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Influence of DNA-Binding Drugs on Chromatin Condensation†

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ABSTRACT: We have used transient electric dichroism to study the ability of DNA-binding drugs to affect the folding of chromatin from the 10- to the 30-nm fiber, either by themselves or in conjunction with multivalent cations. Variables considered include the cationic charge of the drug, the comparative influence of intercalation and groove binding as modes of interaction, and the effect of bis-intercalation compared to mono-intercalation. In parallel with our findings with other cations, we observe that a drug must have a charge of 3+ or greater in order to condense chromatin at concentrations substantially lower than the concentration of chromatin, measured in base pairs. Drugs of low charge, whether groove binders or mono- or bis-intercalators, are unable to condense chromatin on their own. Bis-intercalators of high charge, however, are extremely efficient condensers, being able to cross-link chromatin with greater efficiency than polyamines of corresponding charge. When Mg^{2+} is used in combination with bis-intercalators of high charge, the order of addition of the two determines whether compaction or cross-linking is favored. Finally, the antibiotics actinomycin D, daunomycin, and distamycin, despite varied modes of binding to DNA, all inhibit the compaction of chromatin beyond a critical point in a remarkably similar manner.

A number of small organic molecules are able to bind to DNA, among them dyes and various antibiotics. Many of these have pharmacological properties and can therefore be regarded as drugs. Over the years a great deal of research has been done toward understanding the interaction of these drugs with DNA and the dynamics of the drug–DNA complexes (Neidel & Waring, 1983).

A frequently encountered, and certainly the most-studied, mechanism of binding of small molecules to DNA is intercalation. In this mechanism, flat molecules of polycyclic or heterocyclic aromatic substances can be inserted between the base pairs. Drugs that interact with DNA in this fashion are, among others, ethidium bromide, the actinomycins, and the antitumor drugs of the anthracycline family such as daunomycin and adriamycin. Other drugs, notably oligopeptides such as distamycin and netropsin, attach themselves to the outside of the helix, in the small groove. This kind of binding can be very strong and is frequently several orders of magnitude stronger than intercalative binding.

Other widely accepted mechanisms of binding include the stacking of planar molecules outside of the DNA helix. In all of these different modes of binding, electrostatic interactions between the ligand molecule and DNA provide a variable, and in some cases important, contribution to the binding energy.

In higher cells DNA exists in the form of the nucleoprotein complex chromatin in which the DNA is complexed with histone and non-histone proteins. In order to understand the pharmacology of DNA-binding drugs in higher organisms, it is necessary to understand the interactions of these drugs with chromatin. In most somatic cells, chromatin is a dynamic substance undergoing various modifications of structure in response to the metabolic state of the cell. The most fundamental of these dynamic processes is perhaps the reversible folding and unfolding of the chromatin fiber between the 30-nm-thick condensed form to a form lacking higher order organization, one that is identified with high rates of gene transcription (Labhart & Koller, 1982; Andersson et al., 1982).

In the preceding paper (Sen & Crothers, 1986) we have used electric dichroism to study the condensation behavior of isolated and size-fractionated chromatin fibers in response to a number of physiologically important ions of different charge. Having established the basic physical factors that underlie chromatin condensation in vitro, we are now in a position to study the influence of extrinsic agents such as DNA-binding drugs on chromatin condensation. We have taken three approaches to this problem: to study the effect of intercalative drugs as compared to that of groove-binding drugs; to study the effect of mono-intercalative drugs compared to that of bis-intercalative drugs; to study the effect of cationic charge

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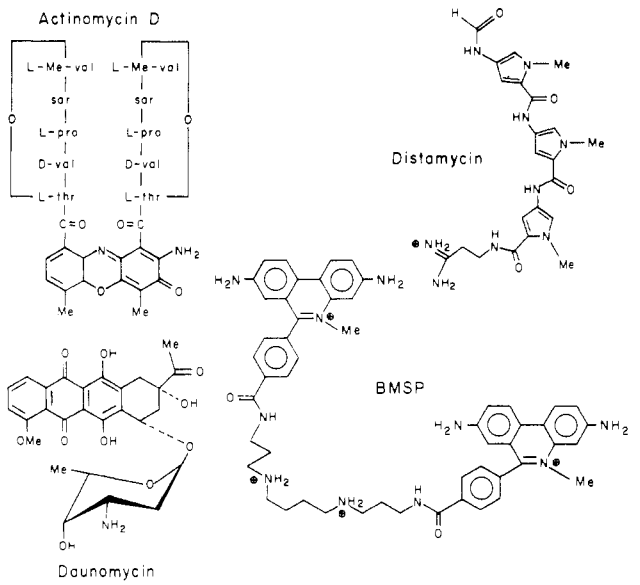

FIGURE 1: Chemical structures of actinomycin, distamycin, daunomycin, and bis(methidium) spermine (BMSp).

of the ligands on condensation. The major drugs employed are distamycin (dis), actinomycin D (ac), and daunomycin (da). All three are important antibiotics, and the latter two are significant antitumor drugs.

Distamycin is an oligopeptide with a cationic guanidinium residue. It binds to the minor groove of the DNA with a preference for A-T base paris and is a potent antibacterial, antiviral, and antineoplastic agent (DiMarco et al., 1962). Daunomycin and actinomycin D are intercalators, daunomycin with a single cationic charge and actinomycin with none at physiological pH. Actinomycin (Holstein, 1976; Sobell, 1973) is a powerful antibacterial and antineoplastic agent, while daunomycin and its relatives are used widely in chemotherapeutic treatment of a variety of cancers (DiMarco et al., 1963).

The bis-intercalating drugs are bis(daunomycin) (Bda) with an oligopeptide linker (D. Phillips, private communication), and two positive charges, and bis(methidium) spermidine (BMSpd) and bis(methidium) spermine (BMSp) (Dervan & Becker, 1978; Becker & Dervan, 1979), with three and four cationic charges, respectively. In addition, we have examined methidium spermine (MSp) with four cationic charges. The structures of some of these drugs are shown in Figure 1.

Materials and Methods

Electric dichroism, a technique highly sensitive to the different degrees of compaction of chromatin, was used to study the hydrodynamic and geometric properties of chromatin fibers. The equipment is described in the preceding paper (Sen & Crothers, 1986) and also by Marini et al. (1982). In summary, electric dichroism experiments for relaxation measurements were carried out by applying a 2-kV exciting pulse across the dichroism cell with a 5-mm electrode gap. Data acquisition and processing were done with a waveform digitizer interfaced with a PDP-11/40 computer. Data were obtained by signal averaging 1000–1000 sweeps, depending on the sample, with reversal of field every eight pulses.

An interactive computer program was used to analyze the decay portion of the dichroism signal to two exponentials for the best fit. The data were presented in the form $y = A_1e^{-t/\tau_1} + A_2e^{-t/\tau_2}$, where $A_1 + A_2 = 1$. We defined $\bar{\tau} = A_1\tau_1 + A_2\tau_2$. As described in the preceding paper (Sen & Crothers, 1986), $\bar{\tau}$ is a highly useful parameter that serves as a suitable index for the overall size and shape of the chromatin particles and changes in reproducible and characteristic ways during compaction and after the onset of aggregation.

High-field experiments for the measurement of dichroism amplitudes (Hogan et al., 1978) were carried out at 16 kV/cm. At this field strength, optimally compacted chromatin fibers are fully aligned in the electric field. Field dependence experiments were carried out between 0 and 25 kV/cm.

Chromatin from chicken erythrocytes was prepared and monitored for integrity as described in the preceding paper (Sen & Crothers, 1986). Experiments, unless otherwise stated, were carried out with chromatin at $A_{260} = 0.2 = 15$ μM DNA base pairs = 30 μM DNA phosphates. Samples were dialyzed into dichroism buffer [0.3 mM NaCl, 0.2 mM tris(hydroxymethyl)aminomethane (Tris),[1] 0.003 mM EDTA, pH 7.5)], with other cations and drugs added as indicated.

Actinomycin D, daunomycin, and distamycin were purchased from Boehringer. Bis(methidium) spermine (BMSp), bis(methidium) spermidine (BMSpd), and methidium spermine (MSp) were the kind gift of Dr. Peter Dervan, California Institute of Technology; bis(daunomycin) was given by Dr. Don Phillips of La Trobe University, Melbourne, Australia. All drugs were used without further purification. Solutions of all drugs were made into aliquots of 100 μM concentration and frozen until required, with the exception of distamycin, which was dissolved in buffer fresh before each experiment, owing to its instability in solution.

Experiments with most drugs were performed at high drug to base pair ratios (up to added drug/base pairs = 0.1). To check that all of the drug was bound to the chromatin, the various drug–chromatin complexes were centrifuged extensively in an Eppendorff centrifuge under salt conditions substantially higher than the maximum used in the experiments, at which salt the chromatin complexes were essentially aggregated out of solution. After centrifugation of the chromatin pellet, the supernatant in all cases yielded essentially no absorbance at either 260 nm or the various maxima of the drug absorbances.

Results and Discussion

The information obtained about the physical processes involved in chromatin condensation [see Sen & Crothers (1986)] can be summarized as follows: the addition of cations to unfolded (10 nm) chromatin leads to the binding of these cations to the chromatin, resulting in a progressive neutralization of the negative charge of the DNA backbone, such that there is a lessening of the repulsive forces between adjacent segments of a given chromatin fiber as well as between separate chromatin fibers. These phenomena manifest themselves as a progressive compaction of individual chromatin fibers and a subsequent tendency of a number of chromatin fibers to aggregate.

Provided experiments are performed on chromatin at reasonably low concentrations (below 120 μM DNA phosphates = 60 μM base pairs = $A_{260} = 0.8$), compaction is initially the dominant process and progresses smoothly until a critical compaction is reached, which we call the optimal compaction and which is reached presumably after the neutralization of a critical fraction (90–100%) of the DNA phosphate charges. Beyond this point, with further added cations, aggregation processes begin to predominate. This type of behavior is evident regardless of the cation used, or the charge of that

[1] Abbreviations: Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid.

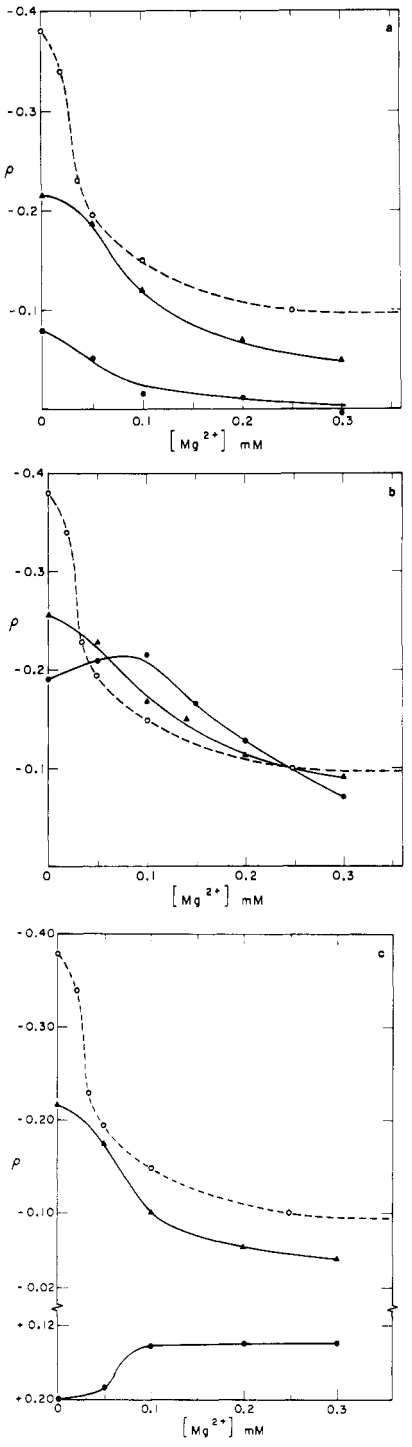

FIGURE 2: (a) Dichroism amplitudes at 15 kV/cm of an actinomycin–chromatin complex (r = 0.05), with increasing Mg^{2+} concentration. (O) Pure chromatin, measured at 266 nm; (▲) chromatin–ac complex, at 266 nm; (●) chromatin–ac complex, at 440 nm. (b) Dichroism amplitudes at 15 kV/cm of chromatin complexed with daunomycin (r = 0.01), with increasing Mg^{2+} concentration. (O) Pure chromatin, measured at 266 nm; (▲) chromatin–da complex, at 266 nm; (●) chromatin–da complex, at 470 nm. (c) Dichroism amplitudes at 15 kV/cm of chromatin complexed with distamycin (r = 0.1), against increasing Mg^{2+} concentrations. (O) Pure chromatin, measured at 266 nm; (▲) chromatin–dis complex, measured at 266 nm; (●) chromatin–dis complex, at 315 nm.

cation. Thus, the polyamine spermine (4+), the complex ion hexaamminecobalt(3+) (with ammonia ligands capable of hydrogen bonding), and the simple divalent ion of hydrated Mg^{2+} all bring about similar effects on chromatin, albeit at different concentrations. If chromatin concentrations higher than about 80 μM phosphate are used, then aggregation

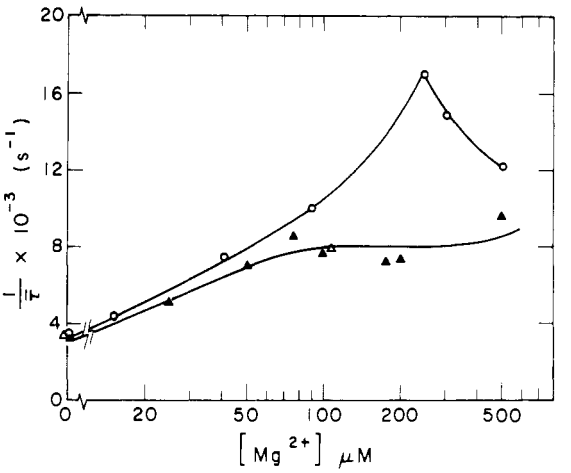

FIGURE 3: Relaxation times of ac–chromatin complexes, plotted as $1/\bar{\tau} \times 10^{-3}$ vs. increasing Mg^{2+} concentrations. (O) Pure chromatin, at 266 nm; (▲) chromatin–ac complex, at 266 nm; (△) chromatin–ac complex, at 440 nm.

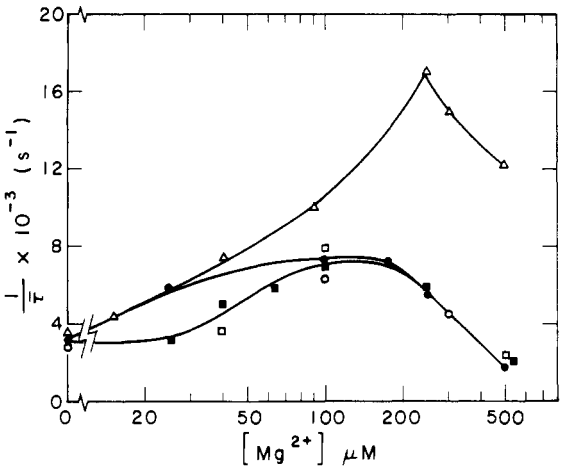

FIGURE 4: Relaxation times of da–chromatin and dis–chromatin complexes, plotted as $1/\bar{\tau} \times 10^{-3}$ vs. increasing concentrations of Mg^{2+}. (△) Pure chromatin, measured at 266 nm; (●) chromatin–da complex, at 266 nm; (O) chromatin–da complex, at 470 nm; (■) chromatin–dis complex, at 266 nm; (□) chromatin–dis complex, at 315 nm.

processes begin to predominate substantially before optimal compaction is reached.

Effect of Drug Charge on Mg^{2+}-Free Chromatin. The first question we asked about DNA-binding drugs was whether they were able, irrespective of charge, to condense chromatin to any extent. The uncharged actinomycin and the singly charged daunomycin and distamycin were added at a high drug to base pair ratio (0.05–0.1) to chromatin with no added magnesium. With all three drugs (correcting for very small drug absorbances at 266 nm), the chromatin dichroism showed a substantial decrease. From the normal unfolded dichroism of approximately –0.38 at 15 kV, we had dichroisms of –0.22 with actinomycin, –0.26 with daunomycin, and –0.22 with distamycin (Figure 2). Yet the relaxation profiles for these three unfolded chromatin–drug complexes (Figures 3 and 4) were very similar to that of unfolded chromatin alone, when monitored at 266 nm.

The complex of these same drugs with naked, rodlike DNA does not exhibit this kind of behavior. The binding of distamycin and daunomycin up to a level of one drug per 10 base pairs and actinomycin at half that level do not significantly affect the limiting dichroism of DNA (Dattagupta et al., 1980; Fritzsche et al., 1982; unpublished results). We performed field dependence experiments on unfolded chromatin by itself

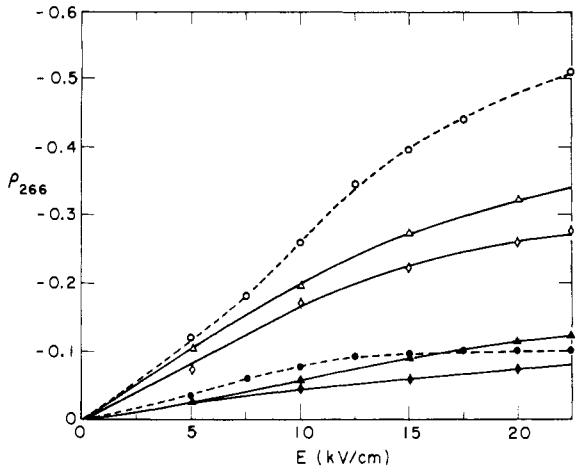

FIGURE 5: Dichroism at 266 nm of chromatin and chromatin–drug complexes vs. applied field strength. (○) Chromatin at zero [Mg^{2+}]; (△) chromatin–da complex at zero [Mg^{2+}]; (◇) chromatin–ac complex at zero [Mg^{2+}]; (●) chromatin at [Mg^{2+}] = 250 M; (▲) chromatin–da complex at [Mg^{2+}] = 250 M; (◆) chromatin–ac complex at [Mg^{2+}] = 250 M.

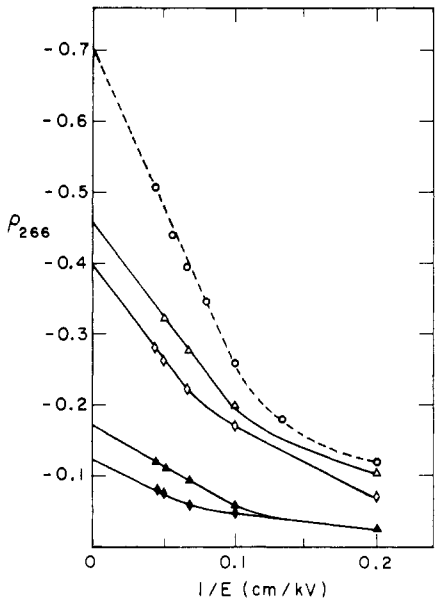

FIGURE 6: Dichroism at 266 nm of chromatin and chromatin–drug complexes vs. the reciprocal of applied field strength. (○) Chromatin at zero [Mg^{2+}]; (△) chromatin–da complex at zero [Mg^{2+}]; (◇) chromatin–ac complex at zero [Mg^{2+}]; (▲) chromatin–da complex at [Mg^{2+}] = 250 Mg; (◆) chromatin–ac complex at [Mg^{2+}] = 250 M.

and chromatin complexed with daunomycin and actinomycin (Figure 5), and when the dichroism amplitudes were plotted vs. $1/E$ (Figure 6), the results were unequivocal in that the limiting dichroism amplitude of pure infolded chromatin was more negative than unfolded chromatin complexed with either drug.

Unfolded chromatin (the "10-nm" fiber) is a loose and flexible structure. When subjected to an electric field, it orients with a geometry that is not fully understood, but the dichroism continues to increase with increasing electric fields, owing presumably to the relatively high flexibility and distortability of the complex structure.

One way to interpret the effect of drugs on unfolded, or partially folded, chromatin is that segments that are normally easily oriented in an electric field are less easily oriented owing to the increased torsional and bending stiffness of the drug-bound DNA, leading to lower overall dichroism amplitudes.

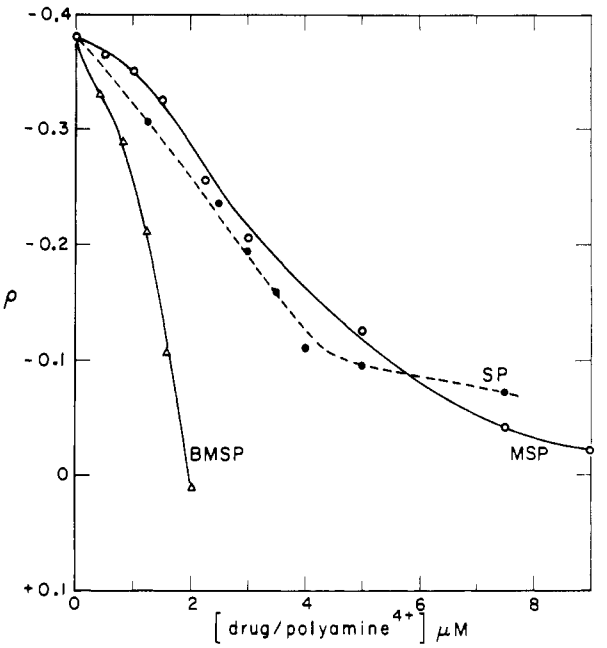

FIGURE 7: Dichroism amplitudes at 15 kV/cm of chromatin complexed with spermine and spermine-based drugs, measured at 266 nm. (●) Chromatin–spermine; (○) chromatin–MSp; (△) chromatin–BMSp.

Thus, the decrease in dichroism amplitude does not represent either substantial compaction or aggregation of the chromatin fibers, but rather a change in the distortion dichroism of the loose fiber. This interpretation is supported by the lack of change in the rotational relaxation time. Thus, we conclude that drugs with zero or low cationic charge are unable to bring about any significant compaction of chromatin on their own.

Mono- and Bis-Intercalation. The question of whether a bis-intercalating drug with low cationic charge (2+) can by virtue of its bidentate nature effect chromatin compaction was investigated by using bis(daunomycin) with chromatin. The behavior of bis(daunomycin) was found to be identical with that of daunomycin (data not shown), if one regards each molecule of bis(daunomycin) as two unconnected molecules of daunomycin.

The drugs bis(methidium) spermine and methidium spermine were then compared with spermine itself for their ability to condense chromatin. With BMSp (Figure 7), there was a rapid decline of the dichroism amplitude with added drug, and by 2 μM added drug (to 30 μM chromatin–phosphate at 0.5 mM monovalent ion concentration), the dichroism had acquired a positive sign, which in our experience is associated with "overcompacted" chromatin [as has been obtained previously by cross-linking chromatin extensively with dimethyl suberimidate (Lee & Crothers, 1982)] or severely aggregated chromatin. MSp, on the other hand, showed a dichroism dependence on drug concentration not dissimilar to spermine itself.

BMSpd, when compared to spermidine, showed an analogous result (not shown) to BMSp and spermine. The relaxation data for all of these drugs is shown in Figure 8. It is evident that the lowest values of $\bar{\tau}$ achieved for all of the drugs are substantially higher than those produced by the polyamines themselves. Up to about 3.0 μM, methidium spermine showed a condensing behavior not dissimilar from spermine. However, aggregation phenomena are presumably present to a much greater degree throughout for MSp compared to spermine, in that well before a fully compacted fiber is obtained with the drug, aggregation phenomena begin to predominate.

With BMSp this effect is notably more pronounced, in that

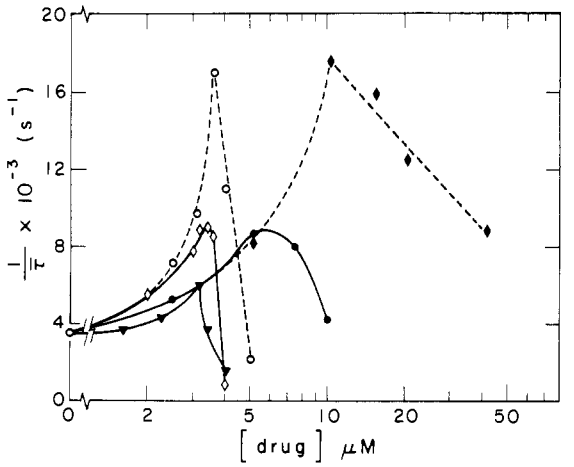

FIGURE 8: Relaxation data of chromatin interacted with spermine and spermidine and drugs based on these polyamines, plotted as $1/\bar{\tau} \times 10^{-3}$ vs. increasing drug/polyamine concentration. All measurements were made at 266 nm. (○) chromatin–spermine; (◇) chromatin–MSp; (▼) chromatin–BMSp; (◆) chromatin–spermidine; (●) chromatin–BMSpd.

hardly any compaction is attained before aggregation phenomena begin to predominate. Since aggregation ought to be sensitive to the concentration of chromatin, it is easy to test the above hypothesis. With a sample in which the chromatin concentration has been halved, the maximum compaction obtained was significantly greater ($\bar{\tau}$ = 100 μs) before the onset of large-scale aggregation.

With BMSpd compared to spermidine, we again obtained analogous results, except unlike spermine and the spermine-based drugs, where maximum compaction occurred at approximately the same molarity of added ligand, BMSpd caused aggregation to begin somewhat earlier than spermidine. This discrepancy is probably not too significant and may lie within a slightly erroneous estimation of the molarity of the BMSpd stock solution. (The extinction coefficient of BMSpd is subject to large changes depending on a number of variables of the solution.)

As with BMSp, halving the concentration of chromatin in a sample enhances the degree of greatest compaction produced by BMSpd. The dramatic decrease in the dichroism amplitudes of chromatin produced by increasing the concentration of the bis-intercalating drugs BMSP and BMSpd can thus be attributed to a strong tendency of chromatin to aggregate in the presence of even small amounts of these drugs.

Thus, cationic charge appears to be the single most important factor in bringing about chromatin compaction. Bis-intercalation in itself seems to have little effect on this process. When combined with a cationic charge of three or more, bis-intercalation contributes enormously to a cross-linking process that leads to chromatin aggregation. Polyamines in themselves, which are efficient cross-linkers of naked DNA (Wilson & Bloomfield, 1979; Widom & Baldwin, 1980), are found not to be such effective cross-linkers of chromatin.

An interesting result, in view of the competing tendencies of chromatin to aggregate and compact, could be seen by adding $BMSp^{4+}$ and Mg^{2+} to the same sample of chromatin. At the ionic strength in which these experiments were carried out, $BMSp^{4+}$ would be expected to have a binding affinity for DNA well in excess of 10^{11} M^{-1} (Becker & Dervan, 1979) and probably a slow dissociation rate. Thus, the order of addition of $BMSp^{4+}$ and Mg^{2+} might be expected to yield nonidentical results.

The concentration of BMSp was kept constant at 1.2 μM, and the concentration of magnesium was varied. The results

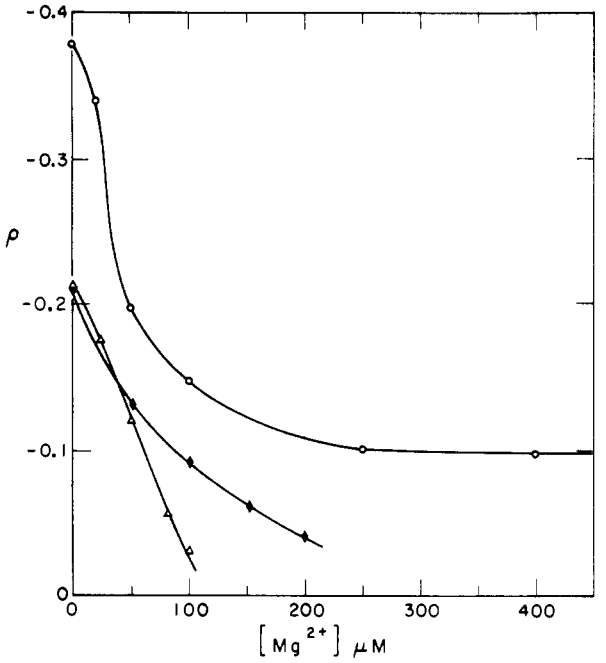

FIGURE 9: Effect of the order of addition of BMSp and magnesium to chromatin. Dichroism amplitudes measured at 266 nm for a field strength of 15 kV/cm plotted vs. increasing [Mg^{2+}]. (○) Chromatin compacted with Mg^{2+} alone; (◆), chromatin, with 1.2 μM BMSp added before magnesium; (△) chromatin, with 1.2 μM BMSp added after magnesium.

are shown in Figure 9. The dramatic decline of the dichroism amplitude of chromatin where Mg^{2+} was added before BMSp is reminscent of the condensation of chromatin by BMSp alone. In the case where BMSp is added before Mg^{2+}, the dichroism profile resembles that of Mg^{2+} itself (taking into account the initial decline of the dichroism at [Mg^{2+}] = 0, due to the added BMSp). It seems that, regardless of whether Mg^{2+} or BMSp is the first added cation, the initial effect is largely one of compaction. However, it seems that aggregation by BMSp is much greater for fibers precompacted by Mg^{2+}.

Effect of Drugs on Magnesium Dependence of Chromatin Condensation. Since it was found that actinomycin, distamycin, and daunomycin had relatively little compacting ability on their own, we were interested in studying the ways high doses (r=0.05–0.1) of these drugs might interact with partially folded chromatin fibers and interfere with that process. All three of these drugs have strong absorption bands in the visible and the near-UV (dis at 315 nm, da at 470 nm, and ac at 440 nm). Thus, the geometry (and rotational relaxation) of drugs complexed to chromatin could be followed along with the geometry and relaxation of the chromatin chromophores.

The dichroism dependence of chromatin with increasing magnesium in the presence of added daunomycin (r = 0.1) is shown in Figure 2b. The corresponding dependence of the distamycin (r = 0.1) and actinomycin (r = 0.05) complex are shown in panels a and b of Figure 2, respectively; the relaxation curves are shown in Figures 3 and 4. As we have noted earlier for all three drugs, the complexes of fully unfolded chromatin show substantial drops in dichroism amplitudes although the relaxation profiles are not substantially different that of from pure unfolded chromatin. With added magnesium there is a trend toward convergence of the ρ_{266} signals for pure chromatin and the complexes. With daunomycin (Figure 2b) the two signals converge very closely, and the signal for the drug dichroism, although substantially lower in the unfolded complex, also approaches the other two signals under conditions of added magnesium.

With distamycin and actinomycin the 266-nm (chromatin) signal of the complexes remains consistently lower than that for pure chromatin. As for the drug dichroisms, distamycin at 315 nm shows that from 0.1 nM Mg^{2+} upward there is no change of the positive drug signal. With actinomycin the drug signal is very small relative to the chromatin signals and approaches zero at high magnesium levels.

From the relaxation data we can see that the drug complexes have certain features in common with each other. At low magnesium concentrations (below ~75 μM) the actinomycin and distamycin complexes relax at rates comparable to that of chromatin itself. The distamycin complex is somewhat more resistant to begin folding and maintains for this low magnesium range a substantially larger $\bar{\tau}$ compared to that of the other two. However, all three complexes between 75 and approximately 200 μM Mg^{2+} reach a plateau of their relaxation times. What is notable about this $\bar{\tau}$ (which is approximately equal for all three) is that it is about twice as long (~125 μs) compared to that of pure chromatin optimally condensed by 250 μM Mg^{2+}. Thus, all three drugs at high drug to base pair ratios present considerable obstacles to the further folding of the chromatin. Beyond 250 μM Mg^{2+}, the distamycin and daunomycin complexes begin to aggregate in a manner similar to each other, much like chromatin itself. Even at this stage, the aggregating fibers are not compacted optimally, as can be seen from the field dependence and inverse-field dependence (Figures 5 and 6) of the dichroism amplitude of the drug–chromatin complexes at this ionic strength (250 μM Mg^{2+}), as compared to that of compacted chromatin in the same buffer. We have reported earlier that the dichroism amplitude of optimally compacted chromatin fibers reaches a plateau at fields equal to and above 15 kV/cm, corresponding to a saturation of the orientation of compacted fibers in the electric field. In contrast, the dichroism amplitudes for both drug complexes shown continue to rise with increasing field, and as such, this behavior is indicative of a greater "looseness" of the structure compared to that of the fully compacted fiber. In the drug–chromatin complexes, increasing alignment of segments of the relatively "loose" structure is possible with increasing orienting field, where in fully compacted chromatin this is no longer so.

Actinomycin shows a somewhat different behavior from distamycin and daunomycin, in that when a plateau for compaction is reached, the complex does not begin to aggregate as readily as the other complexes. This difference is probably simply related to actinomycin D being an uncharged molecule. Daunomycin and distamycin, upon binding to chromatin, would help to neutralize up to 10% of the available negative charges of the chromatin.

Measurements of $\bar{\tau}$ made at the adsorption wavelengths of the three drugs, 315, 440, and 470 nm for dis, ac, and da, respectively (Figures 3 and 4), at various concentrations of magnesium, yield numbers that agree very well with the $\bar{\tau}$ found at 266 nm. This is suggestive of the fact that the drugs are tightly bound to the chromatin, and while bound, their motions are coupled to those of chromatin itself, both in terms of local orientation of segments in the electric field to the overall tumbling of the chromatin fibers.

Conclusions

DNA-binding drugs as a class generally alter DNA function in vivo, affecting transcription, replication, or both. But in most cases it is difficult to establish firmly that their pharmacologic effects are the direct consequences of their interaction with DNA/chromatin, and concomitant interference with DNA function. At best, one can say that DNA and chromatin are primary potential targets for drugs such as actinomycin, daunomycin, and distamycin.

There is also difficulty in relating the in vivo effects of the drugs on DNA and chromatin to the effects observed in vitro. The differences in the two arise from the characteristic differences in the transport properties of living cells, and also their relative ability to metabolize the drugs (Goldberg et al., 1975).

Some of the conclusions we were able to draw about the effects of these drugs from various published studies made in vitro, as well as in prokaryotic and eukaryotic systems, are the following: For a given class of drugs, the effectiveness of a particular member is proportional to its binding constant to DNA (Canellakis & Bellantone, 1976; Canellakis et al., 1976; D. R. Phillips, D. Straney, and D. M. Crothers, unpublished results); the net concentration of a drug within the cell nucleus, as well as the ability of specific chromatins to bind or exclude certain drugs (Ringertz & Bolund, 1969; Pederson & Robbins, 1972), is are also likely to be important. The three drugs, distamycin, daunomycin, and actinomycin, in spite of their different modes of binding to DNA, all act to inhibit the transcription of DNA. Actinomycin and daunomycin do so primarily by inhibiting the elongation of nascent RNA (Gale et al., 1972; Müller & Crothers, 1968; DiMarco et al., 1975), while distamycin acts by inhibiting the initiation of transcription (Puschendorf & Grunicke, 1969; Puschendorf et al., 1976; D. R. Phillips et al., unpublished results).

While the effects of these drugs may be understood in terms of their interactions with naked DNA, it is obviously necessary to understand what further effects the drugs might have at the level of chromatin. We have seen from this study, and from the preceding paper (Sen & Crothers, 1986), that polyamines and drugs with cationic charges of three or more are effective at low concentrations in compacting and cross-linking chromatin. This is true not only in the low ionic strength regime of our experiments but presumably also at physiological salt, where electrostatic interactions are expected to remain of considerable importance. Thus, mono- or bis-intercalating drugs of high-binding affinity to DNA, which affect DNA function at the level of transcription, may prove to be significantly more effective in their function if they are provided with a sufficient cationic charge to effect changes in the chromatin of the target cell, causing compaction or clumping of regions of chromatin that are relatively decondensed for the purpose of transcription. Thus, the study of the effectiveness of analogues of a proven antitumor drug, or its bis analogue, with progressively added cationic groups is probably a worthwhile endeavor.

The structural effects in vitro on the folding equilibrium of chromatin brought about by the binding of daunomycin, distamycin, and actinomycin have a number of features in common. The limiting dichroism amplitude of the unfolded 10-nm fiber is lowered dramatically by the binding of these drugs. This can be attributed to increased local stiffness of the DNA produced by the drug binding, which results in a less complete orientation of the various flexible segments of the 10-nm fiber in the electric field. With progressively increasing magnesium concentration, the drugs do not prevent an initial folding of the fiber, but the most compact fiber produced in each case, at approximately 100 μM magnesium, is substantially less compact than optimally compacted chromatin. The drug–chromatin complexes at magnesium higher than 100 μM show rapid aggregation for the da and dis complexes and somewhat less dramatic aggregation for the ac complex. All of these semicompact fibers show a constantly increasing field dependence of their dichroism amplitudes,

unlike optimally compacted chromatin, and are thus more "distortable" than optimally compacted chromatin.

Registry No. BMSp, 70940-03-3; BMSpd, 93194-39-9; MSp, 86388-76-3; actinomycin D, 50-76-0; daunomycin, 20830-81-3; distamycin, 39389-47-4.

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Metal Ion Requirements and Other Aspects of the Reaction Catalyzed by M1 RNA, the RNA Subunit of Ribonuclease P from *Escherichia coli*†

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ABSTRACT: M1 RNA, the RNA subunit of ribonuclease P from *Escherichia coli*, can under certain conditions catalytically cleave precursors to tRNA in the absence of C5, the protein moiety of RNase P. M1 RNA itself is not cleaved during the reaction, nor does it form any covalent bonds with its substrate. Only magnesium and, to a lesser extent, manganese ions can function at the catalytic center of M1 RNA. Several other ions either inhibit the binding of magnesium ion at the active site or function as structural counterions. The reaction rate of cleavage of precursors to tRNAs by M1 RNA is enhanced in the presence of poly(ethylene glycol) or 2-methyl-2,4-pentanediol. Many aspects of the reaction catalyzed by M1 RNA are compatible with a mechanism in which phosphodiester bond cleavage is mediated by a metal ion.

The RNA subunit of ribonuclease P from *Escherichia coli*, M1 RNA, is a catalyst (Guerrier-Takada et al., 1983; Guerrier-Takada & Altman, 1984a). In vivo this enzyme is responsible for the maturation of the 5′ termini of tRNA molecules, cleaving P–O3′ bonds to produce 5′-phosphate and 3′-hydroxyl end groups. M1 RNA hydrolyzes phosphodiester bonds at specific sites in several different substrates, is unchanged during catalysis, and has a characteristic turnover number. The reaction of M1 with its substrates is different from the cleavage of RNA that is catalyzed by heavy-metal ions (Brown et al., 1983; Rubin & Sundaralingam, 1983) or by RNase A (Richards & Wycoff, 1971) during which P–O5′ bonds are cleaved via cyclic 2′,3′-phosphate intermediates. The self-splicing reaction of *Tetrahymena thermophila* pre-rRNA (Cech, 1983) is similar to cleavage by M1 in that P–O3′ bonds are cleaved and a divalent metal ion is required. However, M1 RNA produces a single cut in each substrate molecule,

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