Intermolecular Cross-Linking of DNA through Bifunctional Intercalation of an Antitumor Antibiotic, Luzopeptin A (BBM-928A)

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ABSTRACT

A bifunctional intercalator may intercalate with DNA in at least two ways. Both intercalating moieties may intercalate with the same DNA molecule (type I, intramolecular cross-linking) or with two separate DNA molecules (type II, intermolecular cross-linking). Production of type I is often assumed. Type II bintercalation has been suggested, but no direct evidence has been reported. In the present study, endonuclease-restricted PM2 phage or pBR322 plasmid DNA fragments were treated with the bifunctional intercalating antitumor antibiotics, luzopeptin A (BBM-928A) and echinomycin, and analyzed by agarose gel electrophoresis. Luzopeptin A treatment produced additional DNA bands which were the products of type II bintercalation. The types of restriction fragments involved were identified. Maximal type II bintercalation occurred at a luzopeptin A/DNA range of 0.14 to 0.18, at which more than 50% of the total DNA molecules were involved. Type II products were converted gradually to type I products upon prolonged incubation at 37°C, probably due to the tendency for intermolecular bonds to disrupt. Echinomycin treatment failed to produce type II products, probably because of a DNA-binding affinity weaker than that of luzopeptin A. Thus, it is possible to use the present gel system to demonstrate the type II bintercalation for strong bintercalators, but milder systems are needed for weak bintercalators.

INTRODUCTION

Luzopeptins (formally BBM-928) are a family of actinoleukin-like antibiotics (12, 13) containing 2 substituted quinoline chromophores linked by a cyclic decapepsipetide (Chart 1, I). Luzopeptin A showed potent antitumor activities against a variety of experimental animal tumor systems with a potency approximately 3-fold greater than that of a structurally related antibiotic, echinomycin (Chart 1, /I), and 100- to 300-fold those of mitomycin C (12). Luzopeptin A intercalates bifunctionally with DNA (9). The apparent affinity constant for DNA binding is $1.93 \times 10^7$ M$^{-1}$, with one binding site per 5 to 6 DNA BPs$^3$ at saturation. Echinomycin binds to DNA with an apparent affinity constant of 1 to $6 \times 10^6$ M$^{-1}$ (14). Echinomycin intercalates with DNA bifunctionally at low ionic strengths but monofunctionally at high ionic strengths (14).

At least 2 types of bifunctional DNA intercalation of a bintercalator can be formed (Chart 2). In one type (type I, intramolecular cross-link), the 2 chromophores of one bintercalator molecule intercalate into 2 separate sites located in one DNA molecule. In the other type (type II, DNA:DNA intermolecular cross-link), the 2 chromophores of one bintercalator molecule intercalate into 2 separate sites located in 2 separate DNA molecules, i.e., one site on each DNA molecule. To date, most experimental data reported for interaction of bintercalators with DNA have been discussed in terms of type I intramolecular cross-links of DNA. Although the possible production of the type II intermolecular cross-links by bintercalators has been speculated (3), no direct demonstration of the production of this type of bintercalation has been reported.

In this report, we present results suggesting the production of DNA:DNA intermolecular cross-links by treatment of restriction fragments of PM2 phage or pBR322 plasmid DNA with luzopeptin A. Comparative studies indicated that, under similar conditions, echinomycin failed to produce detectable amounts of type II intermolecular cross-links.

MATERIALS AND METHODS

Materials. Luzopeptin A (BBM-928A) and echinomycin were obtained from Bristol Laboratories, Syracuse, N. Y. Covalently closed, circular, superhelical PM2 phage DNA was isolated as described previously (8). Superhelical plasmid pBR322 DNA was isolated from Escherichia coli strain J221 according to the procedure of Clewell (5). Restriction endonucleases HindIII, PstI, and Sall were from Bethesda Research Laboratories, Rockville, Md., or Boehringer Mannheim Biochemicals, Indianapolis, Ind. Ethidium bromide, Tris, EDTA, and borate were from Sigma Chemical Co., St. Louis, Mo., and agarose-ME from Miles Laboratories, Elkhart, Ind.

Agarose Gel Electrophoresis. DNA was mixed with an equal volume of a dye/EDTA mixture containing 56% glycerol (v/v), 50 mM EDTA, and 0.05% bromophenol blue (w/v). Aliquots of these mixtures containing 0.8 to 2.0 µg of DNA (for analytic gels) or 50 to 200 µg of DNA (for preparative gels) were layered onto 1.0% agarose slab gels and electrophoresed as described previously (8). After electrophoresis, gels were stained with ethidium bromide (0.5 µg/ml), illuminated with a transilluminator (Ultra-violet Products, Inc.), and photographed with a Polaroid C-U Transparency Film. DNA fragments were cut from the gels and analyzed in a Beckman GS-25 scanning densitometer. Quantitative analysis was made of the gel bands using the program of a scanning densitometer equipped with an integrator.

Preparation and Isolation of PM2 Restriction Fragments. The PM2 DNA was incubated with restriction endonuclease HindIII (2 to 5 units/µg DNA) in a buffer solution containing 10 mM Tris-HCl, 50 mM NaCl, 10 mM MgCl$_2$, and 14 mM dithiothreitol, pH 7.6. After incubation at 37°C for 4 to 10 hr, an aliquot of the reaction mixture was taken out, and the reaction was stopped with the addition of an equal volume of a dye/EDTA mixture containing 56% glycerol (v/v), 50 mM EDTA, and 0.05% bromophenol blue (w/v) before subjecting to agarose gel (1.0%) electrophoresis to ensure complete digestion.

For the isolation of each restriction fragment, the HindIII-digested PM2 DNA mixture, after mixing with the dye/EDTA mixture, was subjected to

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2 To whom requests for reprints should be addressed, Smith Kline & French Laboratories, P. O. Box 7929 (J00), Philadelphia, Pa. 19101.
3 The abbreviation used is: Bp, base pair.
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DNA-DNA Cross-Links by Luzopeptin A (BBM-928A)

DNA in 50 mM Tris-HCl (pH 7.5) and 1 mM EDTA was treated with luzopeptin A (10 μg/ml) for 30 min and then prepared for electron microscopy by Kleinschmidt cytochrome c spreading technique as described by Davis et al. (7). The spreading mixture contained DNA (0.5 to 1.0 μg/ml), cytochrome c (0.5 mg/ml), ammonium acetate (0.5 mM), EDTA (0.1 mM) as the hyperphase, and 0.25 mM ammonium acetate as the hypophase. The grids were coated with nitrocellulose/amyl acetate before use. After samples were attached to the grids, they were rotary shadowed with platinum/palladium (80/20) at a 7° tilt and examined with a Philips 200 electron microscope.

RESULTS

Products of Luzopeptin A-treated DNA Restriction Fragments. Fig. 1 shows the agarose gel electrophoretic pattern of luzopeptin A-treated restriction fragments of phage PM2 DNA produced by HindIII restriction endonuclease digestion. Lane a shows 5 of the 7 restriction DNA fragments untreated with luzopeptin A: A, 5348 BP; B, 2055 BP; C, 863 BP; D, 430 BP; and probably E, 382 BP. Bands F (287 BP) and G (111 BP) were produced but were stained too lightly to be photographed. Lanes

PREPARATION OF pBR322 Plasmid DNA Restriction Fragments. For preparation of HindIII, HindIII plus PstI, or HindIII plus PstI plus Sall-restricted pBR322 DNA fragments, superhelical pBR322 DNA was digested with the endonucleases (3 units/μg DNA) in a buffer solution containing 10 mM Tris-HCl, 50 mM NaCl, 10 mM MgCl2, 14 mM dithiothreitol, and gelatin (0.1 mg/ml), pH 7.6, for 2 to 4 hr at 37°C. After adding the dye/EDTA mixture, the reaction mixture was electrophoresed in 1% agarose gel for 3 hr.

TREATMENT OF DNA RESTRICTION FRAGMENTS WITH DRUGS. The restriction DNA fragments (0.2 to 0.4 mM) were treated with varying amounts of luzopeptin A or echinomycin in a buffer solution containing either 50 mM Tris-HCl (pH 7.5) or 50 mM borate (pH 9.5), and 75 mM NaCl at 37°C for 30 min or longer periods of time as specified. The treated DNA was then mixed with an equal volume of dye/EDTA mixture and subjected to agarose gel electrophoresis.

Electron Microscopic Studies of Luzopeptin A-treated pBR322 DNA Fragments. The preparation HindIII plus PstI-restricted pBR322...
Treatment. To study the origin of the additional DNA bands, we stained the gel with ethidium bromide. As shown in Chart 3, A and B, respectively. Scans started from sample wells (arrows), and prominent peaks at the untreated (A) DNA were Fragments A, B, C, and D. Fragments E, F, and G were less certain. Numbered bands (B), some of the additional bands produced by luzopeptin A treatment. 

b to g show that treatment with increasing luzopeptin A concentrations resulted in 2 types of changes: (a) a decrease in the gel electrophoretic mobility of all the restriction fragments; and (b) the appearance of many additional discrete DNA bands with electrophoretic mobilities slower than those of their corresponding restriction fragments.

The retardation of the mobility of the drug-treated DNA fragments is probably a result of type I intramolecular intercalation which increases the DNA length and rigidity. As will be described in more detail, the additional bands are interpreted by us to be cross-linked products of identical or different DNA restriction fragments resulting from DNA-DNA intermolecular bifunctional intercalation (type II). It is also interesting to note that both the drug concentration-dependent production of additional bands and the mobility reduction reached a maximal extent at a luzopeptin A:DNA ratio of 0.116 (Fig. 1), (Lane f). Results of more than 7 similar experiments (not shown) showed that the maximal effects occurred at a ratio range of 0.14 to 0.18. At concentration ratios greater than 0.18, both the production of additional bands and the retardation of the mobility were reduced (Fig. 1). However, the amount of DNA remaining in the sample wells increased with increasing drug concentration, and this material could be aggregated products.

The results in Fig. 1 also show that the treatment with luzopeptin A, even at very high concentrations, did not significantly reduce the overall fluorescence intensity induced by ethidium bromide staining which is a strong DNA intercalator used in these studies to stain gels for detection of DNA bands. This observation further confirms our previous observation (10) that, under the conditions used, luzopeptin A does not interfere with the intercalative binding of ethidium bromide.

The gel scans of Lanes a (control) and f (treated) of Fig. 1 are shown in Chart 3A, A and B, respectively. Chart 3B shows that the luzopeptin A treatment of DNA fragments produced at least 9 clear additional bands and some indistinct bands. The scans also confirmed that no significant reduction of the total ethidium bromide-induced staining of the gel was observed, although the staining pattern was of course altered.

Identification of DNA Bands Produced from Luzopeptin A Treatment. To study the origin of the additional DNA bands, we have isolated from agarose gels each of the HindIII restriction fragments (A, B, and C). Each type of fragment alone or the various combinations of these purified fragments were then treated with luzopeptin A and analyzed by gel electrophoresis using the whole spectrum of the restriction products as markers. Representative results of such experiments are shown in Fig. 2.

In Fig. 2, A and B, Lane 1 shows several large DNA fragments of the whole mixture of PM2 restriction fragments untreated with drug, and Lane 2 shows the same mixture after treatment with luzopeptin A. The gels with the drug-treated mixture of DNA fragments did not show as many drug-induced DNA bands as did those shown in Fig. 1, since a smaller amount of DNA was applied to the gels. Lane 3 of Fig. 2, A and B, shows untreated A fragment, and Lane 4 shows the luzopeptin A-treated A fragment which produced an additional band moving at a rate slower than that of the A fragment. This additional band is probably a dimer of the A fragment, since no other types of fragments were present. In Fig. 2, A and B, Lanes 5 and 6 show the untreated and the drug-treated B fragments, respectively. The slowly moving DNA bands produced by the drug treatment are probably a dimer of the B fragment.

In Fig. 2A, Lanes 7 and 8 show the untreated and the drug-treated C fragments, respectively. The additional band in Lane 8 is probably a dimer of the C fragment. In Fig. 2B, Lane 7 shows the drug-treated equimolar mixture of A and B fragments. Two major cross-linked DNA bands migrating more slowly than the A fragment were produced. Considering the gel mobility, the 2 bands, in order of decreasing mobilities, probably can be assigned as A-B and A-A. In Fig. 2C, Lane 1 shows DNA bands from the drug-treated whole mixture of HindIII fragments, and Lane 2 shows those from the drug-treated equimolar mixture of A, B, and C fragments. Three cross-linked DNA bands migrating more slowly than the A fragment were obtained from the drug treatment of the mixture of A, B, and C fragments. In the order of decreasing gel mobilities, the first 2 bands are probably A-B and A-A, since the equivalent bands of both can be observed from the drug-treated mixture of the A and B fragments (Fig. 2B, Lane 7). The origin of the third band is less certain. However, we observed a weak band at an equivalent position when the gel was loaded with large quantities of the drug-treated mixture of A and B fragments and photographed with a prolonged time of exposure (not shown). Thus, this band is tentatively assigned as the cross-linked product of two A fragments and one B fragment, since it migrated behind the A-A band.
As shown in Fig. 1, the apparent intensity of ethidium bromide staining decreases with the decrease in the size of restriction DNA fragments. Thus, larger amounts of smaller restriction fragments are needed to obtain observable cross-linked DNA bands. Furthermore, with the isolation procedure we used, the yields were relatively poor for small DNA fragments. Thus, although we have analyzed the drug-treated mixture of B and C, A and C, and C and D in an attempt to assign cross-linked bands, the results (not shown) are inconclusive. However, the size of these fragments suggests that they may obey the linear relationship (11) between the gel mobility and the log of the molecular (particle) weight (or the number of BPs) of DNA fragments (see below).

Assignments of the Drug-induced DNA Bands by Electrophoretic Mobility. The linear relationship between the gel mobility and the log of the molecular (particle) weight (or the number of BPs) of DNA (11) was tested with the DNA fragments with molecular (particle) weight (or the number of BPs) expected. Table 1 shows reasonably good agreement between the calculated and expected values of the assigned dimers.

Reversal of the Luzopeptin A-induced Type II Intermolecular Cross-Links. Fig. 3A shows that the luzopeptin A-induced type II intermolecular cross-linking products of restriction DNA fragments (r = 0.23) gradually disappeared upon further incubation at 37°. The maximal production of the additional bands usually occurred with an incubation time of 20 to 60 min at 37°, as shown in Fig. 3, Lane b, which contained the products from an incubation for 30 min. Incubation for longer periods (4, 6.5, and 22.5 hr) resulted in the disappearance of the additional bands, the restoration of the control-type restriction fragment pattern, and the further retardation in the electrophoretic mobility of the restriction fragments. Usually, total disappearance of the additional bands occurred after an incubation of 24 hr. In Fig. 3, Lane f shows that, after 22.5-hr incubation, neither the composition nor the mobility of untreated DNA fragments differed from those of control fragments.

To unequivocally demonstrate loss of cross-linked oligomers and regeneration of the original fragments, 2 restriction fragments (L, 3579 BP, and S, 783 BP) produced by digestion of pBR322 with restriction endonucleases HindIII and PstI were treated with luzopeptin A-inducing cross-linked oligomers and postincubated for periods of as long as 96 hr. The results are shown in Fig. 3B.

This experiment demonstrated that the formation of some of the higher molecular weight oligomers from these 2 restriction fragments required a longer period (up to 48 hr) than was required for other fragments studied and that, after reaching a maximum in cross-linking, a further incubation resulted in loss of the higher molecular weight oligomers regenerating the L and S fragments. It also demonstrates no loss of luzopeptin from the L and S fragments, since the electrophoretic mobility of the fragments after 96 hr remained equivalent to the retarded mobility observed after 30 min of treatment. However, the bands were slightly more diffuse. This may represent microheterogeneity in the intercalation of these fragments of luzopeptin as the intermolecular cross-links rearrange to intramolecular cross-links.

Electron Microscopic Study of Luzopeptin A-treated Restriction Fragments of pBR322 DNA. The results of a preliminary electron microscopic study of the luzopeptin A-treated HindIII plus PstI restriction fragments of pBR322 DNA are shown in Fig. 4. In this study, the samples were spread with cytochrome c (Kleinschmidt spreading). Fig. 4A shows the untreated long (L, 3579 BP) and short (S, 783 BP) restriction fragments. Under the conditions used, virtually no cross-over products between 2 DNA fragments were observed. Fig. 4, B to D, shows some typical cross-over products after treatment with luzopeptin A. These products are probably produced from cross-linking of 2 long fragments, since the total length is approximately twice that of a long (L) fragment.

Protease Digestions Have No Effect on Luzopeptin A-induced DNA Intermolecular Cross-Linking. The possibility that the residual proteins, if any, of the HindIII restriction PM2 DNA fragments may have contributed to the production of the additional bands was examined. Prior to treatment with luzopeptin A, the PM2 DNA restriction fragments were first digested extensively with protease K and extracted with redistilled phenol (3 times). The results (not shown) indicated that these treatments had no effect on the production of additional DNA bands. In other experiments, protease K was present during the treatment of DNA fragments with luzopeptin A. No significant change was observed (data not shown) on either the formation or the disappearance (after 24 hr at 37°) of the additional bands.

No DNA Cross-Linking by Echinomycin Was Detected. When HindIII fragments of PM2 DNA were treated with echinomycin (Fig. 5) under the conditions equivalent to those for luzopeptin A treatment, we observed a retardation of electrophoretic mobility of all restriction fragments in a manner similar to that of the aforementioned treatment. The results are shown in Fig. 5A, Lane d, which contained the products from an incubation for 30 min. Incubation for longer periods (4, 6.5, and 22.5 hr) resulted in the disappearance of the additional bands, the restoration of the control-type restriction fragment pattern, and the further retardation in the electrophoretic mobility of the restriction fragments. Usually, total disappearance of the additional bands occurred after an incubation of 24 hr.
that of luzopeptin A treatment. However, no additional bands were observed even with a treatment at an echinomycin/DNA concentration ratio of 0.3. It is also interesting to note that, as in the case of luzopeptin A treatment (Fig. 1), the overall fluorescence from the ethidium bromide staining of each of the echinomycin-treated restriction DNA fragments, although slightly more diffuse, was not reduced significantly as measured by densitometric scans (not shown).

DISCUSSION

As illustrated in Chart 2, at least 2 types of bifunctional intercalation of a biintercalator with DNA can exist, depending on whether the 2 chromophores intercalate with only one DNA molecule (type I, intramolecular cross-link) or intercalate with 2 separate DNA molecules (type II, intermolecular cross-link). In most cases, the type I biintercalation has been assumed. To our knowledge, no direct evidence indicating the presence of the type II DNA:DNA intermolecular cross-linking by a biintercalator has been reported, although the possible occurrence of such a type of biintercalation of the diacridines has been discussed (3).

In the present study, we have shown that the treatment of DNA restriction fragments with luzopeptin A, a potent antitumor antibiotic (12, 13) capable of bifunctional DNA intercalation (9, 10), produced type II DNA:DNA intermolecular cross-linkings along with the prominent type I biintercalation. By agarose gel electrophoresis of luzopeptin A-treated DNA restriction fragments, we showed that the type II biintercalation produced additional bands. The DNA restriction fragments involved in the formation of these additional bands were identified. Our studies of the cross-linked products of HindIII plus PstI-restricted pBR322 fragments by electron microscopic observations after cytochrome c spreading confirmed the physical cross-links and the assignments of the cross-linked products. We feel that the present demonstration of type II biintercalation was made possible probably because of the remarkably high affinity constant, \(1.93 \times 10^7 \text{ M}^{-1}\), for DNA at intermediate ionic strengths (9). Our previous studies using the same gel systems (10) indicated that luzopeptin A, which was bound to superhelical PM2 DNA (Form I), did not dissociate appreciably from DNA during electrophoresis, whereas partial and almost complete dissociation occurred for DNA-bound echinomycin and ethidium bromide, respectively. Thus, it may be possible to use the present gel system to demonstrate the occurrence of type II biintercalation for other high-affinity biintercalators such as certain diacridines (3).

The type II cross-linking DNA products produced from treatment of luzopeptin A have several characteristics. The type II biintercalation occurred with various types of restriction fragments. Under the conditions used, as much as almost half of the DNA preparation underwent type II biintercalation. The maximal production occurred at a drug/DNA concentration ratio range of 0.14 to 0.18 which approximates the theoretic value of 0.125 required for a biintercalator to saturate sites on linear DNA molecules obeying the neighbor-exclusion principle for small DNA binding ligands (6). It might also be possible that luzopeptin A, with 2 coplanar quinoline chromophores, may have formed stacked dimers or multimers at high concentrations and thus may have hindered the DNA-drug interactions.

The type II cross-linked DNA products were not very stable. Incubation of these products at 37°C for prolonged periods probably converted these type II products into the type I products, since the intensity of the type II DNA bands gradually disappeared, the intensity of the restriction fragments was restored, and the retardation in gel mobility of the restriction fragments was enhanced (Fig. 3). Thus, as compared to the type I products, the type II products seem to be thermodynamically less favored probably because of the larger constraint encountered in keeping the 2 biintercalated DNA molecules together.

Both type I and type II biintercalations were resistant to protease activity. The pretreatment of DNA with protease or the presence of protease during incubation did not affect the biintercalation. Our earlier studies using fluorescence spectroscopic examination of the response of luzopeptin A chromophores to protease digestion suggest that luzopeptin A shows a resistance to protease—a property not unusual to many cyclized polypeptides (2).

Although we cannot exclude very limited sensitivity of luzopeptin A to proteases, the disappearance of drug-induced DNA bands after prolonged incubation probably is not due to the digestion of luzopeptin A molecules by contaminating proteases in the DNA preparation, since the kinetics of the disappearance, like that of the formation, was not affected by extensive phenol extraction and by the addition of a large amount of protease K. Furthermore, we have observed that the half-molecule or smaller molecular fragments of luzopeptin A produced by acid hydrolysis did not interact with DNA at all as measured by spectrophotometry, circular dichroism, viscometry, and gel electrophoresis. In contrast, the DNA after prolonged incubation migrated with a mobility equivalent to that of the original restriction fragments following incubation with luzopeptin A, suggesting that intact luzopeptin A remained.

The conformational states assumed by luzopeptin A in producing type I and type II biintercalation may be different. X-ray studies of luzopeptin A crystals have been performed (1). Despite some asymmetric carbons in the polypeptide ring, the 2 quinoline chromophores are suggested to be in a cis-configuration with respect to the peptidic ring. If the solution conformations are similar to the crystal conformations, we would speculate that the cis-configuration may favor the production of intramolecular type I biintercalation. However, the 2 chromophores still possess some degree of freedom with respect to the peptide ring and thus may allow the production of DNA:DNA intermolecular cross-linking.

As shown in Figs. 1 and 4 and Chart 3, the overall fluorescence intensity of the ethidium bromide staining of the untreated as well as the luzopeptin A-treated or the echinomycin-treated DNA restriction fragments (including the additional bands) was not reduced significantly even when DNA fragments were treated with very high concentrations of drugs. The results substantiate our earlier suggestion (10) that luzopeptin A (or echinomycin) and ethidium bromide, which is an effective monofunctional DNA intercalator, probably bind to DNA at different sites, since no competition was observed.

The biological consequences of the production of type II biintercalation by luzopeptin A remain to be studied. It is possible that the production of this type of biintercalation in addition to the type I biintercalation may provide luzopeptin A with a wider spectrum of cytotoxicity. It remains to be seen how much the type II biintercalation contributes to the remarkably high potency of luzopeptin A against a variety of experimental tumor systems.
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Fig. 2. Cross-linked products produced from the luzopeptin A treatment of the purified HindIII restriction DNA fragments. Either a single type or a combination of 2 or 3 types of DNA fragments was treated with luzopeptin A (30 min at 37°) at a 0.05/0.07 drug/DNA concentration ratio. Gel A: Lane 1, untreated whole mixture of restriction fragments; Lane 2, treated mixture; Lane 3, untreated A fragment; Lane 4, treated A fragment; Lane 5, untreated B fragment; Lane 6, treated B fragment; Lane 7, untreated C fragment; and Lane 8, treated C fragment. Gel B: Lane 1, untreated whole mixture; Lane 2, treated whole mixture; lane 3, untreated A fragment; Lane 4, treated A fragment; Lane 5, untreated B fragment; Lane 6, treated B fragment; and Lane 7, treated mixture of A and B fragments. Gel C: Lane 1, treated whole mixture; and Lane 2, treated mixture of A, B, and C fragments. Stoichiometry of mixing was the same as that in the whole mixture.

Fig. 3. A, disappearance of the luzopeptin A-induced bands of HindIII-restricted PM2 DNA fragments after prolonged incubation at 37°. Lane a, untreated fragments; Lane b, fragments treated for 30 min at 37° at a drug/DNA concentration ratio of 0.20; Lane c, treated for 4 hr; Lane d, treated for 7 hr; Lane e, treated for 22.5 hr; Lane f, untreated fragments incubated for 22.5 hr at 37°. B, formation and disappearance of the luzopeptin A-induced cross-linking bands of HindIII- and PstI-restricted pBR322 DNA fragments (L, 3579 BP; S, 783 BP). a, untreated fragments; b, treated with luzopeptin A at 37° for 0.5 hr; c, treated for 5 hr; d, treated for 24 hr; e, treated for 48 hr; f, treated for 96 hr. Drug/DNA ratio, 0.5.

Fig. 4. Electron microscopic study of luzopeptin A-treated pBR322 DNA restriction fragments. Long (L, 3579 BP) and short (S, 783 BP) fragments (0.7 μg) produced by HindIII plus PstI restriction (A) were treated with luzopeptin A (10 μg/ml) in 50 mM Tris-HCl buffer containing 1 mM EDTA (pH 7.5) for 30 min at room temperature. The cross-over products (B, C, and D) are probably cross-linked products from 2 long (L) fragments, since their length is approximately twice that of a long fragment, x 19,800.

Fig. 5. Gel pattern of DNA products of echinomycin-treated HindIII restriction fragments of PM2 DNA. Fragments were treated at 37° for 30 min at echinomycin/DNA concentration ratios of 0 (a); 0.01 (b); 0.03 (c); 0.06 (d); 0.18 (e); 0.30 (f); and 0 (g).
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