Stimulation of Rauscher Leukemia Virus DNA Polymerase DNA-directed DNA Synthesis by Cationic Trypanocides and Polyamines

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ABSTRACT

Activated DNA-directed DNA synthesis catalyzed by Rauscher leukemia virus (RLV) and other type C mammalian retroviral DNA polymerases is uniquely stimulated by biologically active polyamines. Cationic trypanocides may act as antagonists of polyamine function. As described here, several cationic trypanocides stimulate RLV polymerase-catalyzed DNA-directed DNA synthesis at concentrations significantly inhibiting eukaryotic DNA polymerases. Such stimulation is negated by polyamines. Kinetic analysis of the stimulation of RLV DNA polymerase by these structurally dissimilar cationic trypanocides (Antrycide, Burroughs-Wellcome Compound 64A, and Bayer Compound 1694) suggests that such stimulation is, in part, due to a drug:DNA structural interaction resembling the polyamine:DNA structural complex recognized by the RLV DNA polymerase.

INTRODUCTION

Polyamines are ubiquitous aliphatic organic cations present in living organisms and virions which complex with nucleic acids and are important for cellular replication. Type C mammalian retroviral DNA polymerases are uniquely stimulated in activated DNA-directed DNA synthesis by the polyamines spermine and spermidine. Stimulation by spermine occurs partly by a mechanism wherein the polymerase interacts with the polyamine:DNA structural complex. No other DNA polymerase is known to so recognize the polyamine:DNA complex. Recently, Bacchi et al. (2, 3) have shown that polyamine function in Trypanosoma brucei is antagonized by cationic trypanocides. Certain cationic trypanocides, although inhibiting eukaryotic DNA polymerases, stimulate RLV DNA polymerase in DNA-directed DNA synthesis (7). In this paper, we present a survey of cationic trypanocides in RLV DNA polymerase stimulation and show by kinetic analysis that stimulation by these compounds may involve a mechanism similar to that of polyamine:DNA complex recognition.

MATERIALS AND METHODS

Reagents. [3H]dTTP was obtained from New England Nuclear, Inc. Unlabeled deoxynucleoside triphosphates were obtained from P-L Biochemicals, Inc. Calf thymus DNA was purchased from Calbiochem-Behring. DNase I (pancreatic) was purchased as "RNase-free" from Worthington Biochemicals, Inc. Purified Rauscher leukemia virus was kindly provided by Dr. J. Cole through the office of Dr. J. Gruber of the National Cancer Institute. Activated DNA (nicked and gapped DNA) was prepared from calf thymus DNA by a modification of the method of Aposhan and Kornberg (1) using an additional step of phenol extraction and ethanol precipitation following inactivation of the DNase.

All of the following compounds were obtained as gifts: pentamidine [1,5-di(4-aminophenoxo)pentane di(2-hydroxyethanesulfonate)], isometamidium [7-(m-aminophenyl)diazooamine]-2-amino-10-ethyl-9-phenylanthridinium chloride-HCl], and amidobalbidil (3,3-diaminocarbiline disethionate) from May & Baker, Ltd.; Antrycide [(quinapyramine), (4-amino-6-methyl-4-pyrimid-4-ylamino)methylquinolinidinum-2-methyl sulfate], from Imperial Chemical Industries, Ltd.; imidocarb [3,3'-bis(2-imidazolin-2-yl)carbanilide dipropionate] and 64A [3,3',5,5'-tetra(2-imidazolin-2-yl)carbanilide-4HCl] from the Wellcome Research Laboratories; Berenil [(diminazene aceturate), p,p'-diamino(2-iminodioxazinobenzene dicacetate)] from Farbwerke Hoechst AG; Bayer 1894 [bis(3-methyl-1,2,3-triazolyl-1-yl)-4-amino)phenyl]urea-2-methylsulfate] from Farbenfabriken Bayer AG; DDUG [4,4'-diacetyldiphenylurea]bis(guanilhydrazone)] from Dr. Carl Porter, Roswell Park Memorial Institute; WR-199,385 [2,5-bis(guanilhydrazinyl)furur-2HCl] from the Walter Reed Army Institute of Research; and NSC 38280 [2-chloro-4,4'-bis(2-imidazolin-2-yl)perethalainilide-2HCl] from the National Service Center for Cancer Chemotherapy. MGBG was from Aldrich Chemical Co.; spermine was from Sigma Chemical Co.

DNA Polymerase Assays. Reactions were carried out in a total volume of 100 µl and consisted of 50 mM Tris-HCl, pH 7.8, 1 mM dithiothreitol, 10 µg bovine serum albumin, 5 mM MgCl2, and 100 ng RLV DNA polymerase purified by affinity chromatography on polycytidine:agarose as described (6). Unlabeled deoxynucleoside triphosphates were present individually at 200 µM and [3H]dTTP at 10 nM. Unless otherwise stated, 2.5 µg of activated DNA were used per assay, reactions were run at 37°for 30 min, and trichloroacetic acid-insoluble radioactivity was collected on glass-fiber filters and quantitated as described (6).

RESULTS

Effects of Various Cationic Trypanocides on RLV Polymerase-catalyzed DNA Synthesis. Drugs studied were tested at concentrations ranging from 2 to 1000 µM. The results of these experiments and structures of the bases are shown in Table 1. Antrycide, an asymmetric compound, effectively stimulates RLV DNA polymerase, as do the symmetrical compounds imidocarb, DDUG, Burroughs-Wellcome Compound 64A, and the bisquaternary heterooligobase Bayer 1694. Because of these findings, it is of interest to compare the compounds which stimulate RLV DNA polymerase with these structurally related compounds either ineffective as stimulants or effective only as inhibitors of RLV DNA polymerase in this system. Both imidocarb and 64A, symmetrical compounds with a ureido bridge between phenyl groups with imidazole ring substitutions at positions 3 and 3.5, respectively, stimulate the DNA polymerase reaction. Increasing
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Table 1

Cationic trypanocides and their effect on RLV DNA polymerase-catalyzed DNA synthesis

All compounds were initially dissolved in water or Tris-HCl, pH 7.8, with the exception of NSC 38280, which was first dissolved in 50% (v/v) dimethyl sulfoxide prior to dilution with aqueous solvent. Dimethyl sulfoxide controls showed no effect on enzyme activity. All compounds were tested on the DNA polymerizing systems at 2, 5, 10, 25, 50, 100, 300, 600, and 1000 M final concentration. Assays were in triplicate as described (5). Compounds were placed in assay tubes, and reactions were begun by addition of cold reaction mixture containing both enzyme and DNA, and, after mixing, placing tubes in a 37° water bath. Control assays lacked enzyme and determined the possible artifactual precipitation of labeled deoxynucleoside triphosphate at the various concentrations of compounds. No precipitation of label in the absence of DNA synthesis was observed for any compound tested. Optimal concentrations given for stimulation are the lowest concentration of compound at which maximal stimulation was achieved.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Effect</th>
<th>Optimal concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antrycide (quinapyramine)</td>
<td>Stimulation</td>
<td>50 µM</td>
</tr>
<tr>
<td>ICI-L</td>
<td>Stimulation</td>
<td>50 µM</td>
</tr>
<tr>
<td>Isometamidium</td>
<td>Inhibition</td>
<td></td>
</tr>
<tr>
<td>Imidocarb</td>
<td>Stimulation</td>
<td>300 µM</td>
</tr>
<tr>
<td>Pentamidine</td>
<td>No effect</td>
<td></td>
</tr>
<tr>
<td>Berenil (diminazene aceturate)</td>
<td>Inhibition</td>
<td></td>
</tr>
<tr>
<td>WR-199-385</td>
<td>Inhibition</td>
<td></td>
</tr>
<tr>
<td>Amicarbalide</td>
<td>Inhibition</td>
<td></td>
</tr>
<tr>
<td>MGBG</td>
<td>No effect</td>
<td></td>
</tr>
</tbody>
</table>

the number of terminal imidazole rings appears to stimulate further. In contrast, amicarbalide, a diarylamidino compound similar to imidocarb, inhibits the DNA polymerase reaction. Aromatic groups may also be important in determining how these drugs affect reactions of DNA synthesis. MGBG, a linear aliphatic molecule, does not affect the DNA polymerase reaction under the conditions used. An aromatic analogue of MGBG, DDUG, which, unlike the aliphatic MGBG, binds to DNA, inhibits DNA polymerases (4) and stimulates RLV DNA-directed DNA synthesis. Additionally, the bisquaternary 1,2,3-triazole (Bayer 1694) stimulates the DNA polymerase reaction, whereas a similar compound, NSC 38280, a terephthalanilide with terminal imidazole groups, inhibits the reaction. At optimal concentrations, all stimulatory compounds caused 8- to 10-fold increases in rates of DNA synthesis, compared to those elicited by optimal concentrations of spermine or spermidine (8). Addition of those stimulatory trypanocides to reaction mixtures already containing stimulatory concentrations of polyamines did not enhance enzyme activity (data not shown).

Kinetics of RLV DNA Polymerase Stimulation. Because the cationic trypanocides which stimulate RLV DNA synthesis are antagonized in vivo by polyamines and their stimulation of the RLV DNA polymerase is negated by polyamines, we carried out kinetic analyses of the stimulation to detect parallels in mechanism to that of the polyamines. Spermine stimulates RLV DNA polymerase in 2 ways: (a) structural interaction with the DNA template; (b) interaction of free spermine with the enzyme or enzyme-DNA complex (2). Double-reciprocal plots of stimulation...
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The mechanism of Antrycide stimulation appeared to be the least complicated and most consistent with that of spermine (Chart 1). At DNA concentrations >250 ng/assay, Antrycide addition inhibited synthesis, but higher concentrations proved stimulatory (Chart 1A), much as observed with polyamines (8). With spermine-pretreated, dialyzed activated DNA as template, addition of Antrycide to reaction mixtures also inhibited the enzyme at low DNA concentrations but, at DNA concentrations where spermine stimulation was seen, Antrycide neither inhibited nor further stimulated RLV polymerase activity (Chart 1B). Compound 64A, structurally unrelated to Antrycide, similarly stimulated activated DNA-directed DNA synthesis at DNA concentrations >250 ng/assay (Chart 2A), with the $K_m$ for the template increasing as the drug concentration increased. In the presence of spermine-pretreated activated DNA, however (Chart 2B), stimulation by 64A was not seen. Bayer 1694 differs in stimulation of activated DNA-directed DNA synthesis from both Antrycide and 64A in that it stimulated at all DNA concentrations used (Chart 3A). Significant stimulation by 1694 persisted, even in the presence of spermine-pretreated activated DNA (Chart 3B), although stimulation in this case decreased with higher concentrations of spermine-treated DNA. Hence, stimulation by cationic trypanocides of RLV DNA polymerase-catalyzed DNA-directed DNA synthesis may denote recognition of a template:ligand complex perhaps similar to that of the polyamines with DNA.

DISCUSSION

In surveying a variety of cationic trypanocides and related compounds, we found that several stimulated the RLV DNA polymerase-catalyzed DNA-directed DNA synthesis reaction. It is of interest that certain compounds which stimulated, e.g., Antrycide and imidocarb, inhibited murine DNA polymerase-α and T. brucei DNA polymerase in the same assay system (7). DeClerq and Dann (5) have also recently shown that certain diaryl amidine derivatives inhibit endogenous RNA-directed and polynucleotide:oligonucleotide-directed DNA synthesis by murine retroviral DNA polymerase. Retroviral DNA polymerases differ in their action with DNA from RNA in that high concentrations of DNA inhibit synthesis, producing double-reciprocal plots suggestive of "substrate inhibition" (Ref. 8; see controls, Charts 1A, 2A, and 3A). This is also one way in which the retroviral enzymes differ catalytically from mammalian DNA polymerases (8). When polyamine:RNA complexes were template primers for the retroviral enzymes (without free polyamines), the
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Chart 3. Effect of Bayer 1694 (for structure, see Table 1) on the apparent $K_m$ and $V_{max}$ of the reaction of RLV DNA polymerase with activated DNA (A) and activated DNA which had been pretreated with 2000 μM spermine (B). Activated DNA concentrations were the same as those described in the legend to Fig. 1. The series of DNA concentrations was assayed in the presence of 1694 at final concentrations of 5 μM (○), 25 μM (△), and 50 μM (□) or in the absence of 1694 (●).

double-reciprocal plot pattern at high DNA concentrations changed to one resembling “substrate activation” (Ref. 8; see controls, Charts 1A, 2B, 3B). A similar phenomenon is seen in the kinetic analyses of 3 stimulatory but structurally unrelated trypanocides (Charts 1 to 3), but no significant stimulation is observed if polyamine-complexed DNA serves as template primer. Therefore, stimulation by the cationic trypanocides may reflect a structural interaction with DNA similar to that of the polyamines.

It is interesting to note that the in vivo trypanocidal activity of compounds stimulating the RLV DNA polymerase is antagonized by concomitant administration of biologically active polyamines (2, 3). Several of the compounds which either do not stimulate, or inhibit polymerase activity, such as pentamidine, amicarbalide, WR-199-385, and isomethamide, however, also have their in vivo trypanocidal action antagonized by polyamines (2, 3). Further work will center on structural requirements for RLV polymerase stimulation by cationic trypanocides, coordinated with DNA-binding studies to investigate the affinities of these compounds for activated DNA. Stimulation of activated DNA-directed DNA synthesis by RLV DNA polymerase may therefore provide an inexpensive screening technique for detecting certain kinds of chemotherapeutically useful polyamine antagonists.

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REFERENCES

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