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Inhibition of the reactions catalysed by a type I topoisomerase and a catenating enzyme of *Trypanosoma cruzi* by DNA-intercalating drugs. Preferential inhibition of the catenating reaction

Setha Douc-Rasy, Alain Kayser and Guy F. Riou*

Laboratoire de Pharmacologie Moleculaire, Institut Gustave Roussy, F-94805 Villejuif Cedex, France

*To whom reprint requests should be sent
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A catenating enzyme and a type I topoisomerase were purified from *Trypanosoma cruzi*. We investigated the inhibitory effect of DNA-intercalating drugs on topoisomerisations catalysed by these enzymes. Inhibition of catenation was detected by electrophoretic analysis in neutral agarose gels. However, the inhibition of relaxation was not readily detectable in these gels since supercoiled DNA, which was relaxed in the presence of an intercalating drug, returned to a supercoiled state when the drug was removed. Thus electrophoretic analyses were made in gels containing chloroquine so that unreacted DNA could be distinguished from DNA relaxed by the enzyme. The results show that the catenation was more sensitive to DNA-intercalating drugs than the relaxation.
Key words: trypanosome/topoisomerases/intercalating drugs/ellipticine/gel electrophoresis

Introduction

Kinetoplast DNA (kDNA) of trypanosomes comprises thousands of circular molecules held together by catenation so that they constitute a network (Englund, 1981). Replication of kDNA is assumed to involve the release of circles, their replication, the re-attachment of the progeny circles followed by the scission of the double-size network. Topoisomerases are involved in many steps of the process and the kDNA system could be the target for potential trypanocidal drugs; at present there is no chemotherapeutic cure for trypanosomiasis.

DNA topoisomerases are able to break and reseal either one (type I) or both strands (type II) of DNA molecules (Gellert, 1981). Thus, topoisomerases can relax supercoiled molecules and can catenate or decatenate circular molecules. We have characterised topoisomerase activities in extracts of *Trypanosoma cruzi* (Riou *et al.*, 1982) and we have partially purified the enzymes responsible for these activities (Riou *et al.*, 1983a, 1983b). Here we report on the *in vitro* inhibition of topoisomerisations catalysed by *T. cruzi* enzymes by ellipticine derivatives and other intercalating compounds. Catenation was generally more sensitive to these drugs than the relaxation by type I topoisomerase.

In addition, we present a system of electrophoretic analysis using chloroquine to detect inhibition of relaxation by DNA intercalating drugs. Supercoiled DNA molecules of natural origin – for instance replicative forms of bacteriophage fd – are heterogeneous in their topological parameter (linking number), which renders them diversely supercoiled in every given medium (Crick *et al.*, 1979). When the medium contains an agent, such as chloroquine, which exerts an unwinding effect on the DNA molecules, the electrophoretic mobility of these molecules is modified. Whereas in gels without chloroquine molecules are highly supercoiled and migrate as a single band, in the presence of chloroquine heterogeneity can be revealed since DNA topoisomers appear as discrete bands (Shure *et al.*, 1977). This pattern generally differs from those obtained with relaxed DNAs. Relaxed DNA, like supercoiled DNA, consist of a heterogeneous population of molecules: this is due to thermal motion (Pulleyblank *et al.*, 1975). DNA relaxed in normal conditions will not remain in the relaxed state when placed into the chloroquine medium: it will appear as a fast-migrating band of supercoiled molecules. However, DNA relaxed in the presence of unwinding agents, such as intercalating drugs, might appear as separate bands. Open circular DNA, in which at least one strand is nicked, cannot become supercoiled and has, regardless of the presence of unwinding ligand, a lower mobilty than supercoiled molecules. This system allowed relaxation to be tested in media containing drugs.

Results

Catenation

The chromatography fractions described in Materials and methods were able to catenate circular supercoiled DNA molecules. When SV40 DNA (Figure 1, lane 1) was treated by the catenating enzyme, it was aggregated and no longer

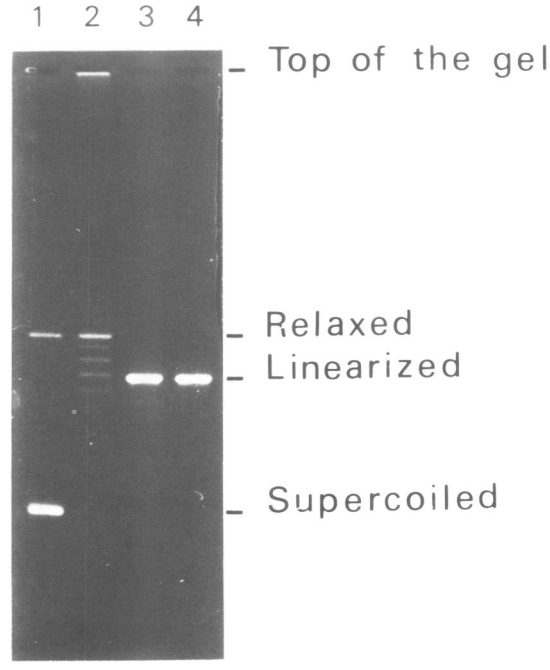

Fig. 1. Catenating activity of *T. cruzi* enzyme. Electrophoretic analysis in a 1% agarose gel of SV40 DNA samples. **(1)** DNA control. **(2)** + 0.5 μl enzyme (~3 units) then treated by SDS, proteinase K and phenol. The catenated DNA remains at the top of the gel. **(3)** as in **(2)** and treated by *Eco*RI. **(4)** DNA treated by *Eco*RI (control).

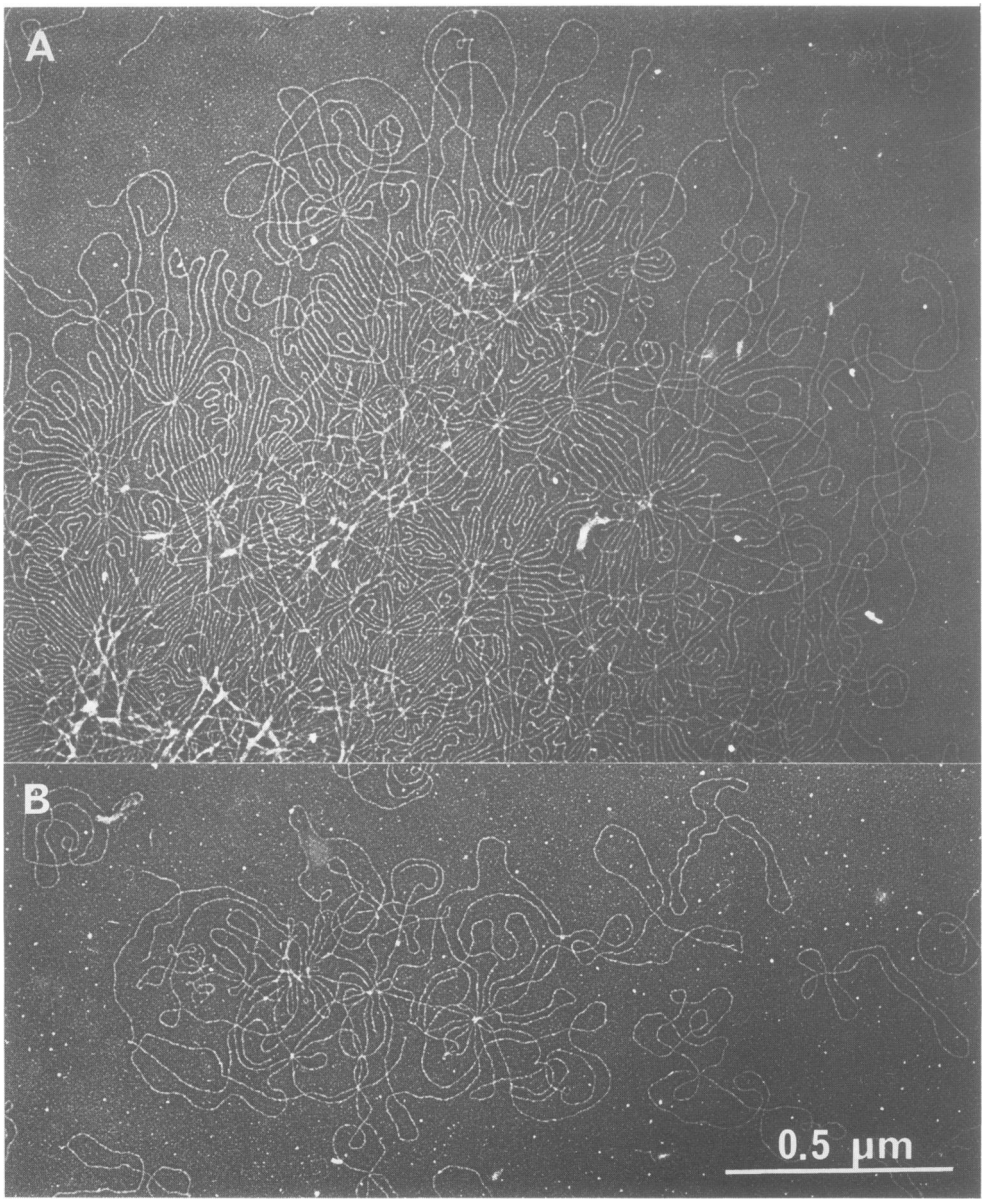

Fig. 2. Networks of DNA rings. fd DNA was incubated with the catenating enzyme. DNA was spread as previously described (Riou and Gutteridge, 1978). **(A)** Part of a network. **(B)** Catenated oligomer.

penetrated gels containing 1% agarose (lane 2). The aggregate was not dispersed by treatments with proteinase K, SDS and phenol. It consisted of circular DNA molecules, which in the electron microscope appeared as networks of variable size (Figure 2). The catenated circles could be dissociated by treatment with the restriction endonuclease *Eco*RI. SV40 DNA molecules were cut once and gave rise to free linearised molecules (form III): see Figure 1, lanes 3 and 4 (control).

Catenation by the *T. cruzi* enzyme required both Mg^{2+} and spermidine but not ATP (Riou *et al.*, 1983b). However, when either Mg^{2+} or spermidine was omitted, relaxation occurred. This relaxation activity could be due to contaminating type I topoisomerase, but the catenating activity did not seem attributable to a type I enzyme, which would only catenate nicked molecules. We have eliminated the possibility that an endonuclease cuts the DNA and allows a type I topoisomerase to act. DNA was analysed in a gel containing chloroquine (Figure 3) where relaxed and nicked DNA occupy different positions. It can be seen that treatment with the enzyme preparation did not generate nicked molecules (form II): compare lane 2 with untreated control (lane 1). DNA relaxed prior to the addition of chloroquine appears in lane 2 as the fast moving band (see Introduction).

Inhibition of the catenating reaction

Inhibition of the catenating reaction by drugs could be analysed in agarose gels. Catenation was completely inhibited when the band disappeared from the top of the gel (Figure 4). We used eight ellipticine derivatives (Figure 5 and 6; Tables I and II) all of which were effective inhibitors (see Table III). We compared their efficiency with that of other intercalating agents such as ethidium and acriflavine (Table III). All of the drugs inhibited catenation at similar concentrations. 2,6-diCH_3-9-OH-$El^m(Cl^-)$ was inhibitory at 1.5 μM and was the most efficient drug. We have also tested homodimers of ethidium, of acridine and of isoellipticinium and one hetero-

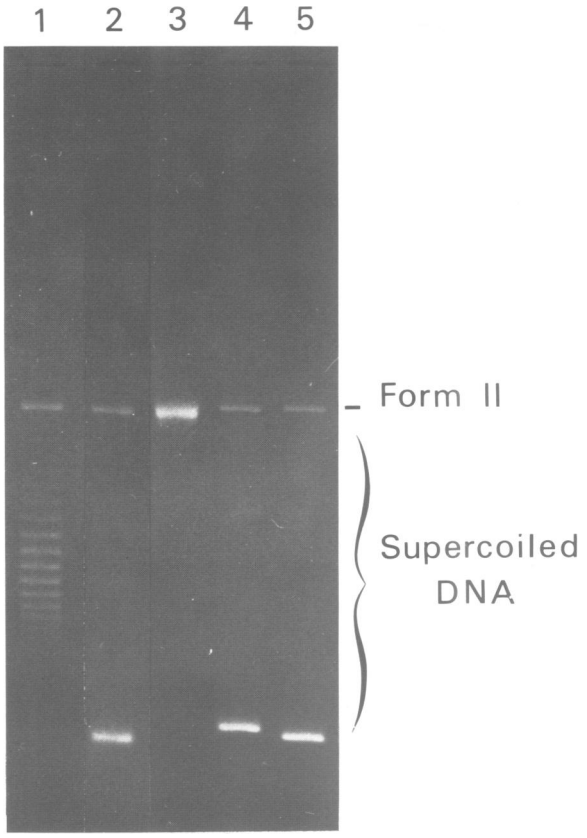

Fig. 3. The enzyme does not nick DNA. Electrophoretic analysis in a 1% agarose gel containing 18 μM chloroquine. **(1)** fd DNA (control). **(2)** + 0.5 μl enzyme (~3 units); reaction mixture without spermidine. DNA was relaxed, then supercoiled by chloroquine intercalation: it migrated as the fast-moving band. Form II DNA was not augmented. **(3–5)** + 3.6, + 18, + 36 μM acriflavine. Relaxation activity was not inhibited.

dimer of ethidium and acridine, because dimeric molecules may have a higher affinity for DNA than monomers depending on the chain linking the intercalating groups (Gaugain *et al.*, 1978; Roques *et al.*, 1979). The results suggest that IsoEl^{m}Di and EtDi which were inhibitory at 0.5 μM are the most efficient compounds.

Inhibition of type I topoisomerase relaxation activity

Inhibition of the relaxation by intercalating drugs cannot be detected in neutral agarose gels. When a DNA molecule is relaxed by a topoisomerase in the presence of ethidium bromide, for instance, and thereafter transferred to a drug-free medium, it will loose the intercalated molecules, thereby returning to the supercoiled state. However, if ethidium inhibits the expected relaxation by the topoisomerase, the DNA remains supercoiled. One cannot determine if a drug has an inhibitory effect unless one can distinguish unreacted substrate from DNA relaxed by the enzyme in the presence of the drug. Such a distinction is possible when DNA is electrophoresed in gels containing an unwinding agent (Keller, 1975). Chloroquine was preferred to ethidium because it had a lower affinity for DNA and gave more reproducible results (Shure *et al.*, 1977). In such gels, the migration of a closed circular DNA depends on the concentration of the ligand.

Partial unwinding of the DNA gave the opportunity to test whether topoisomerisation occurred or not. We used chloroquine sulfate at a concentration of 18 μM. In these conditions the topoisomers of the native sample occupied a medium position, between that of highly supercoiled molecules and that of open circular molecules. This distribution of DNA offered a convenient basis for comparison with enzyme-treated samples. The pattern drawn in Figure 7 presents the various configurations that can appear in neutral gels and gels containing chloroquine for various concentrations (C_1-C_7) of a given intercalating drug.

When DNA was in its native state, it was highly and nega-

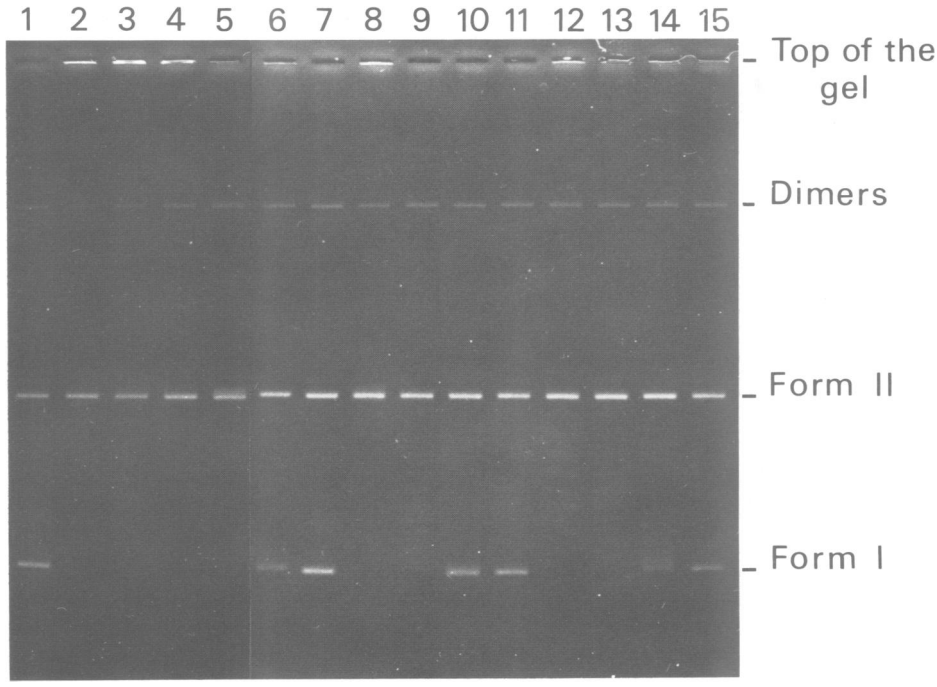

Fig. 4. Inhibition of the catenating enzyme. Electrophoretic analysis in a 1% agarose gel. **(1)** pBR322 DNA. **(2)** + 0.5 μl enzyme (~3 units). **(3–5)** + 0.9, + 1.8, + 3.5 μM acriflavine. **(6–7)** + 0.75, + 1.5 μM 2-6-diCH_3-9-OH-El^{m}. **(8–11)** + 1.75, + 3.5, + 9, + 18 μM El. **(12–15)** + 1.5, + 3, + 8, + 15 μM BrEl. Inhibition occurred in **lanes 5, 7, 9, 10, 11** and **15**.

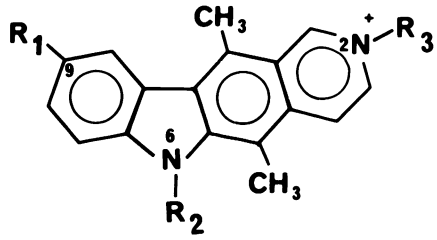

Fig. 5. Structure of ellipticinium derivatives.

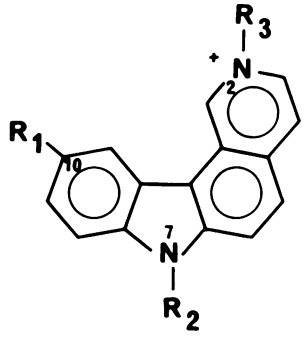

Fig. 6. Structure of isoellipticinium derivatives.

Table I. Structure of ellipticine derivatives

Derivatives	R1	R2	R3
Ellipticine	H	H	–
9-OH-Ellipticine	OH	H	–
9-OCH_3-6-CH_3-Ellipticine	OCH_3	CH_3	–
9-Br-Ellipticine	Br	H	–
2,6-diCH_3-Ellipticinium (I^-)	H	CH_3	CH_3
2-6-diCH_3-9-OH-Ellipticinium (Cl^-)	OH	CH_3	CH_3

Table II. Structure of isoellipticinium derivatives.

	R1	R2	R3
$IsoEl^m$	OCH_3	H	CH_3
$IsoEl^m$Di	OCH_3	H	$(CH_2)_2$-1-piperidyl,Cl^-

tively supercoiled (HS^-) in the neutral gel and less negatively supercoiled (S^-) in the gel containing chloroquine: see lane 1.

When DNA was submitted to the action of topoisomerase, it was relaxed (R). However, when electrophoresed in the presence of chloroquine it was positively supercoiled (HS^+) as a result of chloroquine intercalation and consequently had a higher mobility than the control (S^-): see lane 2.

When DNA was relaxed in the presence of low concentrations of drug (C_1 lane 3, in Figure 7), chloroquine induced less supercoiling than in lane 2. When DNA was relaxed in the presence of more drug (C_2), chloroquine had an effect equivalent to that of the drug and the DNA remained in the relaxed state: see lane 4. As the concentration of drug in the reaction mixture was further increased, the DNA became more negatively supercoiled during electrophoresis: see lanes 5 and 6.

When the DNA was treated with topoisomerase in the presence of higher concentrations of drug, inhibition was

Table III. Inhibitory concentrations (in μM) of intercalating agents on type I topoisomerase relaxing activity and on catenating activity of *T. cruzi*

Drug	K_{ap}[a] x 10^{-5}	Relaxation	Catenation
El	1.5	>140[b]	3.5
9-OH-El	20	30	7
9-OCH_3-6-CH_3-El	20	100	<17
9-Br-El	4	>60[b]	15
2-6-diCH_3-El^m (I^-)	66	25	2.5
2-6-diCH_3-9-OH-El^m (Cl^-)	40	1.5	1.5
Et	–	>100[b]	12
Acriflavine	–	70	3.5
PDI	–	15	7.5
DAPI	–	30	<35
Mepacrine	–	>80[b]	>10[b]
Chloroquine	–	2400	–
$IsoEl^m$	2.9	>85[b]	–
$IsoEl^m$Di	1 x 10^2	5	0.5
EtDi	2 x 10^3	>10[b]	0.5
AcEtDi	–	>10[b]	1
AcDi	5 x 10^3	3.5	1.5

[a]DNA binding constants measured as described in Le Pecq *et al.* (1974), Pelaprat *et al.* (1980a, 1980b), Paoletti *et al.* (1980).
[b]Partial inhibition at the quoted concentration.

detected. This can be seen in lane 7, where a portion of the DNA remained in the stage of the control DNA. The configuration of the molecules in chloroquine was $S^- + HS^-$ which is the superposition of lanes 1 and 6. This indicates partial inhibition. In lanes 8 and 9 all the DNA remained as the control distribution (S^-): this indicates total inhibition.

The testing of three drugs is presented in Figure 8. The topoisomers of fd DNA appeared as a series of >10 bands (lane 1). Uppermost bands were due to nicked (form II) DNA. One sees that reacted DNA presented various distributions, depending upon the concentration of the drug in the reaction mixture. To analyse the molecular form of the DNA without interference from the drug, the drug was extracted with butanol. Lanes 3–9 show the effect of acriflavine: the relaxation was totally inhibited at 71 μM (lane 9). Lanes 10–16 show the effect of isoellipticinium. The patterns are not so diverse since the conditions of lane 10 (1.3 μM $IsoEl^m$) already allowed DNA to remain relaxed in the gel. The reaction was partially inhibited at 85 μM (lane 16). Chloroquine (lanes 17–20) was not inhibitory at 145 μM (lane 20). These results and similar data obtained with other drugs are reported in Table III. It is noticeable that El was inhibitory at concentrations >140 μM, whereas supercoiled DNA was apparent in lane 10, Figure 4. This was, of course, an illustration of the situation of lanes 3–6, Figure 7: the reaction was not inhibited at 9 μM of El.

Discussion

Trypanosomes contain a type I topoisomerase, which is comparable with those already described in eukaryotes (Gellert, 1981). The properties of this enzyme which relaxes positive as well as negative supercoils were reported previously (Riou *et al.*, 1983a). Another enzyme was able to catenate closed circular molecules. As shown above our preparation seemed to

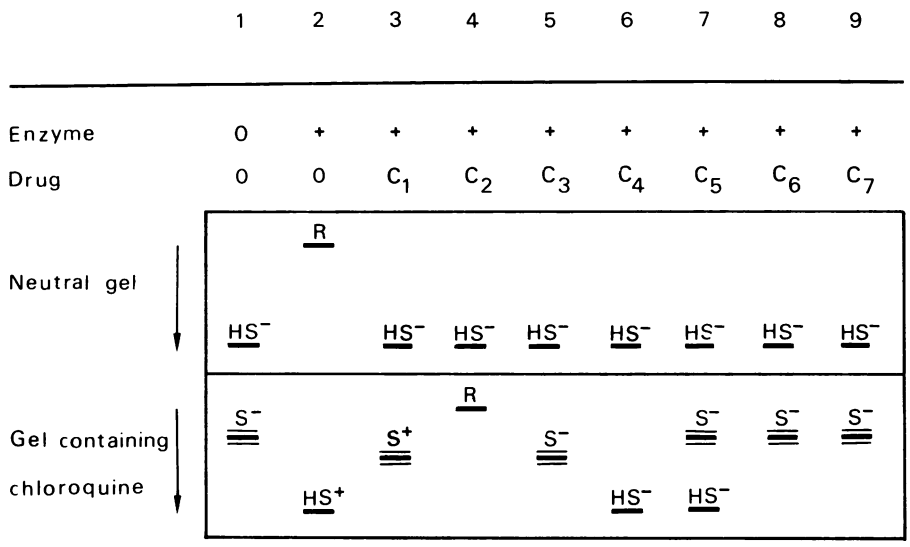

Fig. 7. Schematic representation of electrophoretic analysis in a neutral gel and in a gel containing chloroquine. Search for inhibitory effect of an intercalating drug present in the reaction mixture during topoisomerisation. S = supercoiled (− negatively, + positively); HS = highly supercoiled; R = relaxed; C_1-C_7 = increasing concentrations of a given intercalating drug. Real values depend upon the nature of the drug. Neutral gel: **(1)** supercoiled DNA. **(2)** + enzyme. **(3−9)** + drug: one does not see whether there was an inhibition or not. Gel containing chloroquine: **(1)** supercoiled DNA. **(2)** + enzyme. **(3−9)** + drug at increasing concentrations. **(3−6)** No inhibition. **(7)** Partial inhibition. **(8−9)** Total inhibition.

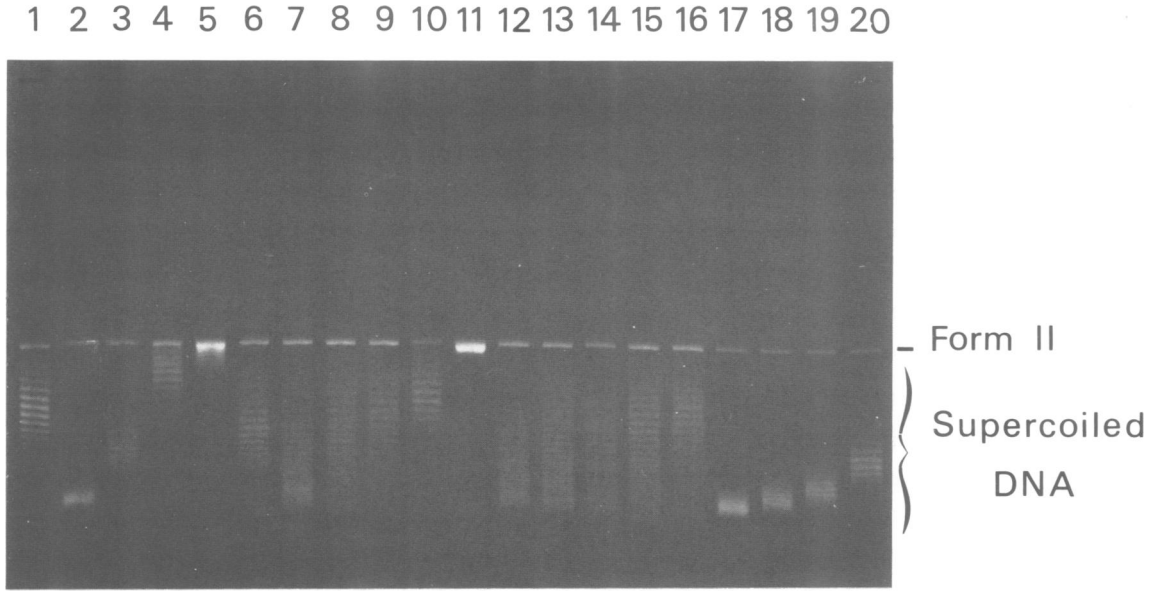

Fig. 8. Inhibition of *T. cruzi* type I topoisomerase activity. Electrophoretic analysis in a 1% agarose gel containing 18 μM chloroquine. **(1)** fd DNA (0.2 μg) (control). **(2)** + 1 μl type I topoisomerase (~3 units). **(3−9)** + 0.9, + 1.8, + 3.6, + 9, + 18, + 36, + 71 μM acriflavine. Relaxation was inhibited at 71 μM. **(10−16)** + 1.3, + 2.6, + 5.3, + 11, + 21, + 43, + 85 μM $IsoEl^m$. Relaxation was partially inhibited at 85 μM. **(17−20)** + 18, + 36, + 72, + 145 μM chloroquine. Relaxation was not inhibited.

be contaminated with type I topoisomerase since relaxation was obtained when the reaction mixture was altered. This relaxing activity was not inhibited by drugs at higher concentrations than the minimal doses inhibiting catenation, which supports our suggestion of contamination: see Figure 3, lanes 3−5. The enzyme responsible for the catenating activity could be a type II topoisomerase. However, enzymes of that type are generally ATP-dependent, catalyse the decatenation of natural catenanes such as the kinetoplast DNA of trypanosomes, and unknot DNA molecules prepared from tailless capsids of the bacteriophage P4. We were unable to find conditions that would allow these reactions to occur when using the *T. cruzi* catenating fractions.

Both *T. cruzi* enzymatic activities were sensitive to intercalating agents, such as many ellipticine derivatives, and to drugs whose interaction with DNA is not so clear, such as DAPI (De Clercq and Dann, 1980), chloroquine (Jones *et al.*, 1979) and 9-bromoellipticine (Le Pecq *et al.*, 1974). Intercalation into DNA is a property of some of the tested drugs which interferes with the measure of their inhibitory power (see Introduction). However, using electrophoresis in gels containing chloroquine, we were able to determine inhibitory concentrations for the relaxing and catenating activities. Dimeric molecules have been synthesised to augment the DNA affinity of the intercalating moieties. Those molecules proved to be potent inhibitors, as shown in a preliminary communication

(Douc-Rasy *et al.*, 1982): the acridine dimer inhibited the relaxing activity at 3.5 μM concentration, which is 20-times less than the minimal inhibiting concentration of the monomer (acriflavine). The isoellipticinium dimer was ~30-times more potent than the monomer. Catenation proved more sensitive to most tested inhibitors than relaxation. The catenation could be inhibited solely as supercoiling was abolished by the intercalating effect of drug, but this does not seem a convincing explanation, since relaxed DNA can be catenated (results not shown). However, in the case of 2-6 diCH_3-9-OH-El^m, we observed inhibitory effects at the same very low concentration (1.5 μM) for both the relaxation and the catenation reactions. This compound was effective at only 60 μM with topoisomerase I from rat liver. However, the reactions catalysed by rat liver type II topoisomerase were as sensitive as those catalysed by *T. cruzi* enzymes (Douc-Rasy *et al.*, 1983). Intercalation of drugs is not a requirement for their efficiency: for instance bromoellipticine, a non-intercalating compound, inhibited catenation.

Among the drugs that we have tested, ethidium is known as a trypanocide in cattle (Brownlee *et al.*, 1950). Chloroquine and mepacrine are used as anti-malarials (Hahn, 1975) and some ellipticine derivatives as anti-tumorals (Paoletti *et al.*, 1980). Investigations *in vitro* with acriflavine (Hajduk, 1978), DAPI (Hajduk, 1978) and ellipticines (Benard *et al.*, 1975; Benard and Riou, 1975) have already revealed the trypanocidal effect of these drugs.

Materials and methods

Abbreviations

El, ellipticine; El^m, ellipticinium; Et, ethidium; DAPI, 4′,6-diamidine-2-phenylindole; PDI, propidium diiodide; EtDi, ethidium homodimer; AcDi, acridine homodimer; AcEtDi, acridine ethidium heterodimer; IsoEl^m, isoellipticinium = 10-OCH_3-2-CH_3-7H-pyrido[4,3c]carbazolium; IsoEl^{m}Di, isoellipticinium dimer.

T. cruzi, Tehuantepec strain from Institut Pasteur, Paris, was grown in LIT medium (Riou and Gutteridge, 1978), collected in the exponential phase, and washed in 0.15 M NaCl + 0.015 M Na citrate. The extraction procedure of type I topoisomerase was described in another paper (Riou *et al.*, 1983a). Briefly, the trypanosomes were treated by Nonidet-P40. The lysate was centrifuged. The pellet was resedimented through a 20% sucrose solution. The nuclear fraction (pellet) was suspended in a Tris buffer and lysed by an equal volume of 2 M NaCl. Nucleic acids were precipitated by slow addition of polyethylene glycol (PEG 6000) and discarded. The liquid phase was loaded onto a hydroxylapatite column. Proteins were eluted by a 0.2–1.0 M potassium phosphate gradient. The catenating enzyme was eluted around 0.4 M and further purified by phosphocellulose chromatography (Riou *et al.*, 1983b). The type I topoisomerase was eluted around 0.5 M. Enzyme fractions were pooled, dialysed against a conservation buffer [50% w/v glycerol, 0.1 M Tris-HCl pH 7.5, 10 mM mercaptoethanol, 0.5 mM dithiothreitol (DTT), 0.1 mM EDTA] and stored at −20°C.

The DNA was pBR322, SV40 or the replicative form of the bacteriophage fd purified in a gradient of CsCl-ethidium bromide. The DNA (0.1 μg for neutral agarose gels and 0.2 μg for gels containing chloroquine) was submitted to topoisomerase action at 30°C for 30 min. Reaction medium for relaxation by topoisomerase I was: 10 mM Tris HCl (pH 7.9), 50 mM KCl, 10 mM $MgCl_2$, 0.5 mM DTT, 0.5 mM EDTA, 15 μg/ml bovine serum albumin. Volume: 20 μl. Reaction medium for catenation assays: 10 mM Tris HCl (pH 7.9) 10 mM $MgCl_2$, 5 mM spermidine, 0.5 mM DTT, 0.5 mM EDTA, 15 μg/ml albumin. Volume 20 μl.

One unit of relaxation activity relaxes half of the supercoiled DNA under the conditions of the assays. One unit of catenation activity catenates half of the DNA under the conditions of the assays, as appreciated by lowering of the quantity of migrating DNA.

Drugs

Ellipticine derivatives were provided by Professor J.B. Le Pecq, Laboratoire de Physicochimie Macromoléculaire, Institut Gustave Roussy, Villejuif and dimers by Professor B.P. Roques, Université René Descartes, Paris. Ethidium bromide and DAPI were from Sigma, USA; PDI from Calbiochem, USA; acriflavine, mepacrine and chloroquine sulfate from Specia, France. Drugs were incubated with the DNA for 15 min (at 30°C) before addition of enzyme.

Electrophoresis

Electrophoresis was performed either in normal gels (40 mM Tris, 20 mM sodium acetate, 2 mM EDTA, pH 7.8) or in chloroquine-containing gels, as described by Shure *et al.* (1977). The buffer, in the latter case, was 50 mM Tris phosphate (6.06 g Tris, 2.85 g 85% H_3PO_4 per liter, 1 mM EDTA, 7.5 μg/ml chloroquine sulfate). The buffer was recirculated. The gels contained 1% agarose. The electric field was 1,4 V/cm and the duration 18 h. Gels were stained by ethidium bromide (10 μg/ml), washed and photographed under u.v. illumination with a polaroid camera, film 665, and a red filter.

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References

Benard,J. and Riou,G. (1975) in Van Den Bossche,H. (ed.), *Biochemistry of Parasites and Host-Parasite Relationships,* Elsevier North Holland Biomedical Press, Amsterdam, pp. 477-484.

Benard,J., Dat-Xuong,N. and Riou,G. (1975) *C.R. Hebd. Seances Acad. Sci. Paris,* **280**, 1177-1180.

Brownlee,G., Goss,M.D., Goodwin,L.G., Woodbine,M. and Walls,L.P. (1950), *Br. J. Pharmacol.*, **5**, 261-275.

Crick,F.H.C., Wang,J.C. and Bauer,W.R. (1979) *J. Mol. Biol.*, **129**, 449-461.

De Clercq,E. and Dann,O. (1980) *J. Med. Chem.*, **23**, 787-795.

Douc-Rasy,S., Kayser,A., Gabillot,M. and Riou,G. (1982) *Special FEBS Meeting Athens 25–29 April,* p. 170.

Douc-Rasy,S., Multon,E., Kayser,A. and Riou,G. (1983) *C.R. Hebd. Seances Acad. Sci. Paris,* **296**, 899-904.

Englund,P.T. (1981) in Levandowsky,M. and Hunter,S.H. (eds.), *Biochemistry and Physiology of Protozoa,* 2nd edition, Vol. **4**, pp. 333-383.

Gaugain,B., Barbet,J., Capelle,N., Roques,B.P. and Le Pecq,J.B. (1978) *Biochemistry (Wash.),* **17**, 5078-5088.

Gellert,M. (1981) *Annu. Rev. Biochem.*, **50**, 879-910.

Hahn,F.E. (1975), in Corcoran,J.W. and Hahn,F.E. (ed.), *Antibiotics III,* Springer Verlag, Heidelberg, pp. 58-78.

Hajduk,S.L. (1978) *Prog. Mol. Subcell. Biol.*, **6**, 158-200.

Jones,R.L., Davidson,M.W. and Wilson,W.D. (1979) *Biochem. Biophys. Acta,* **561**, 77-84.

Keller,W. (1975) *Proc. Natl. Acad. Sci. USA,* **72**, 2550-2554.

Le Pecq,J.B., Dat-Xuong,N., Gosse,C. and Paoletti,C. (1974) *Proc. Natl. Acad. Sci. USA,* **71**, 5078-5082.

Paoletti,C., Le Pecq,J.B., Dat-Xuong,N., Juret,P., Garnier,H., Amiel,J.L. and Rouesse,J. (1980) in Mathé,G. and Muggia,F.M. (eds.), *Recent Results in Cancer Rearch,* Springer-Verlag, Heidelberg, pp. 107-123.

Pelaprat,D., Oberlin,R., Le Guen,I., Roques,B.P. and Le Pecq,J.B. (1980a) *J. Med. Chem.*, **23**, 1330-1335.

Pelaprat,D., Delbarre,A., Le Guen,I., Roques,B.P. and Le Pecq,J.B. (1980b) *J. Med. Chem.*, **23**, 1336-1343.

Pulleyblank,D.E., Shure,M., Tang,D., Vinograd,J. and Vosberg,H.P. (1975) *Proc. Natl. Acad. Sci. USA,* **72**, 4280-4284.

Riou,G. and Gutteridge,W. (1978) *Biochimie,* **60**, 365-379.

Riou,G., Gabillot,M., Douc-Rasy,S. and Kayser,A. (1982) *C.R. Hebd. Seances Acad. Sci. Paris,* **294**, 439-442.

Riou,G., Gabillot,M., Douc-Rasy,S., Kayser,A. and Barrois,M. (1983a) *Eur. J. Biochem.*, **134**, 479-484.

Riou,G., Gabillot,M., Douc-Rasy,S. and Kayser,A. (1983b) *DNA-Topoisomerases of Trypanosomes: Inhibitory Effects, of Chemicals, UCLA Symposia on Molecular and Cellular Biology*, in press.

Roques,B.P., Pelaprat,D., Le Guen,I., Porcher,G., Gosse,C. and Le Pecq, J.B. (1979), *Biochem. Pharmacol.*, **28**, 1811-1815.

Shure,M,. Pulleyblank,D.E. and Vinograd,J. (1977) *Nucleic Acids Res.*, **4**, 1183-1205.

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