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Studies on the interaction of H1 histone with superhelical DNA: Characterization of the recognition and binding regions of H1 histone[1]

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ABSTRACT

The very lysine rich histone, H1, isolated from a variety of sources interacts preferentially with superhelical DNA compared to relaxed DNA duplexes[1-3]. The nature of this specific interaction has been investigated by studying the ability of various purified fragments of H1 histone from calf thymus to recognize and bind superhelical DNA. The data suggest that the globular region of the H1 histone molecule (amino acid residues 72-106) is involved in the recognition of superhelical DNA. Thus, the H1 histone carboxy-terminal fragment, 72-212, resembles native H1 histone both quantitatively and qualitatively in its ability to discriminate between and bind to superhelical and relaxed DNA while the H1 histone carboxy-terminal fragment, residues 106-212, has lost this specificity, binding superhelical and relaxed DNA equally well. Furthermore, under conditions in which the globular region of the intact H1 histone has been unfolded, the molecule loses its ability to discriminate between superhelical and relaxed DNA, and binds both forms of DNA equally.

INTRODUCTION

Of the five major histone fractions present in the chromatin of most eukaryotic cells, four appear to be associated in a core about which the DNA is wrapped[2-4]. This unit of chromatin, commonly called a nucleosome or nu body, consists of two molecules each of the arginine-rich and moderately lysine-rich histones (H3, H4, H2a, and H2b) and is associated with 140-200 base pairs of DNA[5]. The structural relationship of the very lysine-rich histone H1 to this fundamental unit of chromatin is poorly understood and its function completely unknown. Whereas histones H2a, H2b, H3 and H4 have remained remarkably constant in structure throughout eukaryote evolution, the primary sequence of H1 histone has undergone extensive interspecies and interorgan variation as a result of both conservative and non-conservative amino acid replacements[6]. Some species are known to have as many as five H1 histone subfractions within a single organ; the other four classes of histones have no more than two or three[6]. Furthermore, H1 histone is modified through phosphorylation[7]. Distinct sites for

phosphorylation of H1 are reported to be associated with various stages in the cell cycle and hormonal stimulation[7-10].

Calf thymus H1 histone, a protein of 212 amino acid residues with a molecular weight of 21,000, is highly asymmetric with respect to both the distribution of basic amino acid residues and its secondary structure[11,12]. The molecule has a short highly basic tail at the amino terminal end and a globular central core region containing a relatively high proportion of the hydrophobic amino acid residues; the carboxy terminal half of the molecule consists largely of alanine, proline, and lysine residues[12].

Recent reports from this laboratory indicated that H1 histone from a variety of sources interacts with superhelical DNA in preference to relaxed DNA duplexes, and that the efficiency of the binding of DNA to H1 increases with the negative or positive superhelical density of the DNA molecules[13-15]. The other four major classes of histones do not distinguish between the relaxed and superhelical forms of DNA[13]. Böttger and co-workers have confirmed these observations[16].

In this report we present experiments indicating that the globular region of the calf thymus H1 histone molecule is involved in the recognition of superhelical DNA. Various polypeptide fragments of the H1 molecule have been purified and their ability to interact with superhelical and relaxed DNA has been studied.

MATERIALS

All DNA preparations were simian virus 40 labeled with ^{14}C-thymidine. Closed circular duplex superhelical DNA (DNA I) was isolated from purified virions and then purified through two cycles of ethidium bromide-CsCl banding[17] The DNA I sample was divided into aliquots, precipitated with ethanol and stored at -20°C. Prior to use, a single aliquot of precipitated DNA was centrifuged for 1 hour at 10,000 rpm, resuspended in 0.01 X SSC (1.5 mM sodium chloride, 0.15 mM sodium citrate) containing 1 mM EDTA, and dialyzed against the same solution. By handling the DNA I in this way there is less than 10% contamination with nicked, relaxed DNA.

Relaxed closed circular duplex DNA (DNA I^r) was prepared from DNA I by treatment with mammalian DNA-relaxing enzyme[18,19] as follows. DNA-relaxing enzyme was partially purified from mouse LA-9 cells according to the procedure of Germond *et al.*[19], by Dr. M. Bina-Stein. One µl of enzyme extract was used to relax 3 µg of DNA I. A reaction mixture of 160 µg of DNA I in 0.05 M Tris·HCl, pH 7.8, 1 mM EDTA, 0.1 M NaCl, and 1 mg/ml bovine serum albumin (BSA) in a total volume of 0.6 ml was incubated without

DNA-relaxing enzyme for 30 minutes at 23°C. The appropriate volume of enzyme was then added and the reaction mixture incubated for an additional hour at 23°C. The reaction was stopped by adding sodium dodecylsulfate to a final concentration of 1%. The reaction mixture was extracted with an equal volume of buffer saturated phenol. The phenol phase was re-extracted with buffer and the two supernatant fluids combined. The DNA I^r was precipitated with ethanol and stored at -20°C. The reaction product was characterized by agarose gel electrophoresis[20]. Under these reaction conditions all the DNA I was converted to the relaxed closed circular form, DNA I^r.

The specific activity of the two forms of DNA was 11,309 cpm/µg. A single preparation of DNA was used throughout these experiments.

Calf thymus H1 histone was obtained from Worthington Biochemical Corp. and was further purified by gel filtration through a 1.9 cm x 40 cm column of Biogel P-60. Column elution was with 0.05 M NaCl, 0.02 N HCl, 0.02% sodium azide, according to Böhm et al.[21]. The flow rate of the column was 6 ml/hr; 0.6 ml fractions were collected. Peak fractions from the column were pooled and precipitated with 50 volumes of 0.05% HCl-acetone at -20°C. The precipitate was washed twice with acetone, dried in a desiccator, and resuspended in water. The concentration of H1 histone was determined from the absorbance at 230 nm (absorbance of 1 is equivalent to 4.25 mg/ml)[22]. Analysis of this material by gel electrophoresis gave only the expected doublet protein band characteristic of calf thymus H1 histone. ^{3}H-labeled H1 histone was prepared as previously described[15].

METHODS

N-Bromosuccinimide Cleavage of Calf Thymus H1 Histone. Histone H1 contains a single tyrosine at amino acid residue 72[24,25] which can be cleaved by N-bromosuccinimide (NBS)[11]. A modification of the procedure of Bustin and Cole[11] was used for the present work. Calf thymus H1 histone (0.1 mole), purified as described above, was suspended in 0.4 ml of 50% acetic acid. To this, 6 µmoles of NBS (Sigma Chem. Co.) freshly dissolved in 0.4 ml of 50% acetic acid (2.7 mg/ml NBS) were added. The reaction mixture was incubated for 4 hours at 22°C.

The reaction products were separated by gel filtration on Biogel P-60 as described above. The elution of polypeptides was monitored at 230 nm and 260 nm. NBS cleavage of tyrosine results in the formation of a dienone spirolactone which absorbs at 260 nm. Therefore, the amino-terminal fragment (residues 1-72) can be distinguished from the carboxy-terminal fragment (residues 73-106) by absorbance of the amino-terminal fragment at 260 nm.

The Biogel P-60 column elution profile of the NBS-reacted H1 is shown in Fig. 1. Each of the two peaks corresponding to the fragments was pooled and repurified on another Biogel P-60 column. Gel electrophoresis of the separated fragments following the second column chromatography did not reveal any cross contamination (Fig. 2). Amino acid analyses (data not shown) of the two fragments were consistent with the analyses reported by Bustin and Cole[11].

Chymotrypsin Cleavage of Calf Thymus H1 Histone. Calf thymus H1 histone has a unique initial cleavage site for α-chymotrypsin on the carboxy-terminal side of the single phenylalanine residue in the middle of the H1 molecule[26,27]. The cleavage procedure used in these studies is a modification of that described by Bradbury et al.[27]. To a 10 mg/ml solution of calf thymus H1 histone (purified as described above) in 0.05 M Tris·HCl, pH 8.0, α-chymotrypsin (Sigma) was added to a final concentration of 0.02 mg/ml. The reaction mixture was incubated for 10 min. at 26°C. Under these conditions most of the H1 was cleaved into two half-molecules. After longer times of incubation, further digestion of the amino terminal fragment was observed.

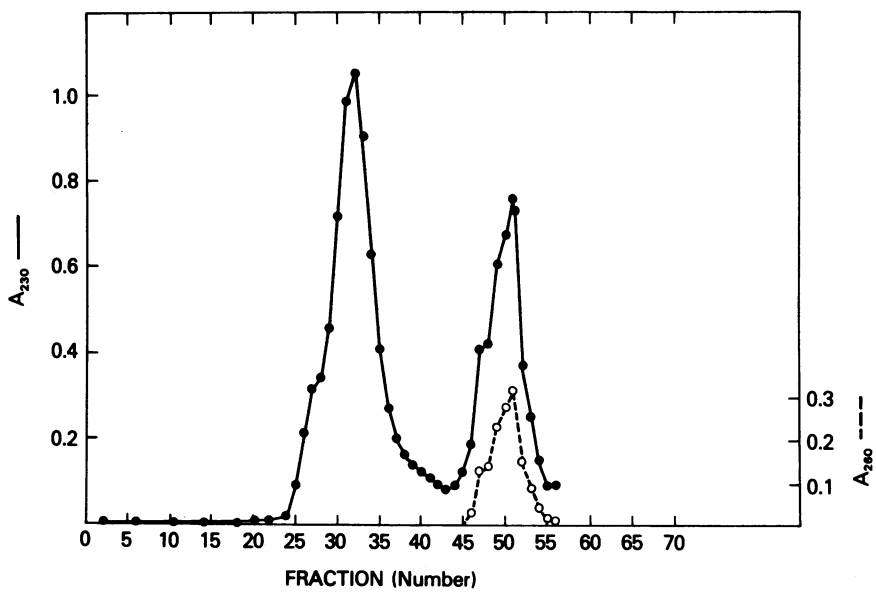

Fig. 1 Separation of NBS digestion products of H1 histone on Biogel P-60. H1 histone was cleaved with NBS and the products resolved on a Biogel P-60 column as described in the section on methods. The first peak contains the carboxy-terminal fragment, residues 73-212; the second peak contains the amino-terminal fragment, residues 1-72.

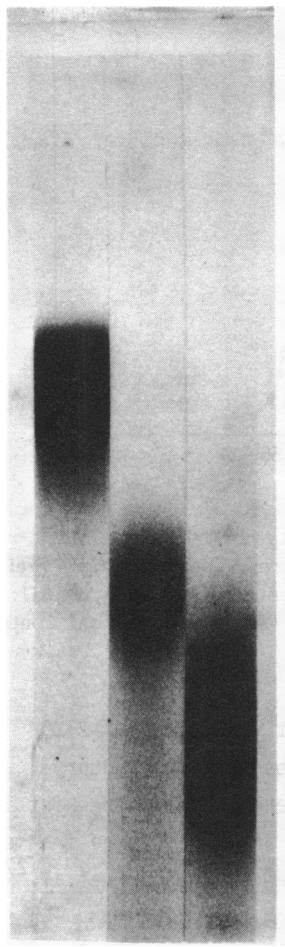

Fig. 2 Gel electrophoresis of purified NBS fragments. Following an initial separation on Biogel P-60 column, each of the two fragments was rechromatographed on the same column. Pooled fractions were subjected to gel electrophoresis as described in the section on methods. The gels were intentionally overloaded (50 μg/gel) to reveal any minor cross contamination. a) Intact H1; b) carboxy-terminal fragment 73-212; c) amino terminal fragment 1-72.

Following exposure to α-chymotrypsin, the reaction mixture was adjusted to pH 2 by the addition of HCl. The digest was then layered onto a Biogel P-60 column. The fragments were eluted as described above. The elution profile of the digest from the column is shown in Fig. 3. The material in the first peak is unreacted histone H1. The material in the second peak is the carboxy-terminal fragment (residues 107-212). The material in the third peak is the amino-terminal fragment (residues 1-106). Each of the fragments was further purified on Biogel P-60 columns. Gel electrophoresis of the separated fragments did not reveal any cross con-

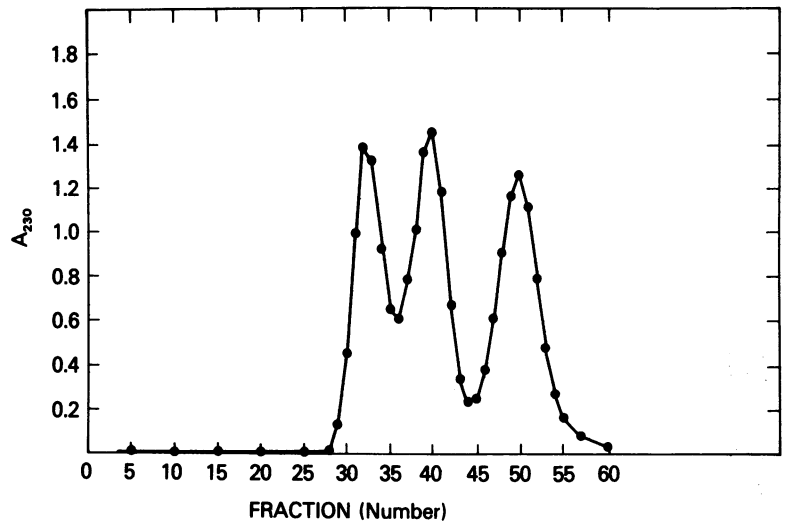

Fig. 3 Elution profile of chymotrypsin digestion products of H1 on Biogel P-60. H1 histone was cleaved with chymotrypsin and the products resolved on a Biogel P-60 column as described in the section on methods. The first peak is unreacted H1; the second peak contains the carboxy-terminal fragment 107-212; the third peak contains the amino-terminal fragment 1-106.

tamination (Fig. 4). The carboxy-terminal fragment (residues 107-212) ran as a doublet (data not shown), as previously observed by Bradbury et al.[27] this presumably reflects the microheterogeneity known to exist in the calf thymus H1 histone fraction[24]. Amino acid analyses of the fragments (data not shown) were consistent with those reported by Bradbury et al.[27] and verified the identification of the fragments.

Binding Assay. Interaction between DNA and H1 histone or H1 fragments was measured by determining the protein-dependent accumulation of ^{14}C-labeled DNA on nitrocellulose filters, as described previously[13]. Standard reaction mixtures (0.1 ml) contained 50 mM Tris·HCl pH 7.8, 1 mM EDTA, 1 mg/ml BSA, 0.1 M NaCl, histone (or fragments) and ^{14}C-labeled DNA as indicated in the text. The mixture was incubated at 23°C for 15 min, and was then diluted with 2 ml of the buffer mixture used for the reaction (50 mM Tris.HCl pH 7.8, 1 mM EDTA, 0.1 M NaCl), filtered, and the filter washed four times with the same solution prior to determining the radioactivity bound to the filter.

The results are expressed as the percentage of input counts retained on the filter as a function of the R value. The R value is defined as the molar ratio of protein to DNA (DNA is expressed as molecules of DNA, not

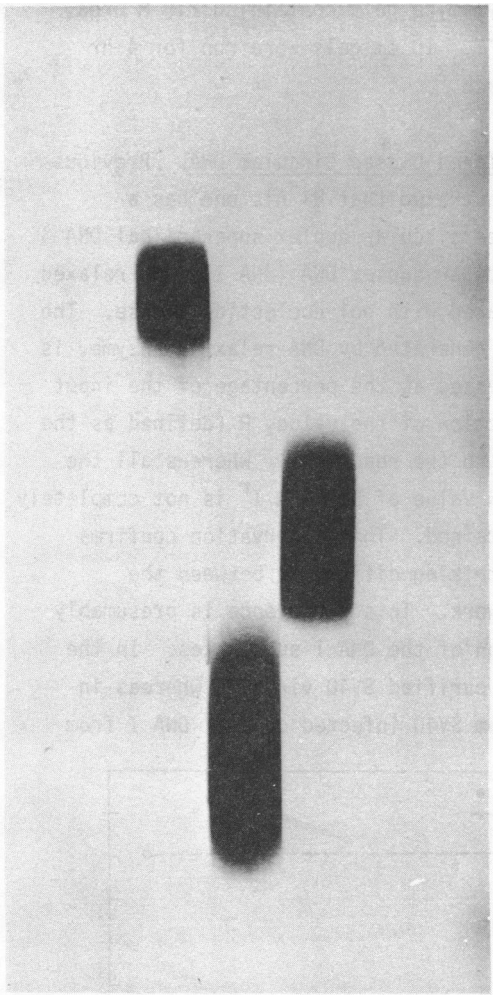

Fig. 4 Gel electrophoresis of purified chymotrypsin fragments. Procedure was as described for Fig. 2. The gels were intentionally overloaded (50 μg/gel) to reveal any minor cross contamination.

nucleotides). Molecular weights of 21,000 for H1 histone, 6,000 for amino-terminal fragment (residues 1-72), 15,000 for carboxy-terminal fragment (residues 73-212), 10,500 for both amino-terminal fragment (residues 1-106) and carboxy-terminal fragment (residues 107-212), and 3.6×10^6 for SV40 DNA[28] were used to calculate the R value. In all cases, the concentration of DNA used was approximately 1 μg/ml; the H1 histone concentration ranged from 0.01 to 0.5 μg/ml.

In the range of R values studied here, H1 forms largely soluble complexes with DNA I. However, at high R values (around 100), H1 histone forms large aggregates with DNA[16], (D. Singer, unpublished observations).

Gel Electrophoresis. 15% polyacrylamide gels containing 2.5 M urea, 0.9 N acetic acid were used throughout[23]. 10 cm gels were run for 4 hr at 150 volts and stained with amido black[27].

RESULTS

Interaction of H1 Histone With Relaxed Closed Circular DNA. Previous studies from this laboratory[13-15] demonstrated that H1 histone has a specific ability to interact with closed circular duplex superhelical DNA (DNA I), compared to either nicked circular duplex DNA (DNA II), or relaxed closed circular duplexes (DNA I^r) prepared with polynucleotide ligase. The interaction of H1 histone with DNA I^r, generated by DNA-relaxing enzyme, is shown in Fig. 5. The results are expressed as the percentage of the input DNA which is bound to filters as a function of the value, R (defined as the molar ratio of H1 histone to DNA added to the reaction). Whereas all the DNA I is retained on the filter at an R value of 50, DNA I^r is not completely retained until an R value of 300 is attained. This observation confirms previous results[15], indicates a more striking difference between the two DNA molecules than in our earlier work. This difference is presumably due to the difference in the preparation of the DNA I substrates. In the present study DNA I was extracted from purified SV40 virions, whereas in earlier studies DNA I was extracted from SV40 infected cells. DNA I from

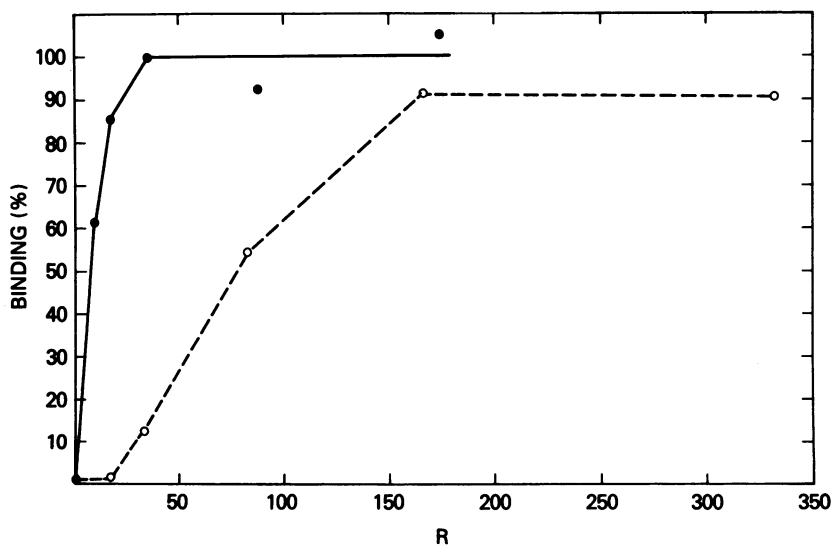

Fig. 5 H1 histone dependent accumulation of ^{14}C-DNA I and ^{14}C-DNA I^r on nitrocellulose filters. R is the molar ratio of H1/DNA. The concentration range of H1 used was 0.05-1.0 μg/ml; ^{14}C-DNA was approximately 1 μg/ml. DNA I. —— ; DNA I^r - - - -.

virions has a higher average superhelical density and a smaller distribution of superhelical turns than does DNA I from infected cells[29]. Therefore, there is a larger difference between the superhelical densities of the DNA I and DNA I^r preparations used in this study than in previous ones.

Interaction of Amino-Terminal Fragments of H1 Histone With DNA I and DNA I^r. The two purified amino-terminal fragments resulting from the NBS and chymotrypsin cleavages were examined for their ability to interact with DNA I and DNA I^r by the nitrocellulose filter technique. The extent of binding of DNA by both amino-terminal fragments 1-72 and 1-106 is markedly reduced compared to the binding by native H1 at comparable R values (Table I). The amino-terminal fragment 1-72 does not complex DNA I^r at the highest concentration tested (R = 568) and only a small amount of DNA I is complexed at an R of 323. The amino-terminal fragment 1-106 complexes DNA I significantly but only at high R values, and has only a very limited ability to complex DNA I^r.

Interaction of Carboxy Terminal Fragments of H1 Histone With DNA I and DNA I^r. Unlike the amino-terminal fragments, both carboxy terminal fragments complexed DNA efficiently (Fig. 6). The carboxy-terminal fragment

TABLE I

The Binding of Amino Terminal Fragments to DNA I and DNA I^r

	DNA I		DNA I^r	
Fragment Conc. (µg/ml)	R	Binding (%)	R	Binding (%)
Amino-terminal Fragment (1-72)				
0	0	0.9	0	0.4
0.05	32.5	1.9	28.6	-
0.10	64.6	1.5	56.9	0.3
0.20	129.2	2.5	113.7	0.3
0.50	322.9	7.2	284.3	0.5
1.0	-	-	568.5	1.0
Amino-terminal Fragment (1-106)				
0	0	0.3	0	0
0.04	14.6	2.8	10.6	0.5
0.08	29.2	6.8	21.1	2.8
0.10	36.5	10.7	26.4	0.2
0.20	73.1	21.6	52.8	4.6
0.50	182.7	24.4	131.9	4.7

†R is the molar ratio of fragment/DNA.

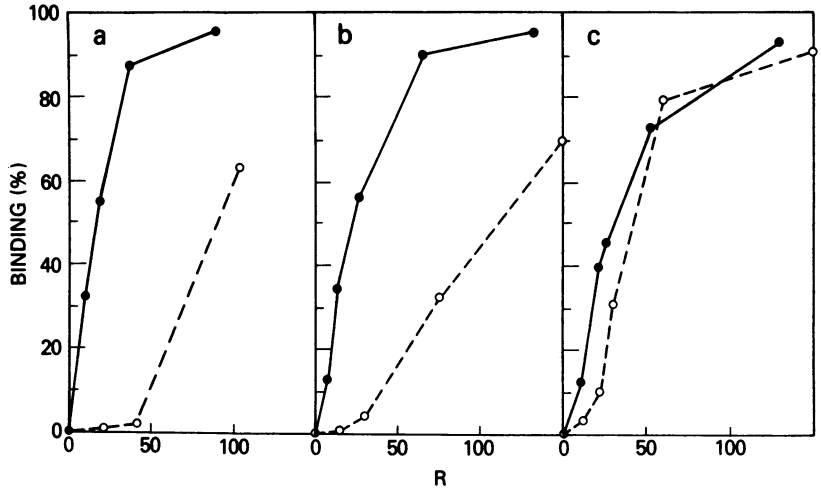

Fig. 6 Binding of H1 and H1 carboxy terminal fragments to DNA I and I^r. The procedure was as described in the section on methods. DNA I: —; DNA I^r: - - -. a) Carboxy-terminal fragment 73-212; b)intact H1; c) carboxy-terminal fragment 107-212.

73-212 complexed DNA I completely at an R value of 52, whereas DNA I^r is not completely complexed until an R value of 260 (Fig. 6a). The carboxy-terminal fragment 73-212 therefore resembles native H1 histone (Fig. 6b) both qualitatively and quantitatively in its ability to discriminate between and bind to superhelical and relaxed DNA. On the other hand, the carboxy-terminal fragment 107-212 binds both forms of DNA equally well; virtually all of the DNA I or DNA I^r is complexed at an R value of about 75 (Fig. 6c). The carboxy-terminal fragment 107-212 apparently has lost the specificity for superhelical DNA molecules and binds DNA I^r with increased efficiency relative to native H1 histone.

Binding of NBS Fragments to Nitrocellulose Filters. It has been demonstrated previously[14] that nitrocellulose filters quantitatively retain ^{3}H-H1 histone in the concentration range used in these studies. The possibility that the differential ability of the various fragments of H1 to complex DNA results from a differential retention by the nitrocellulose filters was investigated. ^{3}H-H1 histone was cleaved with NBS and the two tritium labeled fragments isolated in the same way as before. Various concentrations of the fragments were incubated in a mock binding experiment (standard binding buffer without DNA), and filtered as usual. The results (Table II) demonstrate that over a concentration range of 0.1

TABLE II

The Binding of N-Bromosuccinimide Fragments of ^{3}H-Hl Histone to Nitrocellulose Filters

Fragment Concentration (μg/ml)	^{3}H-Fragment 73-212 Bound cpm	%	^{3}H-Fragment 1-72 Bound cpm	%
0.1	313	80.9	352	98.4
0.5	312	80.6	338	94.0
1.0	293	75.0	336	93.4
2.0	287	73.2	310	85.3
0.5 μg unfiltered	378	100	357	100

^{3}H-Hl (specific activity 800 cpm/μg) was cleaved with NBS and the fragments purified as described in Methods. The fragment concentration was calculated from the specific radioactivity, assuming that the specific radioactivity of the fragments was the same as that of the starting Hl. 0.5 μg of each of the fragments was diluted in the appropriate volume of binding buffer to give the concentrations listed above. The samples were incubated, filtered, washed and counted as in a standard binding assay. 0.5 μg (in 5 μl of binding buffer) was applied directly to a filter, without washing and served as the control for 100% binding.

to 2.0 μg/ml both the carboxy-terminal fragment 73-212 and the amino-terminal fragment 1-72 are quantitatively retained on nitrocellulose filters.

Effect of Salt Concentration on the Interaction of DNA With Hl and Hl Carboxy-Terminal Fragments. Previous work in this laboratory demonstrated that Hl interacts with superhelical DNA optimally at 0.1 M NaCl[15]. This is confirmed by the data in Fig. 7a. Fig. 7a also shows that the extent of interaction of Hl histone with DNA I^{r} (at a constant R value) decreases with increasing salt, as would be expected for a purely electrostatic interaction. The effect of salt concentration on complex formation between the carboxy-terminal fragments and both DNA I and DNA I^{r} was also examined (Fig. 7). The reaction between the carboxy-terminal fragment 73-212 and DNA I was optimal between 0 and 0.1 M NaCl; complex formation with DNA I^{r} decreased with increasing salt concentration (Fig. 7b). Thus the salt dependencies of the reaction with Hl histone and with carboxy-terminal fragment 73-212 are similar. The results obtained with carboxy-terminal fragment 107-212 are markedly different (Fig. 7c). In this case, binding of both DNA I and DNA I^{r} by the carboxy-terminal fragment 107-212 was highest in 0.2 M NaCl. Consistent with the observed loss of specificity for superhelical DNA at 0.1 M NaCl, the carboxy-terminal fragment 107-212 does not distinguish between superhelical and relaxed DNA at any salt concentration tested. It is surprising that this fragment, which is approximately one third lysine, should bind DNA optimally at 0.2 M NaCl. However,

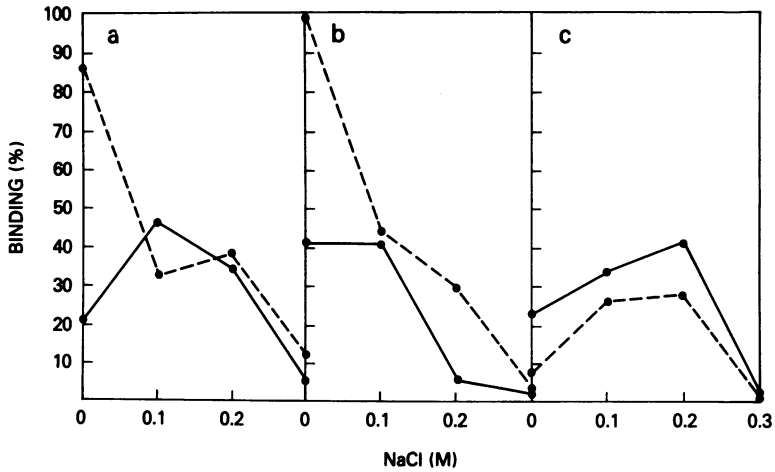

Fig.7 Binding of H1 and H1 carboxy terminal fragments to DNA as a function of salt concentration. H1 and fragments, at constant R values, were incubated in 10 mM Tris, pH 7.8, 1 mg/ml BSA, 1 mM EDTA with either DNA I or DNA I^r at various NaCl concentrations for 15' at 23°. R values for DNA I were: fragment 73-212, 14.8; H1, 15.2; fragment 107-212, 30.4. R values for DNA I^r were: fragment 73-212. 92.4; H1, 95.2; fragment 107-212, 38.0. DNA I:—; DNA I^r:- - -. a) intact H1; b)carboxy-terminal fragment 73-212; c) carboxy-terminal fragment 107-212.

it is known from NMR studies that in the absence of salt, 15% of the lysine residues of this fragment are not bound to DNA, whereas at 0.15 M NaCl, all lysines are bound[27]. Bradbury et al. proposed[27] that at 0.15 M NaCl, intermolecular cross-linking of linear DNA molecules by the carboxy-terminal fragment 107-212 can occur, mediated by the lysine residues which remained unbound in the absence of salt. Alternatively, it is possible that increasing the salt concentration induces a conformational change in the fragment which enables more effective binding of the DNA. The superhelicity of the DNA molecule should not be significantly affected over this range of concentration. It should also be noted that the carboxy-terminus of the intact molecule may also bind DNA optimally at 0.2 M NaCl but that this effect would go largely undetected, due to the marked optimum at 0.1 M NaCl.

Interaction of H1 Histone With DNA in the Presence of Urea. The observation that the carboxy-terminal fragment containing amino acid residues 107-212 has lost the ability to discriminate between superhelical and relaxed DNA, while the carboxy-terminal fragment 73-212 retains this ability, suggested that the region of the native H1 molecule between residues 73 and 106 is involved in the recognition of superhelical molecules.

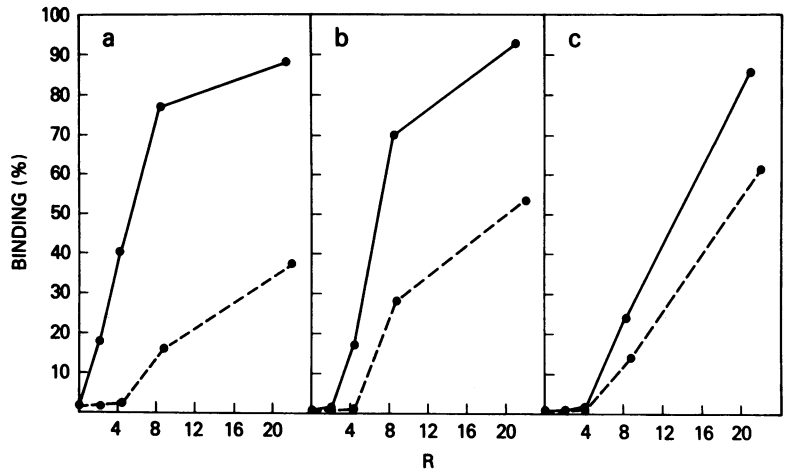

Fig. 8 Binding of H1 to DNA in the presence of urea. Filter binding assays were done as described, except that urea was added to the incubation mixture before the addition of either H1 or DNA. DNA I: —; DNA I^r: - - -. a) no urea; b) 4 M urea; c) 6 M urea.

It is known from both NMR and CD studies that this region of the molecule is globular and contains α-helical structure[12]. If the observed loss of specificity for superhelices results from a loss of the structure in this hydrophobic region, a similar loss might be expected when the binding assay was performed under conditions that denature apolar regions of H1. Bradbury et al.[12] have demonstrated that between 4 and 6 M urea, in 0.15 M NaCl, nearly all of the globular structure of the H1 molecule is disrupted. Therefore, native H1 was tested for its ability to complex DNA I and DNA I^r in the presence of 4 M (Fig. 8b) and 6M (Fig. 8c) urea. The results clearly demonstrate that at both urea concentrations tested, H1 is able to complex DNA. However, as the urea concentration is increased, the H1 molecule loses its ability to discriminate between superhelical and relaxed DNA. At low R values, the binding of H1 to DNA I decreases with increasing urea concentration, whereas at higher R values, the binding of H1 to DNA I^r increases. The net effect is that in 6 M urea, the binding of H1 to DNA I^r is nearly the same as to DNA I. This is the same result as that obtained with the carboxy-terminal fragment 107-212 in the absence of urea (Fig. 6c).

Since urea is known to denature DNA (unwind duplexes), it is possible that some of the effect seen in Fig. 6c results from changes in the superhelical density of DNA I and DNA I^r. Therefore, we studied the relative sedimentation rates of DNA I and DNA I^r compared to nicked relaxed circular

duplex SV40 DNA in sucrose gradients in the presence of 6 M urea. The results (not shown) indicated that there were no gross changes in the relative superhelical densities of either DNA I or DNA I^r.

DISCUSSION

H1 histone extracted either by salt or acid from a variety of tissues, including calf thymus, rat hepatoma cells, cultured monkey cells (BSC-1), chicken erythrocytes and HeLa cells, interacts with superhelical DNA in preference to relaxed DNA duplexes[14,15] (M. Bina-Stein et al, manuscript in preparation; unpublished observ.). Here we have presented experiments suggesting that the globular region of the molecule is involved in determining this specificity. The carboxy-terminal fragment of the molecule, containing residues 73-212, retains both the ability to recognize superhelical DNA and to bind DNA. It is known from other studies that this fragment contains 1) a globular region similar to that in the intact H1, and 2) a non-globular, highly lysine rich region, extending from approximately residue 123 to the carboxy-terminus at residue 212[30]. On the other hand, the carboxy-terminal fragment, containing residues 107-212, which has lost most of the globular region of the intact H1 molecule, binds DNA but no longer distinguishes between superhelical and relaxed DNA. It binds both forms of DNA equally and with an efficiency somewhat lower than that with which the intact H1 molecule binds superhelical DNA. Therefore, while the globular region of the H1 molecule extending from amino acid residues 73 to 106 confers on the H1 molecule the ability to recognize superhelical DNA, it also appears to limit the ability of the molecule to interact with relaxed DNA duplexes. Since the carboxy-terminal fragment 107-212 binds DNA, the binding of the DNA molecule appears to be predominantly mediated by the lysine rich portion of the molecule beyond residue 107.

Results consistent with this model were obtained by studying the interaction of H1 and DNA in 6M urea, where the globular structure of the protein is denatured. In this case, the H1 molecule lost the ability to recognize superhelical DNA (at low R values, see Fig. 8) and gained enhanced ability to complex relaxed DNA (at higher R values, see Fig. 8).

The amino-terminal fragment containing residues 1-106 does bind to DNA, albeit with a low efficiency, and, moreover, displays specificity for superhelical DNA. It is known that the amino terminal end of the molecule contains a small lysine rich stretch from residues 1 to 40[25]. It is possible that this cationic region can interact with DNA and stabilize the

recognition of superhelical DNA by the globular region. In the absence of any globular region (amino-terminal fragment 1-72), this lysine rich portion does not interact efficiently with either DNA I or DNA I^r, under the conditions used here.

The salt dependency curves for both intact H1 and the fragment containing residues 73 to 212 suggest that interaction with DNA I^r is purely electrostatic, whereas interaction with DNA I is more complex. The requirement for 0.1 M NaCl for optimal binding of H1 and fragment 73-212 to DNA I may reflect a salt-induced change in the conformation of the H1 molecule related to its ability to recognize superhelical DNA.

Recent experiments (D. Singer, unpublished) indicate that the complexes formed when H1 interacts with DNA I differ from those formed when H1 interacts with DNA I^r. Velocity sedimentation gradient analyses of the H1-DNA complexes demonstrate that at low H1/DNA ratios (w/w), H1 primarily forms a soluble complex with DNA I, but forms only large aggregates with DNA I^r.

These results suggest that the interaction of H1 with superhelical DNA has two components: 1) recognition of superhelicity by the globular region of the molecule extending from amino acid residues 73-106, and 2) interaction between the DNA and the lysine rich portion of the molecule beyond amino acid residue 106. The overall reaction may also involve a conformational change in the H1 protein such as to stabilize the superhelical turns in the DNA. It should be noted that while this discussion does not imply any particular structural description of superhelical turns, it does suggest that a physical conformation characteristic of superhelicity is recognized by H1. Partial single-strandedness, known to exist in superhelices[31,32], is not likely to be involved since H1 binds much more poorly to single-stranded DNA than to double-stranded DNA [13,35].

It is interesting to note that whereas there is considerable variation in the amino acid sequence of different H1 histones in the regions of amino acid residues 1-40 and 110-212, the amino acid sequence of the globular region of the molecule is nearly invariant (R. Cole, personal communication). Therefore, it may be that recognition of superhelical configurations of DNA by H1 is an important component of the physiological role of H1 histone. This speculation is of particular interest in view of recent proposals for chromatin structure, all of which involve some sort of folding or supercoiling of the DNA duplex about the nucleosome. The nature of the interaction of H1 histone with nucleosomes is currently being investigated.

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a similar placement of phenylalanine in this histone (34) but the precise position is not known. In this paper, the phenylalanine position is referred to as position 106 by analogy with the RTL-3 fraction. The position of the single tyrosine residue in the calf thymus H1 is at residue 72 (11).
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