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Selective cleavage of kinetoplast DNA minicircles promoted by antitrypanosomal drugs

(trypanosome/topoisomerase/pentamidine/ethidium/etoposide)

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ABSTRACT **Pentamidine, diminazene aceturate (Berenil), isometamidium chloride (Samorin), and ethidium bromide, which are important antitrypanosomal drugs, promote linearization of *Trypanosoma equiperdum* minicircle DNA (the principal component of kinetoplast DNA, the mitochondrial DNA in these parasites). This effect occurs at therapeutically relevant concentrations. The linearized minicircles are protease sensitive and are not digested by λ exonuclease (a 5′ to 3′ exonuclease), indicating that the break is double stranded and that protein is bound to both 5′ ends of the molecule. The cleavage sites map to discrete positions in the minicircle sequence, and the cleavage pattern varies with different drugs. These findings are characteristic for type II topoisomerase inhibitors, and they mimic the effects of the antitumor drug etoposide (VP16-213, a semisynthetic podophyllotoxin analog) on *T. equiperdum* minicircles. However, the antitrypanosomal drugs differ dramatically from etoposide in that they do not promote detectable formation of nuclear DNA–protein complexes or of strand breaks in nuclear DNA. Selective inhibition of a mitochondrial type II topoisomerase may explain why these antitrypanosomal drugs preferentially disrupt mitochondrial DNA structure and generate dyskinetoplastic trypanosomes (which lack mitochondrial DNA).**

African trypanosomes are parasitic protozoa that cause sleeping sickness in humans and economically significant disease in cattle (1). *Leishmania* and *Trypanosoma cruzi* are related parasites that cause leishmaniasis and Chagas' disease, respectively. All of these organisms have an unusual mitochondrial DNA [kinetoplast (kDNA)], which is in the form of a network of several thousand topologically interlocked circles.

Many of the drugs currently available to treat trypanosomiasis and leishmaniasis, including ethidium bromide, pentamidine, diminazene aceturate (Berenil), isometamidium chloride (Samorin) (Fig. 1), and suramin, were discovered and developed as a direct result of the work of Paul Ehrlich early in this century (2). Despite their long availability, the molecular mechanism of antitrypanosomal action remains unclear. Ethidium bromide and the diamidines (pentamidine and diminazene aceturate) bind directly to nucleic acids: ethidium is an intercalating agent (3) and the diamidines bind in the minor groove of DNA (4).

We previously examined the effects of specific type II topoisomerase inhibitors, such as the antitumor agent etoposide (VP16-213), on *Trypanosoma equiperdum*, an African trypanosome (5). Etoposide has been shown to stabilize a "cleavable complex" between type II topoisomerases and DNA; denaturation with SDS results in double-stranded cleavage of the DNA with a topoisomerase subunit linked to both 5′ ends of the DNA fragment (6, 7). We found that etoposide promoted the formation of cleavable complexes between kinetoplast minicircle DNA and a mitochondrial type II topoisomerase (5).

In this paper, we report that pentamidine, diminazene aceturate, isometamidium chloride, and ethidium bromide, all used to treat human or cattle infections, also generate minicircle–protein cleavable complexes. In contrast to the effect of etoposide, cleavage induced by antitrypanosomal drugs is specific to kDNA; there is no detectable cleavage of trypanosome nuclear DNA. These results raise the possibility of an unusual topoisomerase activity in trypanosome mitochondria; they also may provide clues to the mechanism by which these drugs selectively kill the parasites.

MATERIALS AND METHODS

Drugs. Immediately before use, drug solutions were prepared in RPMI 1640 medium: 1 mM diminazene aceturate (Berenil; Sigma), 3 mM isometamidium chloride (Samorin; May and Baker, Dagenham, U.K.), 2.5 mM ethidium bromide (Sigma), 2 mM pentamidine isethionate (gift of Lypho Med, Chicago), and 2 mM suramin (Center for Disease Control, Atlanta). Etoposide (VP16-213; gift of Bristol Laboratories) was provided at 34 mM in vehicle (5). Stock solutions (20× final concentrations) were prepared at 37°C.

Isolation of DNA. We used *T. equiperdum* for these studies because they have homogeneous minicircles (8) and because they have been used for many studies on minicircle replication (9). *T. equiperdum* (BoTat 24) were isolated from rat blood on DEAE-cellulose (10), centrifuged, and resuspended in RPMI 1640 medium containing 1% bovine serum albumin (BSA). Trypanosomes were treated (37°C; 5–60 min) with drugs at the indicated concentrations and lysed with SDS. The lysates were digested with proteinase K and then RNase A and T1 followed by phenol extraction, dialysis, and ethanol precipitation (5). DNA was digested to completion with restriction enzymes (New England Biolabs or BRL) under conditions recommended by the manufacturer. DNA (0.1–1 pg) was digested (37°C; 15 min) with exonuclease III (10 units; gift of Bernard Weiss, University of Michigan) or with λ exonuclease (2.5 units; BRL), as described (5). *Hin*dIII-linearized pBR322 DNA (100 ng) was included as a control in both digestions. DNA was fractionated on agarose gels (1.5%; 16–18 hr; 3 V/cm) in 90 mM Tris·HCl/92 mM boric acid/2.5 mM EDTA, pH 8.3, containing ethidium bromide (1 μg/ml).

Probes and Hybridization. The full-length *T. equiperdum* minicircle DNA was excised with *Xba* I and *Sma* I from pJN6 (11), purified, and ^{32}P-radiolabeled by the random primer method (12). Synthetic oligonucleotides [L-329 and L-381 (5)] were 5′-end-labeled with T4 polynucleotide kinase and [γ-

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Abbreviations: kDNA, kinetoplast DNA; BSA, bovine serum albumin; TCA, trichloroacetic acid.

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^{32}P]ATP. DNA was transferred from agarose gels to GeneScreen (DuPont). kDNA fragments on GeneScreen were hybridized overnight at 55°C in 10 ml of 500 mM sodium phosphate, pH 7.2/10 mM EDTA/1% BSA/7% SDS containing 25 ng (10^9 cpm/μg) of probe. Filters were washed four times (300 mM NaCl/30 mM sodium citrate, pH 7/0.5% SDS), 15 min each, at 55°C. Membranes to be probed with oligonucleotides (10 pmol; 10^6 cpm/pmol) were processed as described (5). Autoradiography was at −70°C with XAR-5 film (Kodak). Densitometry of autoradiographs or fluorographs was done on a Zeineh model SL-504-XL scanning densitometer (Biomed Instruments, Fullerton, CA).

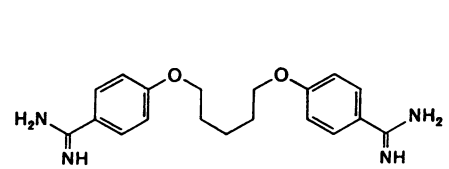
Precipitation of Topoisomerase–DNA Complexes. DEAE-purified trypanosomes (2×10^8) were incubated (37°C; 60 min) with [^{3}H]thymidine (20 Ci/mmol; 346 μCi/ml; 1 Ci = 37 GBq) in 15 ml of RPMI 1640 medium/1% BSA. After washing three times with 5 ml of RPMI 1640 medium/1% BSA, they were resuspended in 20 ml of RPMI 1640 medium/1% BSA. Aliquots (1 ml) were treated with drugs (37°C; 30 min) and the cells were lysed by addition of 1 ml of 1.25% SDS/5 mM EDTA/sheared calf thymus DNA (0.4 mg/ml). The covalent DNA–protein complexes were precipitated by the KCl/SDS method (13).

Alkaline Sucrose Gradient Sedimentation. DEAE-purified trypanosomes (2.6×10^8) were labeled (37°C; 60 min) with [^{3}H]thymidine (20 Ci/mmol; 385 μCi/ml), washed, and resuspended in 13 ml of RPMI 1640 medium/1% BSA. Samples were removed for measurement of trichloroacetic acid (TCA)-insoluble radioactivity (1–50 μl was spotted on 3MM filters for TCA washing and counting), and 4-ml portions (2.3×10^7 per ml) were treated (37°C; 30 min) with either 1 μM isometamidium chloride or 100 μM etoposide (final concentrations). After removal of samples for TCA and KCl/SDS precipitations, 3.5-ml portions were centrifuged; the cells were resuspended in 0.5 ml of supernatant containing 250 ng of λ DNA. The cell suspension was layered over a discontinuous alkaline sucrose gradient (7) in cellulose nitrate tubes prerinsed with 10× Denhardt's solution (14). The gradient contained (from the top) 0.5 ml of freshly prepared lysis solution (200 mM NaOH/10 mM EDTA/1% sodium deoxycholate/0.5 mM phenylmethylsulfonyl fluoride); a 16-ml 5–20% linear alkaline sucrose gradient in 200 mM NaOH/10 mM EDTA/0.2% sodium *N*-lauroylsarcosinate; and a 0.5-ml 50% sucrose cushion containing 200 mM NaOH, 10 mM EDTA. After centrifugation (20 hr; 16,000 rpm, Beckman SW27 rotor; 4°C), fractions (0.8 ml) were collected, starting at 1.5 cm above the bottom of the tube, and neutralized with 500 mM HCl/500 mM Tris·HCl (0.2 ml). Solution remaining at the bottom was sonicated briefly and neutralized.

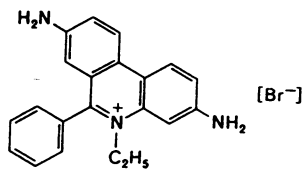
RESULTS

Antitrypanosomal Drugs Promote Minicircle Linearization. To test whether antitrypanosomal drugs promote minicircle cleavage, we exposed freshly harvested *T. equiperdum* to pentamidine, ethidium bromide, diminazene aceturate, or isometamidium chloride (Fig. 1). We tested these compounds at concentrations that could be attained in animals after a curative parenteral dose (2). We also used suramin, an antitrypanosomal drug that does not bind to DNA, and etoposide (VP16-213), an epipodophyllotoxin known to inhibit eukaryotic type II topoisomerases, including a mitochondrial type II topoisomerase in *T. equiperdum* (5). After treatment for 60 min at 37°C, the trypanosomes were lysed with SDS and the purified DNA was resolved by agarose gel electrophoresis. To examine minicircle DNA, a Southern blot was hybridized with a ^{32}P-labeled *T. equiperdum* minicircle probe. Under the conditions of electrophoresis, kDNA networks remain in the slot, and free minicircles, which are intermediates in replication (9), enter the gel. Fig. 2 (lane 1) depicts the normal distribution of free minicircles; the predominant forms are nicked or uniquely gapped circles (II), linearized circles (III), and covalently closed circles (I). As we reported previously, etoposide causes a striking increase in the amount of linearized minicircle DNA (lane 7; form III). Some of the antitrypanosomal drugs, including diminazene aceturate, isometamidium chloride, ethidium bromide, and pentamidine (lanes 2–5, respectively) also caused linearization of minicircle DNA. However, suramin (lane 6) had no detectable effect. The antitrypanosomal drugs that promote minicircle linearization also alter the levels of other free minicircle components, increasing forms I, II, and multimeric minicircle species (lanes 2–5). These changes are different from those induced by etoposide, which produces relatively less form I and results in a more complex pattern of multimeric minicircles (lane 7).

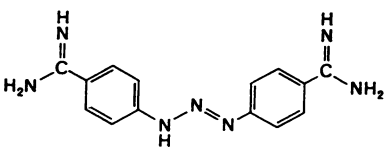
The antitrypanosomal drugs promote minicircle linearization at relatively low concentrations (Table 1). However, unlike etoposide (5), increasing concentrations did not cause a plateau in minicircle linearization. Instead, linearization reached a peak, with less cleavage occurring at higher drug concentrations. With 100 μM etoposide, the linearization of minicircles reaches a maximum within 5 min of drug exposure. However, even at 60 min of treatment with 2 μM



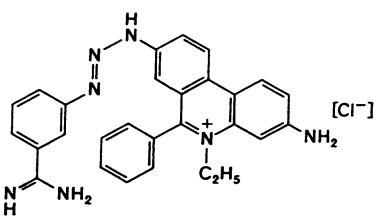
Pentamidine



Ethidium bromide



Berenil



Samorin

FIG. 1. Antitrypanosomal drugs. Pentamidine and diminazene aceturate (Berenil) are diamidines. Isometamidium chloride (Samorin) has the phenanthridium nucleus of ethidium bromide, with a diazoamino benzamidine side chain analogous to that in diminazene chloride.

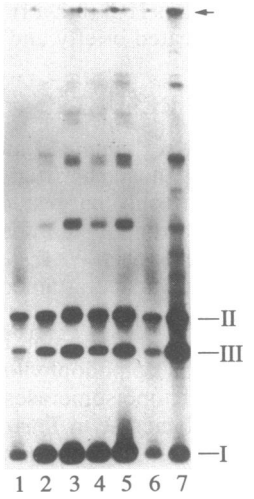


FIG. 2. Linearization of minicircles from drug-treated cells. *T. equiperdum* (2.6×10^7 cells per ml) in RPMI 1640 medium/1% BSA were treated (60 min; 37°C) with drug or solvent. Isolated DNA was fractionated by agarose gel electrophoresis (8×10^6 cell equivalents per lane), transferred to GeneScreen, and probed with ^{32}P-labeled minicircle DNA. Lanes: 1, DNA from control cells; 2, from cells treated with diminazene aceturate (5 μM); 3, from cells treated with isometamidium chloride (1.5 μM); 4, from cells treated with ethidium (5 μM); 5, from cells treated with pentamidine (10 μM); 6, from cells treated with suramin (10 μM); 7, from cells treated with etoposide (125 μM). II, minicircles containing nicks or a small gap; III, linearized minicircles; I, covalently closed minicircles. Bands above form II are minicircle multimers. Arrow indicates slot.

isometamidium chloride, minicircle linearization continues to increase as a function of time (data not shown).

Minicircles Linearized by Drug Treatment Have Protein at Both 5′ Ends. If proteinase K treatment was omitted before phenol extraction of SDS lysates like those in Fig. 2, the aqueous phase (as visualized in Fig. 2) did not contain the linearized minicircles; presumably they were in the phenol phase or the interface. Furthermore, in SDS agarose gels (15), linearized minicircles from cells treated with isometamidium chloride or etoposide (not digested with proteinase K), migrated ≈10% more slowly than form III minicircle markers (data not shown). These experiments indicate that the drug-induced linearized minicircles are linked to protein.

To determine whether protein was bound to the 3′ or 5′ ends of the linearized minicircles, proteinase K-treated DNA from isometamidium chloride-treated cells was digested with either λ exonuclease (a 5′ to 3′ exonuclease) or exonuclease III (a 3′ to 5′ exonuclease). After protease treatment, linearized minicircles that had been linked to protein would presumably contain a residual amino acid or short peptide. As described (5), etoposide-induced linearized minicircles (Fig. 3, lane 3; form III) were digested by exonuclease III (lane 2) but were unaffected by λ exonuclease (lane 4). The isometamidium chloride-induced linearized minicircles behaved similarly (lanes 5–7). pBR322 DNA linearized by *Hin*dIII, an internal monitor of exonuclease activity, was fully digested by λ exonuclease.

The experiment in Fig. 3 indicates that the protein trapped from the mitochondria of isometamidium chloride-treated trypanosomes, like that from etoposide-treated cells, is linked to both 5′ ends of the linearized minicircle.

Map of Cleavage Sites. To evaluate whether the antitrypanosomal drugs promote cleavage at discrete sites in the minicircle sequence, DNA samples from drug-treated or control cells were digested with *Bgl* II, an enzyme that cleaves minicircles only once at nucleotide 345 (8). The fragments were separated by agarose gel electrophoresis, transferred to nylon filters, and probed with ^{32}P-labeled synthetic oligonucleotides (L-329 and L-381, which flank the *Bgl* II site), as described (5). From the sizes of these fragments, and from fragments in *Hin*fI and *Eco*RV digests, we constructed a map of the drug-induced cleavage sites (Fig. 4).

Antitrypanosomal Drugs Do Not Promote Cleavage of *T. equiperdum* Nuclear DNA. To determine whether the antitrypanosomal drugs and etoposide (VP16-213) promote the formation of nuclear DNA–protein complexes, freshly harvested trypanosomes in RPMI 1640 medium/1% BSA were labeled with [^{3}H]thymidine (60 min; 37°C), washed, and treated for 30 min with either 10 μM isometamidium chloride

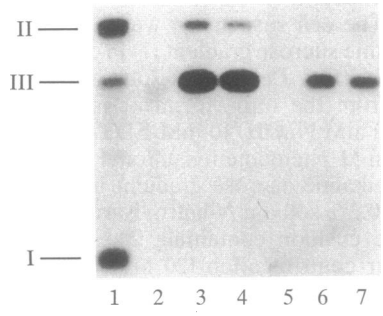


FIG. 3. Exonuclease treatment of drug-induced linearized minicircles. Trypanosomes (1.3×10^8) in 1 ml of RPMI 1640 medium/1% BSA were treated at 37°C for 30 min with 100 μM etoposide or with 10 μM isometamidium chloride (final concentrations) and lysed with SDS. The lysates were digested with proteinase K, RNase A, and RNase T1. After phenol extraction and dialysis, the DNA was sedimented in neutral sucrose gradients (16). Fractions enriched with form III minicircles were isopropanol precipitated and an aliquot (1/10th total fraction) was digested with exonuclease III or with λ exonuclease. Digests also contained 100 ng of *Hin*dIII-linearized pBR322 DNA. After agarose gel electrophoresis, the DNA was transferred to a nylon membrane and probed with ^{32}P-labeled minicircle sequence. To compensate for differences in recovery, for lanes 1 and 5–7, the autoradiograph was exposed for 18 hr; for lanes 2–4, the autoradiograph was exposed for 2 hr. Lanes: 1, *T. equiperdum* free minicircle markers [the faint band between forms I and III is a knotted minicircle (11)]; 2, DNA from etoposide-treated cells, digested with exonuclease III; 3, same as lane 2 but no nuclease treatment; 4, same as lane 2 but digested with λ exonuclease; 5, DNA from isometamidium chloride-treated cells, digested with exonuclease III; 6, same as lane 5 but no nuclease treatment; 7, same as lane 5 but digested with λ exonuclease. There was no detectable *Hin*dIII-linearized pBR322 DNA remaining in the λ exonuclease reactions (not shown). As expected, λ exonuclease did not digest form II minicircles (17).

Table 1. Extent of drug-promoted minicircle linearization

Drug	Minicircle linearization	
	Maximum,* % of total minicircles	Drug concentration at maximum, μM
Etoposide (VP16-213)	12	≥75
Isometamidium chloride	6	1
Pentamidine	5	5
Diminazene aceturate	2	1
Ethidium bromide	2	1

T. equiperdum (2×10^7 cells per ml) in RPMI 1640 medium/1% BSA were treated (30 min; 37°C) with the indicated drugs (0.001–100 μM) and lysed with SDS. The isolated DNA was fractionated by agarose gel electrophoresis, transferred to GeneScreen, and probed with ^{32}P-labeled minicircle DNA. Linearized minicircle bands were quantitated by densitometry of autoradiographs.

*Values were calculated relative to etoposide, which cleaves 12% of the total (free plus network bound) minicircle population. In DNA from control cells, <1% of the total minicircle population is linearized under these conditions.

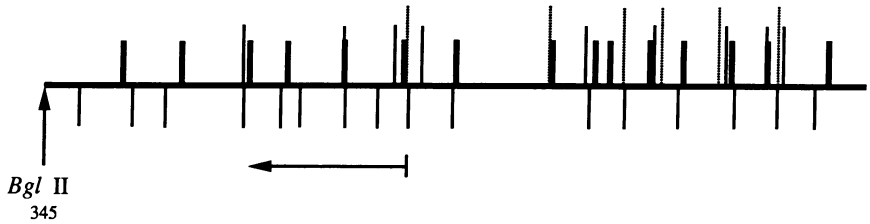


FIG. 4. Map of drug-promoted cleavage sites in *T. equiperdum* minicircle DNA. The minicircle sequence [1012 base pairs (8)] is depicted as linearized at the *Bgl* II restriction site (nucleotide 345). Breaks induced by antitrypanosomal drugs are shown above the line (dotted lines, ethidium; narrow lines, pentamidine and diminazene aceturate; heavy lines, isometamidium chloride); those induced by etoposide are below the line. During minicircle replication, the continuously synthesized L strand initiates at a conserved 12-mer (nucleotides 800–811) and progresses as indicated by the arrow (16, 18).

or 100 μM etoposide. The cells were lysed with SDS and the [^{3}H]DNA was analyzed by the KCl/SDS procedure, a method for precipitation of covalent DNA–protein complexes (13). In six experiments, the KCl/SDS-precipitable radioactivity, as a percentage of TCA-precipitable radioactivity, was as follows (mean ± SD): no drug, 11.1% ± 4.4%; isometamidium chloride, 11.8% ± 3.4%; etoposide, 33.4% ± 16.3%. If samples from etoposide-treated cells were digested with proteinase K, the KCl/SDS-precipitable radioactivity was reduced to control levels. Whereas etoposide clearly generates precipitable DNA–protein complexes, no detectable complexes were formed in the presence of isometamidium chloride. Minicircle DNA from isometamidium chloride-treated cells was linearized in these experiments, but these linears are present in too low a concentration (≈0.06% of total cellular DNA) to contribute significantly to the KCl/SDS-precipitable radioactivity. Treatment from 5 to 60 min with isometamidium chloride, ethidium bromide, diminazene aceturate, or pentamidine (concentrations ranging from 1 nM to 1 mM) did not cause detectable precipitation of nuclear DNA above control levels.

In a second assay for drug-promoted breaks in trypanosome nuclear DNA, cells in RPMI 1640 medium/1% BSA were labeled with [^{3}H]thymidine, washed, and treated with 10 μM isometamidium chloride or 100 μM etoposide. The intact cells were centrifuged on alkaline sucrose gradients, with lysis occurring as the cells entered the gradient. There was no significant difference in the profile of DNA from control or isometamidium chloride-treated cells (Fig. 5), but the DNA from etoposide-treated cells had a much greater proportion of smaller fragments. Furthermore, only 7% of the DNA from etoposide-treated cells remained large enough to sediment to the bottom of the tube, in contrast to 50% from control or isometamidium chloride-treated cells.

From these experiments, we conclude that only the mitochondrial DNA in *T. equiperdum* is sensitive to the antitrypanosomal drugs, whereas both mitochondrial and nuclear DNA are sensitive to etoposide.

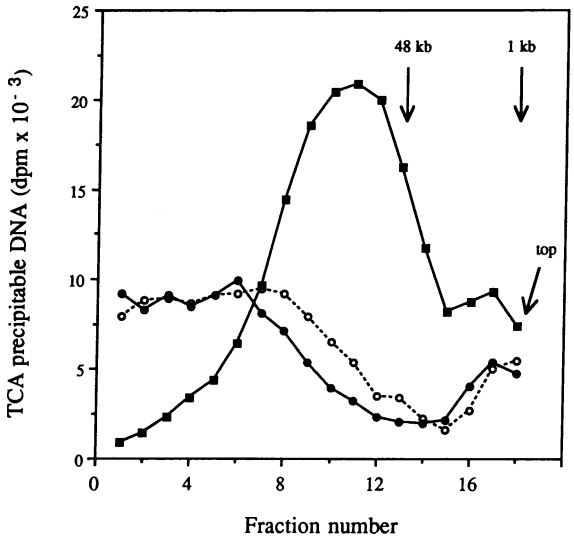


FIG. 5. Cleavage of *T. equiperdum* nuclear DNA by etoposide but not by isometamidium chloride. Trypanosomes in RPMI 1640 medium/1% BSA were labeled with [^{3}H]thymidine and treated with RPMI 1640 medium (○), 1 μM isometamidium chloride (●), or 100 μM etoposide (■). The cells (7×10^7; 4.3×10^6 dpm) were centrifuged on alkaline sucrose gradients. Internal markers were λ DNA [48 kilobases (kb)] and *T. equiperdum* minicircles (1 kb). Aliquots (1/16th of fraction) were precipitated with TCA and counted. Radioactivity in the bottom 1.5 cm of the tubes (not shown on graph) was as follows: no drug, 2.0×10^6 dpm; isometamidium chloride, 2×10^6 dpm; etoposide, 3.4×10^5 dpm. Total recovery of radioactivity was 93–102%. About 95% of total cell DNA is nuclear (19). In control and isometamidium chloride-treated samples, a significant fraction of the radioactivity in the lower half of the gradient may be intact minichromosomes, which are 200 kb or less (20).

DISCUSSION

Type II topoisomerases form cleavable complexes with their DNA substrate in the presence of a variety of drugs (21). Upon denaturation with SDS, a double-strand break is induced in the DNA, and a topoisomerase subunit is linked covalently to each 5′ end (7, 22). We showed previously that the antitumor drug etoposide (VP16-213) promotes linearization of *T. equiperdum* minicircle DNA, with protein bound to both 5′ ends of the DNA (5).

We now report that four major antitrypanosomal drugs, diminazene aceturate, isometamidium chloride, ethidium bromide, and pentamidine, also generate linearized minicircles (Fig. 2). The linearized minicircles were protected from λ exonuclease digestion (Fig. 3, lane 7), indicating that a double-strand break is made and that protein is linked at both 5′ ends of the DNA. These features are the hallmark of type II topoisomerase–DNA complexes. Furthermore, as has been reported for type II topoisomerase inhibitors in trypanosomes (5) and other cells (21), the antitrypanosomal drugs promote cleavage at distinct, but varying, sites in the minicircle sequence (Fig. 4). The linearization of minicircles is not a nonspecific effect of antitrypanosomal drugs. Suramin (Fig. 1, lane 6), bleomycin [an antitrypanosomal drug (23) that intercalates in DNA (24)], or salicylhydroxamic acid plus glycerol [a trypanocidal combination (25, 26)] do not promote minicircle cleavage.

Because strand passing activity was not assayed in these studies, it is possible that the bound protein is not a classical topoisomerase but is some other, unknown, enzyme. DNA–protein complexes could theoretically be formed with any enzyme that cleaves and ligates double strands of DNA by means of a covalent DNA–protein intermediate. Diminazene aceturate and ethidium have been described as inhibitors of mammalian mitochondrial topoisomerase activity (SDS-

induced cleavage was not tested) (27), raising the possibility that mammalian mitochondrial topoisomerase also forms cleavable complexes in the presence of these drugs.

Type I topoisomerases also form cleavable complexes, with a single-strand break induced by SDS treatment. In this case, the topoisomerase is covalently bound to either the 3′ end (in eukaryotes) or to the 5′ end (in prokaryotes) at the site of cleavage (22). Although the minicircles linearized in our experiments could have been generated by a type I topoisomerase acting across from a nick or gap, there was no evidence of protein-bound nicked minicircles (data not shown), and, more importantly, both 5′ ends of the linearized minicircles were protected from λ exonuclease digestion.

The formation of DNA–protein cleavable complexes in trypanosomes by antitrypanosomal drugs appears to be selective for kDNA. SDS-induced nuclear DNA–protein complexes or strand breaks in nuclear DNA (Fig. 5) could not be detected in cells that have demonstrable minicircle DNA–topoisomerase cleavable complexes. This selectivity could be explained if the drug did not enter the nucleus. However, at concentrations of ethidium bromide that generate linearized minicircles, both nuclear and mitochondrial DNA fluoresce, indicating that the drug reaches both compartments. Ethidium bromide also may not affect mammalian nuclear DNA, as it does not promote cleavable complexes with purified calf thymus nuclear type II topoisomerase (21).

Despite differences in chemical structure (Fig. 1) and in the mechanism of interaction with DNA, all four antitrypanosomal drugs, at therapeutically relevant concentrations, create the same pattern of alterations in the pool of free minicircles (Fig. 2, lanes 2–5), suggesting that they target the same topoisomerase. Interestingly, on the basis of light and electron microscopy studies, these drugs also interfere rapidly and preferentially with the structure of kinetoplast, rather than nuclear DNA (28), and they generate "dyskinetoplastic" trypanosomes (29, 30). Dyskinetoplastic cells retain mitochondrial membranes (31), but they lack the characteristic densely staining kDNA disc and have no detectable DNA homologous to minicircle sequences (32). These structural alterations could be caused by selective inhibition of a mitochondrial type II topoisomerase. Suramin, an antitrypanosomal drug that does not alter the free minicircle pool (Fig. 2, lane 6), does not cause selective structural disruption of kDNA (33).

The pattern of free minicircles generated by antitrypanosomal drugs differs from that generated by etoposide (Fig. 2, compare lanes 2–5 with lane 7). This suggests the interesting possibility that these two classes of drugs may target different topoisomerases in the kinetoplast.

These studies reveal a specific molecular effect of four of the classical antitrypanosomal drugs. They also suggest that in trypanosomes there might be differences in the mitochondrial and nuclear type II topoisomerase activities. These findings may prove useful in developing much needed targets for the design of chemotherapy against the kinetoplastid parasites.

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