Inhibition of Retrovirus RNA-dependent DNA Polymerase by Novobiocin and Nalidixic Acid

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(Accepted 10 June 1983)

SUMMARY

Inhibitors of bacterial DNA gyrase and eukaryotic DNA topoisomerase (novobiocin and nalidixic acid) were investigated with respect to their effect on the activity of RNA-dependent DNA polymerases from murine and avian retroviruses. Purified RNA-dependent DNA polymerase from AKR virus was inhibited more than 90% by 0.3 mg/ml and almost completely by 1 mg/ml of the drugs when poly(A)-oligo(dT)12-18 was used as a template–primer. In contrast to the enzyme from AKR virus, purified enzyme from avian myeloblastosis virus was less sensitive, i.e. nearly 50% activity remained even in the presence of 1 mg/ml of the drugs with the same template–primer. RNA-dependent DNA polymerase activity in AKR virus particles was inhibited, but was resistant to low concentrations of the drugs. The inhibition was not due to specific interaction between drugs and the template–primer or labelled precursor, since RNA-dependent DNA polymerase was inhibited by the drugs with activated calf thymus DNA or poly(C)-oligo(dG)12-18 as the template. Endogenous DNA synthesis by AKR virus particles was inhibited by novobiocin to the same extent.

RNA-dependent DNA polymerase (RDDPase) plays a central role during the life cycle of retroviruses. After retrovirus infection of cells, it uses viral RNA as a template for single-stranded DNA and the single-stranded DNA is then converted to linear double-stranded DNA (Verma, 1977, 1981). The linear double-stranded DNA molecules are further converted into covalently closed circular DNA which is subsequently integrated into the host chromosomal DNA. Although the precise mechanism remains unknown, RDDPase may play an important role in the above reaction with the possible cooperation of host-derived factors (Hagino-Yamagishi et al., 1981).

Recently, novobiocin has been found to inhibit the initiation of DNA synthesis in bacteria (Staudenbauer, 1975; Gellert et al., 1976) and mammalian cells (Mattern & Painter, 1979; Mattern & Scudiero, 1981; Mattern et al., 1982; Lavin, 1981). Experiments with novobiocin in eukaryotic DNA-synthesizing systems and with purified DNA polymerase suggest that enzyme systems similar to bacterial DNA gyrase convert the DNA template into a structure suitable for initiating a new DNA strand (Edenberg, 1980). Nalidixic acid also inhibits bacterial DNA gyrase but shows different effects on eukaryotic systems (Cozzarelli, 1977, 1980; Gellert et al., 1977; Gellert, 1981).

Use of these drugs may provide a clue to the role of the viral enzyme from infection to integration. In this report, we present evidence that with isolated virus particles viral DNA synthesis is inhibited by novobiocin and nalidixic acid and that this inhibition is due to the effect of these drugs on RDDPase.
Fig. 1. Effect of novobiocin (○) and nalidixic acid (△) on RDDPase activity in AKR virus particles and purified AKR RDDPase. AKR endogenous virus was obtained from spontaneously virus-producing cells of the AKR-2B derived cell line. Murine cells were maintained in Eagle's minimum essential medium supplemented with 10% newborn calf serum at 37 °C in a 5% CO₂ atmosphere. (a) Inhibition of RDDPase activity in virus particles by the drugs. Viruses were pelleted (100000 g for 2 h from supernatants from cultures of virus-producing cells after the removal of cells and cell debris by low speed centrifugation), dissolved in a solution of 50 mM-Tris–HCl pH 8-3, 10 mM dithiothreitol (DTT) and 0-025% NP40, and enzyme activity was measured in a solution containing 50 mM-Tris–HCl pH 8-3, 10 mM-DTT, 40 mM-NaCl, 0-01% NP40, 3 mM-MnCl₂, 0·3 A unit/ml poly(A), 0·1 A unit/ml oligo(dT)₁₂₋₁₈ and 2.5 μCi [³H]TTP at 37 °C for 2 h. 100% activity corresponds to 22848 cts/min. (b) Inhibition of purified RDDPase by the drugs. AKR virus particles were precipitated from 3 litres of culture supernatant by the addition of 6% polyethylene glycol 20000 (Bronson et al., 1975). Enzyme was purified on a column (1·5 x 6 cm) of phosphocellulose (P-11; Whatman) using a linear gradient of 50 to 500 mM-potassium phosphate. Fractions of 2 ml were collected. Enzyme activity was assayed as in (a); 100% activity corresponds to 34564 cts/min.

The effects of novobiocin and nalidixic acid on the RDDPase activity of AKR murine leukaemia virus particles detected by [³H]TMP incorporation with poly(A)-oligo(dT)₁₂₋₁₈ as a template–primer are shown in Fig. 1 (a). A difference in inhibitory effect of these drugs was not detected. Similar results were obtained in experiments using virions of the Rauscher strain of murine leukaemia virus (data not shown).

AKR virus RDDPase was isolated from virus particles and purified about 50-fold by phosphocellulose column chromatography. Enzyme activity was not affected by aphidicolin at 0.1 mg/ml; thus, the DNA-synthesizing activity of the fraction was not due to contamination by host DNA polymerase-α, which has been found to be sensitive to novobiocin (Edenberg, 1980). The effects of novobiocin and nalidixic acid on the isolated AKR enzyme are shown in Fig. 1 (b). More than 90% inhibition was observed at a drug concentration of 0·3 to 0·4 mg/ml. Enzyme activity was severely affected, especially at low concentration of the drugs, compared with the
Short communication

Fig. 2. Effect of novobiocin (○) and nalidixic acid (△) on RDDPase activity of avian sarcoma virus particles and purified AMV RDDPase. (a) Inhibition of RDDPase activity of avian sarcoma virus particles by the drugs. The Prague-C strain of avian sarcoma virus was propagated in chicken embryonic fibroblast cells. Virus particles were collected by the method described for Fig. 1(a), and dissolved in 50 mM-Tris-HCl pH 8.3, 10 mM-DTT and 0.2% NP40. Incubation was done in 50 mM-Tris-HCl pH 8.3, 10 mM-DTT, 40 mM-KCl, 0.1% NP40, 6 mM-MgCl2, 0.3 A unit/ml poly(A), 0.1 A unit/ml oligo(dT)12-18 and 2.5 μCi [3H]TTP. Incubation was carried out at 37 °C for 2 h. 100% activity corresponds to 6904 ct/min. (b) Effect of the drugs on purified AMV RDDPase. Purified AMV RDDPase was kindly provided by Dr. J. W. Beard (Life Sciences, Inc., St. Petersburg, Fla., U.S.A.); it was assayed as in (a). 100% activity corresponds to 55368 ct/min.

results obtained with virus particles shown in Fig. 1(a). This may result from the presence of certain factors in the virus particles that reduce the effect of the drugs, or alteration of the RDDPase molecule during purification. The dependence of inhibition of purified AKR RDDPase on the drug concentration was similar to that for DNA polymerase-α as reported by Edenberg (1980).

Fig. 2(a) shows the effects of novobiocin and nalidixic acid on the RDDPase activity of isolated Prague-C avian sarcoma virus particles measured with poly(A)·oligo(dT)12-18 as a template–primer. Nearly 60% of the activity persisted in the presence of 1 mg/ml novobiocin. This is in striking contrast to that shown in Fig. 1(a). The RDDPase in avian virus particles seems to be more resistant to novobiocin than the enzyme in murine retrovirus particles.

The effects of novobiocin and nalidixic acid on purified avian myeloblastosis virus (AMV) RDDPase were examined to clarify whether the resistant nature of the avian enzyme is due to distinctive features of avian RDDPase or to the presence of other factor(s) in virus particles. These drugs exhibit the inhibitory effects shown in Fig. 2(b). However, novobiocin and nalidixic acid are less effective toward purified AMV RDDPase than murine retrovirus
Table 1. Effect of novobiocin on RDDPase activity with endogenous viral RNAs as template*

<table>
<thead>
<tr>
<th>Virus</th>
<th>Conc. of novobiocin (μg/ml)</th>
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<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>AKR murine leukaemia virus</td>
<td>100</td>
</tr>
<tr>
<td>Avian sarcoma virus</td>
<td>100</td>
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</table>

* Preparation of murine and avian virus particles was carried out by the methods mentioned in Fig. 1 and 2. Murine virus particles were incubated in a solution of 50 mM-Tris-HCl pH 8.3, 10 mM-dithiothreitol (DTT), 0.01% NP40, 40 mM-NaCl, 3 mM-MnCl₂, 3 mM each of dCTP, dGTP and TTP, 0.3 mM-dATP and 2.5 μCi [³⁵S]dATP (New England Nuclear; 531 Ci/mmol). Avian sarcoma virus particles were incubated in a solution of 50 mM-Tris-HCl pH 8.3, 10 mM-DTT, 0.1% NP40, 40 mM-KCl, 6 mM-MgCl₂, 3 mM each of dCTP, dGTP and TTP, 0.3 mM-dATP and 2.5 μCi [³⁵S]dATP. Incubation was done at 37 °C for 2 h. 100% activity of murine virus corresponds to 91260 ct/min and of avian virus to 38329 ct/min. Data are expressed as activity (%) relative to the control incubated without novobiocin.

RDDPase. The pattern of inhibition closely resembles that of the [³⁵S]dATP incorporation by avian sarcoma virus particles shown in Fig. 2(a). In contrast to murine retrovirus RDDPase, the avian enzyme was more resistant to these drugs. The reason for this difference in sensitivity is unknown, but it may indicate that reverse transcription or the synthesis of double-stranded DNA from single-stranded DNA takes place by different mechanisms depending on the origin of the enzyme.

The inhibition of RDDPase activity by these drugs was not due to a specific interaction between novobiocin and the template–primer poly(A)-oligo(dT)₁₂₋₁₈ or the labelled precursor. The effects of novobiocin on the incorporation of [³⁵S]dATP with activated calf thymus DNA as a template were measured. Murine retrovirus RDDPase activity was reduced to less than 20% in the presence of 0.3 mg/ml novobiocin and nearly all activity was inhibited at 1 mg/ml of this drug. In contrast, AMV RDDPase activity was inhibited only 15% by 0.3 mg/ml, and 50% activity still remained in the presence of 1 mg/ml of the drug. We also measured the effect of novobiocin on the incorporation of [³⁵S]dGMP with poly(C)-oligo(dG)₁₂₋₁₈ as a template–primer. AKR RDDPase activity was inhibited in a manner similar to that when poly(A)-oligo(dT)₁₂₋₁₈ was used. The preference of AKR RDDPase for poly(C)-oligo(dG)₁₂₋₁₈ was very low compared to poly(A)-oligo(dT)₁₂₋₁₈.

Preincubation of the template–primer with the drugs did not increase the inhibitory effect on the enzymes. It is important to confirm whether inhibition by novobiocin occurs in a reverse transcription reaction using endogenous viral RNA as a template. We measured [³⁵S]dATP incorporation into viral DNA. Viral DNA synthesis was inhibited by novobiocin to the same extent as with poly(A)-oligo(dT)₁₂₋₁₈ as a template–primer (Table 1). It has been observed that ATP affects novobiocin inhibition in DNA gyrase and DNA topoisomerase systems (Hsieh & Brutlag, 1980). It was of interest, therefore, to ascertain whether ATP affects the inhibition of RDDPase by novobiocin. The effect of ATP on the inhibition of RDDPase by novobiocin was measured with poly(A)-oligo(dT)₁₂₋₁₈ as the template–primer; the nucleotide did not affect inhibition of enzyme activity. The results preclude involvement of ATP in the inhibition of RDDPase activity. Other ribonucleotide phosphates did not affect the inhibition of RDDPase by novobiocin or nalidixic acid.

The influence of concentration of Nonidet P40 (NP40) on the inhibitory effect of novobiocin was examined to exclude the possibility that the observed differences between murine and avian enzymes were due to the different concentrations of NP40 used; these were 0-01% to 0-1% for the murine system and 0-05% to 0-4% for the avian system. The profile of the inhibition of both murine and avian enzymes by novobiocin in the presence of various concentrations of NP40 was roughly the same as the results shown in Fig. 1 and 2 (data not shown), although the total incorporation varied with the concentration of the detergent. Therefore, the concentration of NP40 was not responsible for the different effects of novobiocin on murine and avian enzymes.

In this communication, we have described the inhibitory effects of novobiocin and nalidixic acid on murine and avian retrovirus RDDPases. The results presented here do not provide any
direct evidence for a DNA topoisomerase activity of retrovirus RDDPase in viral DNA synthesis and integration. However, the results in this paper do not rule out such a possibility.

We are grateful to Drs K. Toyoshima (University of Tokyo), T. Takano (Keio University) and Y. Tomita (Chiba University) for providing viruses and cells. We also thank Dr J. W. Beard for providing purified AMV reverse transcriptase.

REFERENCES


(Received 6 January 1983)