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The Effects of the Antitumor Protein Auromomycin on HeLa S_3 Nuclei

Release of Soluble Chromatin

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SUMMARY

The protein antitumor agent auromomycin releases chromatin from intact HeLa S_3 nuclei by introducing strand scissions in DNA linker regions. The ability of auromomycin to solubilize nuclear chromatin was compared with the well-characterized action of micrococcal nuclease. Production of acid-soluble material (DNA 10–20 base pairs or less) did not increase significantly over a range of drug levels or incubation times. Release of chromatin from auromomycin-treated nuclei was not affected by EDTA but required dithiothreitol for optimal activity. The soluble chromatin, when resolved by agarose gel electrophoresis, consisted of discrete nucleosome bands ranging from monomer to hexamer size and unresolved higher molecular weight oligomers. Sufficiently high concentrations of drug convert most of the higher molecular weight oligonucleosomes to dimer and monomer material. These nucleosome bands were quite broad and could indicate strand scission occurring at random throughout the linker region. The average size of the drug-generated mononucleosomes from multiple experiments was 175 ± 4 base pairs. In contrast to micrococcal nuclease, higher concentrations of drug did not degrade mononucleosomes to core particles. Solubilized chromatin is detectable following drug treatments as short as 5 min. Chromatin produced at early time points was mostly small and very heterogeneous. Chromatin from drug-digested nuclei analyzed under denaturing conditions appeared as discrete bands similar to those observed on native gels. The ratio of single- to double-stranded breaks may be close to 2:1. When chromophore isolated from the holantibiotic was incubated with nuclei, a similar pattern of chromatin degradation was observed.

INTRODUCTION

The antitumor protein auromomycin (also referred to as macromomycin-I) is a highly cytotoxic drug whose mode of action appears to involve degradation of cellular DNA (1–4). Studies on its mechanism of action describe the drug's ability to degrade DNA in both cellular and cell-free systems and the possible relationship between DNA damage and inhibition of DNA replication and cell growth (1–4). Auromomycin has been shown to degrade both linear duplex and superhelical DNA as well as Simian virus 40 virions (2–5). Reaction of drug with cell-free DNA is stimulated approximately 5-fold by dithiothreitol (6, 7). DNA damage appears to be predominantly single-strand in nature, although the ratio of single- to double-strand breaks is only 12:1 (2). Recent data have suggested that this drug cleaves preferentially at guanine sites (8). Our laboratory has recently demonstrated that auromomycin degrades soluble chromatin by cleaving linker DNA (9).

Like another of the antitumor protein drugs, neocarzinostatin, this agent contains a hydrophobically bound chromophore which is responsible for the drug's biological activities (10–12). Another similarity to neocarzinostatin is the degree of amino acid homology between the two proteins (13). Recently it has been shown that neocarzinostatin degrades isolated cellular chromatin, causing double-strand breaks in both linker and core regions (14). Neocarzinostatin can also solubilize chromatin from nuclei (14–16). Nuclear chromatin structure is more highly ordered than soluble chromatin and represents more closely the actual cellular target for this drug. Data obtained in our laboratory have shown that auromomycin is a very powerful agent with regard to degradation of cellular DNA, being almost two orders of magnitude more potent than neocarzinostatin. This study was undertaken to determine how auromomycin alters its cellular target, nuclear chromatin.

MATERIALS AND METHODS

Cell culture. HeLa S_3 cells used for isolation of nuclei were grown in Spinner culture in Joklik's medium supplemented with 5% calf serum. Growth inhibition studies were conducted using HeLa S_3 plate cultures grown in minimal essential medium (Eagle) with 5% calf serum. Cell

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suspensions (5 ml) containing 200 cells were added to 60-mm tissue culture dishes and treated with drug. After 7–9 days of growth, the plates were fixed in methanol, stained with Giemsa stain, and scored for viable colonies.

Preparation and storage of nuclei. Nuclei were prepared according to the method of Fraser and Huberman (17). Briefly stated, cells in mid-log were labeled overnight with [^{14}C]thymidine (New England Nuclear Corporation, Boston, Mass.) (50 mCi/mmole, 0.5 μCi/ml) and collected by centrifugation at 800 × *g* for 3 min. Following two washes with hypotonic Buffer H (2 mM $MgCl_2$/1 mM EDTA/ 10 mM Na_2HPO_4, adjusted to pH 8.0 with KOH) to remove excess medium, cells were placed in this buffer and allowed to swell on ice for 15 min. The cells were homogenized in a 7-ml glass homogenizer (Wheaton Scientific, Millville, N.J.) by 10–15 strokes with a Type A tight-fitting pestle. An equal volume of 20% Ficoll (Sigma Chemical Company, St. Louis, Mo.) in Buffer H was added to the homogenate, and the mixture was layered over 2 ml of 20% Ficoll followed by centrifugation in a Beckman JA 7.5 rotor at 5000 rpm for 12 min. Cell debris was carefully removed, and the pelleted nuclei were washed once and resuspended in the storage buffer, which contained 0.005% Triton X-100, 50 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 0.25 M sucrose, 25 mM KCl, and 1 mM dithiothreitol. The amount of DNA present was measured by its absorbance in 0.1 N NaOH at 260 nm in a Perkin Elmer Lambda 3 UV/VIS spectrophotometer. Nuclei were divided into 50- or 100-μg aliquots and centrifuged at 1000 rpm for 5 min. The supernatant was removed and discarded, and the pellet containing nuclei and residual buffer was frozen at −85°. We found no difference in our results when using these frozen nuclei or nuclei isolated fresh from cells. For each experiment an aliquot of frozen nuclei was resuspended in cold micrococcal nuclease buffer [10 mM Tris-HCl (pH 7.8)/1 mM $CaCl_2$], and the optical density at 260 nm was determined as described earlier. The yield of nuclei after freezing, determined optically or by measurement of [^{14}C]thymidine, was generally 85–95%. Chromatin was prepared by incubating nuclei in 10 mM Tris-HCl (pH 7.8)/1 mM $CaCl_2$ with micrococcal nuclease at 37° for 7 min. For every 6.1 μg of nuclei, 0.183 unit of nuclease was added. At the end of the incubation period, the sample was placed on ice, then centrifuged at 4° in an Eppendorf Microfuge. EDTA (1 mM) (pH 7.5) was added to a volume equal to that of the supernatant removed. The sample was incubated at 4° for 18 hr, then recentrifuged in the Microfuge. The supernatant was removed, and the chromatin concentration in it was determined by reading its absorbance (in 0.1 N NaOH) at 260 nm in a Perkin Elmer Lambda 3 UV/VIS spectrophotometer. Chromatin was stored at −20° in 20% glycerol/1 mM EDTA (pH 7.5).

Drugs and reagents. Crude auromomycin (NSC 170105) was provided by the Developmental Therapeutics Program, Chemotherapy, National Cancer Institute. Auromomycin was purified according to the procedure of Woynarowski and Beerman (18). The nonprotein chromophore was extracted from holoantibiotic as described previously (19). The concentration of chromophore was expressed in microgram equivalents of native drug per milliliter. The ability of drug to convert PM2-I (superhelical DNA) to PM2-II (open circular duplex DNA) was used as a standard measure of drug activity (2).

Micrococcal nuclease was obtained from Boehringer Mannheim Biochemicals (Indianapolis, Ind.). Agarose and ethidium bromide were from Sigma Chemical Company. Cell culture media and sera were obtained from Grand Island Biological Corporation (Grand Island, N. Y.). All other chemicals were reagent-grade.

Native gel electrophoresis. The standard assay for measuring nuclear chromatin degradation contained the appropriate drug or micrococcal enzyme, 10 mM Tris (pH 7.8), 3.0 μg of DNA and, unless otherwise noted, 2 mM dithiothreitol in a total volume of 35 μl. For digestions using micrococcal enzyme, 1 mM $CaCl_2$ was included in the reaction mixture.

After incubation at 37° for various periods of times, the reactions were made 1 mM in EDTA (except where indicated) and placed on ice for 10 min. Samples were then centrifuged in an Eppendorf Microfuge for 5 min. An aliquot of the supernatant was removed and counted in a xylene-Triton X-100/2,5-diphenyloxazole (PPO)-1,4-bis[2-(5-phenyloxazolyl)]benzene (POPOP) mixture (xylene 3 liters, Triton X-100 1 liter, PPO 12 g, POPOP 0.8 g) to determine DNA concentration based upon the specific activity of the DNA. Supernatants containing 1.5 μg of DNA were then treated with 0.1% sodium dodecyl sulfate at 55° for 15 min to remove proteins. The deproteinized oligomeric DNA fragments were separated on native 1.5% agarose submersion gels, stained, and photographed as described previously (14).

Data on acid-soluble DNA were obtained by removing an aliquot from each of the incubation mixtures at the end of the incubation periods but just prior to the addition of EDTA. Each sample withdrawn was added to a volume of cold 1.0 M perchloric acid/1.0 M NaCl equal to 9 times that of the aliquots. The samples were incubated on ice for 30 min and centrifuged in an Eppendorf Microfuge; the radioactivity in the supernatant was then quantitated as described above.

Denaturing agarose gels. Samples were incubated as stated above for native agarose gels. At the end of the reaction, EDTA was added to a final concentration of 1 mM. After incubating on ice for 10 min, samples were centrifuged in the cold in an Eppendorf Microfuge. The supernatants were treated with sodium dodecyl sulfate at 55° and then, with samples at room temperature, NaOH was added to final concentration of 30 mM. The running buffer was 30 mM NaOH/2 mM EDTA, and 1.5% agarose gels were prepared in this buffer. Gels were run at 20 V for 20 hr, then stained with ethidium bromide and photographed as previously described.

RESULTS

Initial experiments were carried out to determine whether auromomycin could solubilize nuclear chromatin and whether it acted similarly to the nucleolytic enzyme micrococcal nuclease (Fig. 1). The mechanism of action of this enzyme is well known: the release of chromatin fragments from nuclei occurs via introduction of double-strand breaks in linker regions (20). The reaction is monitored by measuring the production of acid-soluble material (DNA 10–20 base pairs or less), which increases linearly with either enzyme concentration or time (21). Auromomycin treatment of nuclei also produced acid-soluble material but showed no linear increase with concentration (Table 1). Neither was there a significant increase in acid-soluble material at a fixed drug level when incubation times were varied from 15 to 60 min (data not shown).

Solubilization of chromatin from micrococcal-treated nuclei is stimulated by post-treatment addition of EDTA. We have noted that EDTA enhances chromatin release in HeLa S_3 nuclei treated by micrococcal nuclease as much as 3-fold.[1] Chromatin released in the absence of EDTA apparently consists of material that is enriched in actively transcribing regions (22, 23). This chromatin, because of a more open conformation, is unusually sensitive to micrococcal digestion. Increased levels of auromomycin release increasing amounts of chromatin, but the release of solubilized chromatin is not dependent upon post-treatment addition of EDTA (Table 1). Samples of the drug-solubilized chromatin were analyzed by agarose gel electrophoresis. The lower molecular weight DNA resolved into discrete bands, indicating that DNA strand scissions occur primarily in linker regions (Fig. 1). For comparison purposes, DNA isolated from chromatin solubilized by micrococcal nuclease is shown in the *bottom panel* of Fig. 1. At the lowest drug concentration that resulted in distinct nucleosome bands (37 μg/ml), oligomers up to hexamer sizes are resolved. Increased drug levels converted substantial amounts of these oligomers to dimer and monomer materials as well as sol-

[1] F. Rauscher, III, G. Mueller, and T. Beerman, unpublished data.

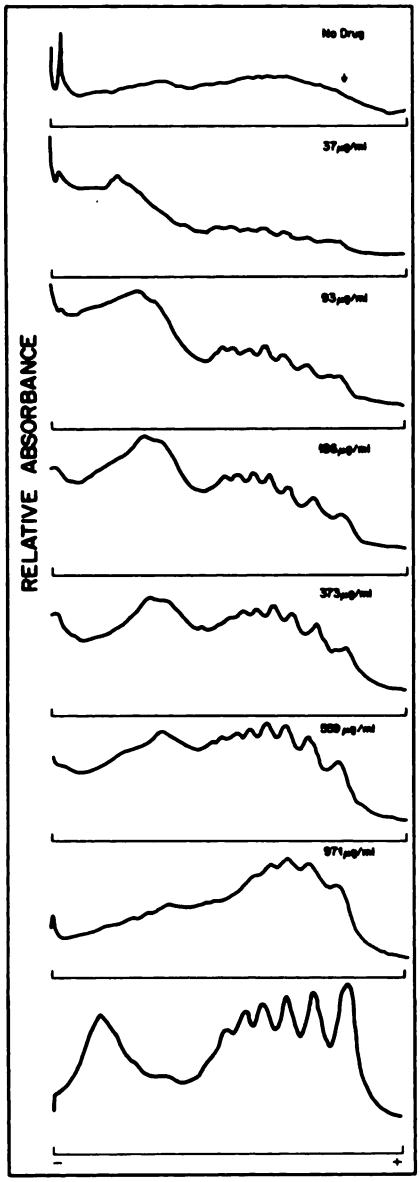

FIG. 1. *Effect of increasing auromomycin concentrations on HeLa S_3 nuclear chromatin*

Isolated nuclei were incubated with the indicated concentration of drug for 1 hr. The chromatin fragments were deproteinized and the DNA was separated on native 1.5% agarose gels, stained, photographed, and scanned as described under Materials and Methods. The *arrow* on the control scan (*top panel*) of this and all subsequent figures indicates the position of the 175-base pair mononucleosome. The *bottom panel* shows DNA oligomers isolated from nuclei treated with micrococcal nuclease for 20 min at 37°.

ubilizing more high molecular weight chromatin. There is also evidence of band broadening, indicating that cleavage in the linker regions may be occurring somewhat randomly. On the other hand, the lack of monomer size material suggests that damage to core DNA is not occurring.

Using a PM2 DNA-Hae III restriction digest as markers, the estimated average size from multiple experiments of the drug-generated monomer is 175 ± 4 base pairs. The addition of more drug causes further breakdown of high molecular weight chromatin but does not change the average size of the monomer unit.

TABLE 1

Solubilization of chromatin from HeLa S_3 nuclei by auromomycin

HeLa nuclei were incubated with auromomycin. The percentage of acid-soluble material and the percentage of soluble chromatin were measured as described under Materials and Methods. Experiments were performed three to five times. Values shown are means ± standard deviation.

Auromomycin concentration	Time	% Acid-soluble	% Counts/min in supernatant	
			−EDTA	+EDTA
μg/ml	*min*			
0	60	3 ± 0.8	5 ± 1	5 ± 1
62	60	3 ± 0.7	44 ± 6	47 ± 5
124	60	4 ± 0.7	68 ± 8	79 ± 6
186	60	4 ± 0.4	71 ± 11	70 ± 8
373	60	4 ± 0.5	88 ± 6	90 ± 5

Chromatin solubilized from nuclei treated with a fixed level of drug from 0 to 60 min was also analyzed by agarose gel electrophoresis (Fig. 2). Treatments as short as 5 min resulted in measurable amounts of solubilized chromatin, although, at these early time points, mostly small and very heterogeneous materials were produced. By 15 min clearly definable oligomers were observed as well as a peak of higher molecular weight DNA. This could be analogous to micrococcal nuclease which, under limited digestion conditions, initially solubilizes chromatin to relatively low molecular weight sizes (24, 25). In the case of micrococcal enzyme, this fraction of chromatin is enriched in transcribing sequences (24, 25).

The strand scission activity of auromomycin with cell-free DNA shows a requirement for dithiothreitol (stim-

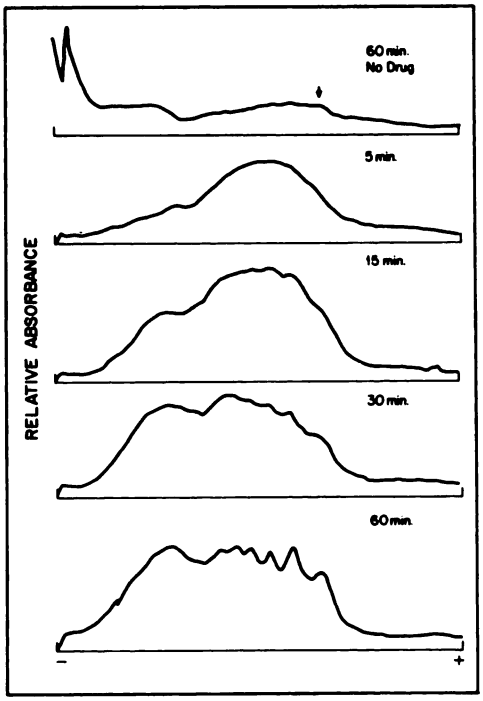

FIG. 2. *Time course of auromomycin activity*

HeLa nuclei were incubated with auromomycin (522 μg/ml) for the times indicated on the graph. The deproteinized DNA was separated on 1.5% agarose gels. Nuclei in control samples showed no significant breakdown over 1 hr.

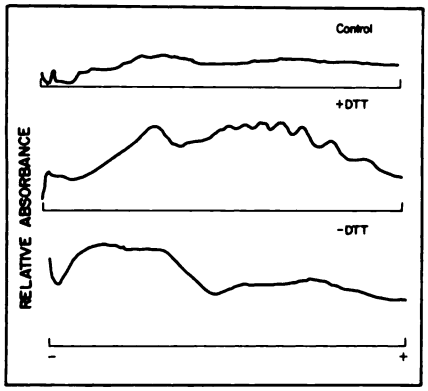

FIG. 3. *Enhancement of auromomycin activity by dithiothreitol*

Nuclei were incubated with auromomycin (372 μg/ml) for 1 hr in the absence of (*−DTT*) or the presence (*+DTT*) of 2 mM dithiothreitol. Nuclei in control samples were unaffected by this concentration of dithiothreitol. Higher dithiothreitol concentrations did not further enhance drug action and in fact increased nonspecific breakdown of nuclear chromatin (data not shown).

ulates the reaction 5-fold) for optimal activity (6, 7). Solubilization of nuclear chromatin by drug is also stimulated by dithiothreitol (Fig. 3). If the amount of drug is increased, chromatin showing discrete nucleosome bands is obtained in the absence of dithiothreitol (data not shown). The presence of EDTA during the reaction does not alter drug activity. These results mark a clear distinction between the activity of the drug and other common cellular nucleases.

Auromomycin cleavage of cell-free DNA produces primarily single-strand scissions, although the ratio of single- to double-strand breaks is unusually low (12:1) (2). Chromatin from drug-treated nuclei, when analyzed under conditions to produce denatured DNA, formed distinct oligomer bands (Fig. 4). Comparison of chromatin structure analyzed under native and denaturing conditions, although there are some differences in gel resolution, does not indicate increased amounts of degradation when single-strand breaks are measured. This lack of increased fragmentation of chromatin analyzed on denaturing gels would indicate a ratio of single- to double-strand scissions near 2:1.

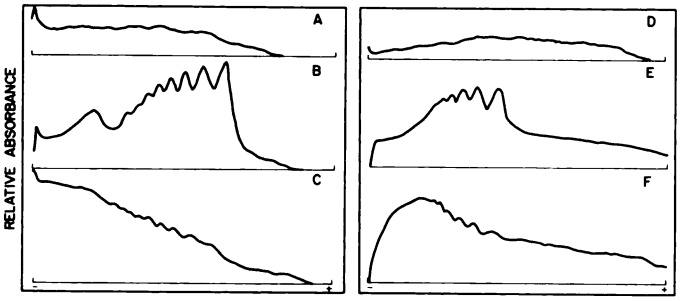

FIG. 4. *Denaturing gel analysis of chromatin released from auromomycin-treated nuclei*

Chromatin obtained from drug-treated nuclei were treated with sodium dodecyl sulfate at 55°, denatured by adding NaOH to a final concentration of 30 mM, and then electrophoresed as described under Materials and Methods. Native samples were also run on a 1.5% native agarose gel (*A–C*) for comparison with the denatured samples (D–F). Nuclei were incubated with no drug (A and D), with micrococcal nuclease at 0.183 unit/6.1 μg of DNA for 20 min (B and E), and with auromomycin (186 μg/ml) (C and F).

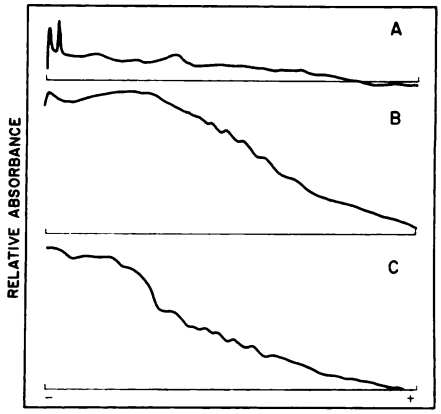

FIG. 5. *Analysis of chromatin from auromomycin- and chromophore-treated nuclei*

HeLa nuclei were untreated (A) or treated with auromomycin at 373 μg/ml for 30 min (B) or with chromophore at 380 μg/ml (this concentration is based upon a comparison of chromophore and holoantibiotic endonucleolytic activity with superhelical PM2 DNA) (C) for 30 min. At the end of the reaction, the volume was doubled by the addition of 10 mM Tris (pH 8), and EDTA was added to a final concentration of 1 mM. Samples were centrifuged at 4° in a Microfuge, the chromatin fragments in the supernatant were deproteinized, and the DNA was separated on a 1.5% agarose gel as described under Materials and Methods.

Finally, we wished to determine whether the chromophore form of auromomycin could solubilize nuclear chromatin in a manner similar to the holoantibiotic. Chromophore levels that are comparable to the haloantibiotic, as measured by the ability of each form of the drug to introduce single-strand scissions into superhelical PM2 DNA, were found to produce soluble chromatin at a rate similar to that of the intact drug (Fig. 5). The oligomer bands patterns were also comparable.

DISCUSSION

Recent studies have analyzed the effects of DNA-reactive antitumor agents on chromatin structure. Isolated chromatin appears to be quite vulnerable to degradation by agents such as neocarzinostatin, bleomycin, and auromomycin (9, 14, 26). Although it is important to understand how these agents interact with isolated chromatin, the development of these drugs as antitumor agents makes it imperative that the level of drug-chromatin interaction also be studied at the nuclear level. In nuclei, chromatin exists as a highly ordered structure, and nuclear chromatin possesses a full complement of non-histone proteins. Both of these conditions may strongly influence drug-chromatin interactions. In addition, drug activity may be altered by the presence of cellular material in nuclei.

The data presented in this paper represent the first published analysis of the effects of the DNA-reactive antitumor drug auromomycin on chromatin in nuclei. Our initial observations reveal that the drug effectively solubilizes nuclear chromatin. Compared with the action of the classical probe of chromatin structure, micrococcal nuclease, auromomycin degrades chromatin in a mark-

edly different manner. From the elecrophoretic profile of drug-solubilized chromatin, it is clear that cleavage of chromatin occurs preferentially in linker regions. At the same time the rather large breadth of the bands suggests that damage to DNA is occurring randomly throughout the linker regions. This contrasts with micrococcal nuclease, which generates bands that are relatively homogeneous. Indeed, digestion with micrococcal enzyme under mild treatment produces a mononucleosome of about 180 base pairs. Increasing the level of enzyme produces a 165-base pair unit (20). This mononucleosome consists of core plus linker DNA regions which, upon further treatment with enzyme, is reduced to a 145-base pair core that is devoid of linker DNA (20). Auromomycin, on the other hand, under mild digestion conditions produced an average-size mononucleosome of 175 base pairs. Increased digestion with drug does not reduce the mononucleosome to smaller sizes. This same chromatin can be converted to core size nucleosomes by treatment with micrococcal nuclease.[1] Thus the nucleosome must consist of the normal core plus linker components.

Unlike micrococcal nuclease, auromomycin release of nuclear chromatin is not dependent upon EDTA (Table 1). At the early time points of drug treatment, the soluble chromatin consists of oligomers that are not resolved into clear bands (Fig. 2). There is also a distinct lack of higher molecular weight DNA. The conditions needed to generate this material appear to be rather specific, as similar fractions of chromatin are not seen when incubations are carried out for longer times but with less drug (Fig. 1). Very mild micrococcal digestion of nuclear chromatin produces small-sized, very heterogeneous chromatin which is enriched in actively transcribing sequences. We are currently investigating the nature of the drug-solubilized chromatin obtained at these early time points.

Unlike drug treatment of cell-free DNA, nearly equal amounts of degradation are observed whether the DNA from solubilized chromatin is analyzed under native or denaturing conditions (Fig. 4). This could result from single-strand breaks being juxtapositioned on opposite DNA strands within 10 base pairs. Strand breakage that occurs selectively on linker regions would enhance the frequency of DNA breaks that occur within close proximity. A similar mechanism has been postulated for neocarzinostatin, where the ratio of single- to double-strand breaks with DNA and chromatin are 30:1 and 5:1, respectively (15, 16). The production of a large amount of double-strand scissions in cellular DNA and the difficulty of repairing such damage could be contributing factors for auromomycin's extreme cytotoxicity (ID_{50} HeLa S_3 cells is 20–40 pM).

Like neocarzinostatin, the active portion of auromomycin is a small (M_r 667) molecular weight chromophore (11, 18). The isolated auromomycin chromophore is similar to the holoantibiotic in its ability to produce soluble chromatin (Fig. 5). When nearly equal amounts of chromophore and holoantibiotic were added to nuclei (the term "equal amounts" refers to drug levels which produce the same amount of cleavage of superhelical PM2 DNA), similar effects were seen on nuclear chromatin.

Auromomycin is one of a number of DNA strand scission antitumor drugs which degrade chromatin structure. Both bleomycin and neocarzinostatin damage chromatin, although they differ in their mode of action on DNA (8). The fact that auromomycin also digests chromatin offers another opportunity to study the effects of a DNA-reactive drug on its cellular target, chromatin. Further studies of the ability of auromomycin to degrade specific fractions of cellular chromatin, i.e., actively transcribing regions, may help to explain the mode of drug action that is responsible for the extreme cytotoxicity of this compound.

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REFERENCES

1. Beerman, T. A. Cellular DNA damage by the antitumor protein macromomycin and its relationship to cell growth inhibition. *Biochem. Biophys. Res. Commun.* **83**:908–914 (1978).
2. Beerman, T. A. Strand scission of superhelical and linear duplex DNA by the antitumor protein macromomycin: relationship of *in vitro* DNA damage to cell growth inhibition. *Biochim. Biophys. Acta* **564**:361–377 (1979).
3. Suzuki, H., T. Nishimura, K. Muto, and N. Tanaka. Mechanism of action of macromomycin: DNA strand scission, inhibition of DNA synthesis and mitoses. *J. Antibiot. (Tokyo)* **31**:875–883 (1978).
4. Sawyer, T. H., A. W. Prestayko, and S. T. Crooke. Purification and mechanism of action of macromomycin. *Cancer Res.* **39**:1180–1184 (1979).
5. Beerman, T. A., and M. W. Gutai. DNA damage in neocarzinostatin-, macromomycin-, and bleomycin-treated SV-40 virions. *Mol. Pharmacol.* **17**:388–394 (1980).
6. Suzuki, H., K. Miura, and N. Tanaka. DNA cleaving potentials of macromomycin and auromomycin: a comparative study. *Biochem. Biophys. Res. Commun.* **89**:1281–1286 (1979).
7. Kappen, L. S., M. A. Napier, I. H. Goldberg, and T. S. A. Samy. Requirement for reducing agents in deoxyribonucleic acid strand scission by the purified chromophore of auromomycin. *Biochemistry* **19**:4780–4785 (1980).
8. Takeshita, M., L. S. Kappen, A. P. Grollman, M. Eisenberg, and I. H. Goldberg. Strand scission of deoxyribonucleic acid by neocarzinostatin, auromomycin, and bleomycin: studies on base release and nucleotide sequence specificity. *Biochemistry* **20**:7599–7606 (1981).
9. Rauscher, F. J., III, M. McHugh, G. Mueller, and T. Beerman. Degradation of HeLa S_3 cell chromatin by auromomycin and its chromophore. *Biochem. Biophys. Res. Commun.* **106**:355–362 (1982).
10. Kappen, L. S., M. A. Napier, and I. H. Goldberg. Roles of chromophore and apo-protein in neocarzinostatin action. *Proc. Natl. Acad. Sci. U. S. A.* **77**:1970–1974 (1980).
11. Suzuki, H., K. Miura, Y. Kumada, T. Takeuchi, and N. Tanaka. Biological activities of non-protein chromophores of antitumor protein antibiotics: auromomycin and neocarzinostatin. *Biochem. Biophys. Res. Commun.* **94**:255–261 (1980).
12. Napier, M. A., B. Holmquist, D. J. Strydom, and I. H. Goldberg. Neocarzinostatin: spectral characterization and separation of a non-protein chromophore. *Biochem. Biophys. Res. Commun.* **89**:635–642 (1979).
13. Sawyer, T. H., K. Guetzow, M. O. J. Olson, H. Busch, A. W. Prestayko, and S. T. Crooke. Amino terminal amino acid sequence of macromomycin, a protein antitumor antibiotic. *Biochem. Biophys. Res. Commun.* **86**:1133–1138 (1979).
14. McHugh, M. M., J. Woynarowski, and T. A. Beerman. Degradation of HeLa cell chromatin of neocarzinostatin and its chromophore. *Biochim. Biophys. Acta* **696**:7–14 (1982).
15. Hatayama, T., and M. Yukioka. Action of neocarzinostatin on cell nuclei: release of specific chromatin. *Biochem. Biophys. Res. Commun.* **104**:889–896 (1982).
16. Beerman, T. A., G. Mueller, and H. Grimmond. Effects of neocarzinostatin on chromatin in HeLa S_3 nuclei. *Mol. Pharmacol.* **23**:493–499 (1983).
17. Fraser, J. M., and J. A. Huberman. *In vitro* HeLa cell DNA synthesis: similarity to *in vivo* replication. *J. Mol. Biol.* **117**:249–272 (1977).
18. Woynarowski, J. M., and T. A. Beerman. Purification and characterization of macromomycin-I, a protein antibiotic containing a non-protein chromophore. *Biochim. Biophys. Res. Commun.* **94**:769–776 (1980).
19. Povirk, L. F., and I. H. Goldberg. Binding of the non-protein chromophore of neocarzinostatin to deoxyribonucleic acid. *Biochemistry* **19**:4773–4780 (1980).
20. Sollner-Webb, B., and G. Felsenfeld. A comparison of the digestion of nuclei and chromatin by staphylococcal nuclease. *Biochemistry* **14**:2915–2920 (1975).
21. Lohr, D., R. T. Kovacic, and K. E. Van Holde. Quantitative analysis of the digestion of yeast chromatin by staphylococcal nuclease. *Biochemistry* **16**:463–471 (1977).

22. Bloom, K. S., and J. N. Anderson. Fractionation of hen oviduct chromatin into transcriptionally active and inactive regions after selective micrococcal nuclease digestion. *Cell* **15**:141–150 (1978).
23. Bellard, M., F. Gannon, and P. Chambon. Nucleosome structure. III. The structure and transcriptional activity of the chromatin containing the ovalbumin and globin genes in chick oviduct nuclei. *Cold Spring Harbor Symp. Quant. Biol.* **42**:779–791 (1978).
24. Whitlock, J. P. Staphylococcal nuclease and pancreatic DNase cleave the DNA within chromatin core particle at different sites. *J. Biol. Chem.* **252**:7635–7639 (1977).
25. Egan, P. A., and B. Levy-Wilson. Structure of transcriptionally active and inactive nucleosomes from butyrate-treated and control HeLa cells. *Biochemistry* **20**:3695–3702 (1981).
26. Kuo, M. T., and T. C. Hsu. Bleomycin causes release of nucleosomes from chromatin and chromosomes. *Nature* (*Lond.*) **271**:83–84 (1978).

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