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Absence of Swiveling at Sites of Intercalator-Induced Protein-Associated Deoxyribonucleic Acid Strand Breaks in Mammalian Cell Nucleoids†

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ABSTRACT: The sedimentation of DNA–nuclear protein complexes in 1.9 M salt–neutral sucrose gradients (nucleoid sedimentation) was used to examine the effects of the DNA intercalator 4′-(9-acridinylamino)methanesulfon-*m*-anisidide (m-AMSA) on mouse leukemia cell DNA. Mild detergent cell lysis and neutral pH make nucleoid sedimentation an extremely gentle, but sensitive, method to detect DNA scission. DNA breaks reduce the compaction of nucleoids and slow their sedimentation. Nucleoids from m-AMSA-treated cells sedimented as did those from untreated cells, indicating no detectable m-AMSA-dependent alterations in compaction despite an apparent underlying DNA break frequency of approximately 3 per 10^6 nucleotides, as measured by alkaline elution with proteinase. Mild proteinase digestion of cell lysates prior to nucleoid sedimentation unmasked some, but not all, of the underlying breaks. The frequency of DNA–protein cross-links in nucleoids from cells treated with m-AMSA was comparable to the single-strand break frequency produced by m-AMSA in whole cells. These results indicate that m-AMSA-induced DNA–protein cross-links conceal DNA breaks so as to prevent swiveling around the breaks within the nucleoids. This unique sort of DNA scission is consistent with the involvement of topoisomerases in the DNA breaks elicited by intercalators in mammalian cells.

When mammalian cells are exposed to intercalating agents, DNA strand breaks are produced. These strand breaks are unusual in that they are protein concealed, i.e., their detection by alkaline elution requires proteinase digestion (Ross et al., 1979; Zwelling et al., 1981). Their production appears to be enzymatically mediated (Zwelling et al., 1981) and to depend somewhat upon the three-dimensional structure of the target chromatin (Pommier et al., 1983). For each intercalator break produced, one DNA-protein cross-link is generated, and it has been postulated that the protein is bound covalently to one of the broken DNA strands that it generates (Ross et al., 1979).

In our previous work characterizing this intercalator-induced DNA strand breakage, we utilized two techniques to quantify DNA scission: alkaline sucrose sedimentation and alkaline elution (Zwelling et al., 1981). Both methods employ lysis of cells by alkali and/or detergent prior to quantification of DNA strand breakage. Despite these treatments, DNA–protein binding was detected in the lysates from intercalator-treated cells, suggesting that this binding was covalent. The possibility that the proteins were bound to *both* DNA termini was deemed unlikely because DNA strand breakage was observed by alkaline sedimentation in the absence of proteinase K (Zwelling et al., 1981). However, it was considered possible that both termini could be bound by the enzyme but that one of them was alkali and/or detergent labile, i.e., noncovalent.

DNA topoisomerases are likely candidates for the proteins that are associated with the intercalator-induced strand breaks. We have hypothesized that the protein-associated scission of DNA in cells incubated with intercalators is similar to or identical with the protein-associated DNA strand breakage produced by DNA topoisomerases (Ross et al., 1979; Zwelling et al., 1981). In the case of topoisomerase-induced DNA breaks, it is believed that the enzyme binds to each side of the DNA break it induces, in such a way that only one bond between DNA and each topoisomerase molecule appears to be covalent and that DNA strand movements at the break site are controlled by the enzyme (Cozzarelli, 1979; Gellert, 1981; Pulleyblank & Ellison, 1982). If the protein-associated breaks produced or stabilized by intercalators in mammalian cells or isolated nuclei (Pommier et al., 1982) are produced by topoisomerases, the protein binding should limit movement of the DNA strands around the break sites within chromatin. This possibility has been assessed in the present study by using the nucleoid sedimentation technique.

Nucleoids are DNA-containing and nuclear protein containing structures isolated by the lysis of cells at neutral pH with nonionic detergents and sedimentation in neutral, 1.9 M salt–sucrose gradients (Cook & Brazell, 1975). Histones and most other nonhistone nucleoproteins remain near the top of the gradients while some structural proteins sediment with the

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DNA (Cook & Brazell, 1975, 1976; Levin et al., 1978; Lebkowski & Laemmli, 1982). Removal of nucleosomes accounts for the negative supercoiling of nucleoids (Cook & Brazell, 1975, 1976). The technique of nucleoid sedimentation can detect small numbers of DNA strand breaks, which remove DNA supercoiling by allowing swiveling at the break sites and lead to relaxed (unfolded) nucleoids that sediment at a slower rate than intact nucleoids. We demonstrate that nucleoids from intercalator-treated cells, despite having DNA single-strand break frequencies in excess of that produced by 2500 rd of X-rays (as quantified by filter elution), sedimented identically with nucleoids from untreated cells. Limited proteinase digestion prior to sedimentation unmasked at least some of the underlying intercalator-induced scission. The retention of nucleoid compaction in intercalator-treated cells despite the underlying strand scission indicated that proteins prevented swiveling of DNA at the break sites within the high-salt gradient. This result is consistent with current structural models for topoisomerase sites and the mechanism of DNA topoisomerase action (Cozzarelli, 1980; Gellert, 1981).

Materials and Methods

Materials. [2-^{14}C]Thymidine (58 mCi mmol^{-1}) and [*methyl*-^{3}H]thymidine (20 Ci mmol^{-1}) were purchased from New England Nuclear, Boston, MA. 4′-(9-Acridinylamino)methanesulfon-*m*-anisidide (m-AMSA) (NSC 249992) was obtained from the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, NCI. A 10 mM stock solution in dimethyl sulfoxide was stored frozen at −20 °C.

Cell Radiolabeling, Irradiation, and Drug Treatment. L1210 mouse leukemia cells were grown in suspension culture in RPMI 1630 medium supplemented with 15% fetal calf serum. Stock cultures were maintained in static bottles without antibiotics and were used to initiate suspension cultures. Cultures used to assess drug effects were in exponential growth phase with a doubling time of 13–15 h.

Cellular DNA was radioactively labeled in exponentially growing cells by incubation with [2-^{14}C]- or [*methyl*-^{3}H]-thymidine for 20 h at 37 °C. For experiments in which single-strand breaks and DNA–protein cross-links were measured by alkaline elution, [^{14}C]thymidine labeling of DNA was with 0.01 μCi mL^{-1}, and the DNA of cells used as internal standards (Kohn et al., 1981) was labeled with [*methyl*-^{3}H]thymidine (0.1 μCi mL^{-1}; 10^{-6} M unlabeled thymidine added). In the nucleoid sedimentation experiments, cellular DNA was labeled with [2-^{14}C]thymidine (0.04 μCi mL^{-1}). In all cases, radioactive label was removed by centrifugation prior to drug treatment or cell irradiation. L1210 cells at concentrations of (1–1.5) × 10^{6} mL^{-1} in iced RPMI 1630 medium plus 15% fetal calf serum were irradiated with either a ^{137}Cs or a 200-kV X-ray source as described previously (Kohn et al., 1981; Zwelling et al., 1981). Cells were maintained at ice temperature until they were assayed either by alkaline elution or by nucleoid sedimentation, m-AMSA treatments were for 30 min at 37 °C. Reactions were stopped by centrifugation and resuspension twice in excess iced drug-free medium. In the studies of reversibility of the DNA effects of m-AMSA, cells were again centrifuged and resuspended in drug-free medium at 37 °C.

Alkaline Elution Assays. The alkaline elution methodology has been described in detail in previous publications (Kohn et al., 1981; Zwelling et al., 1981).

DNA Single-Strand Breaks. Treated (or untreated control) [^{14}C]thymidine-labeled cells (approximately 5 × 10^{5}) were mixed with an equal number of [^{3}H]thymidine-labeled cells that had received no drug treatment but had been irradiated concurrently to serve as internal standard cells (Kohn et al., 1981). Cells were deposited gently onto polycarbonate membrane filters (2-μm pore diameter, Nucleopore Corp., Pleasanton, CA) in a Swinnex 25 filter holder (Millipore Corp., Bedford, MA) and lysed with 0.1 M glycine, 0.025 M disodium ethylenediaminetetraacetic acid (Na_2EDTA), 2% sodium dodecyl sulfate (SDS) (BDH Biomedical Ltd., Poole, England), pH 10.0, plus 0.5 mg mL^{-1} proteinase K. Elution was performed with tetrapropylammonium hydroxide–EDTA–0.1% SDS, pH 12.1. For the high-sensitivity assay, elution was carried out at a pump speed of 0.03–0.04 mL min^{-1} (2 mL h^{-1}), and fractions were collected at 3-h intervals for 15 h as described previously (Kohn et al., 1981). For the low-sensitivity assay (which was required to assay DNA break frequencies exceeding 0.5 single-strand break per 10^{6} nucleotides), elution was at a pump speed of 0.12–0.16 mL min^{-1}, and fractions were collected at 5-min intervals for 30 min (Zwelling et al., 1981). The [^{3}H]DNA internal standard cells received 300 rd of X radiation in the high-sensitivity assay and 1000 rads in the low-sensitivity assay. Single-strand break frequency was calculated by

$$\text{break frequency} = [\log (r_1/r_0)/\log (R_0/r_0)]P_B \quad (1)$$

where P_B is the DNA break frequency produced by the X-ray (300 or 1000 rd equiv) and r_1, r_0, and R_0 are the retentions of DNA from drug-treated, untreated, and 300 or 1000 rd irradiated [^{14}C]thymidine-labeled cells. Retention was quantified when 35% of the [^{3}H]DNA in the high-sensitivity assay or 60% of the [^{3}H]DNA in the low-sensitivity assay remained on the filter. The exact choice of this end point was not critical, since the elution kinetics was nearly first order with respect to time following all drug treatments. Results are expressed in "rd equiv", indicating that the elution rate of DNA from drug-treated cells is equal to the rate produced by a particular X-ray dose.

The percentage of single-strand break resealing after m-AMSA removal was calculated as

$$\% \text{ resealing} = \frac{P_{B0} - P_{Bx}}{P_{B0}} \times 100 \quad (2)$$

where P_{B0} and P_{Bx} are the measured DNA break frequencies (calculated from eq 1) immediately and at a given time after m-AMSA removal, respectively.

DNA–Protein Cross-Links. Drug-treated (or untreated control) [^{14}C]thymidine-labeled cells (or nucleoids from m-AMSA-treated or untreated cells) were X irradiated at ice temperature (4 °C) with 3000 rd. These cells (or nucleoids) were combined with an equal number (approximately 5 × 10^{5}) of [^{3}H]thymidine-labeled cells that had received no drug treatment and had been irradiated concurrently with 3000 rd in ice. Cells were deposited onto 2-μm poly(vinyl chloride) filters (type BS; Millipore, Bedford, MA) and lysed with a solution consisting of 2 M NaCl, 0.2% sarkosyl, and 0.04 M Na_2EDTA, pH 10.0 (5 mL). This lysis solution was removed by washing the filter with 0.04 M EDTA, pH 10.0 (3 mL). DNA was eluted with tetrapropylammonium hydroxide–EDTA, pH 12.1, at a pump speed of 0.03–0.04 mL min^{-1}. Fractions (6 mL) were collected every 3 h for 15 h. Samples were processed and data were computed as described previously (Kohn et al., 1981). DNA–protein cross-linking frequencies (P_x) were calculated from the bound-to-one terminus model of Ross et al. (1979) as

$$P_x = [(1 - r)^{-1} - (1 - r_0)^{-1}]P_B \quad (3)$$

Table I: Comparison of the Effects of m-AMSA on L1210 Cellular DNA When Assayed by Nucleoid Sedimentation (with or without Proteinase K) or Alkaline Elution with Proteinase K[a]

treatment	nucleoid sedimentation distance (% control) −Pro K	nucleoid sedimentation distance (% control) +Pro K (100 ng mL^{-1})	DNA SSB frequency (alkaline elution)[b]
none	100	94 ± 13	0
2 μm m-AMSA	97 ± 17 (n = 8)	39 ± 17 (n = 9)	2692 ± 858 (n = 5)
100 rd of X-rays	47 ± 7 (n = 4)	49 ± 5 (n = 3)	100

[a] Cells were treated with m-AMSA at 37 °C, irradiated at 4 °C, or given no treatment and then washed in medium at 4 °C. Portions of the cultures were assayed simultaneously by nucleoid sedimentation and by alkaline elution for m-AMSA effects on DNA (Pro K, proteinase K; n, number of independent experiments for each value). [b] DNA single-strand breaks expressed in rd equiv (see text).

where P_B is the break frequency (in rd equiv) produced by 3000 rd and r_0 and r are the retentions of DNA on the filter from 3000 rd irradiated [^{3}H]- or [^{14}C]thymidine-labeled cells (or nucleoids), respectively. The degree to which r exceeds r_0 is a measure of DNA–protein cross-linking.

Nucleoid Sedimentation Assays. Following treatment and drug removal, [^{14}C]thymidine-labeled cells were pelleted by centrifugation (1000 rpm for 5 min, 4 °C), and the pellets were resuspended at 2 × 10^5 cells mL^{-1} in 2 mL of neutral lysis solution (0.1% Triton X-100, 0.02 M EDTA, 0.01 M Tris [tris(hydroxymethyl)aminomethane], pH 8.0), at 4 °C. After a 60-min incubation at 4 °C, (1–2) × 10^5 cells were layered onto preformed 15–30% neutral sucrose gradients (36-mL total volume containing 1.9 M NaCl, 0.1 M Tris, and 0.01 M EDTA, pH 8.0, 4 °C) [modification of the procedure of Cook & Brazell (1975)]. For experiments in which nucleoid lysates were digested by proteinase K, the proteinase was incubated with the lysates for the last 10 min of the 60-min lysis period before cells were layered onto the gradients. Proteinase K was dissolved in the above neutral lysis solution at 1 mg mL^{-1}. It was prepared the day before its use and incubated at room temperature until its use to minimize effects of contaminant nucleases (Kohn et al., 1981). The samples were centrifuged in a Beckman L8-80 ultracentrifuge (SW 28 rotor, 4 °C, 15000–17000 rpm for 1.5–2.5 h). Equivalent weight fractions were collected from the bottoms of the tubes, and the trichloroacetic acid insoluble radioactivity contained in each fraction was determined by liquid scintillation spectrometry, as described previously (Mattern & Painter, 1979), and the percentage of control nucleoid sedimentation was calculated for each sample by dividing the nucleoid sedimentation distance (peak fraction) of the treated sample by that of the control and multiplying this ratio by 100.

The percentage restoration of control nucleoid sedimentation after m-AMSA removal was calculated as

$$\% \text{ nucleoid restoration} = \frac{D_x - D_0}{100 - D_0} \times 100 \qquad (4)$$

where D_0 and D_x are the percentage of control nucleoid sedimentation distance immediately and at a given time after m-AMSA removal, respectively.

Results

m-AMSA was selected as the intercalator with which to treat cells to examine the resultant DNA effects by nucleoid sedimentation because it is the intercalator that produces the largest break frequency per micromolar drug or per cytotoxic effect among the intercalators examined previously (Zwelling et al., 1981). m-AMSA at 2 μM for 30 min induced almost three DNA single-strand breaks per 10^6 nucleotides (2692 ± 858 rd equiv) (Table I) as quantified by alkaline elution with proteinase. However, nucleoids from these cells sedimented identically with nucleoids from untreated cells, indicating that there was no major alteration in DNA compaction in nucleoids from m-AMSA-treated cells, despite the large underlying break frequency. This result cannot be attributed to a lack of sensitivity in break detection by the nucleoid sedimentation assay, as nucleoids from cells receiving 100 rd (approximately one DNA single-strand break per 10^7 nucleotides) sedimented to less than half the distance of controls (Table I).

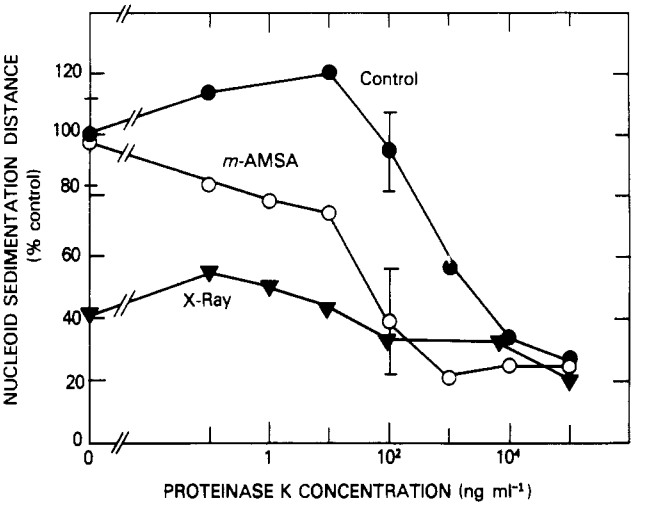

FIGURE 1: Effect of proteinase K treatment on the sedimentation of nucleoids from untreated cells, from cells treated with m-AMSA, or from cells irradiated with 300 rd. Cells were lysed in nucleoid lysis solution for 50 min, after which various concentrations of proteinase K were added for an additional 10 min before the end of the 60-min lysis period. The cells were then layered onto neutral sucrose gradients (1.9 M NaCl), and the rate of nucleoid sedimentation was determined as described in the text. Error bars denote standard deviations of at least four independent experiments. (●) Controls; (○) m-AMSA-treated (2 μM) cells; (▼) X-irradiated cells.

The discrepancy between results obtained by filter elution and those obtained by nucleoid sedimentation following treatment of cells with m-AMSA was resolved, in part, by employing proteinase digestion of nucleoid lysates prior to sedimentation. The proteolysis unmasked an underlying alteration in nucleoids from m-AMSA-treated cells (Figure 1). At proteinase K concentrations of 100 ng mL^{-1} or less, there was a dose-dependent reduction in the sedimentation rate of nucleoids from m-AMSA-treated but not from untreated or X-irradiated cells. The sensitivity of nucleoids from m-AMSA-treated cells to proteinase could be the result of unmasking breaks already present or of creating new breaks in the DNA by the action of contaminating nucleases. Proteinase K incubations (1 μg mL^{-1} or less) performed identically with those employed in nucleoid experiments did not produce detectable breakage in cellular DNA as detected by alkaline elution with proteinase. This indicated that 1 μg mL^{-1} or less proteinase had no detectable nuclease activity (data not shown).

The DNA–protein cross-links produced by m-AMSA in cells were still present in the nucleoids isolated from these cells (Figure 2) at frequencies similar to those of m-AMSA-induced DNA strand breaks quantified by alkaline elution with proteinase. The proteinase-induced decompaction of nucleoids from m-AMSA-treated cells was likely due to the unmasking

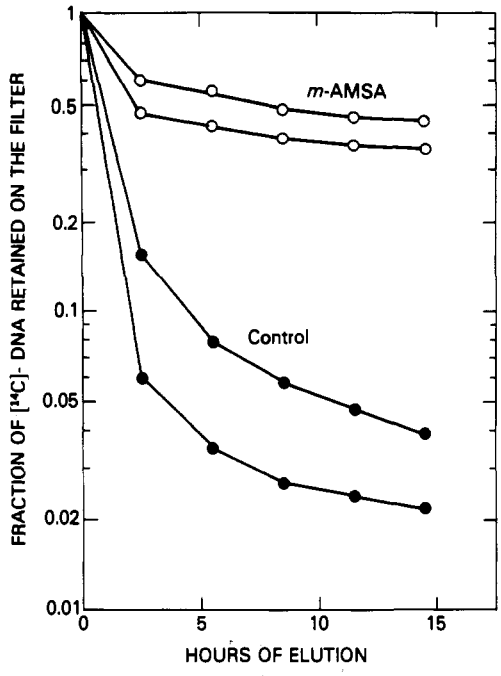

FIGURE 2: DNA–protein cross-linking in nucleoids from m-AMSA-treated cells. Cells were incubated with m-AMSA (2 μM) for 30 min (○) or were untreated (control) (●). Nucleoids were prepared (no proteinase K) by centrifugation, X irradiated (3000 rd) at 4 °C, and assayed for DNA–protein cross-linking by alkaline elution (two independent experiments).

of protein-concealed strand breaks and subsequent swiveling of DNA. These breaks were concealed under the neutral, high-salt conditions of nucleoid sedimentation in the absence of proteinase, indicating that, following m-AMSA treatment, drug-induced DNA scission did not lead to any detectable strand movement (swiveling) because of the m-AMSA-induced DNA-protein interactions associated with the strand breaks.

Nucleoid sedimentation can quantify small numbers of strand breaks but has a limited range of sensitivity (Cook & Brazell, 1975). Nucleoids from L1210 cells containing 200 rd equiv of DNA single-strand breaks are maximally relaxed (Mattern et al., 1982). Additional breaks do not retard sedimentation further. Alkaline elution, by contrast, can be adapted to provide accurate break frequency quantification at break frequencies exceeding 5000 rd equiv. With either of these techniques, however, without the use of proteinase, the results of assays performed with m-AMSA-treated cells resemble those performed with untreated cells (Figure 3). The use of proteinase unmasked DNA strand breaks in both assays; however, fewer breaks were detected with nucleoid sedimentation than with alkaline elution. For example, 0.1 μM m-AMSA produced a break frequency of 350 rd equiv in elution assays but had a smaller effect on nucleoid sedimentation than that of 100 rd (Figure 3 and Table I). Thus, only 25–35% of the intercalator-induced DNA breaks detectable by alkaline elution [or alkaline sedimentation (Zwelling et al., 1981)] was detected in nucleoid sedimentation assays with proteinase. This finding indicates either that a similar yield of strand breaks was present in nucleoids and in cells lysed with SDS and alkali but that the proteinase digestion in the nucleoid assay (which is milder than that in the alkaline elution) was incomplete or that some, but not all, of the DNA–protein binding sites were actually in an open state prior to SDS and/or alkali lysis. This model agrees well with that of Liu et al. (1983). These authors have shown that isolated mammalian topoisomerase II can produce double-stranded DNA cleavage when the enzyme–DNA complex is treated with SDS in an isolated chemical

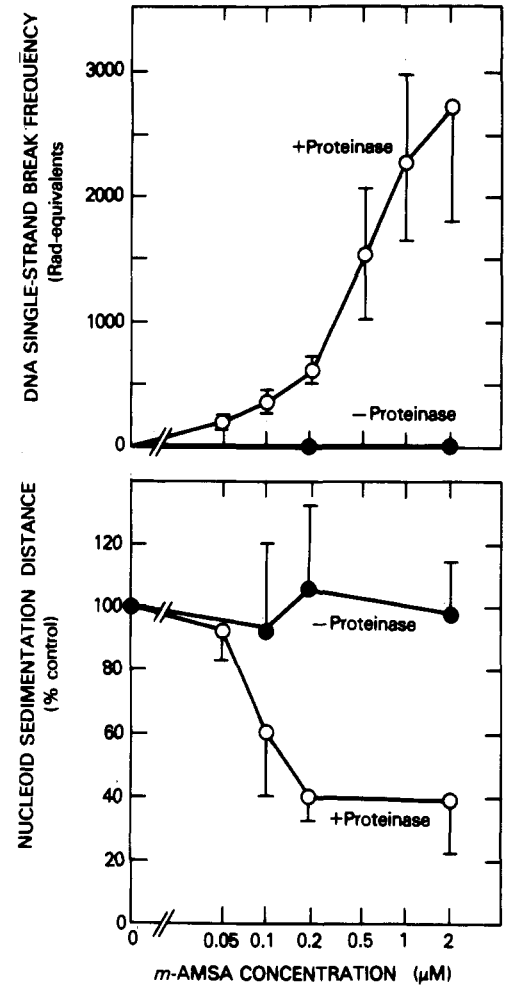

FIGURE 3: Dependence of DNA single-strand break frequency or nucleoid sedimentation rate alterations on m-AMSA concentration. m-AMSA-induced DNA single-strand breaks were quantified by alkaline elution without (●) or with proteinase K (○) (0.5 mg mL^{-1}) (upper panel) or by alterations in nucleoid compactness (lower panel) without (●) or after (○) proteinase K digestion (100 ng mL^{-1}). m-AMSA treatments were for 30 min at 37 °C.

system. If reduced temperature or salt intervened prior to SDS addition, less cleavage was detected. If proteinase acts similarly in cell lysates prior to nucleoid sedimentation, as salt in the isolated enzyme system, this could explain the lower frequency of m-AMSA-induced DNA scissions detected in nucleoids than in alkaline elution where SDS is used to lyse cells.

The DNA scission produced by m-AMSA is rapidly reversible at 37 °C. Following m-AMSA treatment, the rate at which nucleoid sedimentation (using proteinase K) returned to control values (Figure 4) was similar to that of DNA scission rejoining assessed by alkaline elution (Figure 5). The percentage of DNA single-strand breaks rejoined and of nucleoid reconstitution at various times after m-AMSA removal was calculated as described under Materials and Methods (eq 2 and 4). When these calculations were plotted for a series of times, DNA single-strand breaks and unfolding of m-AMSA nucleoids by proteinase K were reversed concomitantly (Figure 6).

Discussion

Protein-associated strand scission has been detected in DNA from several types of mammalian cells exposed to intercalating agents. The methods employed previously (Ross et al., 1979; Kohn et al., 1981; Zwelling et al., 1981, 1982a,c) to quantify this scission included detergent and/or alkali lysis of cells prior

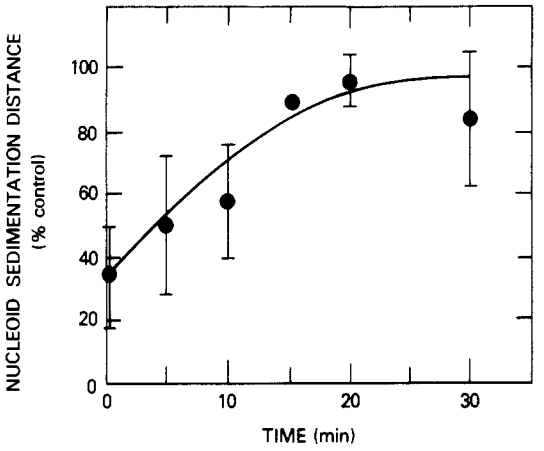

FIGURE 4: Reversal of the effects of m-AMSA on nucleoid sedimentation rate. Cells were incubated with m-AMSA (2 μM) for 30 min at 37 °C, after which the m-AMSA was removed and the cells were incubated in drug-free medium for various times. Aliquots of cells were assayed by nucleoid sedimentation at 4 °C after limited proteinase K digestion (100 ng mL^{-1}).

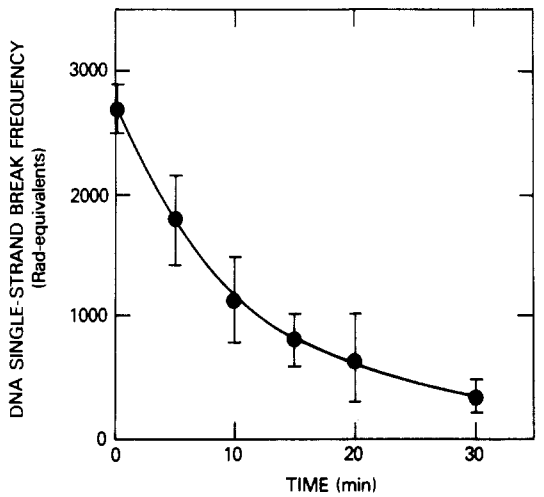

FIGURE 5: Kinetics of resealing of m-AMSA-induced DNA single-strand breaks. Cells were treated as described in the legend to Figure 4. DNA single-strand breaks were assayed by alkaline elution with proteinase K. Error bars denote standard deviations of at least three independent experiments.

to break measurements. As protein-associated DNA scission can be produced in vitro, in the presence of bacterial DNA gyrase and oxolinic acid, by ionic detergent (SDS) or alkali (Gellert et al., 1981), the actual existence of the DNA breaks within m-AMSA-treated mammalian cells prior to lysis could be questioned. Further, the absence of poly(adenosine diphosphoribose) synthesis [a reaction uniformly observed in cells containing broken DNA (Berger et al., 1979; Juarez-Salinas et al., 1979; Nolan & Kidwell, 1982)] in cells permeabilized following m-AMSA treatment and containing alkaline elution detectable DNA breaks (Zwelling et al., 1982b) also raised the possibility that these DNA breaks were produced within the cell lysates by SDS and alkali in vitro, rather than ever having been extant in vivo.

The equivalent sedimentation rates in neutral sucrose gradients of nucleoids from control and m-AMSA-treated cells tend to question further the existence of m-AMSA-induced DNA strand breaks in vivo. However, the result that proteinase K unmasks an underlying DNA strand breakage strongly suggests that the breaks do, in fact, exist in cells and that they are concealed by proteins, which restrict movement of DNA about the breaks (prevent swiveling) within the nucleoid structures. Swiveling of DNA would lead to relaxed nucleoids, which would sediment more slowly than compacted nucleoids in 1.9 M NaCl (Cook & Brazell, 1975, 1976).

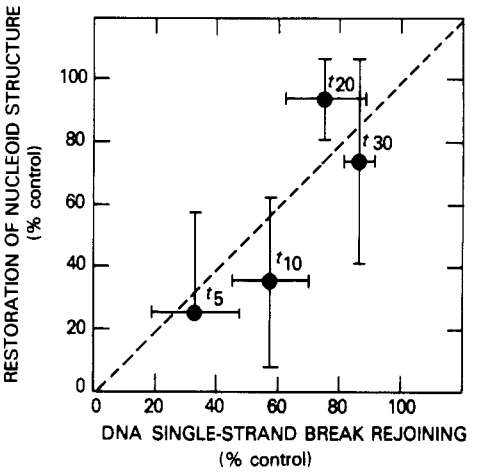

FIGURE 6: Relationship between DNA single-strand break rejoining and restoration of nucleoid structure at various times after m-AMSA treatment. Cells were incubated with 2 μM m-AMSA, and the m-AMSA was removed after 30 min, as described in the legend to Figure 4 and in the text. DNA single-strand breaks remaining in cells at various times after drug removal were determined by alkaline elution and expressed as a percentage of DNA single-strand break rejoining (eq 2). The percentage of restoration of nucleoid structure was determined after limited proteinase K digestion (eq 4). Time following m-AMSA removal (in minutes) is indicated for each point. Error bars denote standard deviations for at least three independent experiments.

The restriction of DNA movement at the intercalator-induced DNA break sites could be due to protein bridging of the breaks. However, only one of the bonds has been shown to resist high salt, alkali, and protein denaturants and, thus, is likely to be covalent (Ross et al., 1979). The other would be alkali-labile and noncovalent, since no bridging was detected in alkaline sucrose gradients in the absence of proteinase or SDS (Zwelling et al., 1981). Swiveling could also be prevented by steric hindrance effected by a protein bound to one terminus only; our data do not exclude this alternate mechanism.

The recent work of Liu et al. (1983), which demonstrated the production of DNA scission by topoisomerase II following SDS treatment, substantiates our cellular model in which m-AMSA-induced DNA scission is mediated through interference with topoisomerase II (Liu et al., 1983; Pommier et al., 1984). In the case of topoisomerase II (Liu et al., 1983), swiveling about the potentially cleavable site was shown to be prevented by the enzyme. Our present finding indicates that m-AMSA probably acts to stabilize a similar DNA–protein complex that is cleaved by SDS, alkali, or proteinase K. Intercalator-induced DNA breaks produced by DNA topoisomerases might represent a stabilized intermediate state of the DNA nicking–closing reaction of the enzymes analogous to that of DNA gyrase in the presence of oxolinic acid (Gellert, 1981). The rapid disappearance of the protein-concealed DNA breaks induced by m-AMSA in cells (Figures 4 and 5; Zwelling et al., 1981), in isolated nuclei (Pommier et al., 1982), and in permeabilized cells (Zwelling et al., 1982b) shows that the m-AMSA-induced or -stabilized DNA–protein complexes are reversible upon drug removal.

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Registry No. DNA topoisomerase, 80449-01-0; 4′-(9-acridinylamino)methanesulfon-*m*-anisidide, 51264-14-3.

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Changes in Deoxyribonucleic Acid Linking Number due to Treatment of Mammalian Cells with the Intercalating Agent 4′-(9-Acridinylamino)methanesulfon-*m*-anisidide†

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ABSTRACT: Treatment of mammalian cells with DNA intercalating agents produces protein-associated DNA strand breaks. These breaks have been proposed to represent the action of a topoisomerase, which would alter the DNA linking number. Changes in DNA linking number in cells treated with the intercalating agent 4′-(9-acridinylamino)methanesulfon-*m*-anisidide (m-AMSA) were studied by ethidium titration of nucleoid sedimentation. m-AMSA treatment was found to produce an increase in DNA linking number. Previously, we had proposed that intercalator-induced protein-associated DNA breaks act to reduce DNA torsional strain that results from the intercalator-induced decrease in DNA twist. In such a model, linking number would be expected to decrease. The finding that the DNA linking number increased following m-AMSA treatment suggests that intercalators may block enzymes that normally decrease linking number. Such enzymes would have DNA gyrase like properties. Consistent with this possibility, a DNA gyrase inhibitor, novobiocin, inhibited the restoration of normal linking number and, to a lesser degree, the reversal of protein-associated strand breaks after removal of intercalator.

Treatment of mammalian cells with DNA intercalating agents results in the formation of DNA strand breaks that have been postulated to result from the action of a DNA topoisomerase like enzyme (Ross et al., 1979; Zwelling et al., 1981). In addition to strand breaks, an approximately equal frequency of apparently covalent DNA–protein links is produced, and this is the case for several structurally different types of intercalators (Zwelling et al., 1981, 1982a,b). The DNA–protein links are localized relative to the sites of strand breakage (Pommier et al., 1984), suggesting that a protein may be linked to one terminus of the strand break, as is generally true for topoisomerases (Cozzarelli, 1979; Gellert, 1981). Consistent with an enzymatic origin, the formation of intercalator-induced strand breaks is temperature dependent, saturable with respect to time and drug concentration (Zwelling et al., 1981, 1982a,b), and mediated in a cell-free system by a 0.35 M NaCl extract from isolated nuclei (Filipski et al., 1983). The previously proposed model (Ross et al., 1979;

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