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SELECTIVITY OF ANTITEMPLATES AS INHIBITORS OF DEOXYRIBONUCLEIC ACID POLYMERASES*

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Abstract—DNA polymerase α from calf thymus was relatively insensitive to the action of partially thiolated polycytidylic acid (MPC) which had been shown previously to be a potent inhibitor of the corresponding enzyme from regenerating rat liver, competitive with the activated DNA template. In contrast, partially thiolated polyuridylic acid (MPU) strongly inhibited the calf thymus enzyme as well, but showed non-competitive kinetics with respect to the activated DNA template. The much more potent inhibitory activity of MPU compared to MPC was attributed to the less rigid conformation of the former. Methyl substitution on the 5-mercapto groups of MPU substantially decreased but did not abolish its inhibitory activity. MPU was also a potent inhibitor of the herpes virus (HSV-1) induced DNA polymerase which, too, showed little sensitivity toward MPC; in this case, the inhibition by MPU was uncompetitive with respect to the DNA template. In preliminary experiments, MPU showed significant (61%) inhibition of the replication of HSV-1, while MPC was inactive. The results demonstrate that the inhibitory activity of partially thiolated synthetic polynucleotides toward certain DNA polymerases is dependent on the base composition.

In our previous work, we demonstrated that the introduction of 5-mercapto groups into some of the cytosine and/or uracil bases of various polynucleotides converted the latter into effective competitive-inhibitory analogues of the functional templates (i.e. "antitemplates") of various DNA and RNA polymerases [1–3]. Potent inhibitory effects of the partially thiolated polynucleotides were noted *in vitro* against DNA-directed RNA polymerases (EC 2.7.7.6) of bacterial [2, 4] as well as mammalian sources [5], and against some *N*-ethylmaleimide (NEM)§ sensitive DNA polymerases such as the DNA polymerase α (EC 2.7.7.7) from regenerating rat liver [6] and the "reverse transcriptases" (EC 2.7.7.7) of oncorna viruses [3, 7, 8]. In comparison, the DNA polymerase β from either regenerating or normal rat liver [6] (which is not sensitive to NEM) was only weakly inhibited, and the DNA polymerase I of *Escherichia coli* (which contains no SH-group at its active site) was not inhibited at all [8] by these modified macromolecules. In these earlier studies, the various types of partially thiolated polynucleotides showed only moderate quantitative differences among themselves; generally, the thiolated tRNA and DNA were the most potent inhibitors at low levels of thiolation (1–3%), while the partially thiolated polycytidylic acid (MPC) and polyuridylic acid (MPU) showed comparable activities only at a higher (7–10%) level of thiolation [4, 6, 8]. Although the unmodified poly(U) itself acted as a weakly inhibitory template analogue in several polymerase systems, particularly certain reverse transcriptases [9], and unmodified poly(C) was either inactive or had a stimulatory effect on the polymerization reaction, both homopolynucleotides became effective inhibitors upon thiolation. Thus, it appeared that the potent inhibitory activities of both MPU and MPC in the previously studied enzyme systems were largely dependent on the binding ability of their ionized 5-SH groups to the active site of the polymerase via ionic or disulfide bond formation.

Because of its well characterized, relatively homogenous structure [10], MPC had been initially selected for biological studies. ^{35}S-Labeled MPC and its double-stranded complex with poly(I) were shown to be taken up *in vitro* by tumor cells [11, 12] and *in vivo* by animal tissues [13]. Both *in vitro* and *in vivo*, MPC decreased the colony-forming ability of murine leukemia bone marrow and spleen cells [14]. Although we failed to demonstrate its activity by the standard L1210 screening test, some other types of biological activities were observed and preliminary clinical trials have been conducted with MPC by Kornhuber and Chandra in Germany against acute

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§ Abbreviations: NEM, *N*-ethylmaleimide; MPU, partially thiolated polyuridylic acid; MPC, partially thiolated polycytidylic acid, MeMPU, polyuridylic acid containing 5-methylmercaptouridylate units; ME, 2-mercaptoethanol; HSV-1, herpes simplex virus type 1; and PFU, plaque forming units.

lymphocytic leukemia in children, and some positive therapeutic results have been reported [15, 16].

More recently, we completed our studies of the enzymatic synthesis and physico-chemical properties of MPU [17] and its *S*-methyl derivative, MeMPU [18]. Results reported in the present paper will show that these modified polynucleotides and MPC show significant quantitative and qualitative differences among themselves in their activities as antitemplates toward DNA polymerases from various sources.

MATERIALS AND METHODS

Materials

The sodium salts of the 2′-deoxyribonucleoside-5′-triphosphates were purchased from P-L Biochemicals, Inc., Milwaukee, WI; [methyl-^{3}H]thymidine-5′-triphosphate tetrasodium salt was obtained from ICN Pharmaceuticals, Chemical and Radioisotope Division, Irvine, CA. Frozen calf thymus gland was a product of Pelfreeze Biologicals, Rogers, AR. Nonidet-P40, a product of the Shell Oil Co., was purchased from Particle Data Inc., Elmhurst, IL. All glassware was siliconized using Prosil-28 organosilane surface treating agent (PCR Research Chemicals, Gainesville, FL). The potassium salt of polycytidylic acid was purchased from Miles Laboratories, Inc., Elkhart, IN. The potassium salt of polyuridylic acid was a product of P-L Biochemicals, Inc.

Activated DNA and modified polynucleotides

Calf thymus DNA and pancreatic DNase were purchased from the Worthington Biochemical Corp., Freehold, NJ. Calf thymus DNA was activated with pancreatic DNase as previously described [6].

Partially thiolated polynucleotides were prepared as previously described [4, 17, 19]. Briefly, the polynucleotides were treated, in the form of their hexadecyltrimethylammonium salts, with methyl hypobromite followed by sodium hydrogen sulfide. In some cases, MPC was prepared by the direct bromination of potassium polycytidylate in aqueous solution followed by treatment with sodium hydrogen sulfide.

Poly(U) containing 5-methylmercaptouridine moieties [18] was prepared enzymatically, using polynucleotide phosphorylase of *Micrococcus luteus*, essentially according to the method previously described for the enzymatic synthesis of MPU [17]. Various ratios of uridine-5′-diphosphate and 5-methylmercaptouridine-5′-diphosphate were polymerized, and the products were purified, characterized, and utilized for biochemical studies (details will be published).

Purification of DNA polymerases

Calf thymus DNA polymerase α: DNA polymerase α from calf thymus gland was prepared according to the method of Chen *et al.* [20] which had been developed originally for the isolation of the DNA polymerase α from mouse myeloma cells. The procedure was followed through the DEAE-cellulose step starting with 55 g of calf thymus gland. The crude extract obtained was batch absorbed to hydroxylapatite which had been pre-equilibrated in buffer B (10 mM potassium phosphate, pH 6.5; 250 mM KCl; 0.1 mM EDTA; 1.0 mM dithiothreitol). The hydroxylapatite was washed in 3 vol. of buffer B and the enzyme eluted with buffer C (150 mM potassium phosphate, pH 7.0; 250 mM KCl; 0.1 mM EDTA; 1 mM dithiothreitol). The enzyme fraction from the hydroxylapatite batch absorption step was dialyzed vs buffer D [150 mM potassium phosphate, pH 7.0, in 1 mM dithiothreitol; 0.1 mM EDTA; 20% (v/v) glycerol]. The enzyme preparation was then applied to a DEAE-cellulose column pre-equilibrated in buffer D. The column was washed with 2 column volumes of buffer D, and the DNA polymerase α was eluted as one large peak with an 80–400 mM linear gradient of KCl in buffer D. The peak of enzyme activity was pooled and stored in aliquots at −20° (sp. act. 150 units/mg protein). The enzyme was determined to be DNA polymerase α, devoid of any DNA polymerase β activity based on its complete inhibition by 2 mM *N*-ethylmaleimide, or strong inhibition by NaCl (at concentrations greater than 25 mM) after the DEAE-cellulose step.

HSV-1 induced DNA polymerase. Mouse LB cells were infected with 3.2×10^8 PFU/ml of HSV-1 strain KOS. Cells were harvested 8 hr post-infection and collected by centrifugation and stored in liquid nitrogen until used.

The viral-induced DNA polymerase was isolated according to the procedure of Powell and Purifoy [21]. However, the final DNA cellulose step was later omitted, because the highly purified enzyme obtained after this step (sp. act. 1800 units/mg) was found to be too unstable for our purposes. The peak of activity from the DEAE-cellulose column was dialyzed vs DE buffer [25% glycerol (v/v); 0.2% (v/v) Nonidet-P40; 50 mM Tris–HCl, pH 7.5; 0.5 mM dithiothreitol] and immediately applied to a phosphocellulose column which had been pre-equilibrated in DE buffer. The column was washed with 2 column volumes of DE buffer and the enzyme eluted from the column in a linear 100–400 mM KCl gradient. The peak of enzyme activity was pooled and dialyzed vs DE buffer containing 50 mM KCl and stored in aliquots at −20°. The specific activity of the enzyme after this step was determined to be 72 units/mg protein. The enzyme was confirmed to be HSV-1 induced DNA polymerase based on its requirement for a high salt concentration in the assay mixture and its sensitivity to phosphonoacetic acid [22]. Assays conducted at low salt concentration [in the absence of $(NH_4)_2SO_4$] showed no enzyme activity, indicating the absence of cellular DNA polymerase α, both in the crude extract and in the DEAE-cellulose fraction.

Enzyme assays

DNA polymerase α: In a total volume of 120 μl were contained: 21 mM Tris–HCl, pH 7.6; 2 mM dithiothreitol; 17 μg/ml of bovine serum albumin; 2 mM $Mg(OAc)_2$; 39 μg of activated calf thymus DNA; 83 μM each of dATP, dCTP, dGTP and dTTP; 20 cpm/pmole of [methyl-^{3}H]dTTP; 0.8 units of enzyme preparation; and the appropriate polynucleotide. Samples were incubated for 45 min at 37°. The reaction was stopped by cooling the tubes

in ice and adding 100 μl of ice-cold 50% (w/v) trichloroacetic acid and then 2 ml of ice-cold 5% trichloroacetic acid, 10 mM sodium pyrophosphate. The acid precipitate was collected on glass fiber filters and processed for radioactivity counting as previously described [6].

HSV-1 induced DNA polymerase. In a total volume of 200 μl were contained: 0.1 mM or 0.25 mM each of dCTP, dATP, dGTP and dTTP; 12–48 cpm/pmole of [methyl-^{3}H]dTTP, or alternatively 30 cpm/pmole of [5-^{3}H]dCTP; 3 mM $MgCl_2$; 100 mM Tris–HCl, pH 8.2; 90 mM $(NH_4)_2SO_4$; 5 mM dithiothreitol; 0.35 units of enzyme; 98 μg of activated calf thymus DNA; and the appropriate polynucleotide. The reaction mixture was incubated for 30 min at 37°. Samples were processed as described above. One unit of enzyme activity equals one nmole of dTMP incorporated per hour.

Protein assays

Protein concentrations were determined using a fluorescent assay as described by Udenfriend *et al.* [23].

Herpes virus inhibition assay

Antiviral activity was determined quantitatively by measuring the amount of virus produced by infected cells during a single cycle of infection in the presence of the compound of interest. Briefly, monkey kidney (CV-1) cells were infected with 20 PFU/cell of herpes simplex virus, type 1 (HSV-1), strain KOS. One hour after infection, cells were rinsed with medium and treated for 30 min with medium made 1% in anti-HSV-1 rabbit antiserum to neutralize unpenetrated virus. Cells were then rinsed with medium and cultured in medium containing the compound of interest. One sample without compound was collected and frozen 4 hr after infection to provide a baseline for measuring net virus production. The remaining samples were collected and frozen 18–20 hr after infection. Virus was titrated in CV-1 cells by a plaque assay as previously described [24].

RESULTS

The effects of unmodified and partially thiolated polycytidylic acid [poly(C) and MPC respectively] on the DNA polymerase α from calf thymus, in the presence of an activated calf thymus DNA template, are shown in Fig. 1. In view of the previously observed potent inhibitory action of MPC on the DNA polymerase α from regenerating rat liver [6], it is surprising to find that this modified polynucleotide had relatively little effect upon the corresponding enzyme from calf thymus. A different MPC sample, containing an unusually high ratio (16%) of 5-mercaptocytidylate units, was considerably more effective, but it still gave only 75% inhibition even at a concentration as high as 250 μM. The unmodified poly(C) showed the same lack of inhibitory activity in this system as it did in all other DNA polymerase systems in which it has been tested thus far [6, 8].

The unmodified poly(U) was only weakly inhibitory toward the DNA polymerase α from calf thymus. However, the partially thiolated polyuridylate

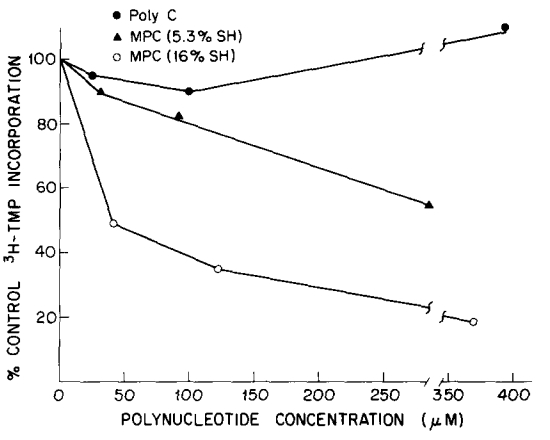

Fig. 1. Effects of polycytidylate and MPC on the calf thymus DNA polymerase α. The polynucleotide was added to the reaction mixture prior to addition of the enzyme. Activated calf thymus DNA was used as the template-primer. Enzyme activity in the presence of various concentrations of the inhibitor is expressed as percentage of [^{3}H]TMP incorporated into acid-insoluble radioactivity relative to a control (100% ≥ 4000 cpm) containing no polynucleotide. All assays were run in triplicate.

(MPU), at either 10.5% or 15.0% 5-mercaptouridylate content, was found to be a potent inhibitor as it caused 75% inhibition at a concentration of 10 μM and essentially full inhibition at 50 μM (Fig. 2).

Kinetic analysis of the inhibitory effect of MPU was performed by graphical methods. Dixon plots of the reciprocal of the reaction rate vs the MPU concentration, at various fixed DNA template concentrations (Fig. 3), indicated that the inhibition was non-competitive with respect to the DNA template. Three different sets of experiments yielded, for MPU, an average K_i value of 4.9 μM. Essentially the same result was obtained when the "single-curve plot" method ("Type F") recommended by Webb [25] was employed, which gave a horizontal line

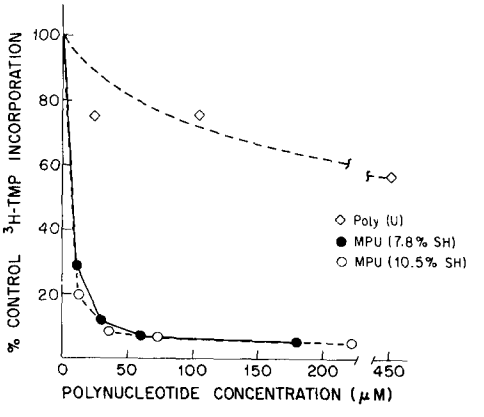

Fig. 2. Effects of MPU and poly(U) on calf thymus DNA polymerase α with activated calf thymus DNA as the primer-template. Enzyme activity is expressed as the percentage of control containing no polynucleotide. The assays were run in triplicate.

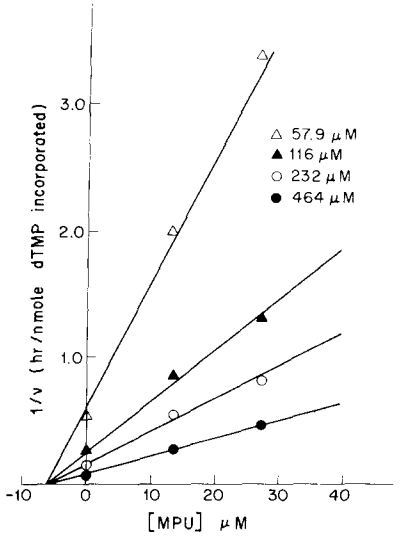

Fig. 3. Dixon plots of the reciprocal of velocity of dTMP incorporation into acid-insoluble radioactivity by calf thymus DNA polymerase α vs MPU concentration, in the presence of the following concentrations activated calf thymus DNA template: 57.9 μM; 116 μM; 232 μM; and 464 μM. All samples were run in triplicate.

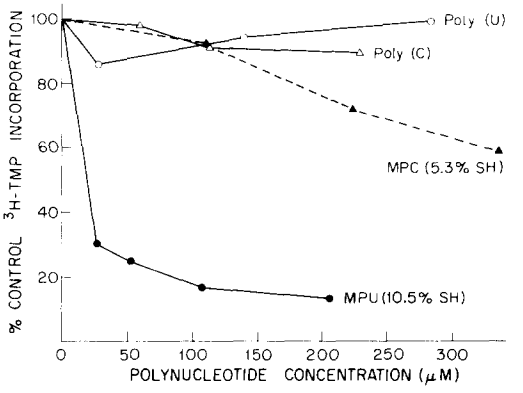

Fig. 5. Effect of polycytidylate, polyuridylate, MPC and MPU on HSV-1 induced DNA polymerase. The polynucleotides were added to the reaction mixture prior to the addition of enzyme. Activated calf thymus DNA served as the template-primer. In this experiment, the concentration of each triphosphate substrate was 0.25 mM [21], and [^{3}H]dTTP was used as the labeled substrate. Enzyme activity is expressed as the percentage of [^{3}H]TMP incorporated into acid-insoluble radioactivity relative to a control (100% ≥ 2000 cmp) containing no polynucleotide. All assays were run in triplicate.

consistent with non-competitive inhibition, regardless of whether the DNA template was considered as the variable substrate (S) or an activator (A) (see Ref. [25], Fig. 5-3F or 5-11F, respectively).

Replacement of the 5-mercapto groups of MPU with 5-methylmercapto groups (either by *S*-methylation of MPU or by enzymatic copolymerization of the diphosphates of uridine and 5-methylmercaptouridine, respectively [18]) dramatically decreased the inhibitory activity; a MeMPU sample containing 9% methylmercaptouridylate units, poly(ms^5U_9,U_{91}), showed even less inhibitory activity than the unmodified poly(U). However, on increasing the ms^5U:U ratio of the MeMPU copolymer, the inhibitory activity increased. Thus, MeMPU containing 26% of the modified monomer, poly(ms^5U_{26},U_{74}), gave 50% inhibition at 100 μM, while the 100% modified MeMPU homopolymer, poly(ms^5U), gave 50% inhibition at 15 μM and 85% inhibition at 100 μM in the calf thymus DNA polymerase α system (Fig. 4).

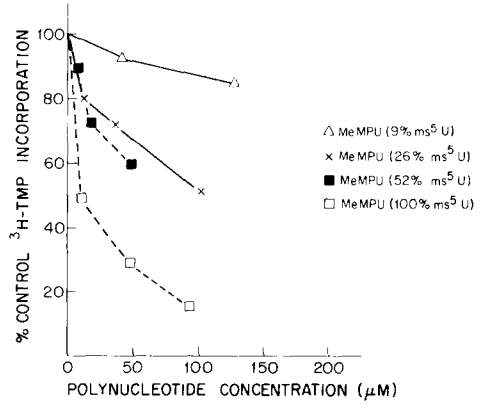

Fig. 4. Inhibition of calf thymus DNA polymerase α by MeMPU. MeMPU with various ms^5 U:U ratios was added prior to addition of the enzyme. Activated calf thymus DNA was used as the primer-template. Enzyme activity is expressed as the percentage of control (no MeMPU). Assays were run in triplicate.

The effects of MPC and MPU, as well as their unmodified counterparts, poly(C) and poly(U), respectively, on the DNA polymerase induced by herpes simplex type 1 virus in mouse LB cells are shown in Fig. 5. Again, activated calf thymus DNA was used as the primer-template. The partially purified enzyme was unaffected by either poly(C) or poly(U) and was only slightly inhibited by MPC. In contrast, MPU was found to be a potent inhibitor of the HSV-1 induced DNA polymerase, producing 70% inhibition at 25 μM and 85% inhibition at 100 μM. The same results were obtained whether the concentration of the triphosphate substrates was 0.1 mM [22] or 0.25 mM [21] each, and whether [^{3}H]dTTP or [^{3}H]dCTP was used as the labeled substrate. Based on the I_{50} values, MPU was about fifty times less active in this system than the potent antiherpes agents, phosphonoacetic and phosphonoformic acids [22]. However, in this comparison, the I_{50} value of MPU was expressed, as usual, in molar concentration based on the molecular weight of the *monomeric* uridylate unit rather than that of the macromolecule. Based on the actual molecular weights of the polymers (>10^5 daltons), all molar concentration data (including I_{50} values) given for these polynucleotides in the present paper would have to be reduced by approximately three orders of magnitude. Thus, one molecule of MPU corresponds in its inhibitory effect to about twenty molecules of phosphonoacetic acid.

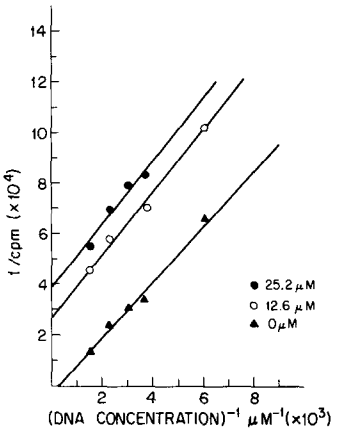

Fig. 6. Double-reciprocal plots of HSV-1 induced DNA polymerase activity with activated calf thymus DNA as the variable substrate. The triphosphate substrates were at 0.25 mM each, to ensure saturation level concentrations [22]. MPU was added to the reaction mixture prior to the addition to the enzyme. The velocity of the reaction is expressed as the total cpm incorporated into acid-insoluble radioactivity per hour. All assays were run in triplicate.

Double-reciprocal plots of the rates of reaction catalyzed by HSV-1 induced DNA polymerase vs variable concentrations of the activated calf thymus DNA template at two fixed concentrations of MPU as the inhibitor (Fig. 6) indicated that the inhibition was uncompetitive. Plotting the same data according to the "single-curve plot" method (Type F, see above) would give at the 25.2 μM concentration a hyperbolic curve consistent with the above conclusion (Ref. [25], Fig. 5-7F).

In a preliminary study of the effects of these polynucleotides on the replication of herpes simplex type 1 virus, MPU gave 41% inhibition at the highest concentration tested (3×10^{-4} M, based on the molecular weight of an average nucleotide unit, or approximately 3×10^{-7} M based on the actual molecular weight of the polymer). However, addition of 2-mercaptoethanol (ME) increased the inhibition by MPU to 61% as compared to the ME control. None of the other polynucleotides tested [poly(U), poly(C), MeMPU] caused measurable inhibition of the viral replication at the concentrations employed.

DISCUSSION

The results obtained in the present study indicate that the calf thymus DNA polymerase α is significantly different from the corresponding enzyme of regenerating rat liver [6] with respect to its sensitivity to various antitemplates, notably partially thiolated polynucleotides. While the latter was fully inhibited by either MPC or MPU and showed approximately the same dose response to both, the calf thymus enzyme was relatively insensitive to MPC but was strongly inhibited by MPU even at very low concentrations of this polynucleotide. Both of these enzymes were inhibited by *N*-ethylmaleimide (NEM), which indicated that they require a free SH-group at their active sites. However, the position of this SH-group relative to the template site may be different due to differences in the subunit structure [26] and conformation between these two α-polymerases. As the enzymic SH-group presumably participates in the binding of the partially thiolated polynucleotides via mixed disulfide bond formation, the relatively low inhibitory activity of MPC toward the calf thymus DNA polymerase α could be explained if the SH-groups of this enzyme were not appropriately juxtapositioned with the thiol groups of MPC molecules that may be reversibly bound at the template site, weakly competing with the DNA template.

It should be pointed out that MPC has an organized secondary structure [10] which makes it more difficult for this macromolecule to interact with any unfavorably positioned thiol group on the enzyme surface. In contrast, MPU has no secondary structure; therefore, it is much more flexible than MPC and could almost as readily react with the enzymic SH-group as the small molecule NEM.

The binding of the MeMPU antitemplates to the DNA polymerase α is, of course, not explicable by interaction with the SH-groups of the enzyme. However, 5-methylmercaptouracil is well capable of complexing with metal ions, particularly Zn^{2+} [18] which is an important constituent of many DNA and RNA polymerases. Such chelating interaction may explain the significantly greater inhibitory activity of poly(ms^5U) as compared to that of poly (U).

These results indicate that the antitemplate selectivity of partially thiolated polynucleotides towards certain DNA polymerases may be manipulated by changing their base composition. Further studies are aimed at exploring the effects of varying the base sequences [1, 27]. It is hoped that, by exploiting the structure–activity relationships of this novel class of inhibitors, their selectively may be substantially increased further.

Our preliminary results relating to the effect of MPU on the viral replication of HSV-1 are of interest. Although MPU may not appear to be a promising antiherpes agent *per se*, these results indicate that the antitemplate activities of partially thiolated polynucleotides toward a viral enzyme may be relevant to their abilities to inhibit viral replication. The *in vitro* and *in vivo* antiviral effects of single stranded polynucleotides in relation to their inhibitory activities toward the viral polymerases, particularly reverse transcriptases, have been reviewed recently [27]. Thus, the antitemplate approach to antiviral chemotherapy may be sound and worth pursuing further.

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REFERENCES

1. T. J. Bardos, in *Topics in Current Chemistry* (Managing Ed. F. L. Boschke), Vol. 52, p. 63. Springer, Berlin (1974).
2. T. J. Bardos, K. Baranski, P. Chakrabarti, T. I. Kalman and A. J. Mikulski, *Proc. Am. Ass. Cancer Res.* **13**, 359 (1972).
3. P. Chandra and T. J. Bardos, *Res. Commun. Chem. Path. Pharmac.* **4**, 615 (1972).

4. A. J. Mikulski, T. J. Bardos, P. Chakrabarti, T. I. Kalman and A. Zsindely, *Biochim. biophys. Acta* **319**, 294 (1973).
5. B. R. Munson, Y. K. Ho and T. J. Bardos, *Cancer Biochem. Biophys.* **2**, 105 (1978).
6. M. P. Kung, Y. K. Ho and T. J. Bardos, *Cancer Res.* **36**, 4537 (1976).
7. B. I. S. Srivastava, *Biochim. biophys. Acta* **335**, 77 (1973).
8. P. Chandra, U. Ebener, T. J. Bardos, P. Chakrabarti, Y. K. Ho, A. J. Mikulski and A. Zsindely, *Ann. N.Y. Acad. Sci.* **255**, 532 (1975).
9. F. W. Tuominen and F. T. Kenney, *Proc. natn. Acad. Sci. U.S.A.* **68**, 2198 (1971).
10. Y. K. Ho, R. J. Fiel, J. Aradi and T. J. Bardos, *Biochemistry* **18**, 5633 (1979).
11. V. Paffenholz, V. H. Le, Y. K. Ho and T. J. Bardos, *Cancer Res.* **36**, 1445 (1976).
12. Y. K. Ho, V. H. Le, V. Paffenholz and T. J. Bardos, *Proc. Am. Ass. Cancer Res.* **17**, 322 (1976).
13. M. P. Kung, Y. K. Ho, D. Lalka and T. J. Bardos, *Proc. Am. Ass. Cancer Res.* **22**, 223 (1981).
14. Y. K. Ho, H. D. Preisler and T. J. Bardos, *Cancer Res.* **39**, 3163 (1979).
15. P. Chandra, L. K. Steel, U. Ebener, M. Woltersdorf, H. Laube, B. Kornhuber, B. Mildner and A. Goetz, *Pharmac. Ther. (A)* **1**, 281 (1977).
16. B. Kornhuber and P. Chandra, *Antiviral Mechanisms in the Control of Neoplasia* (Ed. P. Chandra), Vol. 20, p. 577. Plenum Press, New York (1979).
17. Y. K. Ho, J. Aradi and T. J. Bardos, *Nucleic Acids Res.* **8**, 3175 (1980).
18. T. J. Bardos and Y. K. Ho, in *Chemistry and Biology of Nucleotides* (Eds. R. E. Harmon, R. K. Robins and L. B. Townsend), p. 55. Academic Press, New York (1978).
19. T. J. Bardos, L. Novak, P. Chakrabarti and Y. K. Ho, in *Nucleic Acid Chemistry* (Eds. L. Townsend and R. S. Tipson), Vol. 2, p. 881. John Wiley, New York (1978).
20. Y. C. Chen, E. W. Bohn, S. R. Planck and S. H. Wilson, *J. biol. Chem.* **254**, 11678 (1979).
21. K. L. Powell and D. J. M. Purifoy, *J. Virol.* **24**, 618 (1977).
22. B. Eriksson, A. Larson, E. Helgstrand, N. G. Johansson and B. Oberg, *Biochim. biophys. Acta* **607**, 53 (1980).
23. S. Udenfriend, S. Stein, P. Bohlen, W. Dairman, W. Leimgruber and M. Weigele, *Science* **178**, 871 (1972).
24. R. G. Hughes, Jr. and W. H. Munyon, *J. Virol.* **16**, 275 (1975).
25. J. L. Webb, *Enzyme and Metabolic Inhibitors*, Vol. 1, pp. 150–79. Academic Press, New York (1963).
26. G. Brun and F. Chapeville, *Biochem. Soc. Symp.* **42**, 1 (1977).
27. N. Stebbing, *Pharmac. Ther.* **6**, 29 (1979).