Influence of Elsamicin A on the Activity of Mammalian Topoisomerase I†

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ABSTRACT: The strong effect of elsamicin A on the mobility of DNA in agarose gels has been characterized. This antibiotic forms tight complexes that are resistant to an electrophoretic field, though they are not covalent and can be removed by phenol or 1-butanol extraction. In the presence of mammalian topoisomerase I, elsamicin A behaves as an intercalating agent in unwinding experiments performed with either ϕX174 rf I (double-stranded, covalently closed DNA) or relaxed pUC19. The unwinding assay was used to calculate the apparent unwinding angle per bound antibiotic molecule, $\phi = 19 \pm 2.7°$. Moreover, an apparent binding constant for elsamicin was derived, under the experimental conditions of the topoisomerase I assays, using the Scatchard equation. The effects of elsamicin A on the mammalian topoisomerase I catalytic cycle do not seem to involve inhibition of the enzyme. Neither symptoms of trapping of covalent DNA−topoisomerase I cleavable complexes nor "nonspecific" inhibition, based solely on DNA binding, was apparent. Utilizing an experimental approach based on the use of relaxed plasmid DNA, we suggest that elsamicin might not be a topoisomerase I inhibitor.

Elsamicin A (Figure 1) is a DNA binding antibiotic that binds specifically to GC-rich sequences (Alhambra et al., 1995; Párraga & Portugal, 1992; Salas & Portugal, 1991), and is capable of converting the Z-form of DNA back to the B-form (Jiménez-García & Portugal, 1992). Moreover, it causes DNA breakage in the presence of ferrous ions and a reducing agent (Párraga et al., 1992). The DNA-cleaving activity appears to be partially blocked by the action of superoxide dismutase and catalase. These results indicate that the aglycon moiety is involved in the production of free radicals. Elsamicin displays broad spectrum activities against several murine and human tumors, and it has recently undergone phase II clinical trials under the name of Elsamitrucin (Goss et al., 1994). Alternative mechanisms may account for the antitumor activity of elsamicin. It is worth considering its potency as an inhibitor of mammalian topoisomerases, including human ones, since these enzymes are important for maintaining the superhelical state of chromosomal DNA during cell processes such as transcription or replication (Chen & Liu, 1994). In fact, a biochemical characterization of the effect of elsamicin on topoisomerase II has shown that it is a strong inhibitor. Elsamicin A appears to be a more potent inhibitor than either teniposide (VM-26) or adriamycin (Lorico & Long, 1993). Since elsamicin may be the most potent inhibitor of topoisomerase II yet described (Lorico & Long, 1993), we sought new information on whether this effect could be accompanied by a strong effect on the topoisomerase I functionality. Whereas drugs like elsamicin may act preferentially on topoisomerase II−DNA complexes, their capacity to inhibit other enzymes like helicases (Bachur et al., 1993) or topoisomerase I should promote their antitumor activity. Topoisomerase I is known to reduce the torsional stress of DNA supercoiling by changing the number of twists of one DNA strand about the other (Chen & Liu, 1994; Ralph et al., 1994). DNA relaxation by topoisomerase I consists of several well-characterized steps. Topoisomerase I inhibitors can either induce topoisomerase I-linked DNA breaks, probably preventing their religation, or suppress topoisomerase I-linked breaks, by inhibiting enzyme binding to DNA (Crow & Crothers, 1994; Chen et al., 1993; Hsiang et al., 1985; Ralph et al., 1994).

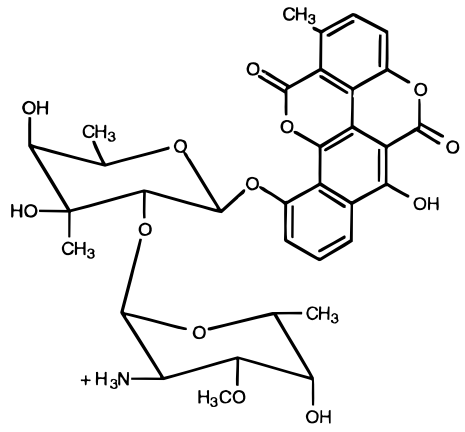

FIGURE 1: Structural formula of elsamicin A.

In this paper, we not only analyze the possible effect of elsamicin on topoisomerase I but also study the intercalating potency of elsamicin using an unwinding assay employing calf thymus topoisomerase I, which allows us to calculate the value of the unwinding angle per intercalated molecule (ϕ) experimentally. Although elsamicin can be fully described as an intercalator, it also contains a large disaccharide moiety, which is deeply accommodated in the minor groove (Figure 1); thus, it is an interesting model in which to study the potential correlation between drugs containing various molecular elements that might work differentially as inhibitors of topoisomerases.

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MATERIALS AND METHODS

Antibiotics, DNAs, and Enzymes. Elsamicin A was kindly provided by Dr. A. M. Casazza (Bristol Myers Squibb) and was prepared as a 1 mM solution in ethanol, and diluted in the desired assay buffer, see below, to the required concentration just before use. Calf thymus type I topoisomerase was purchased from Gibco-BRL. Plasmid pUC19 was prepared by standard methods, and ϕX174 rf I (double-stranded, covalently closed DNA) was purchased from New England Biolabs.

DNA Binding in Vitro. To assay the binding activity of elsamicin A, we studied its ability to change the electrophoretic migration of 0.5 μg of the supercoiled form of the ϕX174 rf I or pUC19 DNA in agarose gels in the presence of 20 mM Tris (pH 7.5) containing 50 mM KCl and 0.1 mM EDTA. Concentrations of elsamicin up to 100 μM were assayed. The samples were incubated together for 30 min at 30 °C and loaded directly on a 1% agarose gel after adding the same volume of 50% glycerol containing 0.02% bromophenol blue.

Quantitative measurements of elsamicin A binding were performed independently of the topoisomerase assays, using established protocols for equilibrium dialysis and fluorescence measurements (Chaires et al., 1982), in the same buffer conditions as the topoisomerase assays (see below). The concentration of the free drug in the dialysate (C_f) was measured directly from the fluorescence intensity (λ_{ex} 266 or 422 nm, λ_{em} 466 nm) using a Perkin-Elmer LS50 spectrofluorometer, with reference to a standard curve prepared from elsamicin solutions of known concentrations. The amount of bound antibiotic (C_b) was determined by the difference:

$$C_b = C_{total} - C_f \quad (1)$$

The number of intercalated elsamicin molecules per nucleotide (r) was calculated using

$$r = C_b/C_{Nt} \quad (2)$$

where C_{Nt} is the nucleotide concentration of the plasmid DNA.

An apparent elsamicin binding constant can be defined from the Scatchard equation:

$$K_{ap} = (r/C_f)/(n - r) \quad (3)$$

where $n = 0.25$, as determined elsewhere (Párraga & Portugal, 1992).

DNA Unwinding Assay Using DNA Topoisomerase I. Reaction mixtures (30 μL) contained 1 μg of topoisomerase II-relaxed pUC19 or ϕX174 rf I DNA and 7 units of topoisomerase I, except when otherwise indicated, in 50 mM Tris (pH 7.5), 50 mM KCl, 10 mM $MgCl_2$, 0.5 mM DTT, 30 mg/mL BSA, and 0.1 mM EDTA. Reactions were performed in the absence or in the presence of the amounts of elsamicin A indicated in the legends to the figures. After 30 min incubation at 30 °C, the reactions were stopped by adding the same volume of phenol, and the sample was extracted twice. This procedure also allowed the separation of the drug from the DNA.

Determination of Elsamicin-Induced DNA Unwinding by a Topoisomerase I Assay. DNA intercalation is characterized by DNA unwinding that occurs irrespective of the supercoiling state of the substrate. An unwinding assay was performed using relaxed pUC19 as substrate in a buffer containing 50 mM Tris (pH 7.5), 50 mM KCl, 10 mM $MgCl_2$, 0.5 mM DTT, 30 mg/mL BSA, and 0.1 mM EDTA. The action of topoisomerase I adjusted the DNA linking number (Lk) to the modified twist (Tw) (Pommier et al., 1987) for an N base-pair closed circular DNA, in such a way that it was possible to measure the unwinding angle (ϕ) produced by the intercalated antibiotic molecule:

$$\phi = 360\Delta Lk/2Nr \quad (4)$$

The change in linking number (ΔLk) was determined by counting the bands on an agarose gel; r, which corresponds to the number of bound elsamicin molecules per nucleotide, was calculated as described above.

Analysis of Topoisomerase Inhibition by Elsamicin A. Reactions (30 μL) contained 5 μM elsamicin A and 0.5 μg of either ϕX174 rf I or pUC19 DNA, as negative-supercoiled or relaxed DNA (this was obtained by treatment of the supercoiled form with topoisomerase II), and 4 units of calf thymus topoisomerase I. The amount of topoisomerase required in the experiment was calculated, by titration of the supercoiled pUC19 plasmid, as the smallest number of units required to obtain fully relaxed DNA in 30 min.

The principle of the assay using these two topologically different closed forms of pUC19 DNA is depicted in Figure 5. Reactions were incubated at 30 °C, and 6 μL aliquots were removed at specific times (Figure 6). These aliquots were mixed with a phenol solution and ethanol-precipitated. Loading buffer (50% glycerol containing 0.02% bromophenol blue) was added, and agarose electrophoresis was then performed as described below.

Agarose Gel Electrophoresis. All gels consisted of 1% agarose in 90 mM Tris−borate (pH 8.3) containing 2 mM EDTA and were run at 4 V/cm for about 16 h. When required, separation of relaxed circular DNA from the comigrating nicked form was accomplished on a gel containing 4 μM chloroquine under the same conditions. All gels were stained with ethidium bromide (0.5 μg/mL) for 30 min, destained in water, and photographed. Densitometric scans of the negatives were carried out in a Molecular Dynamics densitometer.

RESULTS

Increasing amounts of elsamicin A added to either pUC19 plasmid DNA (2686 base pairs long) or the replicative form of ϕX174 DNA (5386 base pairs long) produced a shift in the migration of both supercoiled and relaxed plasmid DNAs on 1% agarose gel. Figure 2 displays the results of the shift induced by elsamicin binding to ϕX174 DNA, about 90% in the supercoiled form (lane 1). Increased amounts of antibiotic resulted in a spectacular shift of the supercoiled form. The effect of elsamicin on the other topological forms was markedly smaller.

At concentrations between 25 and 100 μM, the antibiotic-induced shift produced a major species, which migrated in the vicinity of the relaxed DNA (lanes 7 and 8 in Figure 2) but without reaching its position. Tentatively, lane 8 could correspond to positively supercoiled DNA with a complete relaxation taking place somewhere between 25 and 100 μM. At some concentrations, there was evidence of discrete bands

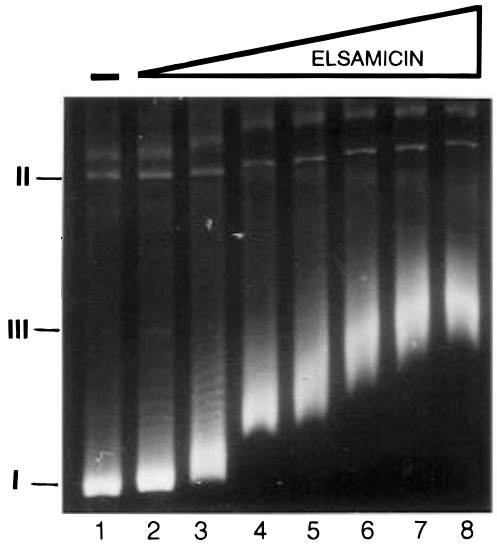

FIGURE 2: Agarose gel (1%) of the shift in electrophoretic mobility of the different topological forms of 0.5 μg of ϕX174 DNA (90% supercoiled form) by the binding of different amounts of elsamicin A. 1, control, no added antibiotic; 2−8, 0.2, 1, 5, 10, 25, 50, and 100 μM elsamicin A. Forms I, II, and III represent supercoiled, relaxed, and linear DNA, respectively.

(e.g., Figure 2, lane 3), which might correspond to DNA species that are likely to differ in writhe number as a consequence of the change in helical twist (unwinding) induced by the intercalation of the elsamicin chromophore. The band broadening at higher antibiotic amounts (lanes 4−8) might reflect the same phenomenon. It is worth mentioning that the DNA−elsamicin complex was hardly dissociated by the electric field during electrophoresis (100 V, 16 h) despite the fact that elsamicin is a small, charged molecule (Figure 1), making the band-shift quite evident (neither the gel nor the buffer contained the antibiotic). Since elsamicin is fluorescent, the presence of drug bound to the DNA during the gel running was directly confirmed using a portable ultraviolet lamp. As a first approximation, these results are reminiscent of the behavior of intercalators as molecules capable of changing the shape, and thereby the hydrodynamic characteristics, of closed-circular DNA (Waring, 1970) and thus an additional demonstration that elsamicin A is an intercalating drug.

Figure 3 displays a DNA unwinding assay using calf thymus topoisomerase I and relaxed covalently-closed circular DNA (form II). The antibiotic was carefully removed from the sample after the topoisomerase I assay since it binds tightly to DNA (see above), and the accuracy of the measurement of the unwinding angle (ϕ) depends on the condition that no antibiotic remains bound during electrophoresis (Pommier et al., 1987). As a matter of routine, phenol was used to remove the antibiotic, though 1-butanol also did so efficiently (data not shown). The alterations of topoisomerase I-mediated DNA relaxation permitted an accurate calculation of the degree of intecalation, and the removal of elsamicin, together with the enzyme inactivation, left DNA in a new topological state (Figure 3). Since elsamicin changed the topoisomerase I-mediated linking number to negative values, ΔLk was measured by band-counting from densitometric scans of Figure 3.

The unwinding angle per intercalated molecule (ϕ) and some parameters of the binding of elsamicin to pUC19 DNA were calculated under the same experimental conditions as the topoisomerase I assays displayed in Figure 3, using the ΔLk and the equations described under Materials and Methods. The derived parameters of binding of elsamicin to pUC19 are displayed in Table 1.

The unwinding experiments, performed as described under Material and Methods, show (Figure 3) the effect of running the resulting reaction products in an agarose gel in both the absence and the presence of chloroquine. Samples run under both conditions correspond to two equal aliquots coming from the same experiment. There were obvious differences in both the shape and positions of the different topoisomers: the changes found in the absence of chloroquine

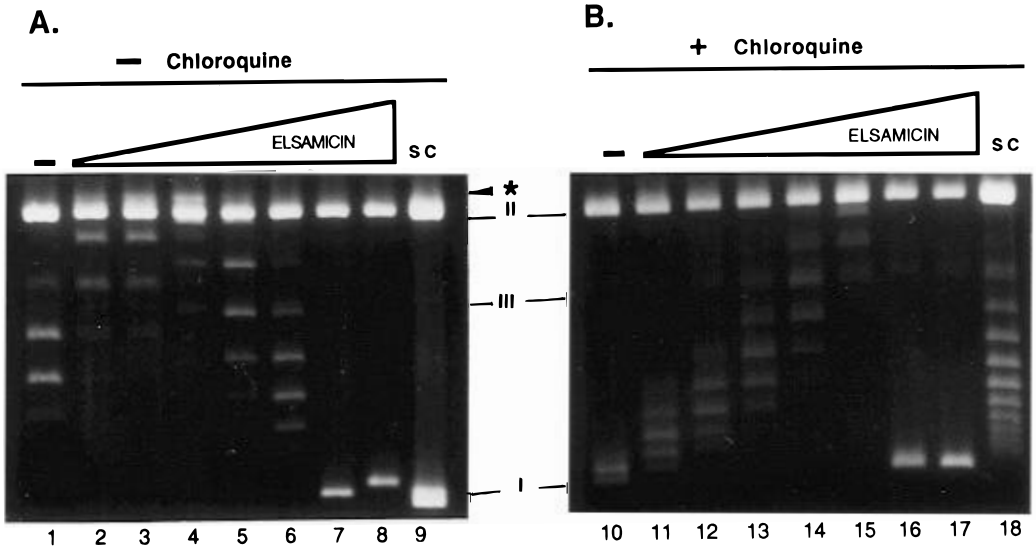

FIGURE 3: DNA unwinding results obtained using different amounts of elsamicin A, with 0.5 μg of relaxed pUC19 as substrate. Panel A depicts an agarose gel run in the absence of chloroquine, while panel B depicts a gel in the presence of 4 μM chloroquine. Samples run under both conditions correspond to two equal aliquots coming from the same experiment. The elsamicin concentrations were 0 μM (lanes 1 and 10), 0.2 μM (lanes 2 and 11), 0.3 μM (lanes 3 and 12), 0.5 μM (lanes 4 and 13), 0.75 μM (lanes 5 and 14), 1 μM (lanes 6 and 15), 5 μM (lanes 7 and 16), and 10 μM (lanes 8 and 17). Lanes 9 and 18 are controls for the effect of chloroquine on the electrophoretic behavior of supercoiled (SC) DNA. Forms I, II, and III represent supercoiled, relaxed, and linear DNA, respectively. The asterisk indicates the location of the relaxed covalently-closed plasmid in the absence of chloroquine.

Table 1: Parameters of Binding of Elsamicin A to pUC19 DNA Derived from the Topoisomerase I Unwinding Assays[a]

C_t (μM)	ϕ	ΔLk	r	k	K_{ap} (M^{-1})
1	19 ± 2.7	7	0.0248	0.471	3.80×10^5

[a] Ct: total (added) concentration of elsamicin A; ϕ, unwinding angle produced by intercalated molecule; r, number of antibiotic molecules bound per nucleotide; ΔLk, change in linking number in the unwinding assay; k, elsamicin-induced unwinding per nucleotide ($k = r\phi$); K_{ap}, apparent binding constant (30 °C; 50 mM KCl, 10 mM $MgCl_2$) calculated from the Scatchard equation.

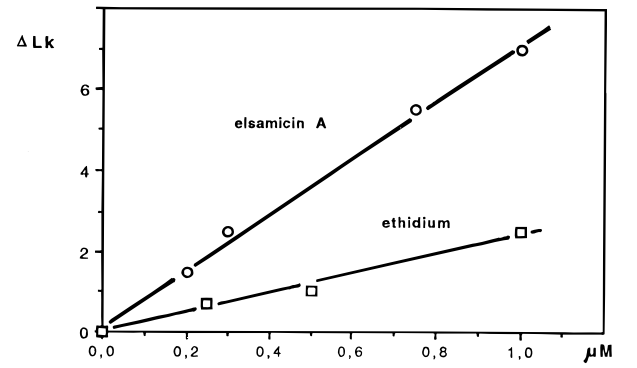

FIGURE 4: Average linking number difference (ΔLk) of the distributions of topoisomers generated by topoisomerase I treatment of pUC19 DNA in the presence of different concentrations of elsamicin A (see Figure 3) or ethidium bromide [adapted from Pons et al. (1991)].

mostly represent negative topoisomers, while those in the presence of chloroquine are mostly positive. The presence of chloroquine affects the mobility of relaxed DNA but not that of the nicked form. There was no significant increase in the amount of nicked DNA compared to control—absence of elsamicin (Figure 3B). This result might indicate that the antibiotic did not stabilize the nicked intermediate [i.e., the cleavable complex (Chen & Liu, 1994; Hsiang et al., 1985; Jaxel et al., 1991)] significantly in the topoisomerase I enzymatic pathway. It is noteworthy that even in the absence of chloroquine (Figure 3A), there were two populations of bands corresponding to form II of the DNA. These are the nicked double strand and the relaxed covalently-closed forms (indicated by an asterisk in Figure 3A). Lanes 5−8, without chloroquine, appear to show negatively supercoiled species, as expected for topoisomerase I-mediated relaxation of positive supercoiling induced by elsamicin bound to the relaxed substrate. In the presence of elsamicin A at concentrations higher than 5 μM, the DNA migrated to approximately the same positions on an agarose gel irrespective of the presence (Figure 3, lanes 7 and 8) or the absence (Figure 3, lanes 16 and 17) of chloroquine, while untreated relaxed or supercoiled DNA (neither topoisomerase I nor elsamicin A) ran as expected in the presence/absence of chloroquine in the gel (cf. lanes 1 versus 9 and 10 versus 18). It is somewhat striking that antibiotic concentrations as low as 5 μM produced topological species that migrated in both the presence and absence of 4 μM chloroquine as if they were highly supercoiled DNA (lanes 7, 8, 16, and 17 in Figure 3). These supercoiled species correspond to pUC19 plasmids with a supercoil density (σ) higher than −0.06.

For the sake of comparison, Figure 4 shows a plot of the elsamicin-induced topoisomerase I change in linking number (ΔLk) determined from the distribution of topoisomers in

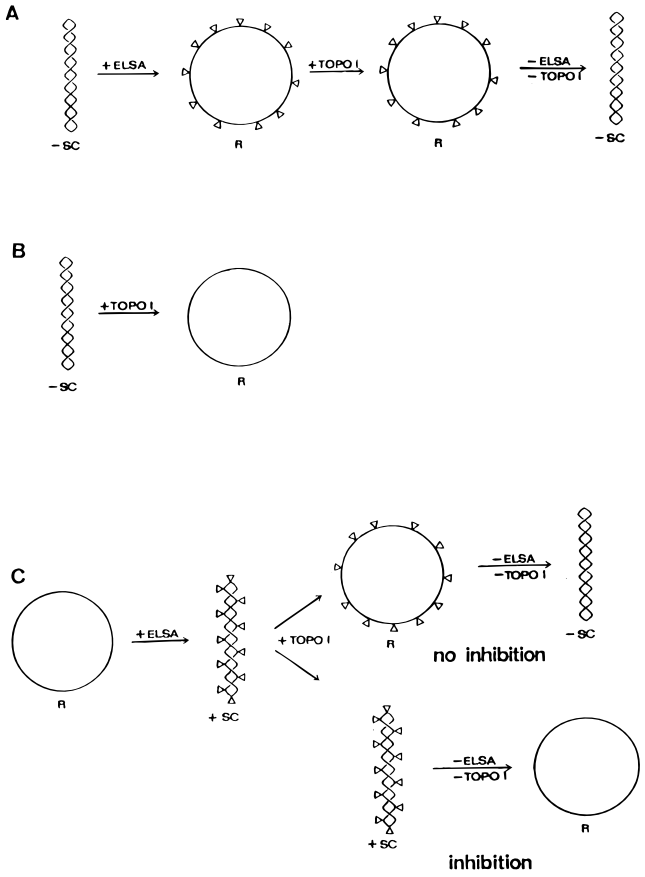

FIGURE 5: Scheme of the assay used to determine whether an intercalating agent acts either as a "nonspecific" inhibitor of mammalian topoisomerase I (i.e., arising from DNA binding) or by changing the topological state. The experimental approach in panel C can distinguish between the nonspecific inhibition and the absence of it ("no inhibition") in the topoisomerase I assay, produced by the change in the supercoil state of the target DNA resulting in the incapacity of topoisomerase to find an appropriate substrate, since relaxed DNA is not an efficient substrate for the enzyme.

Figure 3, together with the results of a similar experiment using ethidium bromide, adapted from Pons et al. (1991). Significantly higher concentrations of ethidium would be required to obtain such higher supercoiled species. The difference is likely to be related to the apparent binding constants for ethidium and elsamicin A, which under the conditions of the topoisomerase I unwinding assay are $5.74 \times 10^4\ M^{-1}$ (Pommier et al., 1987) and $3.80 \times 10^5\ M^{-1}$ (Table 1), respectively. At first glance, the value of the binding constant does not seem particularly high given the lack of dissociation of the drug during the electrophoresis. However, it is well-known that ligand−DNA binding in a gel appears to be stronger than expected (Fried & Crothers, 1981). This stability can arise from a caging effect in which the agarose hinders the spatial separation of DNA and the antibiotic which might dissociate.

Figure 5 displays the principle of an assay using two topologically different closed forms of DNA: supercoiled (Figure 5A) and relaxed (Figure 5C). This assay distinguishes between a general inhibition of the enzyme arising solely from drug binding to DNA, and the absence of topoisomerase I activity because the DNA is relaxed by the the ligand and therefore the topology of the substrate cannot be efficiently recognized by topoisomerase I (Caserta et al., 1990; Chen & Liu, 1994). A control for "appropriate"

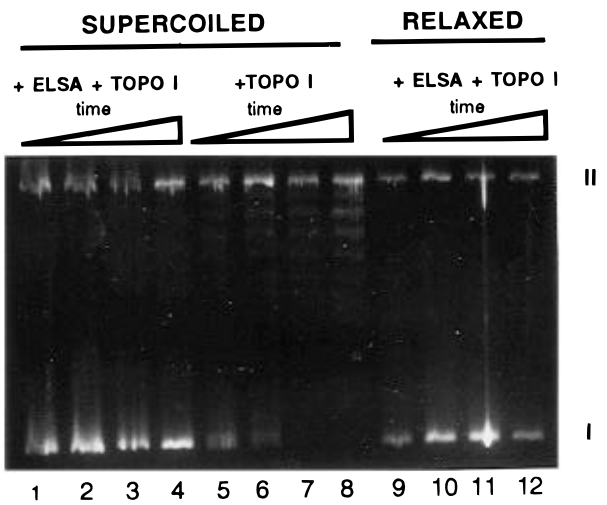

FIGURE 6: Kinetic analysis of the topoisomerase I-mediated effect on supercoiled pUC19 (lanes 1−4, in the presence of 5 μM elsamicin A; lanes 5−8, in its absence) and relaxed pUC19 in the presence of elsamicin (lanes 9−12). The three sets of experiments display the results of reactions stopped, by phenol extraction, after 2, 5, 15, and 30 min, respectively.

conditions for a topoisomerase I assay was also used (Figure 5B). This approach was used to analyze the hypothetical inhibition of topoisomerase I by elsamicin A. The experimental results are displayed in Figure 6. Lanes 1−4 show that supercoiled DNA (>90% in form I) was in the same conformation after treatment with elsamicin and topoisomerase I for 30 min, while in the presence of topoisomerase I alone there was a processive formation of relaxed (form II) DNA. When the substrate was relaxed DNA (obtained previously using eukaryotic topoisomerase II, see Materials and Methods), the presence of elsamicin A (5 μM) and topoisomerase I showed that the DNA was mostly supercoiled (Figure 6, lanes 9−12). According to the experimental protocol shown in Figure 5, these results indicate that elsamicin A is not an inhibitor of the relaxing activity of topoisomerase I, since otherwise the DNA would be relaxed rather than in the supercoiled form.

DISCUSSION

In this paper, we have determined the characteristics of the strong binding of elsamicin A to covalently-closed DNAs. It is a startling observation that elsamicin A does not appear to dissociate during electrophoresis. Phenol extraction restores the behavior of DNA as a drug-free polynucleotide, demonstrating that elsamicin does not form any irreversible (covalent) complex with DNA although it is resistant to an electric field. The results in Figure 2 cannot be produced by "band-shift" due merely to the increment in the mass of the sample (DNA+drug) compared to DNA alone, since the supercoiled (form I) ϕX174 is strongly retarded in a concentration-dependent way, whereas the linear DNA (form III) or the relaxed (nicked) DNA (form II) are only slightly affected, and because the ligand is small. In the experiments in Figure 2, in which there is no antibiotic added to the gel/or the running buffer, we would expect the net positive charge of elsamicin (Figure 1) to be affected by the presence of an electric field; thus, most of the elsamicin molecules should be displaced toward the cathode. This is a well established phenomenon observed, for example, with ethidium or chloroquine, but only when the gel is soaked with the drug during the run, since these dyes dissociate from DNA. One explanation is that the rate of dissociation of the antibiotic from DNA is much slower than for simple dyes like ethidium or chloroquine. Although there are no calculations of the dissociation rate of elsamicin, it is conceivable that it is much slower than for other ligands. For example, elsamicin is a strong impediment to transcription by T7-RNA polymerase (Portugal, 1995), and it has been suggested that the capacity to inhibit the elongation rate of RNA polymerases depends on the relative dissociation rates of the ligands (Phillips & Crothers, 1986). The T7-RNA polymerase achieves a highly processive capacity, and it is able to elongate through large molecular barriers, but the presence of elsamicin A induced the formation of aborted transcripts [see Portugal (1995) and references cited therein]. Furthermore, it is conceivable that a caging effect, like the one detected in protein−DNA complexes (Fried & Crothers, 1981), might help to decrease the dissociation rate of the elsamicin−DNA complex.

The topoisomerase I assay presented in Figure 3 corroborates the view that elsamicin A is an intercalator (Alhambra et al., 1995; Salas & Portugal, 1991). The unwinding angle (ϕ) determined by this assay is in concordance with the value, around 18°, that can be inferred from molecular dynamics studies on oligonucleotide−elsamicin complexes (Alhambra et al., 1995). The measured unwinding angle is larger than for adriamycin, but quite close to the value for m-Amsa (Pommier et al., 1987; Waring, 1970), reflecting the differences in size and shape of the intercalating and sugar moieties. Moreover, the apparent DNA binding constant for elsamicin A (Table 1) calculated using eq 3, under the same experimental conditions of the unwinding assays, was not at variance with the value determined by a fluorencence quenching method using poly(G-C) (Uesugi et al., 1991). The large chromophore of elsamicin A is deeply inserted in the intercalating site (Alhambra et al., 1995), suggesting that the strong inhibition of topoisomerase II (Lorico & Long, 1993) might occur because the aglycon moiety of the antibiotic is well stacked against a base pair, in either the 5′ or the 3′ site of the strand break, in agreement with the general models put forward to explain drug−DNA−topoII complexes [see Ralph et al. (1994) and references cited therein], but showing a stronger effect, tentatively related to the peculiar location of the huge chromophore.

The results presented in Figure 3 indicate that elsamicin does not interrupt the breakage−ligation process of mammalian topoisomerase I since it does not stabilize the topoisomerase I-cleavable complex. The slight increase in nicked DNA at concentrations above 5 μM (Figure 3B) might be accounted for by the formation of free radicals, as described elsewhere (Párraga et al., 1992).

Although it seems evident that elsamicin cannot inhibit topoisomerase I by trapping of covalent DNA−enzyme cleavage complexes, it may do so simply by binding to DNA, which would interfere with the interaction between the enzyme and the polynucleotide. This "nonspecific" inhibition of topoisomerase I by intercalators can be analyzed using supercoiled DNA (Crow & Crothers, 1994; Pommier et al., 1987). However, it is not possible to distinguish between the lack of interaction between DNA and the topoisomerase in the presence of the ligand and the absence of topoisomerase I activity due to the unwinding of supercoiled DNA

after drug binding. This might render the relaxed form of the plasmid, which is not an efficient substrate for topoisomerase I (Caserta et al., 1990; Chen & Liu, 1994) (see Figure 5). Therefore, relaxed DNA was used as substrate. Figure 5C shows that this approach distinguishes between the two possibilities that are not well differentiated using supercoiled (form I) DNA. We conclude that elsamicin A does not inhibit topoisomerase I activity, but produces negatively supercoiled DNA at concentrations as low as 5 μM (Figures 3 and 6). Elsamicin A removal and topoisomerase I inactivation left DNA in a new supercoiled state (cf. Figures 5C and 6), while if the enzymatic inhibition had occurred we would have observed a relaxed plasmid rather than the supercoiled form (Figure 6, lanes 9−12). Nevertheless, higher concentrations of elsamicin could inhibit the catalytic activity of topoisomerase I by impeding the physical access of the topoisomerase to the DNA, though this remains to be demonstrated.

Interestingly, some of the more useful anticancer drugs in clinical practice are intercalators like the anthracyclines. It is not straightforward to generalize our results on elsamicin A to other intercalating agents, which could be nonspecific inhibitors of topoisomerase I (Crow & Crothers, 1994; Pommier et al., 1987). Nevertheless, the approach outlined in Figure 5C might be used to extend the analysis of the effect of intercalators on the catalytic cycle of topoisomerase I. Our results indicate that generalities regarding the details of antibiotic mechanism are sometimes precarious and they might depend on the experimental approach. In any case, the results presented in this paper should prove valuable in investigating to what extent the topological state of the DNA template may influence the cellular response to elsamicin, which is a strong inhibitor of mammalian topoisomerase II (Lorico & Long, 1993).

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