Preferential Binding of H1e Histone to GC-Rich DNA†

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ABSTRACT: We have investigated the binding of a pure H1 histone, the mouse variant H1e, to a 214 bp fragment of DNA from pBR322. Binding was monitored by observing the effects of the protein on melting of the DNA and by a gel-mobility-shift assay. We found, using this highly purified system, that H1e protein binds preferentially and cooperatively to the GC-rich region of the DNA. A chemically synthesized peptide containing 25 residues, corresponding to a region of the carboxyl-terminal domain of H1e, shows the same sequence preference but does not exhibit cooperativity.

The H1 histones, found in most eukaryotes, bind to DNA and, along with the core histones, help it to compactly fold. In the cell, H1 histones play important roles in maintenance, and perhaps induction, of particular levels of chromatin condensation. The H1 histones (and the core histones as well) are generally not considered to bind to DNA with absolute sequence specificity. In their roles as packaging proteins they are assumed to bind readily to many different sequences of DNA. While H1 histones would not be expected to exhibit absolute specificity for one particular sequence, they might show clear sequence preferences arising from the sequence-dependent structural properties of DNA. DNA of different sequences may differ in the width of the major and minor grooves, flexibility, and in electrostatic properties (Pullman, 1981). These features could dictate preferential binding by histones. For several sequences of DNA, for example the 5S ribosomal gene of sea urchin, it has been shown that reconstituted nucleosomes are not randomly distributed on the sequence; rather, they form on specific regions of the DNA (Simpson & Stafford, 1983). Poly(dA)–poly(dT), a polynucleotide known to adopt an unusual right-handed structure, is refractory to formation of nucleosomes (Rhodes, 1979; Kunkel & Martinson, 1981). Other nucleic acid binding proteins, such as DNAse I, also show such "structural" specificity (Lahm et al., 1991). In the past two decades there have been sporadic, and contradictory, reports concerning the sequence preference for the DNA binding of H1 histones (Sponar & Sormová, 1972; Hwan et al., 1975; Renz & Day, 1976; Sevall, 1988; Izaurralde et al., 1989; Suzuki, 1989; Churchill & Suzuki, 1989; see Discussion). In order to clarify the confusion surrounding this issue, we have studied the binding of a specific variant of H1, H1e, to a well-defined, homogeneous fragment of DNA, by monitoring the effects of the protein on the melting of the DNA and by a gel-mobility-shift assay. This system allows us, unambiguously, to separate histone H1 binding from coupled condensation phenomena and provides a clear and direct view of preferred histone H1 binding to GC-rich DNA sequences.

We have also studied the binding of a synthetic peptide whose sequence is found in the carboxyl terminus of H1e. This domain, which has been shown to bind to DNA, is a region of considerable sequence variability among the H1 variants and so could contribute to any differences among the H1 variants in binding to DNA.

MATERIALS AND METHODS

Preparation of DNA. The DNA fragment used in these studies is a 214-base-pair fragment from the plasmid pBR322. Its sequence extends from position 4340 to position 192 and includes the promoter and the transcription start site of the gene that confers tetracycline resistance. 214-mer DNA was synthesized by amplification of the region of pBR322 between base pairs 4340 and 192, using the GeneAmp kit from Perkin Elmer Cetus. The primers were 20 nucleotides long. The amplified 214-mer was dialyzed against 15 mM sodium phosphate buffer, pH 7.0, with 1 mM EDTA (BPE) to remove unincorporated nucleotides. Primers were removed either by chromatography through Sepharose 4B or through Qiagen columns (using gravity flow). Purified 214-mer was dialyzed against BPE.

Purification of Histone H1e. H1e protein (for sequence, see GenBank accession no. L04141) was purified from murine erythroleukemic or BALB/3T3 cells, using reverse-phase HPLC as described (Brown & Sittman, 1993). The concentration of the protein was calculated from its absorbance in water at 205 nm, using $E_{205}^{1\ mg/mL}$ of 31, which was determined from the formula $E_{205}^{1\ mg/mL} = 27.0 + 120\ A_{280}/A_{205}$ (Scopes, 1987).

Synthesis and Purification of Histone H1e Peptide. H1e peptide is a 25 amino acid peptide from the carboxyl terminus of the mouse H1e protein, corresponding to residues 173–197; its sequence is KKAKATKAKKAPKSPAKAKTVKPKA. It is to the carboxyl-terminal side of the highly conserved sequence S(T)PKKAKKP. H1e peptide was synthesized on an Applied Biosystems Model 430 automated peptide synthesizer using standard cycles for *t*-BOC chemistry. The peptide was cleaved from the resin with HF, lyophilized, and dissolved in 10 mM triethylammonium bicarbonate buffer, pH 8. After being desalted on a Sephadex G-10 column, peptide was purified by HPLC on a Vydac C-18 column, 10-μm particle size (The Separations Group, Hesperia, CA). The purified peptide was analyzed by mass spectrometry (Multiple Peptide Systems, San Diego, CA). The molecular weight was 2606, as predicted.

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Thermal Denaturation of DNA. The 214-mer samples used in thermal denaturation studies were in 15 mM sodium phosphate buffer, pH 7.0, and 1 mM EDTA (BPE), at concentrations ranging from 210 to 230 nM (molecule). Small volumes of concentrated solutions of H1e protein or peptide were added to give protein– or peptide–214-mer samples. Thermal denaturation of samples was monitored by measuring their absorbance at 270 nm. Samples were heated at a rate of 1 °C per minute in a Perkin Elmer Lambda 3 spectrophotometer with temperature programmer. The spectra were collected on a Bascom-Turner recorder and digitized using SigmaScan (Jandel). First derivatives of the spectra were calculated and smoothed.

CD Spectra. CD spectra of 214-mer and of H1e–214-mer complexes were measured in a Jasco J-500 spectrophotometer, in a cell with a pathlength of 1 cm. The scan speed was 10 nm/min and the time constant was 8 s. Four repetitive scans were collected and averaged. The protein–DNA solutions used to measure the spectra in BPE were the same as those used in melting experiments; because of absorbance by buffer components, CD spectra were not measured below 220 nm. To form ψ-DNA from the H1e–214-mer sample, 5 M NaCl was added to a final concentration of 0.14 M.

Mobility-Shift Gels. Aliquots of samples used in thermal denaturation experiments were run in mobility-shift gels. Mobility-shift gels of H1e protein complexes were 6% polyacrylamide (acrylamide:bisacrylamide, 30:0.8); gels of H1e peptide complexes were 7.5% polyacrylamide. Gels were run at 4 °C in 3.3 mM sodium acetate, 6.7 mM Tris, and 1 mM sodium EDTA, pH 7.8 (Varshavsky, 1987). Gels were stained with ethidium bromide and photographed. DNA bands were quantitated by scanning densitometry of negatives of stained gels.

RESULTS

The DNA fragment that was used in these studies exhibits distinctive melting behavior (Figure 1A, spectrum 1): it has two sharp helix–coil transitions, with T_m values of 68.5 and 72.5 °C, and a third, minor, transition, with a midpoint that is between those of the two major transitions. The presence of three melting transitions has been confirmed using differential scanning calorimetry. Studies of the melting of 214-mer at different wavelengths, and of the effects of the DNA-binding drugs actinomycin and netropsin on its melting, confirm that the transition at 68.5 °C is melting of a relatively AT-rich portion of the DNA, and that the transition at 72.5 °C is melting of a relatively GC-rich portion of the DNA (L. Falzon, J. C. Dabrowiak, S. E. Wellman, and J. B. Chaires, unpublished experiments). Analysis of the sequence of 214-mer shows that there are GC-rich and AT-rich regions: there is an abrupt change in the average base content at position 101, from about 40% GC base pairs for the region from 4340 to 100, to about 60% GC base pairs for the region from 101 to 192.

Binding of H1e Protein. Thermal denaturation studies of the 214-mer in the presence of increasing amounts of pure histone H1e show unambiguously that the protein binds preferentially to the GC-rich region of 214-mer. At molar ratios of 0.5, 1, or 1.5 (H1e:214-mer), H1e protein did not discernibly affect the transition with a T_m of 68.5 °C, ascribed to the AT-rich region of the DNA (Figure 1A, spectra 2–4; Figure 1B). At these concentrations, however, the transition with a T_m of 72.5 °C (ascribed to the GC-rich region) was decreased in magnitude, and two additional transitions appeared, with T_m values of 77.5 and 81.8 °C. The melting

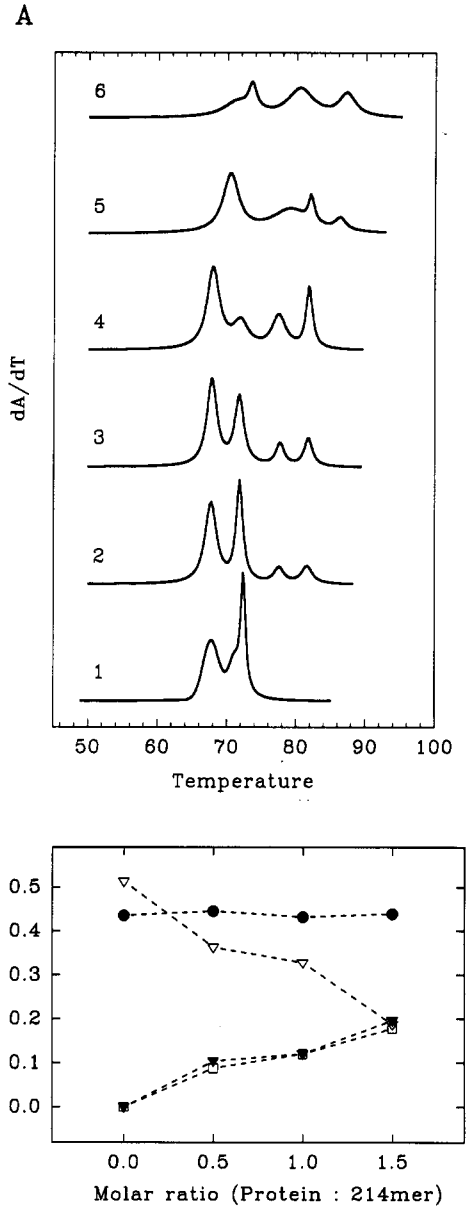

FIGURE 1: Melting of 214-mer in the presence of H1e protein. The samples were in BPE, and the 214-mer concentration was between 210 and 230 nM (see Materials and Methods). (A) First derivatives of the spectra are presented. (1) 214-mer; (2–6): H1e protein and 214-mer at a molar ratio of (2) 0.5, (3) 1, (4) 1.5, (5) 2, or (6) 3. (B) Change in absorbance for each transition, expressed as a fraction of the total change in absorbance for the melt, plotted against molar ratio (H1e protein:214-mer). [Filled circles, transition at 68.5 °C (AT-rich DNA alone); open triangles, transition at 72.5 °C (GC-rich DNA alone); filled triangles, transition at 77.5 °C; and open squares, transition at 81.8 °C (H1e–214-mer complex)].

behavior of 214-mer in the presence of H1e is unusual in that there are no intermediate transitions evident between the transition at 72.5 °C and those at 77.5 and 81.8 °C. Noncooperative ligand binding to DNA is expected to result in broad, biphasic melting transitions at less than saturating amounts of ligand (McGhee, 1976). The absence of these intermediate transitions indicates cooperativity in the protein–DNA interaction; that is, the 214-mer is either entirely free of H1e protein, or two regions are fully saturated with H1e protein resulting in the transitions at 77.5 and 81.8 °C. The transition at 81.8 °C most probably corresponds to the 72.5 °C transition seen in the absence of H1e, while the 77.5 °C transition corresponds to the transition seen as a pronounced shoulder on the 72.5 °C peak in the absence of H1e (Figure

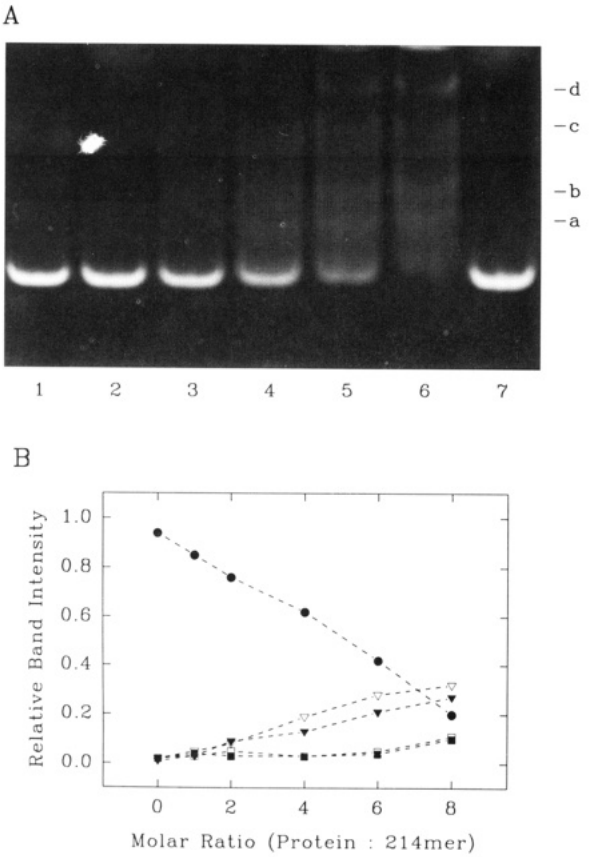

FIGURE 2: Mobility shift of 214-mer–H1e protein complexes. Aliquots of samples from thermal denaturation experiments were run in the gel (see Materials and Methods). (A) Lanes 1 and 7, 214-mer; lanes 2–6: H1e protein and 214-mer at a molar ratio of (2) 1, (3) 2, (4) 4, (5) 6, or (6) 8. The four bands that can be resolved in addition to free DNA are marked a, b, c, and d. (B) Quantitation of band density. Data are plotted as the fraction of total band density versus molar ratio (protein:DNA). (Filled circles, free DNA; open triangles, band a; closed triangles, band b; open squares, band c; closed squares, band d).

1A, spectrum 1). The melting behavior that we observed is reminiscent of that observed by Snyder et al. (1989) in a simpler system, that of the cooperative binding of actinomycin to a deoxyoligonucleotide. They observed no intermediate transitions over a range of drug concentrations, but only transitions arising from unligated and fully ligated species.

At higher concentrations of H1e protein, the melting profile became more complex and the 68.5 °C transition was also altered, indicating that H1e protein also binds to the AT-rich region once the preferred sites within the GC-rich region are fully saturated (Figure 1A, spectra 5–6).

Mobility Shift by H1e Protein. Binding of H1e protein to the 214-mer could also be seen in mobility-shift gels (Figure 2). At the two lowest concentrations of H1e protein, two bands corresponding to complexes were present (bands a and b, visible in Figure 2A, lane 3). At increased concentrations of protein, two additional bands (bands c and d) were present (Figure 2A, lanes 4–6). There may be additional complexes that do not enter the gel; ethidium bromide staining can be seen in the wells, especially in lanes 5 and 6. Identification of the stoichiometry within each shifted band of the gel is especially complicated for H1–DNA interactions. H1 proteins that are bound to DNA may interact (Thomas et al., 1992), resulting in the formation of aggregates. Furthermore, one H1 may be able to bind to two strands of DNA; it has been reported that H1 histones bind to circular DNA with several supercoils in preference to the relaxed form, which was interpreted as a preference for crossovers of the DNA (Krylov et al., 1993). For these reasons it is not possible to assign each band in this gel to a specific complex with any certainty nor to use these data for quantitative estimation of binding constants.

The observed behavior of H1e and DNA in mobility-shift gels is qualitatively consistent with cooperative binding inferred from melting studies. A key point emerging from these mobility-shift data is that, at H1e:214-mer ratios of less than or equal to 6, only free DNA or two bound species exist. Species with intermediate mobilities are not observed, as would be expected for noncooperative binding (and as is seen for the H1e peptide; see below).

CD Spectra of H1e–214-mer Complexes. The H1 histones can produce a condensed form of DNA, the liquid crystalline ψ-DNA (Fasman et al., 1970). It has been reported that either salts or dioxane must be present for this condensation to be produced by H1 histones (Adler & Fasman, 1971). In BPE, the solution that we have used for all melting experiments, the salt concentration is 15 mM, which should be too low for ψ-DNA to form. Nevertheless, in order to show that the phenomenon that we studied was binding of H1 histone and not the linked phenomenon of DNA condensation, we measured the CD spectra of 214-mer and of H1e–214-mer complexes in different solutions. As can be seen in Figure 3A, the CD spectrum of 214-mer with H1e bound, in BPE, was essentially the same as that of 214-mer alone. The negative ellipticity between 220 and 235 nm is due to H1e. In Figure 3B are shown the CD spectra of the same samples with NaCl added to a concentration of 0.14 M. In the absence of H1e, there was no change in the spectrum of 214-mer. If H1e was also present, however, the spectrum that is characteristic of condensed ψ-DNA was produced.

Binding of H1e Peptide. Thermal denaturation studies of the 214-mer in the presence of increasing amounts of H1e peptide show that the peptide, like H1e protein, binds preferentially to the GC-rich region of 214-mer. At a molar ratio of 1 (peptide:214-mer), H1e peptide did not affect the 68.5 °C transition but caused the 72.5 °C transition to become broader (Figure 4A, spectrum 2). At molar ratios of 3, 5, and 7 (peptide:214-mer), both of the transitions were altered (Figure 4A, spectra 3–5). The first transition broadened, and its T_m shifted by 1–3 °C. The second transition continued to broaden, and its T_m shifted by 5–8 °C (Figure 4B). In contrast to binding of H1e protein to 214-mer, binding of H1e peptide to 214-mer resulted in broad, multiphasic transitions, as would be expected if no cooperative interactions occur upon binding of the peptide to the DNA. At the highest concentration of peptide that was used, a molar ratio of 20 (1 peptide/10.7 bp), the two transitions that were apparent had T_m values of 79.5 and 85 °C.

Mobility Shift by H1e Peptide. Mobility-shift gels confirmed that H1e peptide binds to double-stranded 214-mer (Figure 5). Discrete bands corresponding to peptide–DNA complexes were not resolved in the peptide mobility-shift gel, in sharp contrast to the results obtained on the whole H1e protein.

DISCUSSION

Our studies have shown that the histone H1e binds preferentially to a GC-rich region of a homogeneous 214 bp fragment of DNA. Reports in the literature concerning sequence preference of H1 histones are contradictory, with several authors reporting that H1 histones preferentially bind to AT-rich sequences of DNA (Sponar & Sormová, 1972;

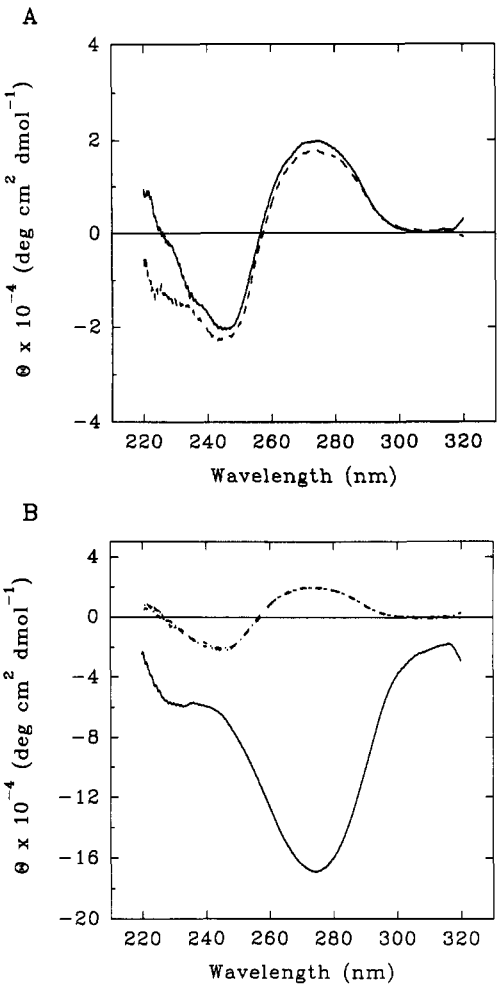

FIGURE 3: CD spectra. The 214-mer concentration was between 210 and 230 nM (see Materials and Methods). (A) Spectra of 214-mer and 214-mer–H1e protein complexes in BPE. Solid line, 214-mer; dashed line, H1e protein and 214-mer at a molar ratio of 8. (B) Same samples as in panel A, with the addition of NaCl to a final concentration of 0.14 M. Dashed line, 214-mer (214-mer in BPE is overlaid for comparison); solid line, H1e protein and 214-mer at a molar ratio of 8. Note that the *Y*-axes in panels A and B differ in scale.

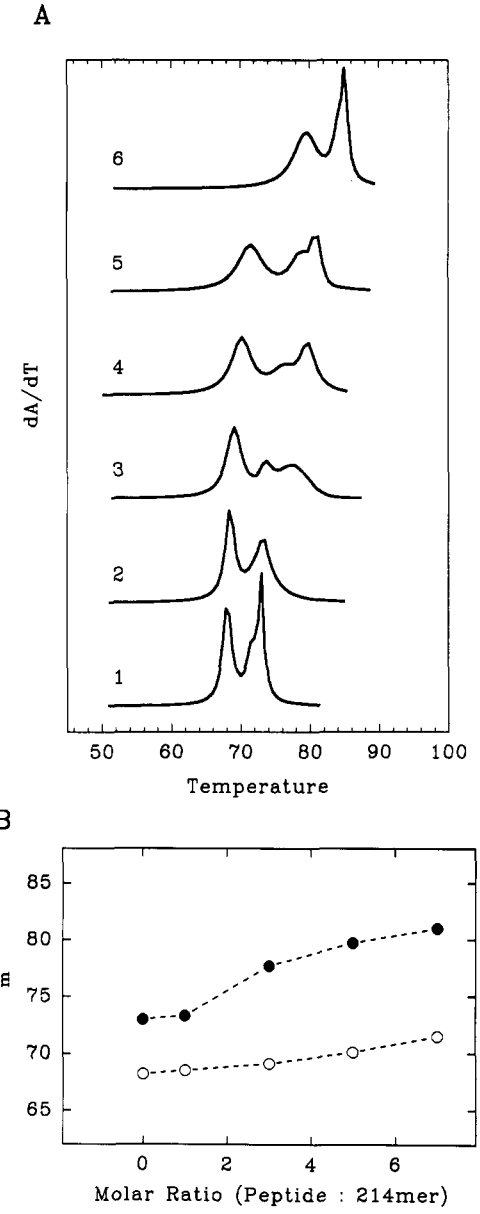

FIGURE 4: Melting of 214-mer in the presence of H1e peptide. The samples were in BPE, and the 214-mer concentration was between 210 and 230 nM (see Materials and Methods). (A) (1) 214-mer; (2–6), H1e peptide and 214-mer at a molar ratio of (2) 1, (3) 3, (4) 5, (5) 7, or (6) 20. (B) T_m was plotted versus molar ratio (H1e peptide:214-mer). (Open circles, AT-rich DNA; closed circles, GC-rich DNA).

Hwan et al., 1975; Renz & Day, 1976; Izaurralde et al., 1989; Suzuki, 1989; Churchill & Suzuki, 1989). However, Sevall (1988) showed by footprinting studies that, while an H1 fraction (containing all of the H1 variants) bound to an overall AT-rich region at the 5′-end of the rat albumin gene, the areas that were actually protected from DNAse cleavage by bound protein were the most GC-rich portions within that region. We have shown that a specific variant, H1e, binds preferentially to GC-rich regions of a homogeneous fragment of DNA, which is consistent with Sevall's results obtained with a mixture of H1 variants. In one of the earliest reports of sequence preference for an H1 (Hwan et al., 1975), the avian erythrocyte-specific variant H5 was shown in melting experiments to bind preferentially to bacterial chromosomal DNA that was AT-rich. Such an observation for large heterogeneous DNA molecules must be interpreted with caution, however, as preferential binding of histones to small GC-rich sections within larger, overall AT-rich regions would not be detected. In most studies, the primary assays that have been used to measure binding of H1 histones to DNA have relied on the formation of large aggregates that could be separated, usually by centrifugation or filter-binding, from the solutions of free proteins and DNA (Sponar & Sormová, 1972; Renz & Day, 1976; Izaurralde et al., 1989). The formation of large aggregates is undoubtedly a separate phenomenon that is preceded by and coupled to simple binding of H1 to DNA. It is difficult to draw inferences about the sequence preference of H1 binding using these methods, since the assays do not directly monitor the primary binding event. In contrast, qualitative interpretation of the melting data presented here is straightforward and unambiguously indicates a preference for GC-rich DNA. The small peptide from the carboxyl-terminal domain of H1e showed the same sequence preference as the whole protein.

In recent work it has been suggested that an H1 variant from sea urchin sperm binds preferentially to AT-rich DNA (Suzuki, 1989; Churchill & Suzuki, 1989). The N-terminal domain of this unique variant has five repeats of the amino acid motif SP(R/K)(R/K). Because an eight-residue peptide with the related sequence SPRKSPRK has been shown to

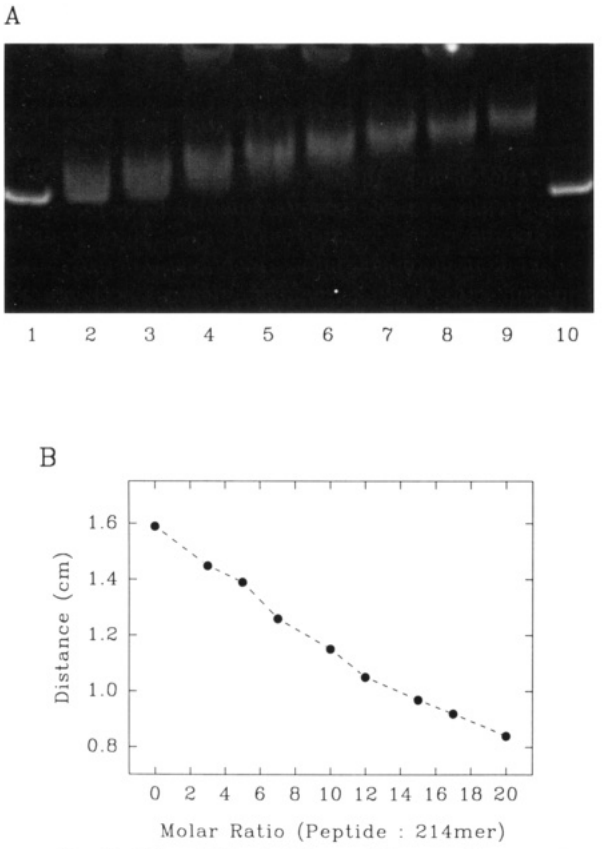

FIGURE 5: Mobility shift of 214-mer–H1e peptide complexes. Aliquots of samples from thermal denaturation experiments were run in the gel (see Materials and Methods). (A) Lanes 1 and 10, 214-mer; lanes 2–9, H1e peptide and 214-mer at a molar ratio of (2) 3, (3) 5, (4) 7, (5) 10, (6) 12, (7) 15, (8) 17, or (9) 20. (B) Distance from well to center of band in each lane plotted versus molar ratio (H1e peptide:214-mer).

bind preferentially to AT-rich DNA, it has been inferred that the entire N-terminal domain of this protein would also show preference for AT-rich DNA. The N-terminal domain of this sperm H1 variant is, however, unlike any domains of the H1 variants of vertebrates, both in predicted secondary structure (β-turn rather than α-helix; Wellman, unpublished data) and in primary sequence. Even if this variant protein were to bind preferentially to AT base pairs, the suggestion that other H1 variants share its sequence preference is therefore unwarranted.

Analysis of the binding of H1e to 214-mer supports previous reports that H1 histones bind cooperatively to DNA (Renz & Day, 1976; Clark & Thomas, 1986; Watanbe, 1986; Thomas et al., 1992). However, several of these studies (Renz & Day, 1976; Clark & Thomas, 1986; Thomas et al., 1992) used conformational changes in the DNA, as monitored by CD, or centrifugation or EM to detect the formation of aggregates, as measures of H1 binding, which again are indirect measures of the primary binding event. The reported cooperativity and salt dependence of cooperativity, then, do not necessarily refer to cooperativity in binding of H1 but may reflect cooperativity in the subsequent aggregation coupled to the primary binding event. We observe cooperative H1 binding to DNA in 15 mM NaCl, which is a lower ionic strength than that reported to be necessary for cooperative binding (Renz & Day, 1976; Clark & Thomas, 1986; Thomas et al., 1992). The H1e peptide does not appear to bind cooperatively under the same conditions.

Considerable effort has been devoted to understanding the H1-induced condensation of DNA. A thorough description of the detailed interactions that occur between H1 histones and DNA is necessary to fully understand the condensation of DNA that they can produce, as well as their interactions with proteins involved in transcription and replication. The simple, homogeneous system that we have described provides considerable insight into the primary interaction between H1 and DNA. This system is especially powerful in that sufficient quantities of the 214 bp fragment may be prepared to allow us to study both the macroscopic (by fluorescence and CD) and microscopic (by footprinting) binding of H1 to DNA, as well as the DNA condensation coupled to such primary binding.

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