Differences among H1 Histone Subfractions in Binding to Linear and Superhelical DNA

SEDIMENTATION VELOCITY STUDIES*

Louise W. Liao† and R. David Cole
From the Department of Biochemistry, University of California, Berkeley, California 94720

H1 histone subfractions exhibit differential abilities in aggregating superhelical DNA, as measured by sedimentation velocity analysis. In 0.15 M NaCl, all the calf thymus H1 subfractions bound to superhelical PM2 DNA to produce nonaggregated H1-DNA complexes as well as rapidly sedimenting, aggregated complexes. Notably, the distribution of the complexes between the nonaggregated and aggregated forms was a function of ionic strength and also depended on which H1 subfraction was complexed to the superhelical DNA. All of the H1 subfractions interacted preferentially with superhelical over relaxed PM2 DNA. The cooperative binding of H1 subfractions to linear T7 DNA produced only aggregated H1-DNA complexes in 0.15 M NaCl, while leaving some DNA free.

The compositional and structural variation between the subfractions of H1 histone serves as a basis for their differential effectiveness in H1-induced aggregation of superhelical DNA. The observed sensitivity of aggregation to NaCl concentration is interpreted in terms of a dependence on hydrophobic interactions, such as the proper folding of the particular H1 subfraction and intermolecular interactions between neighboring hydrophobic regions, as well as on nonspecific shielding of DNA charge. These sedimentation velocity analyses augment previous studies on the interactions of H1 subfractions with linear and superhelical DNA using circular dichroism, viscosity, and filter-binding analyses. The involvement of particular H1 and DNA conformations in producing the various types of H1-DNA interactions characterized in these studies might also apply to chromatin structure.

H1 histones, even when obtained from a single tissue, exhibit a heterogeneity in primary structure (1-3). Attempts to correlate the structural diversity of H1 with functional diversity have focused recently on artificial complexes between individual H1 subfractions and DNA (4-6). The functional diversity seen in these model systems demonstrated the potential for differential interactions with DNA in chromatin. They support the concept that multiple types of H1-DNA interactions can exist in vivo, influencing the degree of chromatin condensation.

We describe here a series of sedimentation velocity analyses of complexes prepared between calf thymus H1 subfractions and DNA, linear and supercoiled. This report characterizes further the H1-DNA interactions that have been examined for these same complexes by circular dichroism analysis (6), and extends our understanding of the functional diversity exhibited by complexes between H1 histones and DNA. These sedimentation velocity measurements allow us to analyze the roles of H1 cooperativity and H1-mediated DNA aggregation in the selective interactions between various kinds of H1 and DNA, which could not be done in the previous study that used the techniques of CD, viscosity, and filter binding.

EXPERIMENTAL PROCEDURES

Preparation of H1 Subfractions—H1 histone was extracted from steer thymus glands with 5% trichloracetic acid and fractionated by chromatography on Bio-Rex 70 as described previously (1). Protein concentration was determined by absorbance at 230 nm assuming ε280 = 18.5 and by amino acid analysis on a Beckman model 121 analyzer.

Radioactive H1 subfractions were prepared by the reductive methylation procedure described by Rice and Means (7), modified as follows. One mg of each H1 subfraction was dissolved in 0.2 M sodium borate buffer, pH 9, in a volume of 0.90 ml. Twenty-five μCi of [3H]formaldehyde (66.0 mCi/mm) were added to 0.40 ml of the pH 9 buffer. Each of the protein samples received 0.10 ml of the radioactive solution, followed 60 s later by four sequential additions, at 15-s intervals, of 2 μl of sodium borohydride (0.38 mg/ml). Each sample received a fifth addition of 10 μl at the next 15-s interval to ensure complete reduction of the formaldehyde. The entire labeling procedure was performed on ice. Samples were dialyzed extensively against 0.25 mM phenylmethylsulfonyl fluoride and then stored at 4 °C. The specific radioactivities of the purified methyl-[14C]-labeled H1 subfractions were 250 to 290 cpm/μg.

Preparation of DNA—Unlabeled DNA was prepared from T7 bacteriophage and PM2 bacteriophage as described previously (6). The DNA concentration was determined by absorbance at 260 nm, assuming ε260 = 200. To obtain radioactive DNA, the phage preparations were grown on thymidine auxotrophs and were labeled in vivo with [3H]thymidine (Schwarz Mann). The specific radioactivity of purified T7 [3H]DNA was 8 × 10⁶ cpm/μg. Purified PM2 [3H]DNA preparations had specific radioactivities of 1.8 to 1.5 × 10⁶ cpm/μg.

Preparation of Complexes of H1 Subfractions and DNA—Complexes were made by step gradient dialysis from a pH 7.6 buffer (5 mM Tris·HCl, 1 mM EDTA) containing the desired final salt concentrations as described previously (6). For all samples the DNA concentration was 100 μg/ml and the histone/DNA weight ratio was 0.4.

Assay for Contamination with Relaxed DNA—Contamination of supercoiled PM2 [3H]DNA with nicked, relaxed DNA was quantitated by assaying retained to nitrocellulose filters (Schleicher and Schuell, BAS, 0.45 μ) after alkaline treatment of the DNA, as described (5). PM2 DNA samples which were assayed within a week of preparation were found to be contaminated with 10 to 15% nicked, relaxed DNA. Samples which were stored at 4°C for 1 to 2 months were comprised of almost equal amounts of supercoiled and relaxed DNA.

Sedimentation Velocity Gradient Analysis—Following overnight

* This work was supported by United States Public Health Service Grants T01 GM 0031 and GM 20338, and by the Agricultural Research Station. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† Present address, Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, CO 80309.

(Received for publication, May 18, 1981)
dialysis in the final NaCl buffer, complexes of H1 with DNA were separated by sedimentation through metrizamide (Accurate Chemicals) gradients. Sample volumes of 0.2 ml were applied to 4.6 ml of linear 5 to 20% metrizamide gradients underlaid with 0.3 ml of 75% metrizamide cushions. All solutions contained 5 mM Tris-HCl (pH 7.6), 1 mM EDTA, and the appropriate NaCl concentration. Cellulose nitrate tubes were precoated with 0.1% gelatin to improve sample recoveries. Sedimentation velocity analyses were performed in a Beckman SW 50.1 rotor at 20,000 rpm at 20 °C for 65 min, essentially as described by Singer and Singer (8). Fractions of 4 drops (approximately 0.1 l) were collected from the bottoms of the tubes and analyzed in a liquid scintillation counter.

**Moving Boundary Sedimentation Analysis**—Sedimentation velocity studies were performed with a Beckman model E ultracentrifuge equipped with a split-beam photoelectric scanning absorption optical system (9). Complexes that had equilibrated overnight in the appropriate buffer were loaded into the sample compartments of double sector cells with 12-mm optical path lengths. Complexes were also diluted to final concentrations of 50 μg/ml and 25 μg/ml just before centrifugation. Samples were centrifuged in an An-F rotor at 28,000 rpm and 20.0 ± 1.0 °C. Scans were recorded every 4 min with the aid of a multiplexer accessory. Absorbance was monitored at 265 nm, a wavelength at which DNA absorbed strongly but at which H1 showed no detectable absorbance at the concentrations used. Corrections were made for radial dilution according to the method described by Schachman (10). Boundary positions were located at the half-concentration levels between the supernatant and plateau regions. Sedimentation coefficients were obtained from a linear least squares analysis of the logarithm of the radial position versus time for six to eight time values for each sample. Sedimentation coefficients are reported as measured and not corrected to water as solvent.

**Liquid Scintillation Counting**—Samples were counted in a Triton-toluene-based scintillation fluid. Quench and spillover were determined for each experiment and the appropriate corrections were made.

### RESULTS

**Interactions of H1 Subfractions with Linear DNA**—Complexes were formed by stepwise salt gradient dialysis of purified [methyl-14C]H1 subfractions and T7 [3H]DNA at a histone/DNA weight ratio of 0.4 and a DNA concentration of 100 μg/ml. (Expressed as a mole ratio, mole of H1 histone to mole of T7 DNA = 480.) After overnight dialysis against buffers containing 0.15 M NaCl for one set and buffers containing no NaCl for the second set, the complexes were characterized by sedimentation velocity analysis through metrizamide density gradients. The sedimentation profiles are shown in Fig. 1. Under the experimental conditions, free H1 remained at the top of the gradient, while free T7 DNA entered the gradient far enough to separate entirely from free H1. As seen in the figure, there was no free H1 in any of the samples; in every case, all of the protein was bound to T7 DNA.

![Fig. 1. Sedimentation velocity gradient profiles of complexes of H1 histone and T7 DNA.](image)

As shown in Fig. 1 (A to C), in the absence of NaCl the complexes CTL-1-T7 DNA, CTL-2-T7 DNA, and CTL-3-T7 DNA gave identical profiles. About 95% of [14C]histone and about 95% of the [3H]DNA were in a peak which sedimented, on the average, slightly faster than free T7 DNA. These are the results expected for a nonaggregated H1-T7 DNA complex. The profiles did not give sufficient resolution to determine the amount of free DNA in the sample; the position of free DNA would overlap that of the H1-DNA complex.

As shown in Fig. 1 (D to F), the complexes also gave identical profiles in 0.15 M NaCl. However, in contrast to the ones observed in the absence of salt, the profiles for complexes in 0.15 M NaCl revealed that the H1 sedimented to the bottom of the gradient. The DNA was distributed between two species; approximately 65% was found in the pelleted complex and approximately 35% was recovered as free DNA. Although our results do not demonstrate noncooperativity at low ionic strength, they are compatible with previous reports (11, 12) that the binding of H1 to linear duplex DNA underwent a transition from a noncooperative to a cooperative interaction as a function of ionic strength. The term "cooperative interaction" refers to the process by which all of the protein molecules bind to only a portion of the DNA molecules.

In order to use an experimental system having a wider range of sedimentation coefficients that could be resolved, duplicates of each of these complexes were sedimented in an analytical centrifuge. Complexes analyzed in the absence of NaCl gave sedimentation velocity patterns with single boundaries but ones which were not as sharp as those observed for free T7 DNA; this might indicate a heterogeneous population. The average S value for the DNA population was 35 S, which compares to a sedimentation coefficient of 30 S obtained for free T7 DNA.

The 12 sedimentation velocity profiles generated by complexes of CTL-1-T7 DNA, CTL-2-T7 DNA, and CTL-3-T7 DNA equilibrated at NaCl concentrations of 0.05, 0.10, 0.15, and 0.20 M were essentially identical. The sedimentation velocity profiles showed that two DNA species were present in each of the complexes. One species sedimented to the bottom of the cell before the first analysis with the photoelectric scanner could be made. It was calculated that the pelleted material had a sedimentation coefficient of 2,000 S. Clearly, this represented extensive aggregation, not merely the compaction of individual DNA fibers. The second DNA species co-sedimented with free T7 DNA. These results agree completely with those observed for sedimentation velocity analyses through metrizamide gradients.
Fig. 2 presents the results of a typical analytical ultracentrifuge run, showing a series of sedimentation velocity patterns obtained for the CTL-1-T7 DNA complex in 0.05 M NaCl. The boundary positions correspond to the sedimentation of free DNA. Already at zero time, chosen to be the time when the rotor speed reached two-thirds maximum, some DNA had pelleted to the bottom of the cell. The $A_{265}$ value of the applied sample was 1.9; since the $A_{265}$ value of the free DNA was 0.85, then slightly more than half of the total DNA was in the pelleted complex. A remarkable feature of these scans is the absence of any minor boundaries, that would indicate complexes of intermediate size. Therefore, the pelleted complex formed between CTL-1 and T7 DNA appears to be the result of a highly cooperative interaction. The analytical ultracentrifuge scans for CTL-2-T7 DNA and CTL-3-T7 DNA complexes also demonstrated the complete absence of intermediate species.

Interactions of H1 Subfractions with Superhelical DNA—

Complexes between [methyl-$^{14}$C]H1 and PM2 [3H]DNA were made in the exact manner as those made using T7 [3H]DNA. Unless stated otherwise, the PM2 [3H]DNA preparations were contaminated with 10 to 15% relaxed DNA. Controls of free H1, free PM2 DNA (85 to 90% supercoiled, 10 to 15% relaxed), and free PM2 DNA (55% supercoiled, 45% relaxed) were analyzed separately to determine their sedimentation positions. H1 sat at the top of the gradient, relaxed PM2 DNA barely entered the gradient, and supercoiled PM2 DNA migrated somewhat farther. Their positions are indicated by the arrows in Fig. 3A.

Sedimentation velocity gradient profiles for samples analyzed in the absence of salt are shown in Fig. 3 (A to C). CTL-1-PM2 DNA, CTL-2-PM2 DNA, and CTL-3-PM2 DNA equilibrated and analyzed in 0 NaCl; in panels D to F, complexes were equilibrated and analyzed in 0.15 M NaCl. The complexes characterized are CTL-1-PM2 DNA (A, D), CTL-2-PM2 DNA (B, E), and CTL-3-PM2 DNA (C, F). The arrows in panel A mark the positions of free supercoiled PM2 DNA (a), free relaxed PM2 DNA (b), and free H1 histone (c). Direction of sedimentation was from right to left.

formed similar types of complexes. Approximately 10% of the DNA population co-sedimented with free, relaxed DNA. About 80 to 85% sedimented with or slightly faster than free, supercoiled PM2 DNA, and a small amount of DNA material pelleted to the bottom of the gradient. The bulk of the protein material (90 to 95% of the recovered $^{14}$C counts) co-sedimented with supercoiled DNA at the position expected for nonaggregated H1-DNA complexes.

The results for complexes prepared and analyzed in 0.15 M NaCl are presented in Fig. 3 (D to E). Again, no free H1 was found. Unlike their behavior in the absence of salt, at this ionic strength CTL-1-PM2 DNA, CTL-2-PM2 DNA, and CTL-3-PM2 DNA complexes each gave a unique profile. For the CTL-1-PM2 DNA complex, 10 to 12% of the recovered DNA was free, relaxed DNA, 65 to 70% was histone-bound, nonaggregated, supercoiled DNA, and about 15% was pelleted material. About 60% of the CTL-1 protein was in the nonaggregated supernatant peak, while about 40% had pelleted. For the CTL-2-PM2 DNA complex, the types of species were similar, but the distribution shifted so that a slightly larger portion was in the pellet. The CTL-3-PM2 DNA complex showed a dramatic increase in the amount of the pelleted material. All of the CTL-3 and 85 to 90% of the PM2 DNA...
were at the bottom of the gradient. These data indicate that the different H1 subfractions possess different abilities to aggregate superhelical DNA. Their relative order of effectiveness is CTL-1 < CTL-2 < CTL-3.

In all of these analyses, the material migrating to a position representing histone-bound, nonaggregated, supercoiled PM2 DNA had a relatively constant ratio of $^3$H to $^{14}$C across the peak. This is indicative of complexes in which all of the nonaggregated supercoiled DNA molecules had H1 bound to them and that H1 bound in a noncooperative manner. At zero ionic strength, the sole interaction with supercoiled DNA was apparently a noncooperative one. At 0.15 M NaCl, the H1 subfractions interacted with supercoiled DNA to produce two types of complexes. The aggregated, pelleted complex was probably the result of a cooperative interaction.

The analyses shown in Fig. 3 (D, E) allow quantitations of the nonrandom distribution of H1 on DNA. Complexes were made at an input weight ratio of histone to DNA (total) of 0.4; thus, the input weight ratio of histone to DNA (supercoiled) was approximately 0.45. The weight ratios calculated for the CTL-1-PM2 DNA and CTL-2-PM2 DNA supernatant complexes were 0.3 and 0.2, respectively, and the pelleted material had a weight ratio of approximately 1.0. (A weight ratio of 1.0 corresponds to a +/− charge ratio of approximately 1 also, so the material would be expected to precipitate.)

Expressed in terms of moles, the input ratio corresponds to moles of histone to moles of DNA (supercoiled) = 140; the histone/DNA supernatant complex ratio = 60 to 95; the histone/DNA pelleted complex ratio = 310. For CTL-3-PM2 DNA in 0.15 M NaCl (Fig. 3F), the ratio in the pellet was essentially the same as the input ratio. Since the sample had reached equilibrium before it was analyzed, no assessment is possible for whether cooperative binding promoted the aggregation.

The effect of ionic strength on the ability of the H1 subfractions to aggregate PM2 DNA was studied further. In addition to the analyses at 0 and 0.15 M NaCl, complexes were analyzed in 0.05 and 0.10 M NaCl. Fig. 4 presents the salt dependence of the distribution of DNA species in the complexes. DNA complexed to CTL-1 exhibited only a small increase in the amount of pelleted material as a function of ionic strength. The effect was slightly more pronounced with DNA formed in a complex with CTL-2. For the CTL-3-PM2 DNA complex, DNA was highly dependent upon the ionic strength; in the transition from 0.05 to 0.10 M NaCl, the DNA fraction shifted from a predominantly nonaggregated supernatant form to one that was almost entirely aggregated. Fig. 5 presents the salt dependence of the distribution of the H1 histone subfractions in the same complexes. Their distributions follow the general patterns observed for DNA. However, a close examination shows that a comparatively larger fraction of the protein was found in pelleted complexes. This is interpreted to be a manifestation of H1 cooperativity, whereby the protein bound extensively to only a portion of the DNA molecules. We suggest that cooperative binding led to the formation of large aggregates.

These studies indicated that H1 histones bound selectively to supercoiled PM2 DNA, leaving relaxed PM2 DNA free. The only possible exception to a selective interaction was observed for CTL-3-PM2 DNA at 0.10 and 0.15 M NaCl, in which cases all the DNA sediments as large aggregates, obscuring any selectivity that might have occurred. Selectivity was investigated further by use of a PM2 $[^{3}H]$DNA preparation that was 55% supercoiled and 45% relaxed; this allowed for substantial competition between the two kinds of DNA. Complexes were made at a histone/DNA weight ratio of 0.4 and a total DNA concentration of 100 μg/ml, as in previous experiments. In these competition experiments, it was found that at all NaCl concentrations the H1 species in CTL-1-PM2 DNA and CTL-2-PM2 DNA complexes had bound exclusively to supercoiled PM2 DNA. With increasing NaCl concentrations there was an accompanying small increase in the amount of DNA.
of pelleted material. This was exactly as observed with complexes made with PM2 DNA preparations which were 85 to 90% supercoiled DNA. In competition experiments with CTL-3-PM2 DNA complexes at 0 and 0.05 m NaCl, the same exclusive interaction with supercoiled PM2 DNA was exhibited. At 0.10 m NaCl, CTL-3 pelleted all of the supercoiled DNA and part of the relaxed DNA. At 0.15 m NaCl, CTL-3 pelleted the total PM2 DNA population. Therefore, it can be concluded that all of the H1 subfractions exhibited a preferential interaction with superhelical DNA over relaxed DNA and that CTL-3 had the greatest ability to pack superhelical DNA into large aggregates.

CTL-1-PM2 DNA, CTL-2-PM2 DNA, and CTL-3-PM2 DNA complexes were characterized further in the analytical ultracentrifuge at ionic strength values of 0, 0.05, 0.10, and 0.15 m NaCl. Two DNA species sedimented slowly enough for their sedimentation velocity patterns to be followed, and the third species migrated to the bottom of the cell before the first pattern could be recorded. The distribution of DNA among these three species was the same as observed when identical complexes were analyzed by sedimentation in metrizamide gradients. This confirmed the previous results. The slowest species had a sedimentation coefficient of 23 ± 1 S and was the free, relaxed PM2 DNA. The DNA species migrating slightly faster had a sedimentation coefficient of 33 ± 4 S and was the histone-bound nonaggregated supercoiled PM2 DNA. The value for the pelleted material was calculated to be >2,000 S. Such a large value precludes the possibility that the pelleted material could have represented collapsed individual DNA molecules; rather, these must have been very large aggregates. There was never any DNA species detected with sedimentation coefficients intermediate between 33 S and 2000 S. Although the data are not shown, the base-lines and plateau regions in the sedimentation velocity patterns were as flat as those recorded for complexes between H1 and T7 DNA. The extent of heterogeneity in the pelleted material could not be measured however, it can be concluded that H1 binding caused an abrupt transition of the DNA from a nonaggregated state to an extensively aggregated one.

**DISCUSSION**

Linear DNA bound all H1 subfractions cooperatively at ≥0.05 m NaCl. Renz and Day (12) reported comparable studies on complexes between unfractionated H1 and linear DNA. In a more detailed analysis of salt dependence, they observed a transition from noncooperative to cooperative binding in the range of 0.02 to 0.04 m NaCl. Consistent with the results reported here, they observed that above the transition most of the DNA sedimented rapidly, with H1 attached while the remainder sedimented as free DNA. An apparent discrepancy between the two studies is that the sedimentation coefficients reported for the rapidly sedimenting material are different. Renz and Day observed that the sedimentation coefficient increased from 83 S to about 1000 S with an increase in NaCl concentration from 0.04 to 0.25 m NaCl, while we observed a sedimentation rate corresponding to >2000 S for similar ionic values. We propose that the discrepancy is due to the different methods employed for complex preparation. For the study conducted here, complexes were prepared by slow salt gradient dialysis from 0.5 m NaCl to the final salt concentration, followed by overnight dialysis to ensure that H1-induced DNA conformational changes reached equilibrium. Renz and Day, however, prepared their H1-DNA complexes by direct mixing in the ultracentrifuge cell, followed immediately by sedimentation velocity analysis. It seems likely that their results included kinetic effects on the salt-dependent aggregation. This notion is supported by the results of Malarska and colleagues (13) who investigated the methods used to prepare H1-DNA complexes. They found that slow salt gradient analysis led to formation of rapidly sedimenting complexes with a sedimentation coefficient of approximately 5000 S. When they passed through the range of 0.42 to 0.34 m NaCl rapidly (1 min), the resultant complexes sedimented with intermediate S values.

Supercoiled PM2 DNA interacted with H1 histones differently than did linear DNA. Although a salt-dependent transition from an apparent noncooperative to a cooperative interaction occurred, a major fraction of the superhelical DNA was bound to H1 without being aggregated, whereas in the case of linear DNA all of the H1-DNA complexation led to aggregation. Evidence that this phenomenon was due principally to the superhelical nature of PM2 DNA, and not to differences with T7 DNA in size, (A + T) content, or some other factor, comes from studies by Singer and Singer (8). They observed that complexes between unfractionated H1 and supercoiled SV40 DNA sedimented as two types of complexes, a nonaggregated complex and a rapidly sedimenting, aggregated complex. Preparations of H1 and linear or relaxed, closed circular SV40 DNA sedimented as free DNA and H1-DNA aggregates.

Other workers (14) have prepared complexes between unfractionated H1 and supercoiled DNA by direct mixing immediately prior to sedimentation velocity analysis and observed intermediate-sized species in 0.05 m NaCl buffers. In the present work, all complexes were prepared by salt gradient dialysis, and no species of sizes intermediate between 33 S and 2000 S were observed at any salt concentration tested. We suggest that a relatively slow kinetic process leads to the formation of large aggregates, and that an early phase of that process was monitored by those investigators, whereas a late phase was observed in our studies.

H1 histones bound selectively to supercoiled PM2 DNA, as compared with relaxed PM2 DNA. In competition experiments between supercoiled SV40 DNA and relaxed SV40 DNA, a similar selective aggregation of the supercoiled form by unfractionated H1 was reported by others (15). These sedimentation velocity analyses complement filter binding studies by Vogel and Singer (16, 17), who found that unfractionated H1 preferentially retained supercoiled DNA on filters, as compared with relaxed circular DNA or linear DNA, and that the degree of the retention increased with increasing superhelicity, regardless of whether the superhelicity was negative or positive.

The present sedimentation velocity analysis showed that H1 subfractions differ among themselves in their interactions with supercoiled DNA. The relative order of effectiveness of calf thymus H1 histones in aggregating DNA into rapidly sedimenting complexes was the same as reported earlier for their effectiveness in distorting the circular dichroism spectrum of supercoiled DNA (6). Previously, differences were found between rabbit thymus subfractions in their abilities to retain supercoiled DNA onto nitrocellulose filters (5). In the case of linear DNA, sedimentation velocity analyses failed to disclose differences between H1 subfractions in their interactions with the DNA, but previous investigations by CD analysis (4, 6) and filter binding (5) demonstrated that they differ among themselves in binding to linear DNA as well.

Increasing ionic strength promoted the histone-induced aggregation of superhelical DNA, and the sensitivity of the aggregation to salt depended on which H1 subfraction was complexed to the DNA (Fig. 4). Presumably, one factor in this salt dependence is the folding of H1 which Smerdon and Isenberg (18) have shown as an ionic strength-dependent transition occurring at slightly different salt concentrations.
from one subfraction to the next. However, the ionic strength dependence of subfraction folding does not match very well the dependence of H1-induced aggregation of superhelical DNA observed in the present work. The discrepancy might include the effect of salt in shielding charges on DNA, but the magnitude of the difference between H1 subfractions in their abilities to aggregate DNA suggests that the shielding factor is not dominant. Note that complexes with CTL-1 and CTL-2 require the binding of about half the input H1 to aggregate about 15% of the PM2 DNA even with the help of 0.15 M NaCl. In contrast, at only about 0.06 M NaCl, the binding of about half the input CTL-3 would have aggregated about half of the DNA. Charge shielding must occur but it appears to be a minor effect under our conditions. It might be pertinent to add that Stratling (19) found upon increasing the ionic strength an H1-dependent contraction of oligonucleosomes did not show this behavior, the nonspecific shielding of DNA charge does not appear to be an adequate explanation.

Another possible factor in the salt dependence of superhelical DNA aggregation by H1 is the stabilization of hydrophobic interactions between hydrophobic regions of different H1 molecules. We favor this notion, in which the coalescence of nonpolar cores of H1 molecules could produce cooperative; noncovalent cross-linking of neighboring DNA strands or neighboring folds along individual strands. The types of aggregation and condensation clearly differ between linear and superhelical DNA and might well differ again with nucleosomal bound DNA. H1-H1 interactions within chromatin could be the basis for the homopolymers of H1 produced by chemical cross-linking (20, 21).

The nonpolar globular region of the H1 molecule, which we speculate to be involved in noncovalent cross-linkage of H1-DNA fibers, has been demonstrated to dominate the interactions of H1 and DNA as studied by filter binding and circular dichroism (22, 23). However, since its amino acid sequence is invariant among subfractions, an interplay between different regions of H1 must be invoked to explain the variation among H1 subfractions in their condensation of DNA as revealed by circular dichroism, viscosity, sedimentation, and perhaps by filter binding. Most of the amino acid sequence differences that have been detected thus far between H1 subfractions of rabbit or calf thymus are confined to a short stretch of the NH2-terminal region which is immediately followed by the highly conserved nonpolar region. The sequence differences could modulate the folding of the globular region or directly modulate the coalescence of two globular regions, or both. In the studies presented here, CTL-3 was the most effective subfraction in aggregating supercoiled DNA. We speculate that the NH2-terminal region of this particular subfraction permitted a more efficient coalescence of globular regions than was possible for CTL-1 or CTL-2. We suggest that the differences among H1 subfractions in their interactions with DNA may also apply to their interactions with chromatin.

REFERENCES

1. Kinkade, J. M., and Cole, R. D. (1966) J. Biol. Chem. 241, 5798-5805
2. Bustin, M., and Cole, R. D. (1968) J. Biol. Chem. 243, 4500-4505
3. Panyim, S., and Chalkley, R. (1969) Biochem. Biophys. Res. Commun. 37, 1042-1049
4. Welch, S. L., and Cole, R. D. (1979) J. Biol. Chem. 254, 662-665
5. Welch, S. L., and Cole, R. D. (1980) J. Biol. Chem. 255, 4518-4518
6. Liao, L. W., and Cole, R. D. (1981) J. Biol. Chem. 256, 6531-6535
7. Rice, R. H., and Means, G. E. (1971) J. Biol. Chem. 246, 831-832
8. Singer, D. S., and Singer, M. P. (1978) Biochemistry 17, 2096-2096
9. Schachman, H. K., and Edelstein, S. J. (1966) Biochemistry 5, 2681-2705
10. Schachman, H. K. (1969) Ultracentrization in Biochemistry, pp. 70-75. Academic Press, New York
11. Renz, M. (1975) Proc. Natl. Acad. Sci. U. S. A. 72, 733-736
12. Renz, M., and Day, L. A. (1976) Biochemistry 15, 3229-3232
13. Malarska, K., Pucienniczak, A., and Skowronski, J. (1979) Biochim. Biophys. Acta 561, 324-333
14. D'Anna, J. A., Sistrone, G. F., and Gurley, L. R. (1979) Biochemistry 18, 943-951
15. Bottger, M., Scherneck, S., and Fenske, H. (1976) Nucleic Acids Res. 3, 419-429
16. Vogel, T., and Singer, M. F. (1975) Proc. Natl. Acad. Sci. U. S. A. 72, 2597-2600
17. Vogel, T., and Singer, M. F. (1976) J. Biol. Chem. 251, 2334-2338
18. Smerdon, M. J., and Iseberg, I. (1976) Biochemistry 15, 4233-4242
19. Stratling, W. H. (1979) Biochemistry 18, 596-603
20. Hardison, R. C., Zeithier, D. P., Murphy, J. M., and Chalkley, R. (1977) Cell 12, 417-427
21. Ring, D., and Cole, R. D. (1979) J. Biol. Chem. 254, 11688-11695
22. Fasman, G. D., Valenzuela, M. S., and Adler, A. J. (1971) Biochemistry 10, 3795-3801
23. Singer, D. S., and Singer, M. S. (1976) Nucleic Acids Res. 3, 2531-2547

\(^2\) M. W. Hsiang and R. D. Cole, unpublished results.