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ADRIAMYCIN-INDUCED COMPACTION OF ISOLATED CHROMATIN

HELEN WALDES and MELVIN S. CENTER*
Division of Biology, Kansas State University, Manhattan, KS 66506, U.S.A.

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Abstract—Adriamycin and certain other agents such as daunomycin, rubidazone and 1,4-dihydroxyl-5,8 bis [[2-[(2-hydroxyethyl)amino]ethyl]amino]-9,10-anthracenedione (DHAQ) were capable of inducing a considerable increase in the sedimentation rate of isolated chromatin. In contrast, *N*-trifluoroacetyl-adriamycin-14-valerate (AD-32) was without effect in this system. Electron microscope studies of chromatin incubated with adriamycin, rubidazone and DHAQ showed that all of these drugs were capable of inducing the formation of highly condensed DNA structures. When chromatin was treated with 0.4 M NaCl, its ability to serve as a substrate for adriamycin-induced compaction was abolished. However, the salt-treated chromatin could still be condensed by DHAQ and rubidazone. The results of this study, therefore, show that a variety of closely related antitumor agents are capable of condensing isolated chromatin. However, certain of these compounds had distinct substrate requirements for the compaction reaction.

Adriamycin, an anthracycline antibiotic, is currently being used as an effective therapeutic agent in the treatment of patients with certain leukemias or solid tumors [1]. The exact mechanism of action of the drug is unknown but its cytotoxic effect probably occurs through its ability to bring about an inhibition of both RNA and DNA syntheses [2–4]. The action of adriamycin in inhibiting macromolecular synthesis presumably occurs through the ability of the drug to interact with, and disrupt, the structure of nuclear DNA. Previous studies have shown that adriamycin intercalates into native DNA [5–7] and, when added to cells in culture, brings about the formation of aberrant chromosomal structures [8, 9] that contain high levels of DNA strand breaks [10–13].

Recent studies have also shown that adriamycin is capable of inducing certain structural transitions in isolated chromatin. It has been observed that the drug is capable of inducing the formation of DNA single-strand regions [14] and also of highly condensed chromatin structures [15].

In the present study, we have examined further the condensation of chromatin by adriamycin and certain other antitumor agents. The results suggest a novel specificity of drug action in the condensation of isolated chromatin.

MATERIALS AND METHODS

Drugs. Adriamycin and other anthracycline derivatives and DHAQ were provided by the Developmental Therapeutics Program of the Division of Cancer Treatment, NCI.

Cell culture. Chinese hamster lung cells (HT-1) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum. Labeling of DNA was carried out by growing cells for 16–20 hr in medium containing 0.5 μCi/ml of [^{3}H]thymidine (70 Ci/mmole).

Preparation of nuclei and chromatin. Nuclei and chromatin were prepared essentially as described previously [14, 15]. Prior to use in these studies, the chromatin pellet fraction in buffer A [10 mM Tris–HCl (pH 7.6), 2.0 mM $MgCl_2$, 0.5 mM EDTA and 5 mM β-mercaptoethanol] was kept on ice for about 72 hr and therafter centrifuged for 5 min at 2500 *g*. Unless indicated otherwise, the chromatin, contained in the supernatant fraction was used for the studies described. We have observed that during the incubation period at 0° chromatin undergoes a progressive conversion to 70–90S structures. We have found this material particularly suitable for measuring drug-induced changes in the sedimentation rate of the DNA. Chromatin, analyzed within 24 hr of preparation, contains a heterogeneous population of larger molecular weight material.

Effects of antitumor agents on the sedimentation rate of chromatin. Isolated chromatin was incubated in a reaction mixture containing 0.03 M Tris–HCl (pH 7.6) and various agents in a reaction volume of 0.12 ml. Control tubes contained identical reaction constituents except that buffer was added in place of drug. After an incubation period of 20 min at 37°, the entire reaction mixture was layered on a 4-ml 5–20% neutral sucrose gradient which overlaid a 0.7 ml CsCl shelf (1.2 g CsCl/ml of 20% neutral sucrose). The sucrose was prepared in 0.01 M Tris–HCl (pH 7.6)–1 mM EDTA–0.05% NP40 (Nonidet-P-40, a nonionic detergent). Centrifugation was carried out for 30 min at 30,000 rpm and 4° in the Spinco SW50.1 rotor. At the end of the centrifugation, fractions were collected from the bottom of the tube directly into scintillation vials containing glass fiber filter paper.

Electron microscopy. In these experiments, chromatin was incubated in the absence and presence of

* To whom correspondence should be sent.

adriamycin. rubidazone or DHAQ under standard conditions. At the end of the incubation period EDTA was added to 0.01 M, and an aliquot of the reaction mixture was spotted on a parlodion coated grid. After 4 min, the grid was blotted, stained with 2% uranyl acetate (in water), and washed briefly in water. The dried grids were visualized in a Philips 201 electron microscope.

DNA concentration. The concentration of DNA was determined according to the procedure of Burton [16]. Native calf thymus DNA was used as standard.

RESULTS

Effects of various antitumor agents on the sedimentation rate of chromatin. We have shown previously that incubation of adriamycin with isolated chromatin results in a considerable increase in the sedimentation rate of the DNA [15]. It was also shown that the increase in sedimentation rate was due to the formation of highly compacted chromatin structures [15]. To examine in greater detail drug specificity for the compaction reaction, we have determined the effects of a variety of antitumor agents on the sedimentation rate of isolated chromatin. As shown in Fig. 1, incubation of adriamycin with isolated chromatin resulted in the formation of DNA structures having an extremely fast sedimentation rate. In contrast, AD-32 was not active in converting chromatin to fast sedimenting forms. The results obtained with a variety of other drugs using the sedimentation assay for measuring chromatin compaction are given in Table 1. These drugs can be grouped into three classes with regard to activity in inducing chromatin compaction. One class, which contains the most active drugs, includes adriamycin, daunomycin, rubidazone and DHAQ. A second class includes aclacinomycin which compacted chromatin, but the activity was less than that observed with adriamycin. A third class contains AD-32, which was not active in this system. The inability of AD-32 to induce chromatin condensation may be related to the results of previous studies which show that the drug has considerably less affinity for DNA binding than does adriamycin [17].

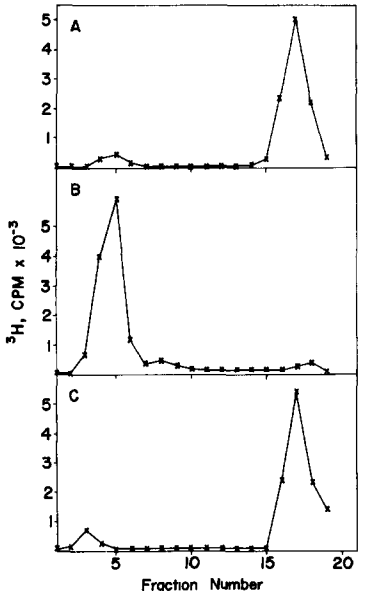

Fig. 1. Effect of adriamycin and AD-32 on the sedimentation rate of isolated chromatin. Isolated chromatin was incubated under standard conditions in the absence (panel A) or presence of adriamycin (panel B) or AD-32 (panel C). The reaction mixture contained 6 μg DNA and 2 μg drug. Sedimentation in neutral sucrose gradients was carried out as described in Materials and Methods.

Table 1. Effects of various antitumor agents on the sedimentation rate of isolated chromatin*

Agent	Fast sedimenting chromatin (%)
Adriamycin	78
Daunomycin	75
Rubidazone	70
AD-32	5
Aclacinomycin	35
DHAQ	70

* Chromatin was incubated with the indicated agents under standard assay conditions. At the end of the incubation period, centrifugation in sucrose gradients was carried out as described in Materials and Methods. The amount of DNA which exhibited a fast sedimentation rate is tabulated as the percentage of total radioactivity recovered from the sucrose gradient. All drugs were used at a concentration of 20 μg/ml.

Electron microscope visualization of drug-induced chromatin compaction. In these experiments, adriamycin at various concentrations was incubated with chromatin for 20 min at 37° and the DNA structures were thereafter examined in the electron microscope. As shown in Fig. 2A, untreated chromatin was characterized by a complex network of DNA fibers that were heavily coated with protein. Essentially all of the chromatin material had this same appearance, but the structures were quite heterogeneous in size. As shown in Fig. 2B, chromatin incubated with adriamycin underwent major structural changes. The most prominent features of drug-treated chromatin were a considerable thickening of the DNA fibers and the presence of regions which appeared to be highly condensed (Fig. 2, panels C and D). Additional studies have been carried out to examine chromatin incubated in the presence of rubidazone and DHAQ. DHAQ is a recently developed antitumor agent [18, 19] which is currently undergoing Phase II clinical testing [20]. The results of these studies clearly demonstrated that both drugs were also capable of producing structural changes in chromatin that were essentially identical to those observed with adriamycin (not shown).

Experiments have also been carried out in which chromatin was incubated with 0.4 M NaCl and then isolated after centrifugation in a sucrose gradient. The dialyzed material was incubated with adriamycin, DHAQ or rubidazone, and the resulting structures were examined in the electron microscope. As shown in Fig. 3A, the salt-treated chromatin that

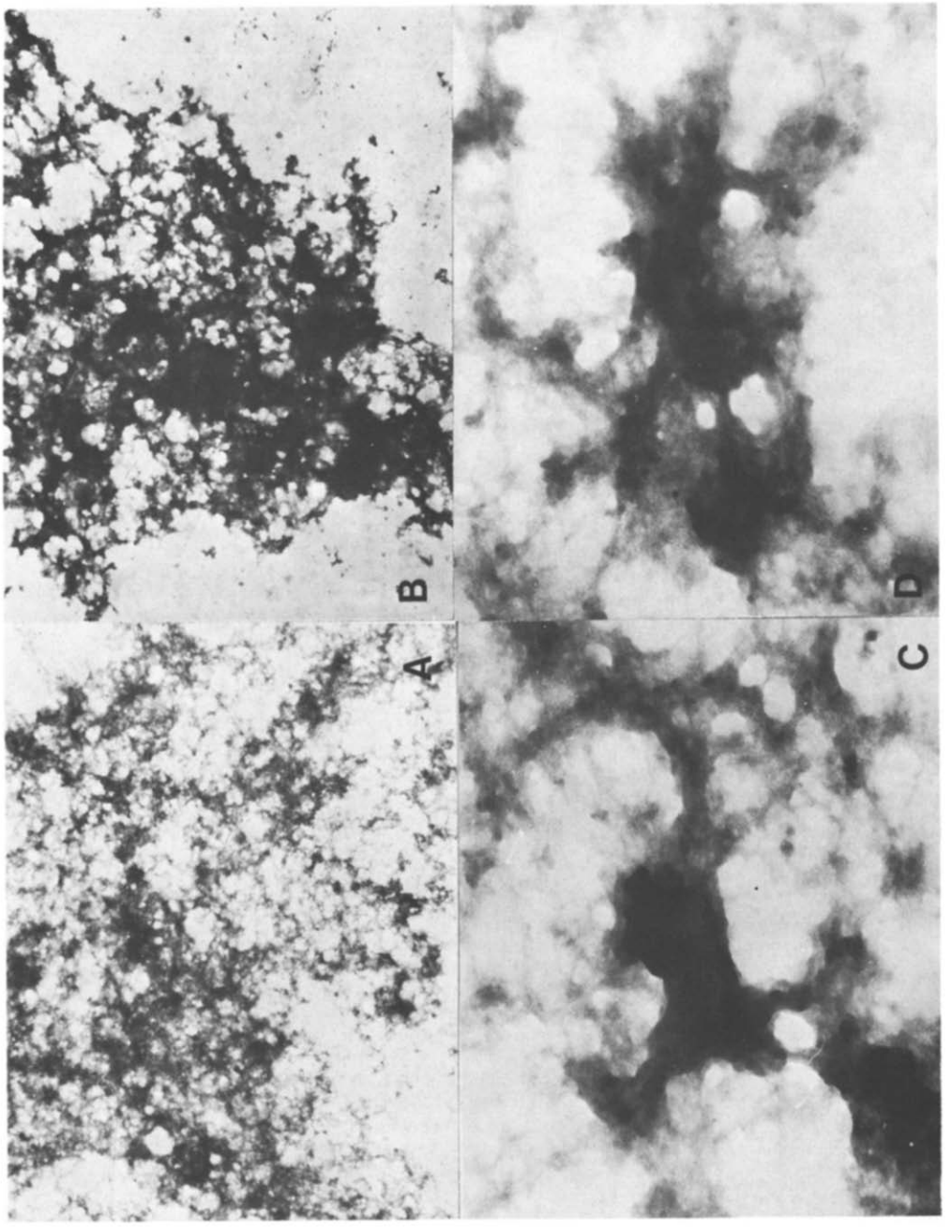

Fig. 2. Electron microscopy of chromatin incubated with adriamycin. Chromatin was incubated in the absence of drug (panel A) or in the presence of 2 μg adriamycin (panels B, C, and D). The DNA and drug concentrations were 100 and 20 μM respectively. Final magnification for panels A and B was 7020×; final magnification for panels C and D was 43,000×.

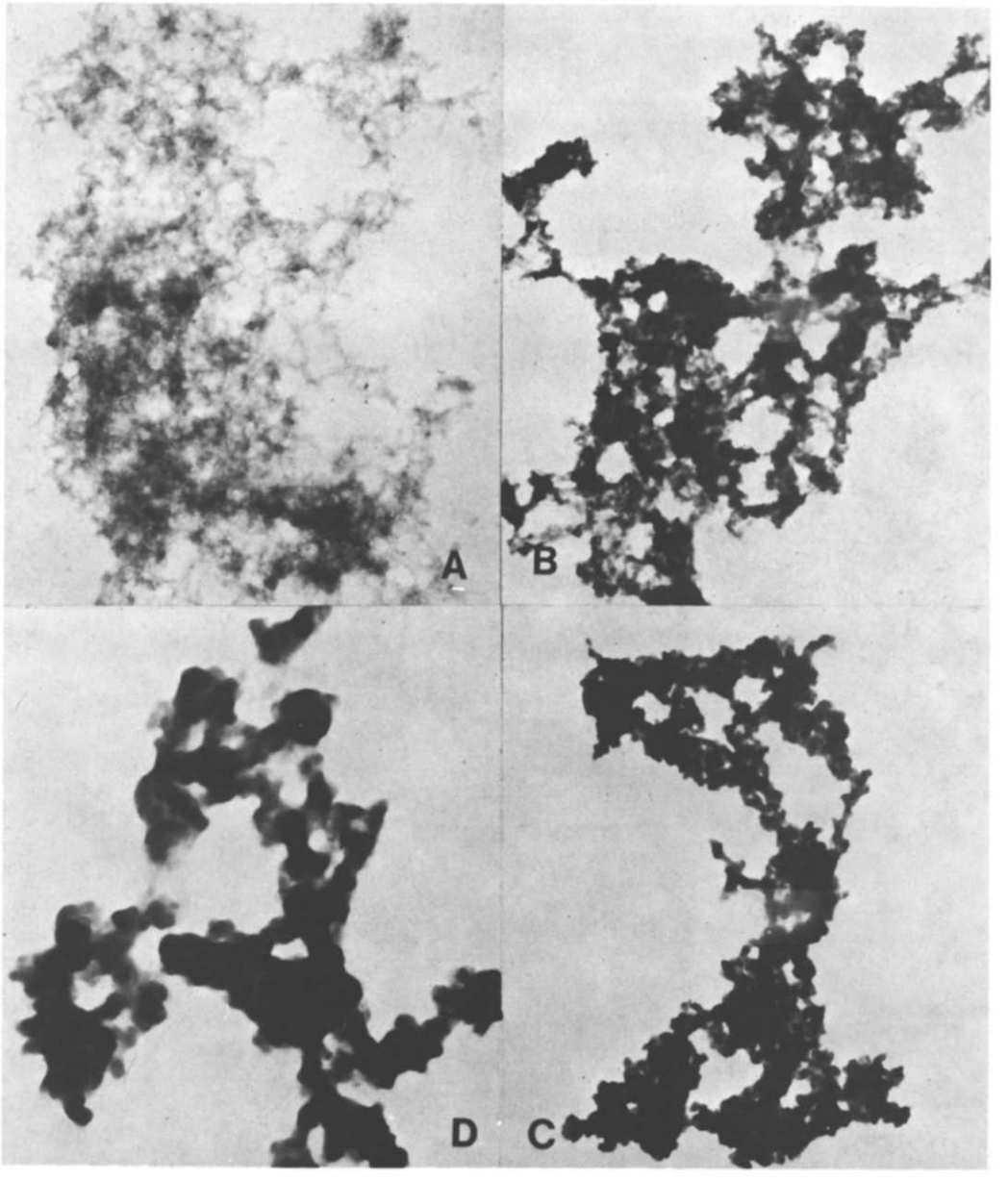

Fig. 3. Electron microscopy of salt-treated chromatin incubated with adriamycin, rubidazone or DHAQ. The isolated chromatin fraction was incubated with an equal volume of 0.8 M NaCl, and after holding 1 hr on ice the solution was centrifuged in a 5–20% sucrose gradient. Sucrose was prepared in 0.01 M Tris–HCl (pH 7.6)–0.4 M NaCl–1 mM EDTA. Centrifugation was for 15 hr at 30,000 rpm and 4° in the Spinco SW50.1 rotor. Fractions were collected from the bottom of the tube and those containing the major radioactivity (from [^{3}H]thymidine) were pooled and dialyzed for 8 hr against 0.01 M Tris–HCl (pH 7.6). Portions of the dialyzed material were incubated with adriamycin (panel A), DHAQ (panel B) or rubidazone (panels C and D). The DNA and drug concentrations were 30 and 7 μM respectively. Although not shown, chromatin not treated with drug appeared essentially identical to that shown in panel A. Final magnification was 18,810× for panels A, B and C, and 43,300× for panel D.

was incubated with adriamycin was completely resistant to any detectable structural changes. However, when the chromatin was incubated with DHAQ (Fig. 3B) or rubidazone (Fig. 3, panels C and D), considerable folding and compaction of the chromatin were observed.

DISCUSSION

In the present study we have examined a structural transition induced in chromatin incubated with adriamycin and certain other antitumor agents. The results of sedimentation analysis and electron microscopy suggest that certain anthracycline derivatives and DHAQ (an anthracenedione) are capable of inducing a condensation and compaction of isolated chromatin. The condensation reaction leads to a considerable folding and thickening of the nucleofilament. This probably occurs through an extensive supercoiling of the DNA fiber. Based on the known

DNA–drug interactions of the anthracyclines [5–7] and DHAQ [19], intercalation must play an important function in the compaction reaction. Previous studies have shown, however, that ethidium bromide is inactive in this system [15], suggesting that factors in addition to intercalation are required. The nature of these factors is unknown, but ionic interactions or inter- and intrastrand crosslinks may be important. Unexpectedly, certain agents within a class of compounds that can condense chromatin were found to have distinct substrate requirements for mediating this reaction. This is based on the finding that chromatin treated with 0.4 M NaCl was resistant to the action of adriamycin but susceptible to condensation by DHAQ and rubidazone. The basis of this result is unknown but it could be that there was a loss of certain proteins from the salt-treated DNA which are required for adriamycin-induced condensation of chromatin.

The relationship of the *in vitro* drug-induced compaction of chromatin to events that occur when adriamycin is added to living cells remains to be determined. It is well documented, however, that adriamycin and daunomycin are capable of inducing the formation of aberrant chromosomal structures [8, 9]. Thus, chromosomal structural rearrangements occurring *in vivo* may be related to an ability of the drug to induce chromatin compaction.

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