm/pmole). Acid-insoluble radioactivity was measured as described previously (8, 10). The data in Figure 2 were obtained using reaction mixtures containing 16 µg/ml template, 8 µg/ml primer, 50 mM Tris-HCl, pH 8.3, 60 mM NaCl, 20 mM dithiothreitol, 0.05% Triton X-100, 1 mM $MnCl_2$ and murine leukemia virus (Moloney strain) at final protein concentration of 20 µg/ml. Reaction products were measured as described previously (8).

The cell toxicity and antiviral activity of polymers were measured using AKR-2B cells plated in 90% McCoy's medium and 10% fetal bovine serum at a concentration of 3.5×10^5 cells per petri dish (60 mm diameter). For antiviral testing (12, 13): At 24 hr after plating, the cell monolayer was treated with DEAE-dextran (20 µg/ml) at 37° during the period from 3 hr to 2 hr before the infection. Then the cells were washed and incubated, either with the medium containing polymer or with medium alone for 2 hr at 37°. After washing, the cells were infected with AKR-L1 virus at a multiplicity given in Table II, and after 2 hr at 37° the cells were washed and layered-over with medium alone or with medium containing polymer. In experiment 1, the cell monolayer was used directly as a basal layer in the UV-XC test which was performed 5 days after infection. In experiment 2, the yield of virus released into the medium was determined 36 hr post-infection. In the measurement of [^{3}H] virus uptake, cells were treated with DEAE-dextran as above and then with medium containing polymer 1 as described above. Cells were then exposed to medium containing [^{3}H]uridine-labeled virus for a given period at 37°.

RESULTS

The structures of poly(9-vinylpurine) - designated polymer 1 - and the purine ring 6 position substituted analog - designated polymer 2 - are shown in scheme 1. No evidence for hypochromic complex formation was found when a mixture of polymer 1 and either poly(rU) or poly(rA) was examined by the continuous variation method (2). However, polymer 1 is capable of complex formation with poly(rA), since it was retained on a column of poly(rA)-Sepharose much more strongly than on a column of unsubstituted Sepharose. Complex formation between poly(rA) and poly(9-vinyladenine)-polymer 3 in scheme 1 - has been reported (2), and binding of another polynucleotide analog had previously been detected using similar methods (2, 14). Polymer 2 also did not form hypochromic complexes with either poly(rU) or poly(rA), however it was not retained on either poly(rA)-Sepharose or unsubstituted Sepharose.



SCHEME 1

Inhibition of Virus Replication. We found that polymers 1 and 2 did not alter the growth of mouse AKR cells in culture when the cells were exposed continuously for three days at the polymer concentrations used for anti-viral testing. As shown in Table I polymer 1 inhibited the production of infectious virus after these cells had been infected with murine leukemia virus (MLV). Polymer 2 did not reduce production of infectious virus (Table I). The inhibition by polymer 1 did not appear to be at the level of virus uptake

Table I

Inhibition of Murine Leukemia Virus Replication in AKR Cells by Poly(9-vinylpurine)

Experiment	Polymer Added	Period of Polymer Treatment (hr relative to infection)	Virus Yield (pfu per plate)	% (of control)
I [a]	None	-	300	(100)
	250 µg/ml Poly(9-vinylpurine)	-2 → 0	52	17
	500 µg/ml Poly(9-vinylpurine)	-2 → 0	34	11
	250 µg/ml Poly(9-vinylpurine)	2 → 24	76	25
	500 µg/ml Poly(9-vinylpurine)	2 → 24	54	18
II [b]	None	-	3×10^5	(100)
	150 µg/ml Poly(9-vinylpurine)	-2 → 0	0.9×10^5	30
	150 µg/ml Poly(9-vinylpurine)	2 → 24	1.9×10^5	63
	190 µg/ml Polymer 2	-2 → 0	3.7×10^5	123
	190 µg/ml Polymer 2	2 → 24	3.6×10^5	120

[a] Virus infection was at 10^{-3} pfu/cell; virus yield was measured directly with the cells as the basal layer in the UV-XC test 5 days after infection.

[b] Virus infection was at 1 pfu/cell; virus released into the medium was measured 36 hr after infection by the UV-XC test in SC-1 cells.

by the cells, since polymer 1 slightly increased absorption of radiolabeled virus (Figure 1); furthermore, as shown in Table I, inhibition of viral replication was observed even when the polymer was added after viral absorption. Comparable antiviral activity of polymer 3 has already been reported, and a

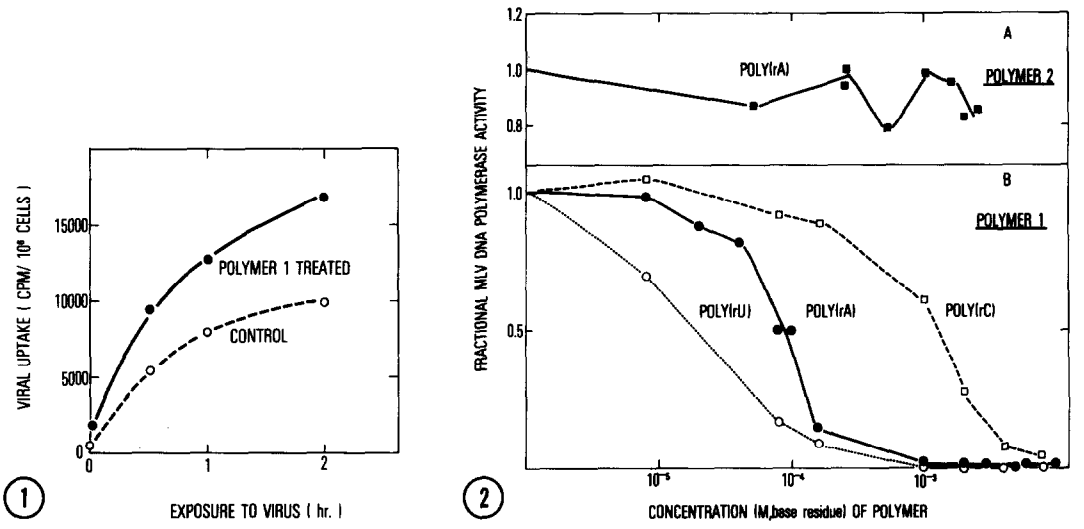


Fig. 1. The effect of 150 µg/ml poly(9-vinylpurine) on the uptake of [^{3}H] murine leukemia virus by AKR-2B cells.

Fig. 2. The effect of polymers 1 and of polymers 2 on the DNA polymerase activity of disrupted murine leukemia virus. Rates of enzyme activity were measured either with poly(rA) ___, poly(rU)..., or poly(rC) --- as the template and a base-pair complementary deoxyoligonucleotide as the primer. Molar concentrations of the vinylpurine monomer in the final reaction mixtures are shown on the abscissa, i.e., 10^{-4} M polymer 1 equals to 14.6 µg/ml.

review of the data from equivalent testing systems indicates that polymers 1 and 3 exhibit greater inhibition than poly(rU) and about the same as poly(rA) (3).

Inhibition of DNA Polymerase Activities. To obtain some insight into the biological action of polymer 1, its effect upon in vitro activity of DNA polymerases from MLV and also from murine cells was studied. In the first set of experiments, the effect of polymer 1 or polymer 2 on the activity of unpurified MLV DNA polymerase was compared (Fig. 2). Polymer 1, but not polymer 2, was a potent inhibitor when poly(rA)•oligo(dT) was the template-primer. In reactions containing poly(rU)•oligo(dA) as template-primer, polymer 1 inhibited even more efficiently, whereas inhibition was much less in reactions containing poly(rC)•oligo(dG) as template-primer. It had been found previously (11) that polymer 3 inhibited MLV DNA polymerase in reactions containing either poly(rA)•oligo(dT)

Table II

Comparison of the Effect of Poly(9-Vinylpurine) on the Activity of MLV and Mouse Cellular DNA Polymerases

Template-Primer, Substrate and Inhibitor		-Amount of DNA Formed[a]- DNA Polymerase			
		Alpha	Beta	Gamma	MLV
			Percent		
A. Poly(rA)•oligo(dT), dTTP					
Control		N.A.[b]	100(0.4)	100(1.4)	100(7)
Poly(9-vinylpurine)	10 µg/ml	-	151	91	90
	50 µg/ml	-	208	38	72
	100 µg/ml	-	91	45	56
B. Poly(dT)•oligo(rA), dATP					
Control		100(16)	100(0.3)	100(0.9)	N.A.[b]
Poly(9-vinylpurine)	10 µg/ml	89	140	98	-
	50 µg/ml	45	130	63	-
	100 µg/ml	36	100	49	-

[a] Values representing 100% are shown in parentheses and are pmoles [^{3}H]dNMP incorporated per reaction.

[b] N.A. = Not active under this reaction condition.

or poly(rU)•oligo(dA) as template-primer, but not in reactions containing poly(rC)•oligo(dG). In a second set of experiments with poly(dT) as template, polymer 1 inhibited the cellular DNA polymerases alpha and gamma, but did not inhibit the cellular beta-polymerase (Table II). With poly(rA) as template both the cellular gamma-polymerase and the virus DNA polymerase were inhibited, whereas the beta-polymerase was stimulated at low concentrations and was not significantly inhibited at the highest concentration tested.

DISCUSSION

DNA replication *in vitro* and the replication of murine leukemia virus are inhibited by a number of polyanions that appear to interact with a crucial site on a polymerase (15). Inhibition of *in vitro* DNA replication by polymers 1 and 3 probably occurs through blocking of the template rather than through a direct interaction with the DNA polymerase, because the inhibition is sensitive

to the kind and amount of template used (10). Both polymers are capable of complex formation with poly(rA). The ring substituted analog, polymer 2, does not complex with poly(rA) and does not inhibit virus replication. There is the suggestion from these findings that the antiviral action of polymers 1 and 3 may be due to interaction with viral nucleic acid and that this interaction can occur even when complete Watson-Crick type base pairs could not be formed.

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