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

References

Berger, N. A., Sikorski, G. W., Petzold, S. J., & Kurohara, K. K. (1979) *J. Clin. Invest. 63*, 1164–1171.

Cook, P. R., & Brazell, I. A. (1975) *J. Cell Sci. 19*, 261–279.

Cook, P. R., & Brazell, I. A. (1976) *J. Cell Sci. 22*, 287–302.

Cozzarelli, N. R. (1979) *Science (Washington, D.C.) 207*, 953–960.

Cozzarelli, N. R. (1980) *Cell (Cambridge, Mass.) 22*, 327–328.

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

Juarez-Salinas, H., Sims, J. L., & Jacobson, M. K. (1979) *Nature (London) 282*, 740–741.

Kohn, K. W., Ewig, R. A. G., Erickson, L. C., & Zwelling, L. A. (1981) in *DNA Repair: A Laboratory Manual of Research Techniques* (Friedberg, E. C., & Hanawalt, P. C., Eds.) pp 379–401, Marcel Dekker, New York.

Lebkowski, J. S., & Laemmli, U. K. (1982) *J. Mol. Biol. 159*, 325–344.

Levin, J. M., Jost, E., & Cook, P. R. (1978) *J. Cell Sci. 29*, 103–116.

Liu, L. F., Rowe, T. C., Yang, L., Tewey, K. M., & Chen, G. L. (1983) *J. Biol. Chem. 258*, 15365–15370.

Mattern, M. R., & Painter, R. B. (1979) *Biochim. Biophys. Acta 563*, 293–305.

Mattern, M. R., Zwelling, L. A., Kerrigan, D., & Kohn, K. W. (1982) *Biochem. Biophys. Res. Commun. 112*, 1077–1084.

Nolan, N. L., & Kidwell, W. L. (1982) *Radiat. Res. 90*, 187–203.

Pommier, Y., Kerrigan, D., Schwartz, R., & Zwelling, L. A. (1982) *Biochem. Biophys. Res. Commun. 107*, 576–583.

Pommier, Y., Zwelling, L. A., Mattern, M. R., Erickson, L. C., Kerrigan, D., Schwartz, R. E., & Kohn, K. W. (1983) *Cancer Res. 43*, 5718–5724.

Pommier, Y., Mattern, M. R., Schwartz, R. E., Zwelling, L. A., & Kohn, K. W. (1984) *Biochemistry* (following paper in this issue).

Pulleyblank, D. E., & Ellison, M. J. (1982) *Biochemistry 21*, 1155–1161.

Ross, W. E., Glaubiger, D. L., & Kohn, K. W. (1979) *Biochim. Biophys. Acta 562*, 41–50.

Zwelling, L. A., Michaels, S., Erickson, L. C., Ungerleider, R. S., Nichols, M., & Kohn, K. W. (1981) *Biochemistry 20*, 6553–6563.

Zwelling, L. A., Kerrigan, D., & Michaels, S. (1982a) *Cancer Res. 42*, 2687–2691.

Zwelling, L. A., Kerrigan, D., Pommier, Y., Michaels, S., Steren, A., & Kohn, K. W. (1982b) *J. Biol. Chem. 257*, 8957–8963.

Zwelling, L. A., Michaels, S., Kerrigan, D., Pommier, Y., & Kohn, K. W. (1982c) *Biochem. Pharmacol. 31*, 3261–3267.

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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Zwelling et al., 1981) was that the intercalation-induced local DNA unwinding generates torsional tension that is propagated along the DNA helix and stimulates a topoisomerase to open a DNA strand and allow a passive compensatory change in linking number. In the present work, we find that treatment of mammalian cells with the intercalating agent 4′-(9-acridinylamino)methanesulfon-*m*-anisidide (m-AMSA) results in a change in DNA linking number in the opposite direction from that which would be expected from the above model. We also report the inhibitory effects of novobiocin on this process and propose a modified hypothesis.

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. 4′-(9-[9-^{14}C]Acridinylamino)methanesulfon-*m*-anisidide ([^{14}C]-m-AMSA) (19.6 mCi mmol^{-1}) was synthesized by SRI International, Menlo Park, CA, and was obtained through the Chemical Resource Section, NCI. This drug was also dissolved in dimethyl sulfoxide and stored frozen at −20 °C. Novobiocin and 3-aminobenzamide (3-ABA) were purchased from Sigma Chemical Co. (St. Louis, MO) and Pfaltz and Bauer Co. (Stamford, CT), respectively. Novobiocin was dissolved at 10 mg mL^{-1} in water and 3-ABA at 5 mM in RPMI 1630 medium supplemented with 15% fetal calf serum. Novobiocin stock solutions and 3-ABA-containing medium were prepared immediately prior to use.

Cell Radiolabeling, Irradiation, and Drug Treatment. L1210 mouse leukemia cells were grown in RPMI 1630 medium supplemented with 15% fetal calf serum and labeled with [2-^{14}C]thymidine (0.01–0.04 μCi mL^{-1}) or [*methyl*-^{3}H]thymidine (0.1 μCi mL^{-1}) as described previously (Pommier et al., 1984). A total of (1–1.5) × 10^{6} cells mL^{-1} was X irradiated in ice-cold RPMI 1630 medium and maintained in ice until assayed by either alkaline elution or nucleoid sedimentation (Pommier et al., 1984). m-AMSA treatments (2 μM) were for 30 min at 37 °C. Reactions were terminated by centrifugation and resuspension in drug-free medium (ice temperature). For the reversibility studies, cells were centrifuged again and resuspended in drug-free medium kept at 37 °C. In some experiments, cells were treated with novobiocin (200 μg mL^{-1}) for 1 h, followed by novobiocin plus m-AMSA, and then with novobiocin alone for various times at 37 °C. The effects of 3-ABA on the reversal of intercalator effects were determined by washing the m-AMSA-treated cells twice and resuspending them in medium containing 5 mM 3-ABA.

Measurement of DNA Single-Strand Breaks by Alkaline Elution. The alkaline elution methodology has been described in previous publications (Kohn et al., 1981; Zwelling et al., 1981). [^{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 received 1000 rd of X radiation to serve as internal standard cells (Zwelling et al., 1981). Cells were deposited gently onto 2-μm polycarbonate filters (Nucleopore Co., Pleasanton, CA) and lysed with 5 mL of a solution containing 0.1 M glycine, 0.025 M disodium ethylenediaminetetracetic acid (Na_2EDTA), 2% sodium dodecyl sulfate (SDS), pH 10, plus 0.5 mg mL^{-1} proteinase K. This solution was washed out with 0.04 M Na_2EDTA, pH 10 (10 mL). DNA elutions were then carried out with tetrapropylammonium hydroxide–EDTA–0.1% SDS, pH 12.1, 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). DNA single-strand break frequency was calculated as described previously (Kohn et al., 1981; Zwelling et al., 1981).

Measurement of Changes in DNA Linking Number by Nucleoid Sedimentation. A modification of the procedure of Cook & Brazell (1975) was used. Details have been published (Mattern & Painter, 1979; Pommier et al., 1984). Cell lysates were layered at 4 °C onto 15–30% neutral sucrose gradients [1.9 M NaCl, 0.10 M tris(hydroxymethyl)aminomethane (Tris), 0.01 M EDTA, pH 8.0] containing 0–10 μg mL^{-1} ethidium bromide. Following centrifugation, the percentages of control nucleoid sedimentation rate were calculated as described previously (Mattern & Painter, 1979). "Controls" for each run were nucleoids sedimenting in gradients containing no ethidium. Ethidium titration of nucleoid sedimentation (Cook & Brazell, 1976) provides a relative measure of DNA linking number, a topologically invariant quantity (Bauer et al., 1980).

Nucleoid DNA tends to be underwound; i.e., it tends to have a linking number less than the helical twist (Bauer et al., 1980), mainly due to the loss of all nucleosomes in the 2 M NaCl solvent. Intercalation by ethidium reduces the twist. At the ethidium concentration at which twist equals linking number, the DNA loops are maximally extended, and the nucleoid sedimentation velocity is at a minimum. The ethidium concentration that produces minimum sedimentation is assumed to be a relative measure of DNA linking number.

The percentage restoration of control DNA linking number after the removal of m-AMSA was calculated as

$$\%\ \text{of control DNA linking number} = \frac{D_x - D_0}{100 - D_0} \times 100$$

where D_0 and D_x are the percentages of control nucleoid sedimentation distance in gradients containing 0.2 μg mL^{-1} ethidium bromide immediately and at a given time after the removal of m-AMSA, respectively (see Results).

Measurement of m-AMSA Retention in Sedimenting Nucleoids. L1210 cells, whose DNA had been labeled with [*methyl*-^{3}H]thymidine (0.1 μCi mL^{-1}), were treated with [^{14}C]-m-AMSA (2 μM = 0.04 μCi mL^{-1}) for 30 min at 37 °C. Reactions were terminated as in the case of the treatments with nonradioactive m-AMSA by centrifugation and resuspension in drug-free medium at 4 °C. Cells were lysed and nucleoids sedimented as above. Gradients were collected from the bottom of the tubes, and each fraction was counted for ^{14}C and ^{3}H. The amount of [^{14}C]-m-AMSA retained per DNA base pair in nucleoids was estimated from the ratio ^{14}C/^{3}H in the nucleoids and the rate of incorporation of [^{3}H]thymidine per cell.

Results

We have previously reported that m-AMSA-induced DNA strand breaks are not free to swivel under the conditions of nucleoid sedimentation (Pommier et al., 1984). In accord with the previous findings, nucleoids from m-AMSA-treated cells sedimented (in the absence of ethidium) similarly to nucleoids from control cells and exhibited the normal rewinding phase when sedimented in high ethidium concentrations (Figure 1). Thus, there was no evidence of swiveling, which would have relaxed the nucleoids and reduced their sedimentation and which would have prevented the rewinding phase of increasing sedimentation at higher ethidium concentration. The nucleoids in these experiments contained approximately 2700 rd equiv of DNA strand breaks, as assayed by alkaline elution with

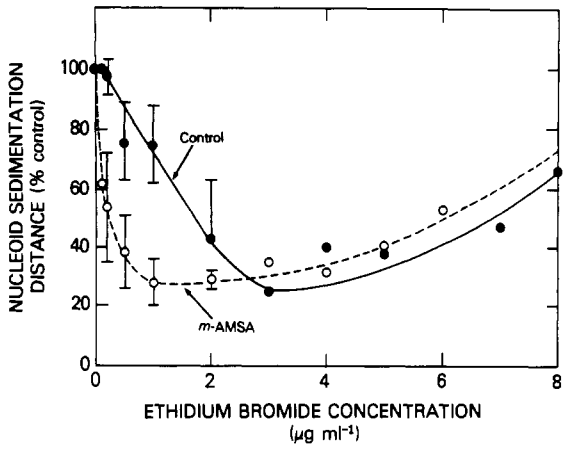

FIGURE 1: Ethidium bromide titration curves of nucleoids from untreated (control) and m-AMSA-treated cells (m-AMSA). m-AMSA treatment was with 2 μM m-AMSA for 30 min at 37 °C. Drug was removed by centrifugation prior to lysis of the cells at 4 °C. Nucleoids were then sedimented in 15–30% neutral sucrose gradients (1.9 M NaCl) containing various concentrations of ethidium bromide. The sedimentation distances of nucleoids from untreated and from m-AMSA-treated cells were expressed as the percentages of control sedimentation distance in the absence of ethidium bromide. Errors bars denote standard deviation for at least three independent experiments.

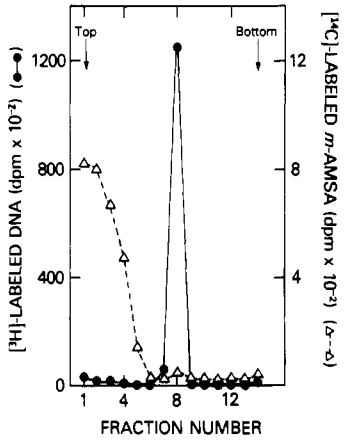

FIGURE 2: m-AMSA retention in sedimenting nucleoids. Cellular DNA was labeled with [^{3}H]thymidine (0.1 μCi mL^{-1}) for 15 h. Cells were chased for 2 h in [^{3}H]thymidine-free medium and then treated with [^{14}C]-m-AMSA (2 μM = 0.04 μCi mL^{-1}) for 30 min at 37 °C. Drug was removed, and nucleoids were sedimented as described under Figure 1. Gradients were collected, and each fraction was counted for [^{14}C]-m-AMSA (△) and [^{3}H]thymidine (●).

proteinase K, and the DNA at these break sites apparently does not swivel.

As a further investigation of the effect of m-AMSA on DNA topology in cells, ethidium titration of nucleoid sedimentation was carried out. These experiments measure the concentration of ethidium required to relax the DNA supercoils fully. It is important that no m-AMSA be carried over into the nucleoids where it could contribute to the relaxing effect and give erroneous ethidium titration estimates. Although previous data had indicated that m-AMSA is readily washed out of cells and that the protein-associated strand breaks are then readily reversed (Zwelling et al., 1982c), a direct test of the removal of m-AMSA was nevertheless carried out. The procedures for drug treatment and nucleoid sedimentation were exactly the same as in the other experiments, except that the m-AMSA was labeled with ^{14}C. The [^{14}C]-m-AMSA associated with the nucleoid band was found to be near the detectability limit (Figure 2). The calculated upper limit for contamination of the nucleoid band by m-AMSA was one molecule per 20 000 base pairs.

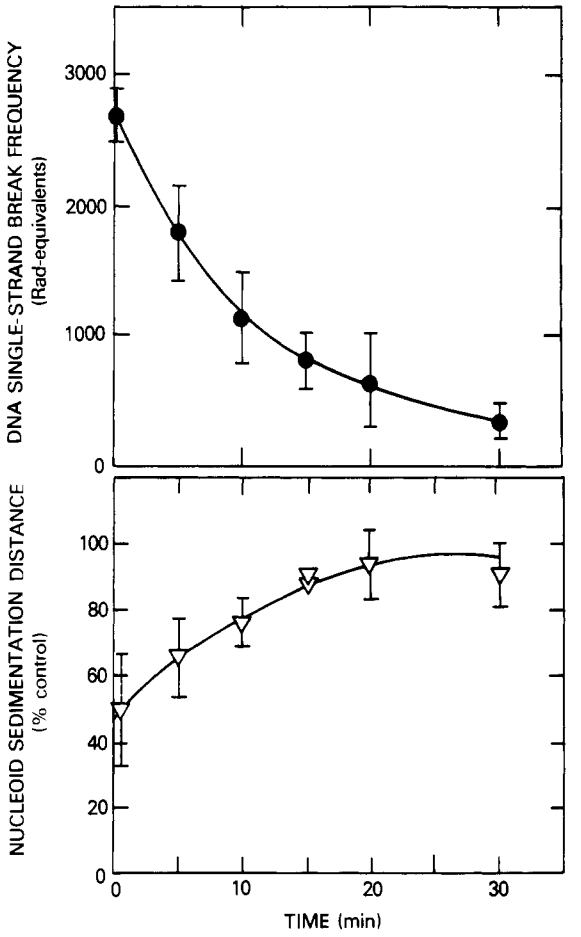

FIGURE 3: Reversal of the effects of m-AMSA on cellular DNA. Cells were incubated with m-AMSA (2 μM) for 30 min at 37 °C, after which the m-AMSA was removed and the cells were incubated in drug-free medium at 37 °C for various times; aliquots of cells were then assayed both by alkaline elution for DNA single-strand breaks (●) (upper panel) and by nucleoid sedimentation at 4 °C in the presence of 0.2 μg mL^{-1} ethidium bromide for DNA linking number alterations (▽) (lower panel). Error bars denote standard deviations for at least three independent experiments.

Despite this negligible amount of m-AMSA within the nucleoids, less ethidium was required to maximally relax these nucleoids than to maximally relax nucleoids from control cells (Figure 1). Maximum relaxation corresponds to the sedimentation minimum in the titration curve, which occurred at a reduced ethidium concentration after treatment with m-AMSA (Figure 1). Since ethidium reduces the average twist of the DNA helix, nucleoids from m-AMSA-treated cells were maximally relaxed when the average twist was less reduced than in the case of untreated cells. Thus, the average twist in maximally relaxed nucleoids was higher in the nucleoids from m-AMSA-treated cells. Because in relaxed DNA twist equals linking number, m-AMSA must have increased the linking number. Since linking number is a topological invariant, the increase must have occurred in the m-AMSA-treated cells.

The difference between nucleoids from untreated and m-AMSA-treated cells was greatest at very low ethidium concentrations (Figure 1). An ethidium concentration of 0.2 μg mL^{-1} reduced the sedimentation of m-AMSA-nucleoids by 48% but did not affect the sedimentation of control nucleoids (Figure 1). This ethidium concentration was used to monitor the return of DNA linking number to normal following removal of m-AMSA. Cells were treated with 2 μM m-AMSA for 30 min and then washed to remove m-AMSA. Figure 3

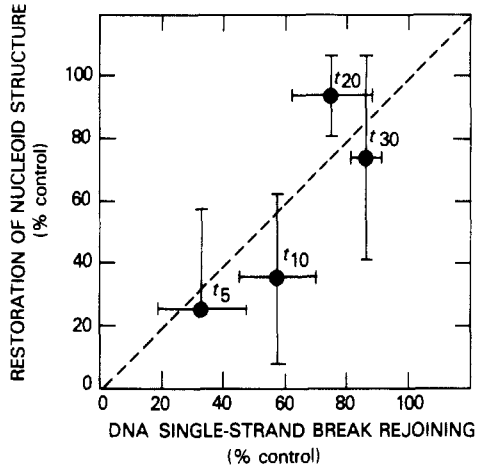

FIGURE 4: Relationship between DNA single-strand break rejoining and restoration of DNA linking number after m-AMSA removal. Cells were incubated with 2 μM m-AMSA, and the m-AMSA was removed after 30 min as described in the legend to Figure 2. DNA single-strand breaks remaining in cells at various times after drug removal were determined by alkaline elution and expressed as percentage of break rejoining. Restoration of linking number was determined by ethidium bromide titration. The percentage of DNA break rejoining for a given post-m-AMSA incubation time is plotted vs. the percentage or reversal of DNA linking number for the same time. Time in minutes is indicated for each point. Error bars denote standard deviations for at least three independent experiments.

(lower panel) shows the gradual return of DNA linking number to that of untreated cells over a period of 20–30 min following drug removal. During the same time period, the DNA strand breaks (assayed by alkaline elution with proteinase K) were resealed (Figure 3, upper panel). When the restoration of linking number was plotted against rejoining of DNA strand breaks at various times after drug removal, the mean data points were fit by a regression line going through the origin and of slope 1 (Figure 4). This would indicate that the m-AMSA-induced DNA strand breaks and the topological alterations reverse concurrently. The limits of error of the measurements however do not exclude the possiblity that strand rejoining may precede restoration of linking number by up to 5 min.

Because of the postulated role of a topoisomerase in the production of the intercalator-induced strand breaks, we examined the effects of novobiocin, an inhibitor of type II topoisomerases (Cozzarelli, 1979; Gellert, 1981). Treatment of cells with novobiocin (200 μg mL^{-1} for up to 2 h) produced neither DNA strand breaks nor DNA–protein cross-links. Novobiocin did not inhibit the formation of DNA strand breaks by m-AMSA but did increase slightly the plateau level of these breaks (Figure 5). This small increase was seen in several independent experiments and was associated with a similar increase in the frequency of m-AMSA-induced DNA–protein links (data not shown). The increase in the steady-state level of protein-associated strand breaks may be due to an inhibition by novobiocin of the rate of resealing of these breaks. This is suggested by the finding that novobiocin reduced the rate of resealing after removal of m-AMSA by approximately a factor of 2; the half-time for rejoining was increased from 7–8 to 16 min (Figure 6). By 30–60 min, however, more than 90% of the breaks were resealed even in the presence of novobiocin.

Novobiocin had a more striking effect on the restoration of nucleoid sedimentation in 0.2 μg mL^{-1} ethidium (Figure 7). In these experiments, novobiocin (200 μg mL^{-1}) was added 1 h prior to m-AMSA treatment and was present in media during and after m-AMSA treatment. In the presence of

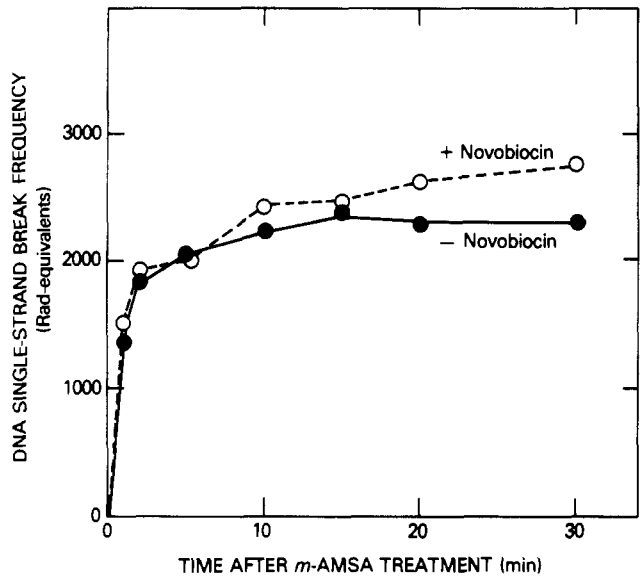

FIGURE 5: Increase in the plateau level of m-ASMA-induced DNA single-strand breaks by novobiocin. Cells were treated at 37 °C with either 2 μM m-AMSA for 30 min (●) or with novobiocin (200 μg mL^{-1}) for 60 min and then with m-AMSA in the presence of novobiocin (○). At the indicated times, aliquots of cells were assayed for DNA single-strand breaks by alkaline elution.

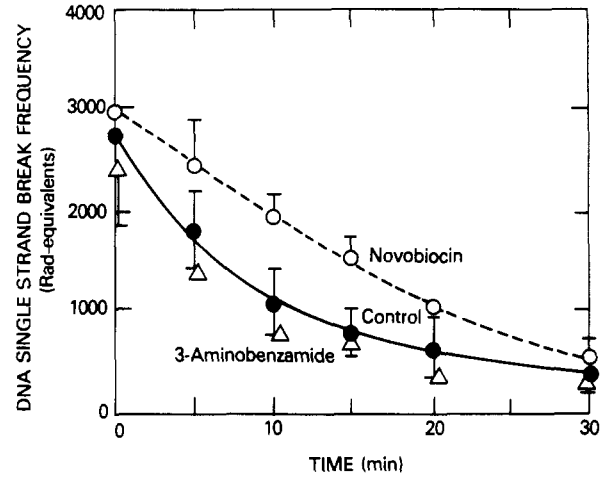

FIGURE 6: Effects of novobiocin or 3-aminobenzamide (3-ABA) on the resealing of m-AMSA-induced DNA single-strand breaks. Cells were treated either with 2 μM m-AMSA for 30 min or with novobiocin (200 μg mL^{-1}) for 60 min followed by m-AMSA in the presence of novobiocin for 30 min. m-AMSA was removed by centrifugation, and the cells that had been treated with novobiocin were resuspended in novobiocin-containing medium (○), and cells treated only with m-AMSA were resuspended either in drug-free medium (●) or in medium containing 3-ABA (5 mM) (△). At the indicated times, aliquots of the same cell suspensions were analyzed by alkaline elution. Error bars denote standard deviations for at least three independent experiments.

novobiocin, the linking number did not begin to recover until after 30 min following removal of m-AMSA, at which time almost all of the strand breaks had been resealed (Figure 8).

Similar experiments showed that 3-aminobenzamide, an inhibitor of poly(adenosine diphoshoribose) synthetase (Durkacz et al., 1980) affected neither the resealing of m-AMSA-induced strand breaks (Figure 6) nor the restoration of DNA linking number (Figure 7).

Discussion

The experiments reported here and in the preceding paper (Pommier et al., 1984) were intended to test the previous working model for the mechanism by which DNA intercalating

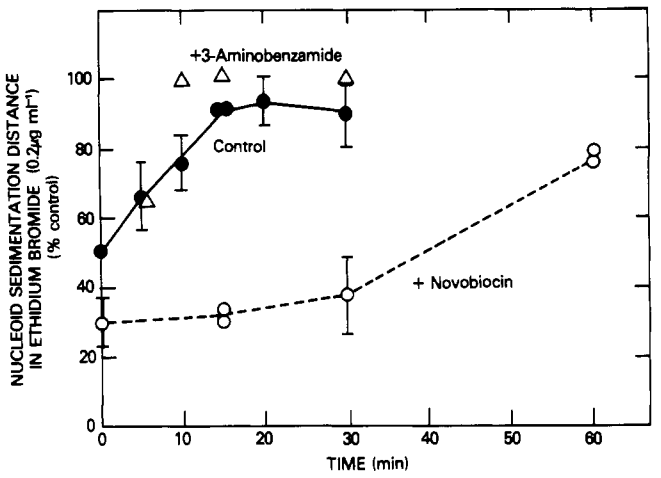

FIGURE 7: Effects of novobiocin and 3-ABA on the reversal of the increase of DNA linking number induced by m-AMSA. Cells were treated as described in the legend to Figure 5. At the indicated times, aliquots of cells in drug-free medium (●) or in novobiocin- (○) or 3-ABA- (△) containing medium were assayed by nucleoid sedimentation in gradients containing 0.2 μg mL^{-1} ethidium bromide.

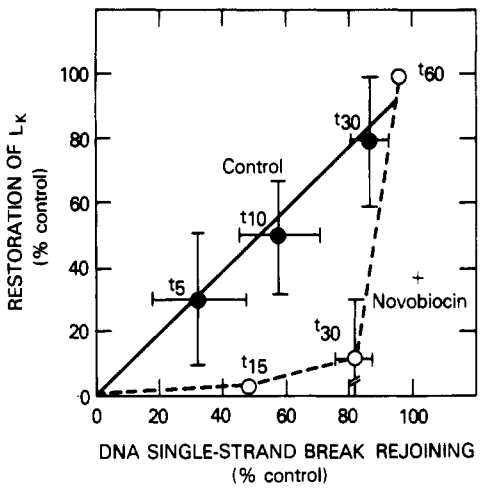

FIGURE 8: Effect of novobiocin on the relationship between DNA single-strand break rejoining and restoration of DNA linking number after m-AMSA removal. Cells were treated as described in the legend to Figure 5; data were processed as in Figure 4. Cells were resuspended in drug-free medium (●) or in medium containing novobiocin (○) after the removal of m-AMSA. Error bars denote standard deviations for at least three independent experiments.

agents induce protein-associated DNA strand breaks in mammalian cells (Ross et al., 1979; Zwelling et al. 1981). This model is diagramed below (Figure 9) in order to facilitate discussion of the several ways in which it is contradicted by the current findings.

In the preceding paper (Pommier et al., 1984), it was found that the m-AMSA-induced breaks were not free to swivel (at least under the conditions of nucleoid sedimentation) unless the nuclei had been previously digested with proteinase K. Unless there is some essential difference between the state of these strand breaks in nucleoids and in intact cell nuclei, this result would argue against the possibility that intercalator-induced strand breaks could function to relieve torsional strain (Figure 9, step 4).

We have also provided evidence (Y. Pommier et al., unpublished results) that torsional strain is not required for the formation of m-AMSA-induced strand breaks and protein cross-links (Figure 9, step 2). The formation of the breaks and protein links in isolated nuclei was unaffected by the presence of X-ray-induced strand breaks, which would have

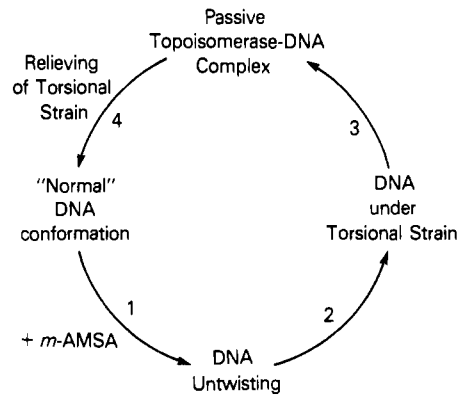

FIGURE 9: Possible interactions between DNA intercalating drugs and DNA topoisomerases. Intercalation of m-AMSA produces DNA untwisting (1), which, in turn, creates torsional strain (2). This torsional strain stimulates DNA topoisomerase to bind to DNA (3) and to relieve torsional strain by transient protein-associated DNA breaks (4).

been expected to prevent the development of torsional strain.

The work of this paper tested whether m-AMSA produces the change in DNA linking number that would be expected if intercalator-induced torsional strain was relieved by a topoisomerase. Intercalation reduces the average twist of the DNA helix (Waring, 1976) (Figure 9, step 1), and this would be expected to alter any torsional strain present in a topologically constrained DNA helix (Figure 9, step 2). Torsional strain introduced in this way could be relieved by a topoisomerase-mediated reduction in DNA linking number in order to preserve chromatin structure (Figure 9, step 4). The observation that m-AMSA caused an increase rather than a decrease in linking number thus lends no support to the idea that intercalator-induced torsional strain is relieved passively through the action of topoisomerase. In view of these accumulated findings, we conclude that the scheme proposed in Figure 9 must be abandoned.

There are two possible explanations for the intercalator-induced increase in DNA linking number. The first is that it is a response to the intercalation-induced change in chromatin structure, e.g., partial unwrapping of DNA from nucleosomes (Center, 1979; Grimmond & Beerman, 1982; Wu et al., 1980). The resulting free negative supercoils could be relaxed by the action of a topoisomerase, or its equivalent, leading to a net increase in linking number. This process would have to be in addition to the action that results in the protein-associated strand breaks.

The second possibility is that the intercalator inhibits a eukaryotic topoisomerase DNA enzyme that normally functions to actively reduce DNA linking number in a fashion analogous to bacterial gyrase (Gellert, 1981). Although attempts to detect DNA torsional strain that might result from the action of a DNA gyrase in mammalian cells have been unsuccessful (Sinden et al., 1980), it is possible that considerable changes in linking number could occur without producing detectable torsional strain. This possibility is attractive because it can explain essentially all of our findings on the basis of a single site of intercalator action. The protein-associated single- and double-strand breaks could be due to the trapping of an enzyme–DNA–intercalator complex in an intermediate state in which either one or both strands of the helix are cleaved (Figure 10, steps 5 and 6). This complex would have to be reversible, since the protein-associated strand breaks reverse within minutes after removal of intercalator (Zwelling et al., 1981). Both the formation and reversal of the covalent complex would have to occur in the absence of an energy

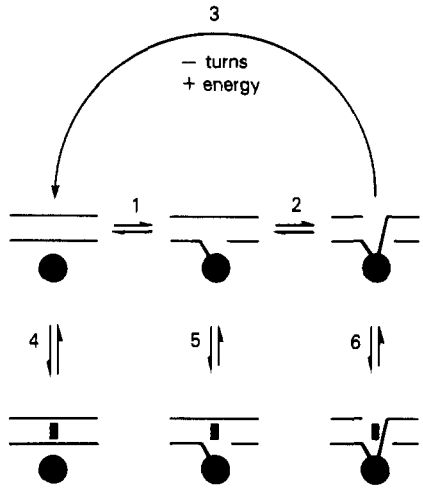

FIGURE 10: Possible interactions between DNA intercalating agents and DNA topoisomerases. Intercalation of m-AMSA inhibits a gyrase-like enzyme that normally functions to actively reduce DNA linking number (1–3) through transient protein-associated DNA breaks (1 and 2). m-AMSA inhibits the topoisomerase–DNA complex either before the DNA breaking reaction (4) or during this reaction when either only one DNA strand is interrupted (5) or both strands are interrupted (6).

source, since both reactions readily occur in isolated nuclei without added nucleoside triphoshates (Pommier et al., 1982). DNA gyrase requires an energy source for active unwinding of the DNA but not necessarily for the production or resealing of individual strand breaks (Cozzarelli, 1979; Gellert, 1981). Thus, an interaction of intercalators with a DNA gyrase like enzyme (or with the DNA segment to which the enzyme is bound) could explain both the protein-associated strand breaks and the increase in linking number.

This hypothesis is consistent with the observed effects of the DNA gyrase inhibitor novobiocin: (1) a reduced rate of resealing of the intercalator-induced strand breaks and (2) a more prolonged inhibition of the recovery of DNA linking number. It is reasonable that, as observed, the simple resealing would be less profoundly inhibited than the repeated cycling that would be required for the recovery of normal linking number.

What might be the structure of the intercalator–gyrase–DNA complex? The structural possibilities of an intercalator–DNA complex are normally restricted by two intact sugar–phosphate chains. When one or both of the chains are broken, as they would be in the hypothesized ternary complex, additional intercalation conformations could become available that might have more favorable stabilities of stacking. The accumulation of protein-associated single-strand breaks in intercalator-treated cells might be due to a stabilization by intercalator of a gyrase–DNA intermediate state in which only one of the two strands is cut (Figure 10). The relative stabilities of possible single-cut and double-cut intermediates with bound intercalator might differ for different types of intercalators and thus could account for the variation observed in the ratios of single- to double-strand breaks produced by different intercalators (Ross & Bradley, 1981; Zwelling et al., 1981, 1982a,b).

Registry No. m-AMSA, 51264-14-3.

References

Bauer, W. R., Crick, F. H. C., & White, J. (1980) *Science (Washington, D.C.) 243*, 118–133.

Center, M. S. (1979) *Biochem. Biophys. Res. Commun. 89*, 1231–1238.

Cook, P. R., & Brazell, I. A. (1975) *J. Cell Sci. 19*, 261–279.

Cook, P. R., & Brazell, I. A. (1976) *J. Cell Sci. 22*, 287–302.

Cozzarelli, N. R. (1979) *Science (Washington, D.C.) 207*, 953–960.

Durkacz, B. W. Omidiji, O., Gray, D. A., & Shall, S. (1980) *Nature (London) 283*, 593–596.

Filipski, J., Yin, J., & Kohn, K. W. (1983) *Biochim. Biophys. Acta 741*, 116–122.

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

Grimmond, H. E., & Beerman, T. (1982) *Biochem. Pharmacol. 31*, 3379–3386.

Kohn, K. W., Ewig, R. A. G., Erickson, L. C., & Zwelling, L. A. (1981) in *DNA Repair: A Laboratory Manual of Research Techniques* (Friedberg, E. C., & Hanawalt, P. C., Eds.) pp 379–401, Marcel Dekker, New York.

Mattern, M. R., & Painter, R. B. (1979) *Biochim. Biophys. Acta 563*, 293–305.

Pommier, Y., Kerrigan, D., Schwartz, R., & Zwelling, L. A. (1982) *Biochem. Biophys. Res. Commun. 107*, 576–583.

Pommier, Y., Mattern, M. R., Schwartz, R. E., & Zwelling, L. A. (1984) *Biochemistry* (preceding paper in this issue).

Pulleyblank, D. E., & Ellison, M. J. (1982) *Biochemistry 21*, 1155–1161.

Ross, W. E., & Bradley, M. O. (1981) *Biochim. Biophys. Acta 654*, 129–134.

Ross, W. E., Glaubiger, D. L., & Kohn, K. W. (1979) *Biochim. Biophys. Acta 562*, 41–50.

Sinden, R. S., Carlson, J. O., & Pettijohn, D. E. (1980) *Cell (Cambridge, Mass.) 21*, 773–783.

Waring, M. J. (1976) *Eur. J. Cancer 12*, 995–1001.

Wu, H.-M., Dattagupta, N., Hogan, M., & Crothers, D. M. (1980) *Biochemistry 19*, 626–783.

Zwelling, L. A., Michaels, S., Erickson, L. C., Ungerleider, R. S., Nichols, M., & Kohn, K. W. (1981) *Biochemistry 20*, 6553–6563.

Zwelling, L. A., Kerrigan, D., & Michaels, S. (1982a) *Cancer Res. 42*, 2687–2691.

Zwelling, L. A., Michaels, S., Kerrigan, D., Pommier, Y., & Kohn, K. W. (1982b) *Biochem. Pharmacol. 31*, 3261–3267.

Zwelling, L. A., Kerrigan, D., Michaels, S., & Kohn, K. W. (1982c) *Biochem. Pharmacol. 31*, 3269–3277.