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ALTERATION OF CHROMATIN STRUCTURE INDUCED BY THE BINDING OF ADRIAMYCIN, DAUNORUBICIN AND ETHIDIUM BROMIDE

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Abstract—The results reported in this paper show the changes in chromatin structure caused by the binding of adriamycin (ADR), daunorubicin (DR) and ethidium bromide (EtdBr) to DNA in chromatin, either isolated or in nuclei or whole cells. Micrococcal nuclease was used as the structural probe of chromatin. The binding of the drugs to chromatin DNA induced two structural changes. First, it produced an unfolding of the overall chromatin structure as evidenced by the increased production of acid-soluble oligonucleotides for the drug-treated samples above the level of the control sample. Second, it caused a disruption of the core particle structure with increased production of DNA of subnucleosomal size and smearing of the nucleosome pattern. The effects were greatest for daunorubicin, followed by adriamycin and ethidium bromide.

The anthracycline antibiotics adriamycin (ADR) and daunorubicin (DR) are used to treat leukemias and solid tumors, but the cumulative cardiac toxicity of these drugs limits their use as therapeutic agents [1–4]. They have been shown to inhibit nucleic acid synthesis by intercalation with DNA and to cause DNA strand scission by forming free radicals [5–7]. Recent studies have suggested that ADR produces single-strand regions in DNA, rendering the DNA susceptible to hydrolysis by single-strand specific nucleases [8], and others have shown that chromosomal proteins form cross-links with DNA when chromatin is incubated with ADR [9, 10].

The interaction of ADR and DR with DNA and chromatin has been studied by spectrophotometric methods [11] and by sedimentation in sucrose gradients [12]. The spectrophotometric studies were concerned with questions of drug–nucleic acid interactions, since the biological activity of these drugs is dependent on their ability to bind DNA, and also with considerations of the degree of therapeutic effectiveness of ADR and DR. Therefore, we decided to investigate the alterations of chromatin structure when ADR and DR are incubated with chromatin, either isolated or in nuclei or whole cells. Micrococcal nuclease was used as the structural probe in these studies.

A comparative study of the effects of ADR and DR was made with that of ethidium bromide (EtdBr), since, a substantial body of information exists on changes in chromatin structure as examined through micrococcal nuclease digestion of EtdBr–nucleosome [13], EtdBr–chromatin [14] and EtdBr–nuclei [15] complexes. The results we obtained will help to provide further insights into the actions of ADR and DR on chromatin structure and function. Also the EtdBr results will be of comparative interest because, while it intercalates, it is of little use as a therapeutic agent. It also differs from the anthracyclines in lacking a quinone function and, in theory, would be unlikely to mediate free radical type reactions.

MATERIALS AND METHODS

ADR and DR were provided by Dr. Ven L. Narayanan of NCI, and EtdBr was obtained from the Sigma Chemical Co., St. Louis, MO. The drugs were dissolved in 10 mM Tris–HCl (pH 7.4) at a concentration of 1 mg/ml and kept frozen in dark containers. Dilutions of stock were made for use in experiments.

Cell culture and labeling. HeLa S_3 cells were maintained in suspension using Eagle's minimum essential medium (MEM) supplemented with 5% calf serum (GIBCO Laboratories, Grand Island, NY). Cells were labeled with 0.5 μCi/ml [^{14}C]thymidine (New England Nuclear Corp., Boston, MA) (50 mCi/mmole) for 20 hr, collected by centrifugation at 800 *g* for 3 min, washed with phosphate-buffered saline (PBS) to remove excess label, and used in the preparation of labeled nuclei, chromatin and DNA for hydrolysis experiments.

Preparation of nuclei, chromatin and DNA. Nuclei were prepared essentially by the method of Fraser and Huberman [16]. Briefly, cells were collected by low speed centrifugation (800 *g* for 3 min), washed twice with PBS to remove excess medium, placed in hypotonic buffer H (2 mM $MgCl_2$, 1 mM EDTA, 10 mM Na_2HPO_4, adjusted to pH 8.0 with KOH), and allowed to swell on ice for 15 min. The buffer was made 1.0 mM with respect to α-toluenesulfonyl fluoride as a protease inhibitor just prior to use. The cells were homogenized in a 7-ml glass homogenizer by six to ten strokes with a tight-fitting pestle. An

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equal volume of 20% Ficoll (Sigma Chemical Co.) in buffer H was added to the homogenate and mixed with a wide bore pasteur pipette; the mixture was layered over a 1-ml volume of 20% Ficoll for centrifugation in a Beckman JA 7.5 rotor at 5000 rpm for 12 min. Cell debris and any unbroken cells were carefully removed by pipetting and the sides of the tubes were washed with micrococcal nuclease buffer. The nuclei were resuspended by gentle homogenization using a wide bore pasteur pipette. Chromatin was prepared by the method of Noll *et al.* [17] except that the resuspended digested nuclear pellet was allowed to sit on ice in 0.2 mM EDTA for longer periods to obtain complete release of micrococcal nuclease solubilized chromatin. The mixture was centrifuged in an Eppendorf centrifuge and the supernatant fraction was collected and used as soluble chromatin. A second aliquot of nuclei was used to prepare DNA, which was done essentially by the procedure of Blin and Stafford [18].

Drug binding. Cells were incubated with drugs in spinners for 2 hr at 37° washed with PBS to remove excess drug and media, and then used in the preparation of nuclei which served as material for whole cell experiments. Nuclei and chromatin from untreated cells were incubated with drugs for periods of 5–30 min at the time of experimentation. All experiments were done at least in triplicate.

The binding of drugs to DNA in chromatin was monitored by changes in the absorption spectra and by measurement of fluorescence changes. The *r* values (the number of moles of drug per mole of DNA nucleotide) were established by quantitatively removing the drugs from DNA by the silver nitrate–isoamyl alcohol extraction procedure [19]. The concentration of bound drug was estimated from standard curves which were obtained from fluorescence measurements of each drug. Samples of drugs were taken through the extraction procedure to correct for fluorescence quenching (anthracyclines) and fluorescence enhancement (EtdBr) of the drugs.

Sample digestion by micrococcal nuclease. Nuclei obtained from cells not previously treated with drugs were resuspended in 10 mM Tris–HCl (pH 7.4), 0.32 M sucrose and 1 mM $CaCl_2$ to give a final concentration of 10 μg of DNA measured in 0.1 N NaOH at 260 nm. Drugs were then added at the desired concentration. Reaction mixtures were in a final volume of 50 μl. Micrococcal nuclease was added to the suspensions (3 units enzyme/10 μg samples) and incubation was for the time indicated. Nuclei obtained from pretreated cells were similarly prepared and digested. Digestion was terminated by the addition of 5 μl of concentrated electrophoresis buffer [10X: 0.36 M Tris–HCl, 0.30 M NaH_2PO_4, 0.01 M EDTA and 1.0% sodium dodecylsulfate (SDS), pH 8.4 (pH 7.8 after dilution)] except in those experiments in which the digests were to be analyzed as nucleoprotein complexes. In these experiments, digestion was terminated by the addition of EDTA to give a final concentration of 2 mM. Digestion was carried out at 37°.

Chromatin–drug complexes were in 10 mM Tris–HCl (pH 7.4), 0.2 mM EDTA–1 mM $CaCl_2$, and were digested with micrococcal nuclease enzyme at 3 units/10 μg DNA for 5–15 min. The reactions were stopped by the addition of concentrated electrophoresis buffer or EDTA (nucleoprotein complex) as stated previously.

Perchloric acid (PCA) solubility. ^{14}C-Labeled chromatin and DNA were resuspended in the appropriate buffer at at a concentration of 250 μg/ml DNA and incubated with the required concentration of the drugs (*r* values of 0.02, 0.03, 0.04 and 0.05) at 37° for 5 min. Micrococcal nuclease was added at 75 units/ml, and digestions were carried out for 10 min. At the end of the digestion period, the reactions were terminated by the addition of 1.0 M PCA to the reaction mixtures to give a final concentration of 0.4 M. The tubes were placed on ice for 30 min and then centrifuged in an Eppendorf centrifuge for 15 min at 4°. Volumes of 121 μl of PCA soluble material were withdrawn and counted in xylene–Triton X-100, PPO–POPOP mixture (xylene, 3 liters; Triton X-100, 1 liter; PPO, 12 g; POPOP, 0.8 g).*

Gel electrophoresis. Vertical slab gel electrophoresis (15 × 12 × 0.3 cm) was performed to separate nuclease digestion products. Acrylamide gels (4%, 20:1, acrylamide:N,N_1-methylene bisacrylamide) used 0.1% SDS. 36 mM Tris, 30 mM sodium phosphate, 10 mM EDTA (pH 7.8) as the buffer system to separate samples as DNA. Samples (5–10 μg of nuclear DNA) in 50 μl gel buffer containing 1% SDS and 0.25% bromophenol blue were heated in a 50° water bath for 30 min, loaded onto the gel, and run at room temperature at 30 V for 14 hr with recirculation of buffer. The gels were pre-electrophoresed at 100 V for 1 hr prior to sample loading. SDS was extracted from the gels with 50% methanol for 5 hr with three changes. The gels were stained in 0.5 μg/ml EtdBr for 1 hr and then photographed using ultraviolet light; the negatives were scanned on a Quick Scan densitometer (Helena Laboratories, Beaumont, TX).

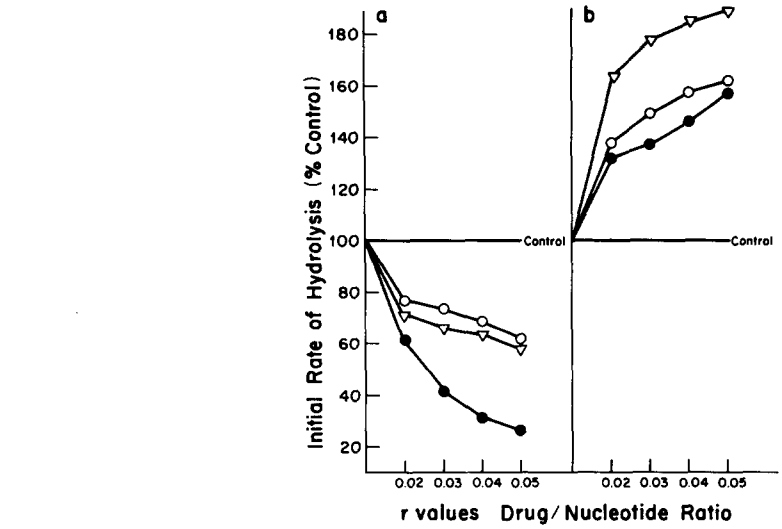
Nucleoprotein complexes were separated by electrophoresis at 4° using 2.5% acrylamide, 0.5% agarose slab gels (acrylamide:N,N^1-methylene bisacrylamide, 20:1, 15 × 10 × 0.3 cm) and 6.4 mM Tris, 3.2 mM sodium acetate, 0.32 mM EDTA (pH 8.0) as the buffer system. Gels were run at 20 mA for 4 hr, stained, and photographed as stated above [20].

In some experiments DNA fragments were separated on 1.8% agarose gels in TAE buffer (40 mM Tris, 5 mM sodium acetate, 1 mM EDTA, pH 8.0). Proteins were removed from DNA by incubation with 0.4% Sarkosyl NL-30 (Ciba–Geigy, Greensboro, NC) [21].

RESULTS

Effects of drugs on DNA digestion with nuclease. Figure 1a shows that, when ^{14}C-labeled DNA was digested with micrococcal nuclease in the presence of increasing concentrations of ADR, DR and EtdBr, there was a progressive reduction in the amount of acid-soluble material with increasing drug

* Abbreviations: PPO, 2,5-diphenyloxazole; and POPOP, 1,4-bis-[2-(4-methyl-5-phenyloxazolyl)]benzene.



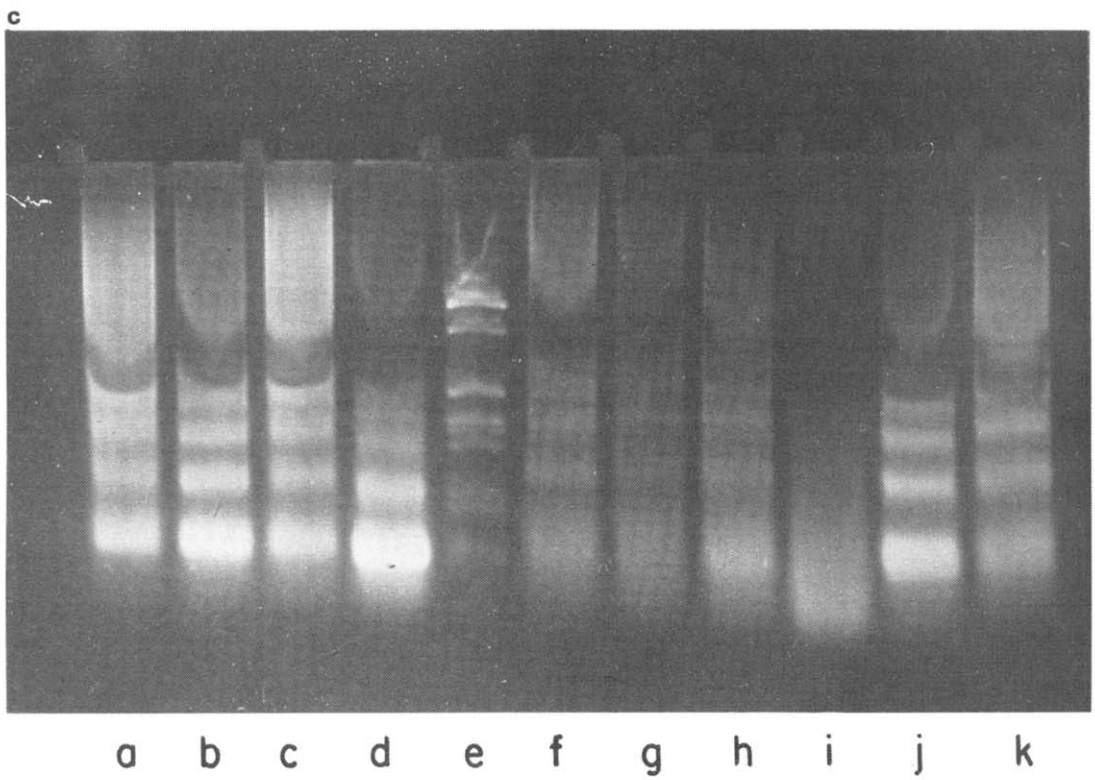


Fig. 1. Percentage increase of the initial rates of degradation of chromatin or DNA by micrococcal nuclease as a function of r as determined through acid-soluble release. (a) Hydrolysis of [^{14}C]DNA and (b) [^{14}C]chromatin. Key: (▽) DR-treated samples; (○) ADR-treated samples, and (●) EtdBr-treated samples. The value 100% of the ordinate axis represents the initial rate of degradation of the control (DNA 30% or chromatin 20%). (c) 1.8% agarose gel electrophoresis of enzymatic digests of chromatin and chromatin–drug complexes. Slots a and c: soluble chromatin; slot b: control 10-min digest; slot d: control 20-min digest; slot e: Hae III digest of PM2 DNA; slot f: ADR (0.02) 10-min digest; slot g: ADR (0.05) 10-min digest; slot h: DR (0.02) 10-min digest; slot i: DR (0.05) 10-min digest; slot j: EtdBr (0.02) 10-min digest; and slot k: EtdBr (0.05) 10-min digest.

concentration. This suggests that there was an inhibitory effect by the nuclease on the rate of DNA hydrolysis in the presence of the drugs. Preincubation of the enzymes with the drugs in the absence of DNA had no apparent effect on the enzyme activity. The drug–enzyme complexes were dialyzed extensively to remove the drugs, and the enzyme was then used to hydrolyze DNA. Comparison of the extent of DNA hydrolysis by control enzyme, which was dialyzed but not treated with drug, with those of the dialyzed drug–enzyme complexes showed a variation of between 5 and 8% above the control value. This variation was well within the region of experimental differences.

It is known that intercalation produces an unwinding of the DNA helix, as well as introducing a steric hinderance in regards to accessibility to drug bound DNA sites. These events probably cause a modification of the micrococcal nuclease sensitive sites in the presence of the bound drugs and so inhibit the enzyme activity. This situation is analogous to that found by Goodman *et al.* [22] who showed the inhibitory effects of ADR and DR on DNA synthesis.

Effects of drugs on digestion of chromatin by micrococcal nuclease. In contrast to increasing inhibition of DNA hydrolysis by nuclease in the presence of drugs, there was an increase in the amount of acid-soluble oligonucleotides, with increasing drug concentration, when chromatin was digested by micrococcal nuclease. As shown in Fig. 1b, the percentage of acid-soluble material obtained by digestion of DR–chromatin complex was greater than that for the ADR–chromatin and the EtdBr–chromatin complexes. This enhancement in the production of acid-soluble oligonucleotides from chromatin–drug complexes by the nuclease was probably caused by unwinding of the chromatin structure, an event which makes more nuclease sensitive sites available. This unwinding must have been very extensive because these drugs inhibited nuclease digestion of already unwound (naked) DNA. That is, there must have been a very large increase in the number of nuclease-sensitive sites as a consequence of the drug binding.

Effects of enzymatic digestion. When the products associated with digestion of chromatin–drug complexes were analyzed by nondenaturing agarose gel electrophoresis (Fig. 1c), it was clearly seen that the increased production of acid-soluble material was not solely an effect of rate. In the presence of the drugs, chromatin was degraded faster to the core particle, and digestion of the core itself was facilitated. At comparable levels of acid-soluble material, there was clearly a more pronounced disruption of chromatin structure in drug-treated samples than in control. This effect was very pronounced for the ADR- and DR-treated samples and to a lesser extent for the EtdBr-treated samples.

Chromatin–drug complex digests. Since anthracyclines have been reported to cause damage to cellular DNA and in some cases to cell-free DNA [7], we wished to see whether the drugs, in the absence of enzyme, produced changes in the chromatin structure. To achieve this, we analyzed chromatin–drug complexes on acrylamide-urea and acrylamide-formamide denaturing gels (data not shown). Strand scission as a consequence of drug intercalation did not occur on a detectable scale. In the preceding section, electrophoretic data were presented showing that micrococcal digestion of drug-treated chromatin resulted in enhancement of the rate of production of smaller oligonucleotides. Limitations of the agarose gel technique used in this analysis did not allow for visualization of changes that might have been occurring within the various nucleosome units. Further studies were done using acrylamide gels which have the ability to detect changes in the size distribution of nucleosomes. Micrococcal nuclease digests of chromatin in the presence of the drugs were analyzed as DNA fragments on 4% polyacrylamide–0.1% SDS gels. Nuclease digestion of chromatin solubilizes portions of chromatin which differ in the number of nucleosomes. This reflects the multinucleosomal organization of chromatin, the accessibility of different sites to the enzyme, and the time of contact with the enzyme [23–27]. Figure 2 shows this characteristic band pattern of DNA fragments from control and drug-treated chromatin digests. The drugs caused a broadening of the nucleosome bands, with this effect being more pronounced at the higher drug concentration, and DR-treated samples showed the greatest extent of degradation followed by ADR and EtdBr.

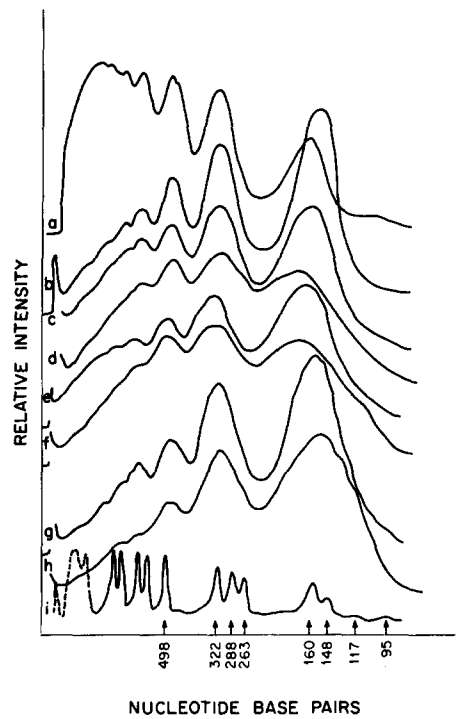


Fig. 2. Chromatin samples. Control and drug-treated samples were digested with micrococcal nuclease and electrophoresed on a 4% polyacrylamide–0.1% SDS DNA gel using Tris–Na_2PO_4–EDTA–SDS buffer. Key: (a) soluble chromatin prepared by the method of Noll *et al.* [17], and aliquots used for samples (b–h); (b) control; (c) ADR at *r* value of 0.2; (d) ADR at *r* value of 0.05; (e) DM at *r* value of 0.02; (f) DM at *r* value of 0.05; (g) EtdBr at *r* value of 0.02; (h) EtdBr at *r* value of 0.05; and (i) Hae III digest of PM2 DNA.

There was also a reduction in the trinucleosome bands of the drug-treated samples with increased production of mononucleosomal fragments. These changes are consistent with an unfolding of chromatin structure, which thereby makes available more nuclease sensitive sites. However, the new sites appeared to be randomly spaced, since no new bands appeared, and the mono- and dinucleosome bands were broadened.

Nuclei–drug complex digests. When drugs were bound to chromatin in nuclei and digested by nuclease, the same set of structural changes was observed as when the drugs were bound to isolated chromatin and then digested with the nuclease. However, in contrast to chromatin–drug digests, the nuclei–drug digests (Fig. 3) showed a higher proportion of subnucleosomal DNA fragments. In the case of nuclei, the nucleosomes may be in a configuration which permits binding of the drugs to DNA in the nucleosome core region [28], and thereby makes available new digestion sites for the nuclease. EtdBr-treated samples were not significantly different from the control sample as compared to the effects seen for ADR- and DR-treated samples.

Whole cell–drug complex digests. We carried out experiments to determine the extent of drug-induced breakdown of chromatin in whole cells. As in the case with isolated chromatin, no evidence of strand scission was found (data not shown). However, in these experiments damaged DNA may have been lost during the procedure of nuclei preparation and chromatin isolation from drug-treated cells since this chromatin is rather small compared to the material used to measure cleavage of cellular DNA [7]. Further experiments did show that the incubation of whole cells with the drugs had a marked effect on chromatin structure as seen by the alteration of micrococcal nuclease digestion of the nuclei from these cells (Fig. 4). In these studies, EtdBr-treated cells did not exhibit the extensive degradation of chromatin compared to that seen for the anthracycline-treated cells. Also, there seemed to be a concentration-dependent effect for ADR which resulted in the accumulation of mononucleosomes over the 165 to 145 base pair regions. This was a reproducible phenomenon which, though unexpected, did not appear to be an artefact. It is possible that cytoplasmic factors may have contributed to the effects seen for cells. Incubation of whole cells with ADR is thought to produce highly reactive free radicals which could produce further changes in cellular chromatin structure [6].

Nucleoprotein complexes. The electrophoretic analysis so far has been concerned with examining chromatin structure in DNA gels. Since anthracyclines are reported to perturb protein–DNA associations [9, 10], it was of interest to repeat these studies but to perform the analysis on nucleoprotein complexes. This type of study would indicate if these drugs alter chromatin structure via induced changes in protein–protein, protein–DNA or DNA-interstrand interactions. Figures 5–7 show the distribution

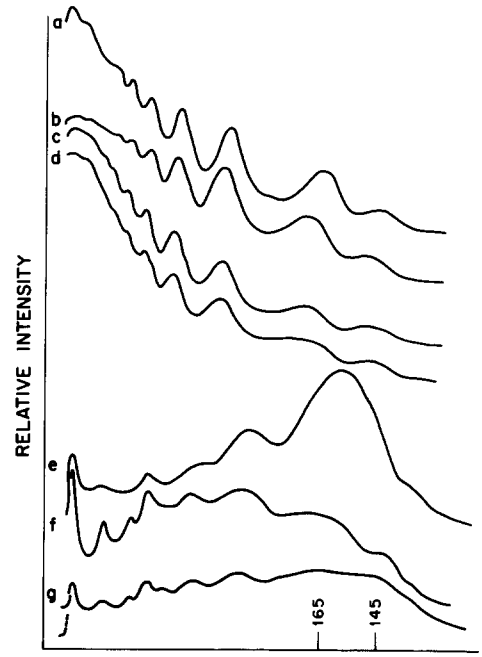


Fig. 4. Whole cells. Control and drug-treated samples were digested for 10 min at 37° and digests were electrophoresed on a 4% polyacrylamide–0.1% SDS DNA gel. Key: (a) control; (b) EtdBr: $r = 0.02$; (c) EtdBr: $r = 0.05$; (d) ADR: $r = 0.02$; (e) ADR: $r = 0.05$; (f) DM: $r = 0.02$; and (g) DM: $r = 0.05$.

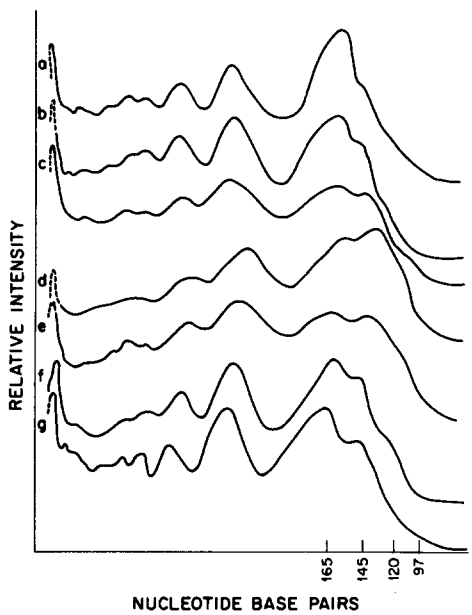


Fig. 3. Nuclei. Control and drug-treated samples were digested for 10 min at 37° and then electrophoresed on a 4% polyacrylamide–0.1% SDS DNA gel. Key (a) control; (b) ADR: r = 0.02; (c) ADR: r = 0.05; (d) DM: $r = 0.02$; (e) DM: $r = 0.05$; (f) EtdBr: $r = 0.02$; and (g) EtdBr: $r = 0.05$.

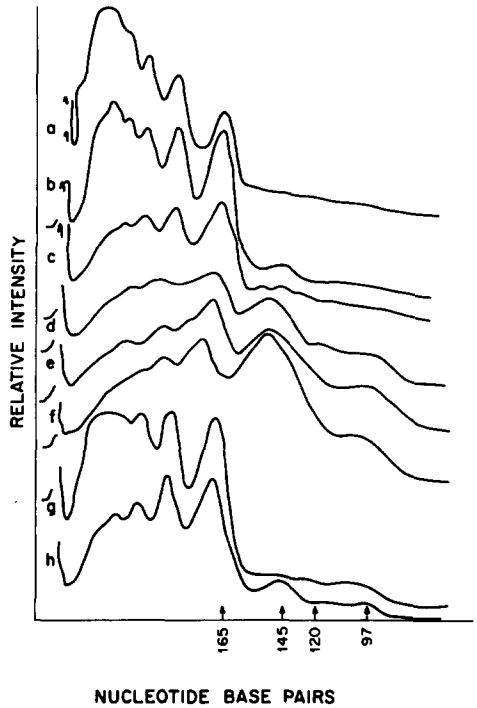


Fig. 5. Chromatin samples. Control and drug-treated samples were digested with micrococcal nuclease and electrophoresed on a nucleoprotein gel of 2.5% polyacrylamide–0.5% agarose using TAE buffer. Key: (a) soluble chromatin prepared by digestion of nuclei with nuclease, by the method of Noll *et al.* [17]. Aliquots of this chromatin were used for samples (b–h); (b) control; (c) ADR at r value of 0.02; (d) ADR at r value of 0.05; (e) DM at r value of 0.02; (f) DM at r value of 0.05; (g) EtdBr at r value of 0.02; and (h) EtdBr at r value of 0.05.

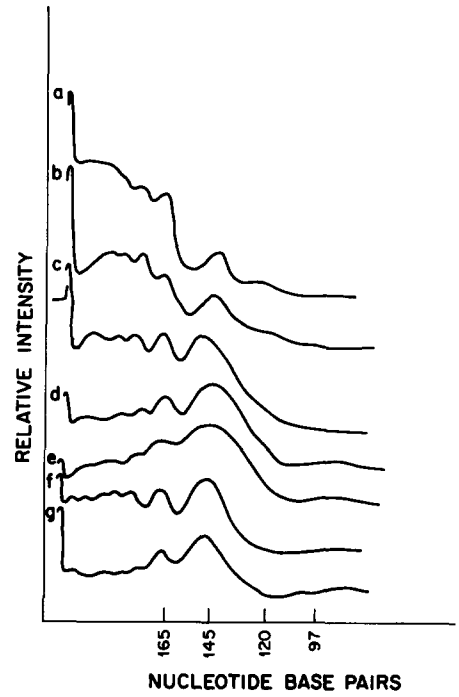


Fig. 6. Nuclei. Control and drug-treated samples were digested for 10 min at 37° and then electrophoresed on a 2.5% polyacrylamide–0.05% agarose nucleoprotein gel. Key: (a) control; (b) ADR: $r = 0.02$; (c) ADR: $r = 0.05$; (d) DM: $r = 0.02$; (e) DM: $r = 0.05$; (f) EtdBr: $r = 0.02$; and (g) EtdBr: $r = 0.05$.

of nucleoprotein complexes separated under non-denaturing conditions. These gels gave very good resolution of complexes up to trinucleosome and also the subnucleosome fragments. The data indicate that there was differential accessibility and solubilization of material by the nuclease in the presence of the drugs. As in the case of DNA fragment separation, there was an increase in the relative amounts of mononucleosomes in the drug-treated samples: chromatin (Fig. 5), nuclei (Fig. 6) and whole cells (Fig. 7). There were changes in the patterns of degradation of the higher multimers (di-, tri- and higher) for ADR 0.05 and DR 0.02 and 0.05, with spreading of the core particle band and subnucleosome pattern. The EtdBr-treated samples did indicate an enhancement in the degradation of nuclei and whole cell samples but not to the extent seen for the anthracyclines. The analysis of nucleoprotein complexes at low ionic strength in acrylamide–agarose gels did not produce the clear-cut evidence for protein–DNA or DNA-interstrand cross-links seen by others employing the alkaline elution methodology [9, 10], but it is useful in giving some indication of the heterogeneity of nucleosome species seen in Fig. 4e. This heterogeneity was demonstrated previously as "whisker" pattern by Levinger and Varshavsky [29].

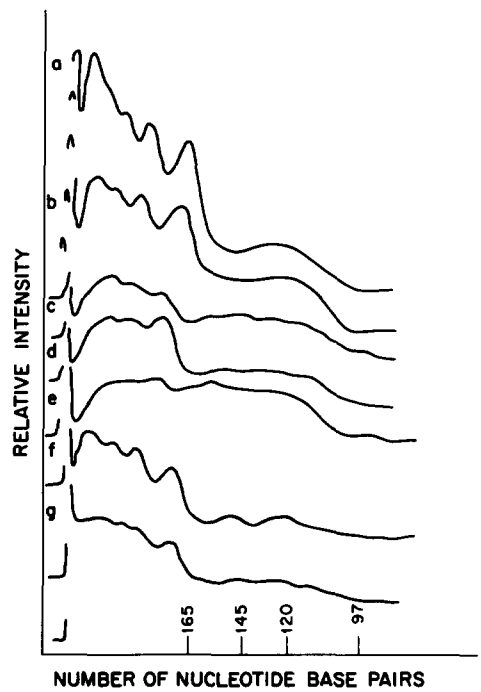


Fig. 7. Whole cells. Samples, as in Fig. 4, were digested for 10 min at 37° and then electrophoresed on a 2.5% polyacrylamide–0.5% agarose nucleoprotein gel. Key: (a) control; (b) ADR: $r = 0.02$; (c) ADR: $r = 0.05$; (d) DM: $r = 0.02$; (e) DM: $r = 0.05$; (f) EtdBr: $r = 0.02$; and (g) EtdBr: $r = 0.05$.

DISCUSSION

The present study investigated the effects of the antitumor anthracycline antibiotics, ADR and DR, and the phenanthridine trypanocide, EtdBr, on chromatin structure at three levels of DNA organization: (i) isolated chromatin, (ii) isolated nuclei, and (iii) whole cells. The results show significant differences between the anthracyclines and EtdBr in their abilities to cause increased production of mononucleosomes and subnucleosome fragments by micrococcal nuclease digestion, particularly at the whole cell level.

The binding of the drugs to DNA was monitored by the changes which occurred in the absorption spectra of the drugs. When the DNA–drug complexes were digested with micrococcal nuclease, it was observed that there was a progressive decrease in the amount of acid-soluble oligonucleotides with increasing r values (Fig. 1a). This suggests that intercalation produced an unwinding of the DNA helix with consequent removal of nuclease sensitive sites. Digestion of DNA with nuclease which was incubated with drugs indicated that the enzyme itself was not affected by the drugs. The inhibitory effect of digestion was, therefore, caused by distortion of the DNA substrate conformation and steric factors due to drug-occupied sites. In contrast to the reduction in the amount of acid-soluble material produced by digestion of DNA–drug complexes, the digestion of chromatin–drug complexes was accompanied by an increase in the amount of acid-soluble oligonucleotides (Fig. 1b). This increase in acid-solubility suggests that the drugs caused an unfolding of chromatin structure and, thereby, made available more nuclease sensitive sites. The unfolding must have been extensive since, as stated above, binding of drugs to free DNA (linker regions in the case of chromatin) inhibited the production of acid-soluble material.

When the products associated with kinetic digestion of chromatin–drug complexes were analyzed by non-denaturing gel electrophoresis (Fig. 1c) it was seen that the increased nuclease sensitivity was not solely a rate effect. The degree of breakdown seen when chromatin was digested in the presence of anthracyclines was well beyond the degradation seen for controls that were digested twice as long as the drug-treated samples. Also, the DR effects were greatest, producing smaller subnucleosomal size DNA, followed by those for ADR and EtdBr.

Micrococcal nuclease digestion of chromatin–drug complexes, nuclei–drug complexes, and nuclei–drug complexes from treated whole cells solubilized portions of chromatin with the unmasking of new sites for nuclease within the nucleosomal core particle DNA. The appearance of new nuclease sites and the generation of subnucleosomal fragments occurred without obvious loss of histones from the core particle (H. E. Grimmond and T. Beerman, unpublished observation). This is in agreement with previous findings on the binding of EtdBr to chromatin [30]. As seen in Fig. 4, there was an apparent concentration effect for ADR which showed an accumulation of mononucleosomes with little internal breakdown (Fig. 4e). While we do not understand this effect, the massive degradation of multimeric forms could be suggestive of some form of cytoplasmic "activation" of ADR as this agent is known to be influenced by cellular factors [6].

The analysis of chromatin fragments as nucleoprotein species is given in Figs. 5–7. These species migrated significantly slower than their counterpart DNA fragments (Figs. 2–4). However, electrophoretic separation of nucleoprotein components gave discrete resolution of monomer bands and subnucleosomal species and demonstrated the more rapid degradation of drug-treated nucleosomes to core particle and sub-core species by the nuclease in comparison to control samples. The nucleoprotein patterns reveal a smear of the particles in the 145 to 97 base pair regions (Fig. 7c, d, e and g). This is suggestive of minor unresolved particles generated by the nuclease in the presence of the drugs, but not adequately separated by this method. Experiments are underway to isolate these mononucleosomal and subnucleosomal species from drug-treated whole cells and to analyze them by one- and two-dimension electrophoresis and by fluorography.

Another characteristic feature of drug-intercalated chromatin was the changed pattern of micrococcal nuclease digest as shown by gel electrophoresis in Figs. 2–4 (chromatin, nuclei and whole cells respectively). The broadening of the nucleosomal bands (Fig. 2) of drug-treated samples and the appearance of core particles and subnucleosomal fragments (Figs. 3 and 4) provide evidence of the changed patterns. It is also clear that DR and ADR showed a greater effect than ethidium bromide. The large DNA peak observed in Fig. 4e, is replaced by a smaller 165 base pair band and larger more diffuse band (145 to 97 base pairs) in Fig. 7c. This apparent discrepancy is not clearly understood and we have not been able to find a simple explanation compatible with our present understanding of nucleosome structure, though the data suggest some form of drug-induced effect between chromatin DNA and protein. The studies under way should help to enhance our understanding of this phenomenon.

In conclusion, the data indicate that the drugs produced some unwinding of chromatin DNA which led to increased accessibility of core particle DNA to nuclease. The observed changes were greatest for DR, followed by ADR and EtdBr. It is possible that part of the cytotoxic effects of anthracyclines on cells might be the increased exposure of chromatin DNA that is normally protected in nucleosomal core structure. Although a precise description of a mechanism of drug action is not yet possible, it is easy to envision that these newly opened sites, which normally are not involved in interactions with cellular DNA reactive enzymes such as nucleases and topoisomerases, now become accessible. Studies are now under way to further define what region(s) of core structures is altered by anthracycline binding.

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