Selectivity of action of an antiherpetic agent, 9-(2-hydroxyethoxymethyl)guanine

(antiviral chemotherapy/virus-specified thymidine kinase/herpes simplex virus/virus-specified DNA polymerase/ acycloguanosine triphosphate)

GERTRUDE B. ELION*, PHILLIP A. FURMAN*, JAMES A. FYFE*, PAULO DE MIRANDA*, LILIA BEAUCHAMP†, AND HOWARD J. SCHAEFFER†

Departments of * Experimental Therapy and † Organic Chemistry, Wellcome Research Laboratories, Burroughs Wellcome Company, Research Triangle Park, North Carolina 27709

ABSTRACT   A guanine derivative with an acyclic side chain, 2-hydroxyethoxymethyl, at position 9 has potent antiviral activity [dose for 50% inhibition (ED_{50}) = 0.1 μM] against herpes simplex virus type 1. This acyclic nucleoside analog, termed acycloguanosine, is converted to a monophosphate by a virus-specified pyrimidine deoxynucleoside (thymidine) kinase and is subsequently converted to acycloguanosine di- and triphosphates. In the uninfected host cell (Vero) these phosphorylations of acycloguanosine occur to a very limited extent. Acycloguanosine triphosphate inhibits herpes simplex virus DNA polymerase (DNA nucleotidyltransferase) 10–30 times more effectively than cellular (HeLa S3) DNA polymerase. These factors contribute to the drug's selectivity; inhibition of growth of the host cell requires a 3000-fold greater concentration of drug than does the inhibition of viral multiplication. There is, moreover, the strong possibility of chain termination of the viral DNA by incorporation of acycloguanosine.

The identity of the kinase that phosphorylates acycloguanosine was determined after separation of the cellular and virus-specified thymidine kinase activities by affinity chromatography, by reversal studies with thymidine, and by the lack of monophosphate formation in a temperature-sensitive, thymidine kinase-deficient mutant of the KOS strain of herpes simplex virus type 1 (tsA1).

The goal of antiviral chemotherapy is the discovery of antiviral agents that are specific for the inhibition of viral multiplication without affecting normal cell division. In general, compounds that have affected the multiplication of DNA viruses have also affected the replication of uninfected cells and have, therefore, been also useful in the treatment of neoplastic disease, e.g., cytosine arabinoside, adenine arabinoside, and trifluorothymidine. Iododeoxyuridine, an antiherpetic agent, is also incorporated into host cell DNA. The closely related 5-iodo-5'-amino-2',5'-dideoxyuridine, on the other hand, has been reported to inhibit DNA synthesis of herpes simplex virus type 1 (HSV-1) selectively with little, if any, cytotoxicity to the host cells (1). Differential toxicity for herpes-infected cells has also been reported for 1-β-D-arabinofuranosyl thymine (2) and 5-ethyl-2'-deoxyuridine (3). The present paper reports the selectivity of action of a new class of antiviral agent that has extremely low toxicity for normal cells while having an inhibitory activity against HSV which is greater than that of any hitherto known compound. The most potent of these compounds is 9-(2-hydroxyethoxymethyl)guanine, a guanine derivative containing an acyclic side chain at the 9-position. This compound may be regarded as an analog of guanosine (Guo) or deoxyguanosine (dGuo) in which the 2 and 3 carbon atoms of the

sugar moiety are missing (Fig. 1). It will be referred to as acycloguanosine (acyclo-Guo) in this report because of biochemical analogies.

The discovery that 9-β-D-arabinofuranosyl derivatives of 2,6-diaminopurine and of guanine possessed good antiviral activity against DNA viruses (4) suggested that purine derivatives with bases other than adenine might be potential antiviral compounds. The studies of Schaeffer et al. (5) had shown that acyclic side chains may substitute for sugar moieties in binding to enzymes, e.g., in a group of adenine derivatives that served as substrates for adenosine deaminase. In a continuation of this series of investigations, Schaeffer et al. synthesized the 9-hydroxyethoxymethyl derivatives of adenine, diaminopurine, and guanine (6), which showed activities against HSV-1 in the plaque-reduction assay with a dose required for 50% inhibition (ED_{50}) of 34, 12, and 0.1 μM, respectively. This antiviral activity of acyclo-Guo was more than two orders of magnitude greater than that of adenine arabinoside (ED_{50} = 12 μM) or guanine arabinoside (ED_{50} = 22 μM). Moreover, the compound was essentially nontoxic to the host Vero cells, showing an ED_{50} of 300 μM in 72-hr growth studies. This represents a therapeutic index in tissue culture of approximately 3000. A further examination of the specificity of this compound revealed that, although it was strongly inhibitory for both HSV-1 and HSV-2, it had essentially no activity against vaccinia virus or adenovirus. Thus, the specificity was evident not only between virus and cell, but likewise among various DNA viruses.

Acycloguanosine was active in vivo in mice with herpes encephalitis when the drug was given either subcutaneously or orally (6). A 1% ophthalmic ointment of acyclo-Guo cured an established herpetic infection in the eyes of rabbits (6). Herpetic lesions of guinea pig skin could be cured by topical application of the drug (6).

Metabolic studies in mice, rats, and dogs indicated that the compound was not catabolized to any significant extent in vivo but was excreted unchanged (ref. 6; P. de Miranda, T. Creagh, R. Sigley, H. Krasny, and G.B. Elion, unpublished). A study was, therefore, undertaken to determine the basis for the antiviral activity and high selectivity of this compound.

MATERIALS AND METHODS

Cells and Viruses. Vero cells and HeLa S-3 cells were grown in Eagle's minimal essential medium and Joklik's modified minimal essential medium, respectively. The media were

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supplemented with 10% fetal calf serum and antibiotics (50 units of penicillin plus 50 μg of streptomycin per ml). Stocks of HSV-1 strains H29 (kindly provided by D. J. Bauer), MacIntyre (kindly provided by J. S. Pagano), and KOS and a temperature-sensitive mutant of KOS, tsA1 (ts343) (kindly provided by Priscilla A. Schaffer) were prepared in Vero cell cultures and stored at −70°. Virus titrations were performed in Vero cell cultures as described by Collins and Bauer (7).

Preparation of Cell Extracts for Nucleotide Analyses by High-Pressure Liquid Chromatography. Vero cells were infected with HSV-1 with a multiplicity of infection of 5–10. One hour after infection, the 10 ml of medium was replaced by medium containing acyclo-Guo and the mixture was incubated at 36° for the time indicated. The medium was removed and the cells were trypsinized, washed with phosphate-buffered saline, and extracted with 9 volumes of cold 3.5 perchloric acid. An internal standard of 25 μl of 2 mM ITP was added to the extract to measure recovery. The acid-soluble extract was neutralized with KOH and centrifuged; the supernatant was concentrated to dryness under reduced pressure, redissolved in 0.75 ml of water, and analyzed with a Varian Aerograph LCS-1000 high-pressure liquid chromatograph fitted with a Whatman Partisil PXS 10/25 SAX column (0.46 × 25 cm). Elution was with a linear gradient of KH_2PO_4, 0.02–1.0 M at pH 3.5 with a flow rate of 30 ml/hr. Fractions (0.2 ml) were collected every 2 min for determination of radioactivity. The UV absorption was monitored at 254 and 280 nm and recorded with a dual pen Honeywell Electronik 194 recorder.

Enzyme Purifications. *Separation of Viral from Host Cell Kinases.* The thymidine (dThd) kinases from HSV-1 (MacIntyre)-infected Vero cells were separated by a modification of the method of Lee and Cheng (8). The ammonium sulfate precipitation step was eliminated and most contaminating proteins were eluted from the dThd-agarose column with a high salt wash (0.8 M Tris·HCl, pH 6.8). A linear gradient of dThd containing 1 mM ATP and 1 mg of bovine serum albumin per ml eluted the dThd kinases in separate peaks.

Separation of Viral from Host Cell Polymerases. Cell extracts of HSV-1 (H29)-infected HeLa cells and mock-infected HeLa cells were prepared by the methods of Weissbach *et al.* (9) and Huang (10). The viral and cellular DNA polymerases (DNA nucleotidyltransferases) were purified by passing the extracts through a DEAE-cellulose column, and the enzyme fractions from the second peak were pooled and applied to a phosphocellulose column (10). Samples from each fraction eluted from the column were assayed for DNA polymerase activity. The viral DNA polymerase was differentiated from the cellular α-DNA polymerase by their activities when different templates for polynucleotide synthesis were used or when 0.05 M ammonium sulfate was present in the reaction mixture (9).

Enzyme Assays. Levels of nucleoside phosphorylating activity were determined with 1 mM [^{14}C]dThd, [^{14}C]acyclo-Guo, and [^{14}C]dCyd by a procedure similar to that of Kessel (11) except that Tris·HCl, pH 7.5/5 mM $MgCl_2$/10 mM ATP was used. For assays with acyclo-Guo the DEAE-paper was washed once with water, twice with 70% ethanol/2 mM ammonium acetate/1 mM guanosine, and once with 95% ethanol. Column fractions that contained dThd were treated with 5 mM arsenate and 60 international units of thymidine phosphorylase before they were assayed for phosphorylation of acyclo-Guo or dCyd. Slices of polyacrylamide gel after electrophoresis at pH 7.5 or 8.9 (12, 13) were assayed in a similar manner. Relative reaction rates of nonradioactive nucleoside phosphorylation were determined by coupling phosphorylation to phosphate transfer
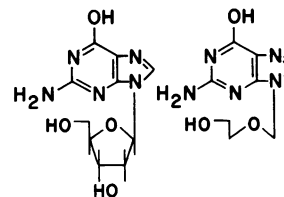

FIG. 1. Formulas for (*Left*) 2′-deoxyguanosine and (*Right*) 9-(2-hydroxyethoxymethyl)guanine.

from [^{14}C]phospho*enol*pyruvate to ADP (ref. 14; J. A. Fyfe, unpublished).

The DNA polymerase was assayed with the reaction conditions of Weissbach *et al.* (9) and the assay procedure of Altman and Lerman (15). Briefly, a 50-μl reaction mixture containing 100 μM Tris·HCl (pH 7.8), 10 mM $MgCl_2$, 1 mM dithiothreitol, 100 μM dATP, dCTP, and dGTP, 11 μg of activated calf thymus DNA, 12.5 μl of enzyme (or as stated), and 100 μM [^{3}H]-dTTP (specific activities as stated) was incubated at 37°.

Materials. The synthesis of 9-hydroxyethoxymethylguanine will be reported elsewhere. The [8-^{14}C]acyclo-Guo had a specific activity of 18 Ci/mol. The [^{3}H]acyclo-Guo was labeled in the two terminal carbons of the acyclic side chain; it had a specific activity of 51 Ci/mol. The acycloguanosine triphosphate (acyclo-GTP) used for the kinetic studies with DNA polymerase was synthesized chemically by Janet Rideout (P. A. Furman, M. St. Clair, J. Rideout, J. A. Fyfe, P. Keller, and G. B. Elion, unpublished). It was identical in all respects with the triphosphate isolated by high-pressure liquid chromatography.

RESULTS AND DISCUSSION

Anabolism of acycloguanosine in HSV-infected cells

Because of the structural resemblance of acyclo-Guo to dGuo (Fig. 1), attention was first given to enzymes that convert dGuo to its nucleotides, e.g., the deoxycytidine kinase from calf thymus, for which dGuo is a good substrate (16). Even at acyclo-Guo concentrations of 1 mM, no activity was found with this kinase (T. A. Krenitsky, unpublished). There was likewise no phosphorylation with the adenosine kinase from rabbit liver (J. Fyfe and R. Miller, unpublished). Incubation mixtures of acyclo-Guo with various cell lines were then examined. High-pressure liquid chromatography of extracts of Vero cells incubated with 1 mM acyclo-Guo, labeled either in the 8 position of the guanine ring with ^{14}C or with ^{3}H in the acyclic side chain, revealed the presence of radioactive material in the mono-, di-, and triphosphate regions of the chromatogram (Fig. 2A) in amounts too small for positive identification. However, the nucleotide profile of HSV-infected Vero cells treated with acyclo-Guo at a concentration of 500 μM showed a very different result (Fig. 2 *B* and *C*). Here, the amounts of mono-, di-, and triphosphates formed from the acyclic nucleoside were extremely high. The identity of the triphosphate was established in a variety of ways. The ultraviolet absorption spectrum was consistent with that of a derivative of acyclo-Guo. The specific activity of the radioactive triphosphate was the same as that of the acyclo-Guo used. In addition, peak-shift experiments showed that the radioactive triphosphate could be converted to the corresponding monophosphate derivative by the use of snake venom phosphodiesterase and to acyclo-Guo by alkaline phosphatase. It was, therefore, apparent that HSV infection had induced in these cells one or more enzymes capable of transforming acyclo-Guo into a triphosphate. Cells infected with vaccinia virus did not show this capability.
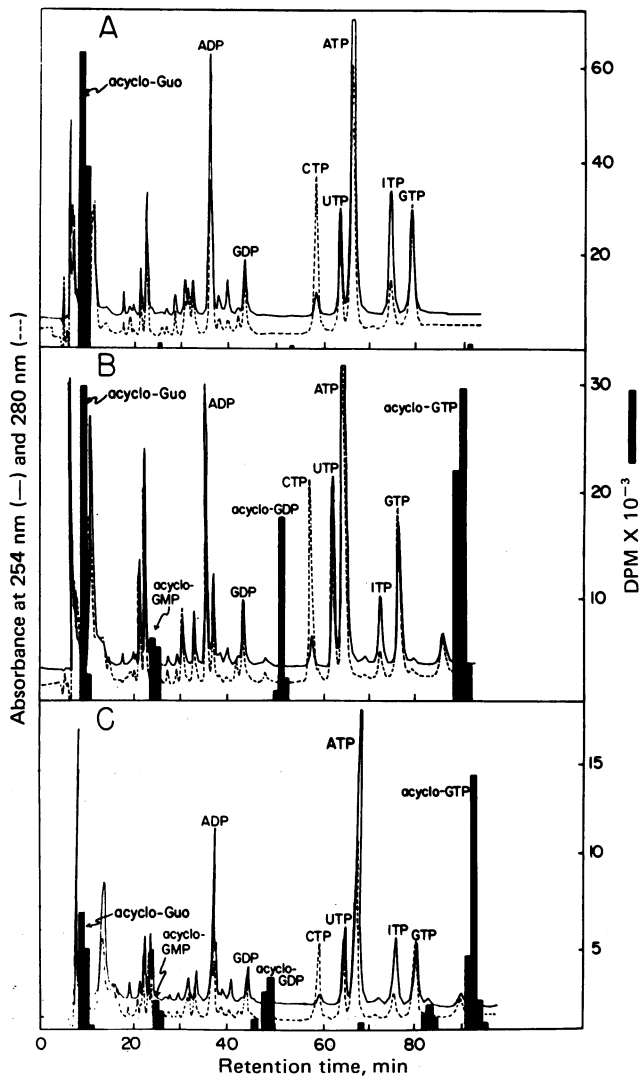
FIG. 2. High-pressure liquid chromatography profiles of extracts of uninfected and HSV-1 (H29)-infected Vero cells incubated with acyclo-Guo for 6 hr. (A) Uninfected cells treated with [³H]acyclo-Guo (1.0 mM); (B) infected cells treated with [³H]acyclo-Guo (0.5 mM); (C) infected cells treated with [8-¹⁴C]acyclo-Guo (0.5 mM).

Table 1. Effect of drug concentration on the concentration of acyclo-GTP

Acyclo-Guo, μM	Acyclo-GTP, pmol/10⁶ cells*	
	Vero	HSV-infected Vero
0.5	0.11	4.0
5.0	0.17	3.5
100.0	1.4	49.0
500.0	8.7	308.0

* Vero cells were infected with HSV-1 (H29), with a multiplicity of infection of 5–10. Drug was added 1 hr after infection or after uninfected cells were plated. Cells were harvested 7 hr after addition of drug.

The time course of the formation of acyclo-GTP in HSV-1-infected Vero cells was investigated. Cells were infected with HSV-1 (H29) and exposed to [8-¹⁴C]acyclo-Guo for various times. Acyclo-GTP formation was detectable 2 hr after infection, reached a maximum at 8 hr, and then declined but was still present in appreciable quantity at 16 hr. These findings are consistent with the fact that the enzymes involved in DNA synthesis are expressed early in the replicative cycle of HSV-1 (9, 17–19).

The extent of formation of acyclo-GTP was dependent upon the concentration of acyclo-Guo in the medium. The large difference between the uninfected and HSV-1-infected cells occurred over a wide range of drug concentration (Table 1). At 0.5 μM, which is higher than the ED₁₀₀ for acyclo-Guo for preventing plaque formation in HSV-1-infected Vero cells, there was a 40-fold greater amount of acyclo-GTP in the infected than in uninfected cells.

Infection of Vero cells with HSV-1 resulted in a large increase in the capability of the cell to take up acyclo-Guo from the medium. The amount of uptake, as well as the degree of conversion to mono-, di-, and triphosphates, varied with the strain

of HSV-1 used. Typical results for uninfected cells and cells infected with two different strains of HSV-1 and exposed to 100 μM acyclo-Guo for 7 hr are shown in Table 2. Conversion to the phosphates appears to facilitate the uptake of acyclo-Guo.

Nucleoside phosphorylation in extracts of HSV-1-infected Vero cells

Since it was now clear that virally induced enzymes were responsible for the substantial conversion of acyclo-Guo to its phosphates, an investigation of the nature of the enzymes responsible for this conversion was undertaken. Although it has been reported (20–24) that HSV infection induces a virus-specified pyrimidine deoxynucleoside kinase, it seemed unlikely, on the basis of structural considerations, that this enzyme would be implicated. Nevertheless, cytosol fractions of uninfected and HSV-1 (H29)-infected Vero cells, prepared by the method of Cheng and Ostrander (25) but modified by the substitution of dithiothreitol for 2-mercaptoethanol and the omission of dThd, were examined for their ability to phosphorylate dThd, dCyd, and acyclo-Guo at substrate concentrations of 1 mM. The extract from uninfected cells showed a high rate of phosphorylation of dThd, a low rate for dCyd, and a barely detectable rate toward acyclo-Guo (2600, 30, and 4 pmol/min per mg of protein, respectively, at pH 7.5). However, the extracts of cells that had been infected 17 hr previously with HSV-1 (H29) showed an approximately 70-fold increase in the rate of phosphorylation of dCyd and acyclo-Guo, while maintaining a high rate of activity toward dThd (Fig. 3).‡ The activity toward dGuo in a similar extract did not increase after HSV infection.

Properties of acyclo-Guo kinase

There is strong evidence that the HSV-specified deoxypyrimidine kinase that phosphorylates both dThd and dCyd is one enzyme (25–30). It was important to determine whether this enzyme was also the one responsible for the phosphorylation of acyclo-Guo. Electrophoresis on polyacrylamide gel of the cytosol of HSV-infected Vero cells at pH 7.5 or 8.9 showed that the enzyme activities toward dThd and acyclo-Guo were in the same fractions.

Thymidine kinase from HSV-1 (MacIntyre)-infected Vero cells was purified with a thymidine-agarose affinity chromatography column. This method separated host and virus enzyme activities from other proteins and from each other. At least 95%

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‡ Other experiments conducted at pH 6, the optimum pH for HSV-specified thymidine kinase (26), showed that the phosphorylation of dThd in the extracts from HSV-infected cells was due principally to HSV-specified thymidine kinase rather than to the Vero cellular enzyme. The cellular enzyme has only 11% of the activity at pH 6.0 as at pH 7.5, whereas HSV-specified enzyme is 120% as active at pH 6.0 as at pH 7.5.

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Table 2. Concentration of acyclo-Guo and its derivatives in uninfected and HSV-1-infected Vero cells*

Form in extract	pmol/10⁶ cells		
	Vero	HSV-1 (H29)-infected	HSV-1 (MacIntyre)-infected
Acyclo-Guo	3.4	15.1	20.1
Acyclo-GMP	2.6	14.9	4.0
Acyclo-GDP	2.0	41.5	13.4
Acyclo-GTP	1.8	71.2	90.5
Total	9.8	142.7	128.0

* Vero cells were exposed to 100 μM acyclo-Guo. Conditions were the same as in Table 1.

of the protein was eluted before a dThd gradient was introduced. Two peaks of thymidine kinase activity were eluted; the first was eluted 7 ml after the gradient was started, the second after 17–18 ml. Fractions from the first peak showed phosphorylating activities toward dThd, dCyd, and acyclo-Guo of 29, 14, and 7 pmol/hr per 20-μl sample, respectively; fractions from the second peak had activities of 2, <0.3, and <0.1, respectively. In a similar fractionation of the cytosol from uninfected Vero cells, the maximum activity with dThd as substrate occurred at 17–18 ml after the start of the gradient, with no detectable activity (level less than 2% of peak level) in fractions 6–8. The relative reaction velocities of phosphorylation of several nucleosides (at 1 mM) with the purified HSV(MacIntyre)-specified thymidine kinase were: dCyd (190), dThd (100), acyclo-Guo (36), dGuo (5), guanine arabinoside (<3), and adenine arabinoside (<3).

The evidence that acyclo-Guo was indeed being phosphorylated by the virus-specified kinase was further strengthened by several experiments showing that acyclo-GMP formation from 0.1 mM acyclo-Guo was completely prevented by 0.3 mM dThd or 8 mM dCyd, that the antiviral activity of 5 μM acyclo-Guo in Vero cells was prevented by the addition of 20 μM dThd, and that dThd interfered with the formation of acyclo-GTP in HSV-1-infected cells. In all of these studies the concentration of dThd required to produce these reversals was 3–5 times the concentration of acyclo-Guo present. By contrast, the reversal of the antiviral activity of acyclo-Guo by dGuo in the plaque-reduction assay was incomplete (25%) even at a ratio of 100:1 of dGuo:acyclo-Guo.

Finally, strong confirmatory evidence was obtained with a temperature-sensitive thymidine kinase-deficient (TK⁻) mutant of the KOS strain of HSV-1 (tsA1), which behaves as a wild-type TK⁻ virus at 34° (31–33). The cytosol extract of tsA1 did not phosphorylate acyclo-Guo more than the extract from uninfected cells (<10 pmol/min per mg of protein), whereas the extract from the KOS strain under the same conditions formed 1100 pmol of acyclo-GMP/min per mg of protein. In addition, extracts of Vero cells infected with the KOS strain showed a very high conversion of acyclo-Guo (100 μM) to mono-, di-, and triphosphates (80, 284, and 1236 pmol/10⁶ cells, respectively, in 7 hr), whereas cells infected with tsA1 at the permissive temperature (34°) formed essentially no phosphate derivatives (total <4 pmol/10⁶ cells).

Effects of acyclo-GTP on viral and cellular DNA polymerases

Although it was clear that significant quantities of acyclo-GTP were formed only in HSV-1 (TK⁺) infected cells, it remained to be determined whether this compound was responsible for the antiviral activity of acyclo-Guo. Suggestive evidence that
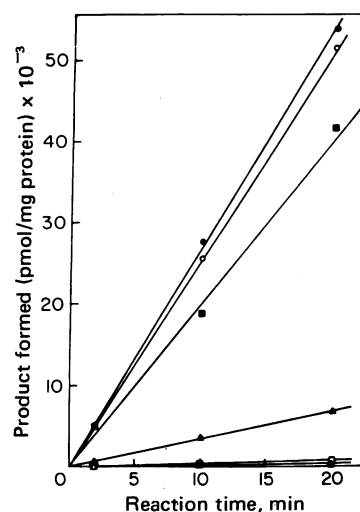

FIG. 3. Phosphorylation of nucleosides by cytosol fractions from uninfected and HSV-1 (H29)-infected Vero cells. Activities with (O,●) dThd, (□,■) dCyd, and (△,▲) acyclo-Guo were measured from uninfected (open symbols) and infected (closed symbols) cells.

acyclo-GTP was required for antiviral activity came from the findings that the tsA1 (TK⁻) mutant of HSV-1 (KOS) described above was insensitive to acyclo-Guo at the permissive temperature. Moreover, the inability of vaccinia-infected cells to phosphorylate acyclo-Guo (J. Fyfe, unpublished) and their inability to form more acyclo-GTP than uninfected cells correlate with the lack of inhibition of the growth of vaccinia virus by 100 μM acyclo-Guo.

In order to determine the effect of acyclo-GTP on both the herpes-specified and cellular DNA polymerases, it was necessary to isolate and purify the DNA polymerase activities. The viral DNA polymerase could be differentiated from the cellular α-polymerase by the difference in their activities when different templates for polynucleotide synthesis were used or when 0.05 M ammonium sulfate was present. The cellular polymerase showed poor incorporation of [³H]dTTP into acid-insoluble material with dA·dT$_{12-18}$ as template, but high incorporation with calf thymus DNA or dC·dG$_{12-18}$; the activity was depressed by ammonium sulfate. The HSV-1-specified polymerase showed greatly increased activity with either dA·dT$_{12-18}$ or dC·dG$_{12-18}$ as template, compared with calf thymus DNA, and was stimulated by ammonium sulfate. These properties are in accordance with those previously reported for these respective polymerases (9).

The inhibitory effect of acyclo-GTP upon the isolated viral and cellular DNA polymerases was investigated by using [³H]dTTP incorporation as a measure of enzyme activity. The Lineweaver–Burk plots of the data are shown in Fig. 4, with calf thymus DNA as template. From these data, which showed competitive inhibition between dGTP and acyclo-GTP, the kinetic parameters were derived. The apparent K_i (0.08 ± 0.03 μM) for acyclo-GTP for the HSV-1 (H29) polymerase was approximately $\frac{1}{30}$ that for the α-DNA polymerase of the host cell, HeLa S-3 (K_i = 2.1 ± 0.8 μM). The apparent K_m for dGTP for the viral DNA polymerase was 0.38 ± 0.13 μM, only one-third that for the cellular DNA polymerase (K_m = 1.08 ± 0.01 μM).

From the above data, it is possible to calculate whether the amount of acyclo-GTP present in HSV-1-infected cells is of the right order of magnitude to bind significantly to the viral DNA polymerase. Assuming that the concentration of acyclo-GTP
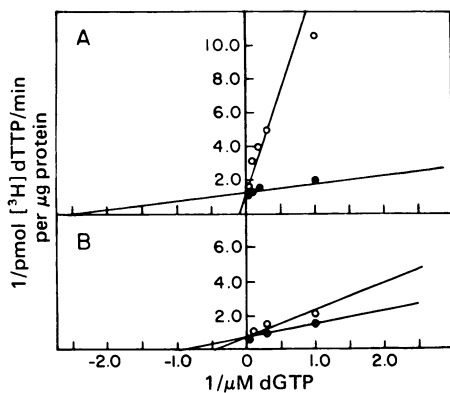
FIG. 4. Lineweaver–Burk plots showing inhibition by acyclo-GTP of (A) HSV-1 (H29) viral DNA polymerase and (B) HeLa S3 cellular DNA polymerase. ●, No acyclo-GTP; ○, 2.55 μM acyclo-GTP. The activity of the polymerase was measured by the rate of incorporation of [^{3}H]dTTP (specific activity = 50 cpm/pmol in A and 36 cpm/pmol in B) into acid-insoluble material.

in HSV-1-infected HeLa cells is similar to that in infected Vero cells, and using a packed volume of 0.005 ml for 10^6 cells, one can calculate that a concentration of acyclo-GTP of 4 pmol/10^6 cells (found when the acyclo-Guo concentration in the medium was 0.5 μM, see Table 1) is equivalent to 0.8 μM. A concentration of 0.8 μM acyclo-GTP would be approximately 10 times greater than the apparent K_i value for the HSV-1 polymerase. On the other hand, the concentration of acyclo-GTP in uninfected cells would be 0.02 μM under the same conditions, or $\frac{1}{100}$ that of the apparent K_i for the cellular DNA polymerase.

Preliminary data indicate that acyclo-GTP is not only an inhibitor of HSV-1-specified DNA polymerase, but also a substrate for this enzyme. Incorporation of acyclo-Guo into viral DNA would be expected to be chain-terminating since no hydroxyl group corresponding to the 3′-hydroxyl group of deoxyribose is available for chain elongation. Although preliminary experiments with alkaline sucrose gradients indicate that the HSV-1 DNA is of lower molecular weight after exposure to acyclo-Guo, verification of DNA chain termination by acyclo-Guo must await the synthesis of radioactive material of higher specific activity than that presently available.

The high selectivity of acyclo-Guo for HSV-1 compared with normal host cells is now explicable on the basis of the specificity of the virus-specified thymidine kinase for acyclo-Guo and the very low activity of cellular kinases toward this compound. The specificities of the kinases that convert acyclo-GMP to acyclo-GDP and acyclo-GTP have not been examined. The virus-infected cells accumulate much more acyclo-Guo, and make approximately 40-fold more acyclo-GTP than the uninfected cells. In addition, there is a much greater sensitivity of the viral DNA polymerase compared with the cellular polymerase [30-fold in HSV-1 (H29) and HeLa cells] to acyclo-GTP. These data help to explain the 3000-fold difference between the ED$_{50}$s of acyclo-Guo for Vero cells and HSV-1 (H29).

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