Condensation of DNA and Chromatin by an SPKK-Containing Octapeptide Repeat Motif Present in the C-Terminus of Histone H1[†]

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ABSTRACT: Several DNA binding motifs have been described in the C-terminus of histone H1 (Churchill & Travers, 1991), of these the S/TPKK repeat (Suzuki, 1989) often occurs as a part of an octapeptide repeat of the type XTPKKXKK. We have studied in detail the DNA and chromatin condensing properties of a consensus octapeptide KSPKKAKK (8 mer) present in many histone H1 subtypes and its imperfect repeat ATPKKSTKKTPKKAKK (16 mer TPKK) as it occurs in the C-terminus of rat histone H1d. The 16 mer TPKK peptide containing two S/TPKK motifs was able to condense both rat oligonucleosomal (2−5 kbp) DNA and histone H1-depleted chromatin as revealed by circular dichroism spectroscopy. The 8 mer peptide, however, was unable to condense either the DNA or the histone H1-depleted chromatin. Both the 8 mer peptide and the 16 mer TPKK peptide displaced distamycin A from the drug−DNA complex, although with different efficiency, indicating that while these two peptides could bind DNA, only the 16 mer (TPKK) peptide could bring about condensation of DNA and histone H1-depleted chromatin. A mutant 16 mer (TAKK) peptide wherein two proline residues are replaced by alanine, was ineffective in bringing about condensation of both DNA and histone H1-depleted chromatin. These results suggest that the two β-turn structures present in the 16 mer (TPKK) peptide could be important in facilitating binding to different regions of duplex DNA thereby bringing about close packing and condensation. The condensation property of the 16 mer (TPKK) peptide was very similar to that of histone H1 in terms of (a) its preference for AT rich DNA, (b) cooperativity of condensation, and (c) salt dependence of condensation. The 16 mer (TPKK) peptide, but not the 8 mer peptide or the 16 mer (TAKK) peptide, could form complexes with a polynucleosomal 5S DNA core resulting in retarded mobility similar to the complexes formed with histone H1 on agarose gel electrophoresis.

DNA in the eukaryotic cell nucleus is organized into nucleosomal array which is packaged into different levels of ordered structures (van Holde, 1988). The folding of a linear polynucleosome fiber is dependent on ionic strength and histone H1. Several models have been proposed to explain the organization of the observed 30 nm fiber (Finch & Klug, 1976; Stanyov, 1981; McGhee *et al*., 1983; Woodcock *et al*., 1984; Worcel *et al*., 1984; Williams *et al*., 1986; Zlatanova & van Holde, 1995; Bednar *et al*., 1995). One of the inherent challenges in explaining these models is to define the role of histone H1 in the folding of polynucleosomal fibre. Histone H1 can be subdivided into three domains, namely, the N-terminal nose, the globular domain, and the basic C-terminal tail. The amino acid sequences of the globular domain of different mammalian histone subtypes are fairly well conserved and hence serve the unique purpose of organizing the nucleosomal structure. In contrast to the globular domain, the C-terminal tails of the various histone H1 subtypes show considerable divergence in their amino acid sequences. The C-terminal tail of histone H1 is implicated in the maintenance of the higher-order structure of chromatin (Allan *et al*., 1986), and therefore it is quite likely that the different histone H1 subtypes might generate subtly different higher-order structures. At the level of protein−DNA interaction in vitro, individual histone H1 subtypes have different DNA (Liao & Cole, 1981; Khadake & Rao, 1995) and chromatin (Khadake *et al*., 1994; DeLucia *et al*., 1994; Khadake & Rao, 1995) condensing properties. The condensation property of histone H1 has been localized to its C-terminal tail (Moran *et al*., 1985). A more direct evidence for the role of histone H1 in the condensation of interphase chromatin, in vivo, has come from the recent work of Shen *et al*. (1995). They observed an increase in the nuclear volume of the macronucleus and the micronucleus of *Tetrahymena thermophila* when their respective histones H1 were disrupted.

Recently, we reported a detailed analysis of the DNA and chromatin condensing properties of the rat testis specific histone H1t in comparison with the somatic histone H1bdec (Khadake & Rao, 1995). To our surprise, we observed that histone H1t was a poor condenser of DNA and chromatin despite the presence of more arginine residues in its C-terminal tail (Drabent *et al*., 1991). A comparison of the amino acid sequence of histone H1t with that of histone H1d revealed the presence of a direct repeat of an octapeptide motif K/AS/TPKKAKK in histone H1d which is absent in histone H1t. This octapeptide motif contains one S/TPKK

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unit which has been shown to be an important DNA binding motif (Suzuki, 1989). Tandem repeats of SPKK are found in the N-terminus of sea urchin spermatogenous histones H1 and H2B (von Holt *et al*., 1984; Poccia, 1987). Bailly *et al*. (1993) showed by using circular dichroism spectroscopy that $(SPKK)_2$ can condense poly(dA-dT)·poly(dA-dT) but not salmon sperm DNA. Earlier, Erard *et al*. (1990) had shown that the peptide $(KSPKKAKKP)_2$ can also bring about condensation of calf thymus DNA. To further substantiate our hypothesis that the octapeptide repeat motif of the type KSPKKAKK, present in the C-terminal tail of histone H1d, may be responsible for imparting the DNA condensation property of histone H1, we have carried out a detailed analysis of the interaction of two peptides, KSPKKAKK (having one unit of SPKK) and ATPKKSTKKTPKKAKK (having two units of TPKK), that are present in the C-terminal tail of majority of histone H1s with DNA and histone H1-depleted chromatin. Results presented here clearly show that only the 16 mer peptide, but not the 8 mer peptide, has DNA and chromatin condensing property. Replacement of proline residues by alanine in the 16 mer peptide abolished this DNA and chromatin condensing property.

MATERIALS AND METHODS

All of the fine chemicals were purchased from Sigma Chemical Company. Synthetic polynucleotides poly(dA-dT)·poly(dA-dT), poly(dG-dC)·poly(dG-DC), poly(dA)·poly-(dT), and poly(dG)·poly(dC) were purchased from Pharmacia Biotech Limited, Sweden. Plasmid p5S 207-12 DNA, containing 12 tandem repeats of 5S DNA, was a kind gift from Dr. Jeffery Hansen. The two peptides KSPKKAKK (8 mer) and ATPKKSTKKTPKKAKK (16 mer) were synthesized by the solid phase peptide synthesis method of Merrifield using the BOC-protected amino acid (Erickson & Merrifield, 1976). The peptides were cleaved off the resin using trifluoromethylsulfonic acid and trifluoroacetic acid. Purification of the peptides was carried out on a Pharmacia Pep RPC^m HR5/5 column using a linear gradient of 0−30% acetonitrile in 0.1% trifluoroacetic acid for the 16 mer peptide and 0−45% acetonitrile in 0.1% trifluoroacetic acid for the 8 mer peptide. Authenticity of the peptides was confirmed by amino acid sequencing on an Applied Biosystems 477A sequencer. The mutant (Pro→Ala) 16 mer peptide was commercially obtained from Biosynthesis Inc., Lewisville, Texas, U.S.A.

Rat liver oligonucleosomal DNA (2−5 kbp) and histone H1-stripped chromatin were prepared as previously described (Khadake & Rao, 1995). Authenticity of the histone H1-depleted chromatin was ascertained by (a) analysis of acid soluble proteins, (b) micrococcal nuclease digestion pattern, and (c) histone to DNA ratio.

Circular Dichroism Spectroscopy

The circular dichroic spectra of the three peptides were recorded in a buffer containing 10 mM sodium phosphate buffer, pH 7.0/0.1 mM EDTA with or without 1 M NaCl in a JASCO J-700 spectropolarimeter attached to a computer using a 1 mm path length cuvette and a slit width of 0.5 mm. The molecular weights of the three peptides were used to calculate the molar ellipticity.

All the synthetic polynucleotides were dialyzed overnight at 4 °C against 10 mM Tris-HCl, pH 7.5, and 20 mM NaCl in water obtained from a Millipore Milli Q system. The circular dichroism spectra of each of the nucleic acid and the complexes obtained after adding increasing aliquots of the peptide solution in 150 mM NaCl/10 mM Tris-HCl, pH 7.5/0.1 mM EDTA and incubating at room temperature for 10 min were recorded in JASCO J-20 C spectropolarimeter. There was no change in the spectrum after 10 min, indicating that it represents complex formed at equilibrium. The concentrations of the peptides were calculated based on amino acid analysis in a Shimadzu amino acid analyzer. The final concentrations of each of the nucleic acids were calculated using the following molar extinction coefficients (per mole of phosphate at 260 nm and 25 °C and expressed as molar concentration of base pair): rat oligonucleosomal DNA, 6500; poly(dG-dC)·poly(dG-dC) and poly(dG)·poly-(dC), 6350; poly(dA-dT)·poly(dA-dT) and poly(dA)·poly-(dT), 9750. A mean residue weight of 330 was used to calculate the mean residue ellipticity, θ, of nucleic acids. The absorbances of peptide−nucleic acid complexes were routinely checked at 320 nm and were in the range 0.001−0.002, indicating that the spectra recorded were of soluble complexes of the peptides with DNA.

To study the effect of peptides on condensation of histone H1-depleted chromatin, the depleted chromatin was mixed with increasing concentration of the peptide in 80 mM NaCl/10 mM Tris-HCl, pH 7.5/0.1 mM EDTA. The spectra were recorded after the sample was kept at 4 °C overnight. Again, no insoluble aggregates were formed as judged by absorbance at 320 nm.

Displacement of Distamycin A from the Drug−DNA Complex by the Peptides

Distamycin A was prepared in 20 mM NaCl, 10 mM Tris-HCl, pH 7.5, 1 mM EDTA to a concentration of 1 mM using an extinction coefficient of 33×10^3 at 303 nm. Increasing concentration of the drug was added to rat oligonucleosomal DNA (2−5 kbp) in 150 mM NaCl, 10 mM Tris-HCl, pH 7.5, 0.1 mM EDTA, and incubated at room temperature for 10 min. The circular dichroic spectra of the resulting complex were recorded in a Jasco J-20 C spectropolarimeter using a cuvette of 1 cm path length at 25 °C. Molar ellipticity was calculated using a mean residue weight of 330 for DNA. The increase in ellipticity, $\Delta\theta$, at 325 nm was plotted against the distamycin concentration, and a saturating concentration of 2 μM distamycin A was used for the subsequent displacement studies. Increasing aliquots of the three peptides were added to distamycin A−DNA complexes in 150 mM NaCl, 10 mM Tris-HCl, pH 7.5, 0.1 mM EDTA, and incubated at 25 °C for 10 min, after which the circular dichroic spectra were recorded.

Interaction of Peptides and Histone H1 with Polynucleosome Core Assembled on a 5S DNA Template

The plasmid p5S 207-12 DNA was prepared from HB 101 cells harboring this plasmid by the alkali lysis method (Sambrook *et al*., 1989). Plasmid DNA was digested with restriction endonuclease *Hha*I (Bangalore Genei) at 37 °C (1 unit/3 μg of DNA for 4 h), which liberated the 2.5 kbp insert containing the 12 tandem repeats of the 5S gene (207 bp). The insert was purified from the digest on a linear 11 mL gradient of 5−15% sucrose in $T_{10}E_1$, 70 mM NaCl in a SW 41 rotor centrifuged at 38 000 rpm for 16 h at 4 °C.

Half-mL fractions were collected, and alternate fractions were analyzed by electrophoresis on a 1% agarose gel in 1× TAE; peak fractions containing the 2.5 kbp insert were pooled and used for reconstitution with liver core histones. The repeat nature of the 2.5 kbp fragment was ascertained by digestion with *Eco*RI, which liberated the individual 207 kbp fragment.

Nucleosomal core histones were prepared from rat liver nuclei as described by Simon and Felsenfeld (1979) with minor modifications. Briefly, the soluble chromatin was prepared as described earlier and adjusted to 20 A_{260} units/mL with 0.25 mM EDTA (pH 7.0). After adjusting the NaCl concentration to 0.35 M by the dropwise addition of 4 M NaCl, CM Sephadex G-25 beads (12 mg of beads/mL) were added to the chromatin suspension and the mixture was kept on ice for 2 h with constant gentle shaking. Under these conditions histone H1s were selectively bound to the CM Sephadex-25 resin and the supernatant obtained after centrifugation at 12000*g* for 10 min was loaded on a hydroxylapatite (Bio-Rad) column equilibrated with 0.35 M NaCl in 10 mM potassium phosphate buffer, pH 6.8, and the core histones were eluted with 2.2 M NaCl containing 10 mM potassium phosphate buffer, pH 6.8. The symmetrical peak containing equimolar amounts of histones H2A, H2B, H3, and H4 as judged by electrophoresis on SDS−15% PAGE (Laemmli, 1970) were stored in aliquots at −70 °C.

Nucleosome core histones were reconstituted with the 2.5 kbp 5S DNA template essentially according to the method Hansen *et al*. (1989) without any modifications. The reconstitution was checked by micrococcal nuclease digestion.

The binding of the three peptides to the 5S DNA polynucleosome core template was done as follows: the peptides were added to 5S chromatin at *r*-values of 0.1, 0.2, 0.3, and 0.4 (weight/weight) in 80 mm NaCl, 10 mM Tris-HCl, pH 8.0, 1 mM phenylmethylsulfonyl fluoride (PMSF) and incubated at 25 °C for 10 min. The complexes were analyzed on a 1.4% agarose gel in 20 mM Tris-acetate, pH 7.4, and 0.5 mM EDTA as described by Simpson *et al*. (1985). The DNA was visualized after staining with 1μ g/mL of ethidium bromide in the presence of 0.1% SDS. Similarly, binding of salt-extracted rat liver histone H1bdec and testis specific histone H1t [prepared according to the procedure described in Khadake and Rao (1995)] to 5SDNA polynucleosome core template was also monitored using this gel mobility shift assay. The histones were added to reconstituted polynucleosomal 5S template at wt/wt ratios of 0.05, 0.10, 0.15, and 0.20.

RESULTS

We recently showed that the testis specific histone variant H1t is a poor condenser of DNA and chromatin (Khadake & Rao, 1995) when compared to somatic histone H1bdec. While comparing the amino acid sequences of rat histone H1d (Cole *et al*., 1990; Drabent *et al*., 1993) we observed that it has a sequence ATPKKSTKKTPKKAKK followed by KSPKKAKA with a spacing of 10 amino acids. The former 16 mer peptide sequence has two S/TPKK motifs separated by four amino acids while the latter 8 mer peptide sequence has only one S/TPKK motif. These S/TPKK motif containing sequences are completely absent in the amino acid sequence of histone H1t (Cole *et al*., 1986). To evaluate the DNA and chromatin condensing properties of these peptide motifs we synthesized the following three peptides (see Figure 1): (a) KSPKKAKK, an 8 mer peptide representing a consensus octamer unit with one SPKK motif; (b) ATPKKSTKKTPKKAKK, a 16 mer peptide as it occurs in the C-terminus of histone H1d, containing two TPKK motifs; and (c) ATAKKSTKKTAKK, a control 16 mer peptide wherein the two proline residues of the 16 mer peptide b have been replaced by alanine residues. We will be referring to these peptides subsequently in this paper as 8 mer, 16 mer (TPKK), and 16 mer (TAKK), respectively.

K**SPKK**AKK (8 mer peptide)

144 160

A**TPKK**STKK**TPKK**AKK (16 mer TPKK peptide)

ATAKKSTKKTAKKAKK (16 mer TAKK peptide)

FIGURE 1: Sequences of the three peptides used in the present study.

Interaction of Peptides with Rat Oligonucleosomal DNA

The DNA condensing property of histone H1 has been studied extensively by employing the circular dichroism spectroscopic technique (Fasman *et al*., 1970; Jordan *et al*., 1972; Liao & Cole, 1981). The basic parameter that is studied in this technique is the decrease in the positive ellipticity at 270 nm of the DNA spectrum upon interaction with histone H1. Increasing concentrations of histone H1 brings about a progressive decrease in this positive ellipticity which ultimately shows a steep negative θ_{270nm} value. The spectrum generated is often referred to as "psi"-type of spectrum and reflects the optical property of the soluble DNA−histone complexes formed. It is generally believed that the psi-type of spectrum is a result of conformational changes in the DNA (base tilting), change of DNA hydration and formation of side by side aggregates of DNA duplexes (Liao & Cole, 1981). We have used the decrease in the positive ellipticity at 270 nm, termed $\Delta\theta_{270nm}$, as a measure of DNA condensation. Initially, we used rat oligonucleosomal DNA for checking the DNA condensation properties of these peptides. Figure 2A−C shows the CD spectra of rat oligonucleosomal DNA upon interaction with increasing concentrations of 8 mer, 16 mer (TPKK), and 16 mer (TAKK) peptides, respectively. It is clearly evident from the figure that among the three peptides, only the 16 mer (TPKK) peptide could bring about condensation of DNA. Neither the 8 mer peptide nor the 16 mer (TAKK) peptide could change the DNA spectrum at equivalent peptide/DNA ratios (mole/base pair).

S/TPKK containing peptides have been shown to adapt β-turn structures (Suzuki *et al*., 1993). Such a structure has been implicated in the binding of the peptide motif to the narrow minor groove of AT rich DNA based on the ability of $(SPKK)_6$ to displace Hoechst 33258 from the drug−DNA complex (Suzuki, 1989). The observation that the 16 mer (TAKK) peptide did not bring about condensation of DNA suggests that the β-turn of the S/TPKK motif is important for binding to the DNA and bringing about the ensuing DNA condensation. We have recorded the CD spectra of the three peptides (Figure 3). The shape of the spectra of the 8 mer and 16 mer (TPKK) peptide has been interpreted to be characteristic of a type I β-turn structure (Hollosi & Fasman, 1985). The molar ellipticity of −60 000 for the 16 mer (TPKK) peptide is approximately 1.7-fold that of −35 000

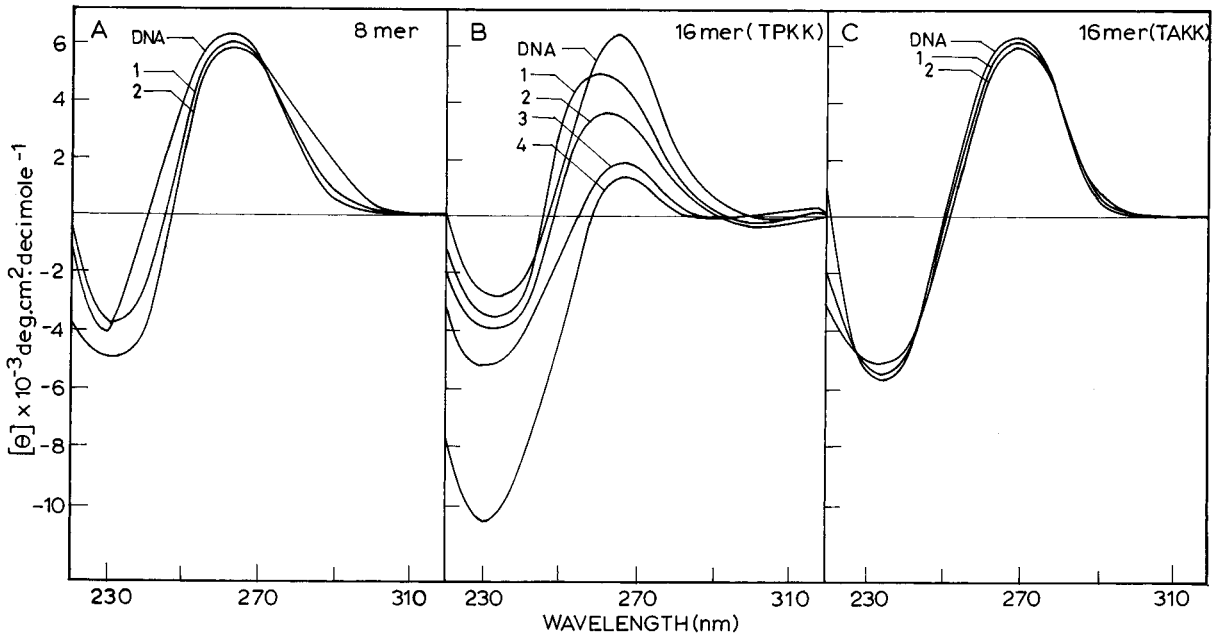

FIGURE 2: Effect of the 8 mer and the 16 mer peptides on the circular dichroism spectrum of rat oligonucleosomal DNA. Rat oligonucleosomal DNA prepared from rat liver nuclei (2−5 kbp) was mixed with increasing concentration of the peptide in 10 mM Tris-HCl, pH 7.5, 0.1 mM EDTA containing 150 mM NaCl. After 10 min of each addition the spectra were recorded in a Jasco 20 C spectropolarimeter. Curves 1 and 2 in the 8 mer panel A corresponds to spectra obtained at a mol of peptide/base pair ratio of 0.225 and 0.375 respectively. Curves 1, 2, 3, and 4 in the 16 mer (TPKK) peptide panel B represent the spectra obtained for a mole peptide/base pair ratio of 0.075, 0.225, 0.300, and 0.375, respectively. Curves 1 and 2 in panel C represent the spectra obtained for the 16 mer (TAKK) peptide at mole of peptide/base pair ratios of 0.225 and 0.375, respectively.

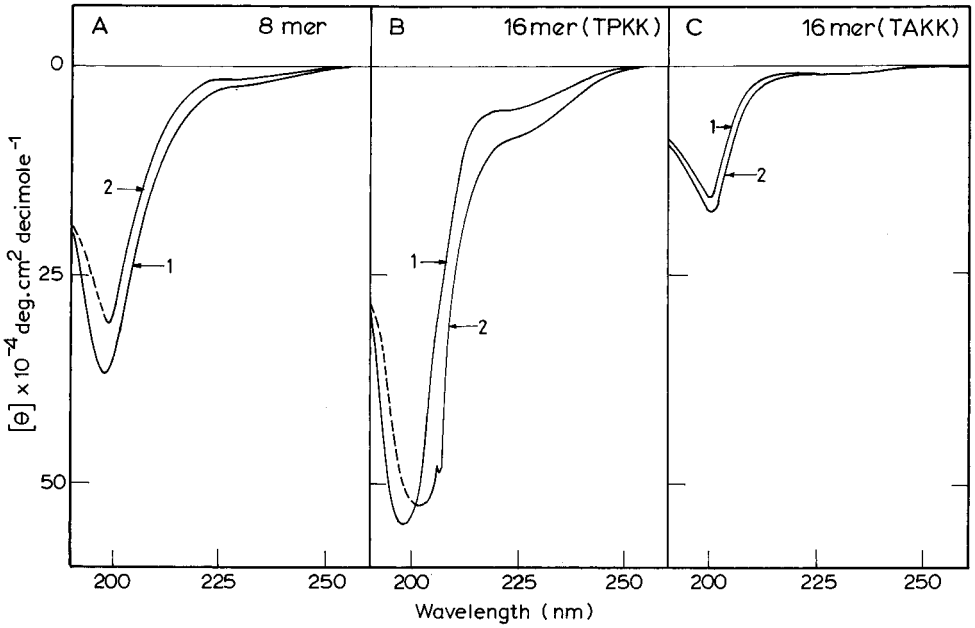

FIGURE 3: Circular dichroism spectra of the peptides. The spectra were recorded in 10 mM sodium phosphate buffer, pH 7.0, 0.1 mM EDTA with (curve 2) or without (curve 1) 1 M NaCl. Panel A, 8 mer peptide; panel B, 16 mer (TPKK) peptide; and panel C, 16 mer (TAKK) peptide.

for the 8 mer peptide which correlates with the presence of two TPKK motifs in the 16 mer (TPKK) peptide. The negative θ for the 16 mer (TAKK) peptide is only −15 000, suggesting that the peptide does not adapt β-turn structure correlating with the absence of proline residues in the sequence. One can argue that the inability of the 8 mer peptide to condense DNA as against the 16 mer (TPKK) peptide can be either due to the differences in the number of SPKK binding motif present in these two peptides or the inability of the 8 mer peptide to bind DNA. To check these two possibilities, we carried out experiments to see whether these peptides can displace DNA-bound distamycin. Distamycin A is an oligopeptide that binds to the narrow minor groove of AT rich DNA (Cole *et al.*, 1987). Binding of this drug to DNA can be easily monitored by circular dichroism spectroscopy. Upon binding of distamycin to DNA there is a decrease in the positive ellipticity at 270 nm with the concomitant appearance of an induced CD band at 325 nm which is very specific to the drug−DNA complex. Displacement of the drug from DNA by any competitor reduces the θ at 325 nm and regenerates the positive ellipticity at 270 nm. We have used this technique to

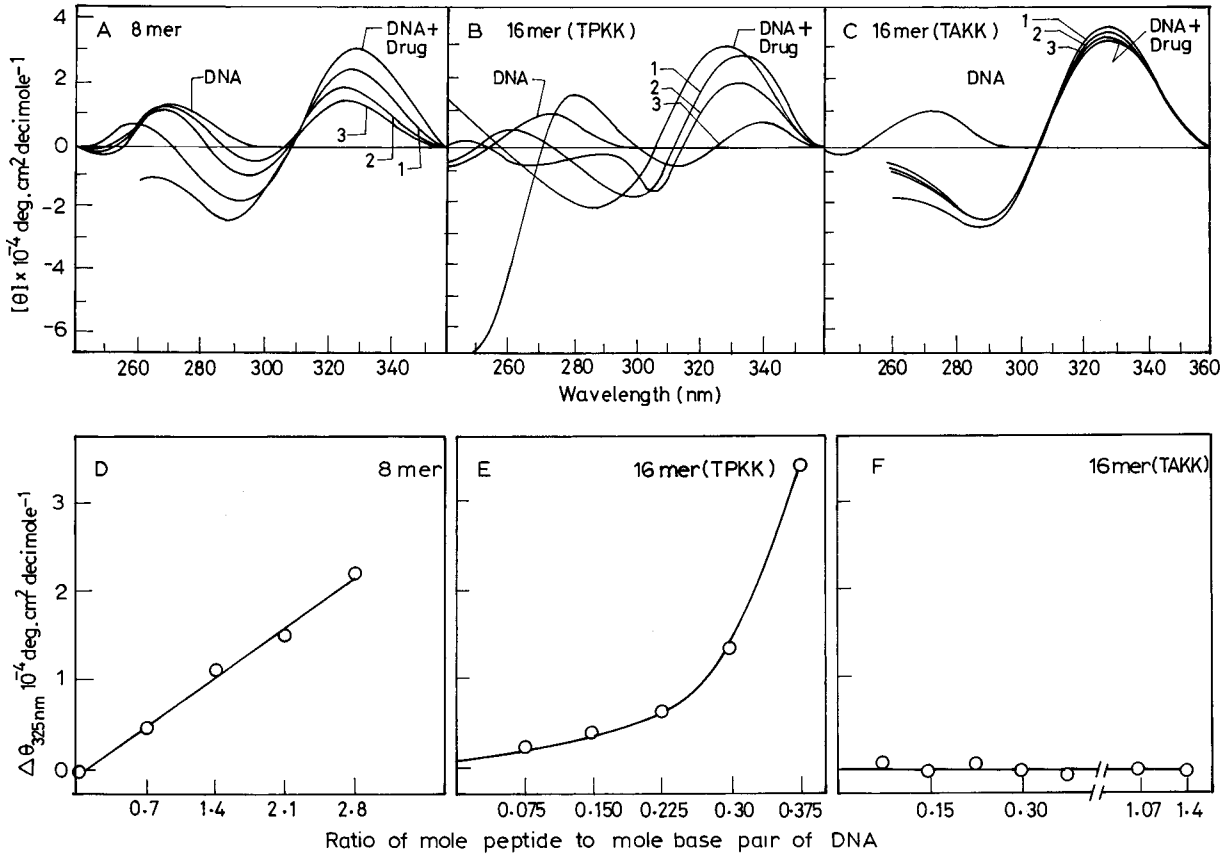

FIGURE 4: Displacement of distamycin A from the drug−DNA complex by peptides. The circular dichroism spectra of 2−5 kbp rat oligonucleosomal DNA was recorded after adding increasing aliquots of peptides to a distamycin A−DNA complex in 150 mM NaCl, 10 mM Tris−HCl, pH 7.5, 0.1 mM EDTA and incubating at room temperature for 10 min. Panels A−C: spectra obtained on adding increasing amounts of 8 mer (curves 1, 2, and 3 correspond to spectra obtained at 0.7, 1.4, and 2.1 mol of peptide/base pair), 16 mer TPKK (curves 1, 2, and 3 correspond to spectra obtained at 0.225, 0.300, and 0.375 mol of peptide/base pair), and 16 mer TAKK (curves 1, 2, and 3 correspond to spectra obtained at 0.225, 0.300, and 1.07 mol of peptide/base pair), respectively. Panels D−F: Derived plots of change in θ at 325 nm ($\Delta\theta_{325nm}$) as a function of increasing ratios of the moles of peptide/base pair. 8 mer (D), 16 mer (TPKK) (E), and 16 mer (TAKK) (F) expressed as moles of peptide/base pair DNA.

compare the binding properties of the 8 mer, 16 mer (TPKK), and 16 mer (TAKK) peptides (Figure 4). As can be seen from Figure 4A and 4D, addition of the 8 mer peptide displaces distamycin from the drug−DNA complex in a concentration dependent manner. This experiment shows that the 8 mer peptide does bind to DNA, and the lack of its ability to condense DNA as against the 16 mer (TPKK) peptide must be due to the differences in the number of S/TPKK units. These experiments are interpreted only to demonstrate the ability of binding of a single S/TPKK-containing octapeptide to DNA. The kinetic parameters of binding of distamycin A are much more complex as has been demonstrated recently (Rentzeperis *et al.*, 1995). Two distamycin molecules are shown to bind A_3T_2-containing oligonucleotide in a side by side manner. It can be seen from Figure 4D and 4E that while the displacement of the drug by the 8 mer peptide is observed between peptide/DNA ratio 0.7 to 2.8 and is linear, the displacement by the 16 mer (TPKK) peptide is observed at a much lower peptide/DNA ratio and is also highly cooperative in nature. The mechanistics of this cooperative interaction needs to be addressed in a separate study. In contrast to the 16 mer (TPKK) peptide, the mutant 16 mer (TAKK) peptide could not displace distamycin from the drug−DNA complex (Figure 4C and 4F) stressing the importance of the β-turn generated by the S/TPKK motif for binding to DNA and bringing about its condensation.

Interaction of Peptides with Histone H1-Depleted Chromatin

The experiments described above were concerned with the DNA condensation properties of the three peptides. Since histone H1 interacts, in vivo, with the polynucleosome fiber, we then investigated the condensation properties of the three peptides with histone H1-depleted chromatin as template. The basic characterization of the histone H1-depleted chromatin used here has been described earlier (Khadake & Rao, 1995). Condensation of histone H1-depleted chromatin upon addition of each of the three peptides was studied by circular dichroism spectroscopy. Addition of the 8 mer peptide to histone H1-depleted chromatin did not appreciably affect the spectrum as shown in Figure 5A. On the other hand, there was a concentration dependent decrease in the positive ellipticity at 275 nm on addition of the 16 mer (TPKK) peptide (Figure 5B). It can also be noted that addition of the 16 mer (TPKK) peptide at ratio of 0.187 (mol/bp) resulted in a greater decrease in the positive ellipticity at 270 nm than that of histone H1 containing native chromatin. It is possible that additional DNA binding sites in the C-terminus of histone H1 molecule might prevent close packing of the polynucleosome as has been observed with the 16 mer (TPKK) peptide. As expected, the 16 mer (TAKK) mutant peptide did not bring about condensation of histone H1-depleted chromatin at equivalent peptide/DNA

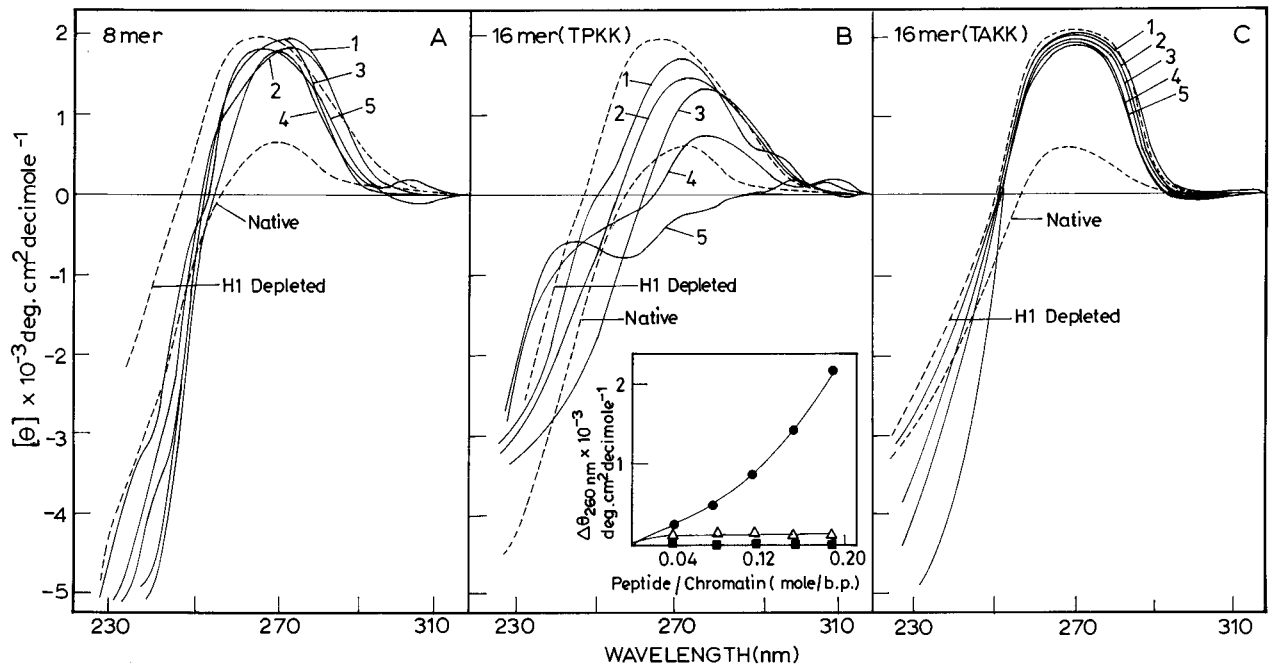

FIGURE 5: Circular dichroism spectroscopy of histone H1-depleted chromatin and its complexes with 8 mer and 16 mer peptides. Polynucleosomes (10−30 mer) prepared from rat liver nuclei were incubated with increasing concentrations of the peptides, overnight at 4 °C, in 80 mM NaCl, 10 mM Tris-HCl, pH 7.5, and 0.1 mM EDTA, and the CD spectra were recorded. The curves 1−5 represent the spectra obtained after incubation with 16 mer peptide at mole to base pair ratio of 0.0375, 0.075, 0.112, 0.15, and 0.187, respectively. Panel A, 8 mer peptide; panel B, 16 mer (TPKK) peptide; and panel C, 16 mer (TAKK) peptide. The inset in the 16 mer (TPKK) peptide panel shows the ellipticity changes, $\Delta\theta_{260nm}$, observed as a function of peptide concentration.

ratio (Figure 5C) again stressing the importance of the SPKK motif in causing condensation of both DNA and histone H1-depleted chromatin.

Interaction of Peptides with Synthetic Polynucleotides

It is now well documented that histone H1 has a preference for binding to AT rich sequences (Kas *et al*., 1989). Therefore, we carried out a detailed analysis of the DNA condensation properties of the 8 mer and 16 mer (TPKK) peptides with synthetic polynucleotides having defined sequences. For this purpose, we have used two alternating copolymers poly(dG-dC)·poly(dG-dC) and poly(dA-dT)·poly(dA-dT) and two homoduplexes poly(dG)·poly(dC) and poly(dA)·poly(dT) for our analysis. After the spectra of the nucleic acids were recorded, increasing concentrations of the 16 mer (TPKK) peptide were added and the spectra were recorded after 10 min intervals. There was no increase in the absorbance at 400 nm under these experimental conditions, ruling out any interference due to formation of large insoluble aggregates. The relative condensation brought about by the peptides on different types of nucleic acids, as determined by the decrease in the positive ellipticity at 270 nm, $\Delta\theta_{270nm}$ was plotted as a function of the input peptide to DNA concentration (Figure 6A). Three important observations can be made from this figure: (a) The degree of condensation measured as θ_{270nm} varies with the type of nucleic acid for the same concentration of the input peptide and in the following order: poly(dA-dT)·poly(dA-dT) > poly(dG-dC)·poly(dG-dC) > poly(dA)·poly(dT) > poly(dG)·poly(dC). (b) The condensation is highly cooperative in nature, and the ratio of peptide/DNA at which 50% of the maximum condensation is observed with each of the nucleic acid ranges from 0.15 to 0.25 mol/base pair. We would like to draw attention to the fact that the displacement of distamycin from the drug−DNA complex by the 16 mer (TPKK) peptide also showed a cooperative behavior. (c) Although condensation was observed with poly(dA)·poly(dT) even at a peptide/DNA ratio of 0.1, subsequently the net condensation showed a decrease. This kind of a behavior might be influenced by the rigid nature of this duplex due to bifurcated hydrogen bonds (Nelson *et al*., 1987). All of these spectral studies were carried out at physiological ionic strength of 150 mM NaCl. We then investigated the effect of NaCl concentration on the condensation property of the 16 mer (TPKK) peptide choosing poly(dA-dT)·poly(dA-dT) as a representative example (Figure 6B). There was an initial small degree of condensation observed at 40−80 mM NaCl, after which there was a steep increase in the degree of condensation reaching a maximum at 150 mM NaCl. The original B-type spectrum of poly(dA-dT)·poly(dA-dT) was regenerated at 250 mM NaCl. A similar salt dependent condensation of DNA was also observed with histone H1 (Khadake & Rao, 1995).

Interaction of Peptides with a Defined 5S DNA Polynucleosome Core Template

All of the experiments described above measured the condensation properties of the three peptides using either free DNA or histone H1-depleted chromatin as templates by employing the circular dichroism technique. Recently gel retardation assays have been developed to study the interaction of histone H1 with core histone-reconstituted DNA templates. We have prepared a polynucleosome core of a defined length using 2.5 kbp DNA containing 12 tandem repeats of *Lytechinus variegatus* 5S DNA (Simpson *et al*., 1985). Since the 5S DNA has a nucleosome positioning signal, the reconstituted polynucleosome core gives us a well-defined system to study the effect of the three peptides. After reconstitution with liver core histones as described in methods, the polynucleosome core was digested with mi-

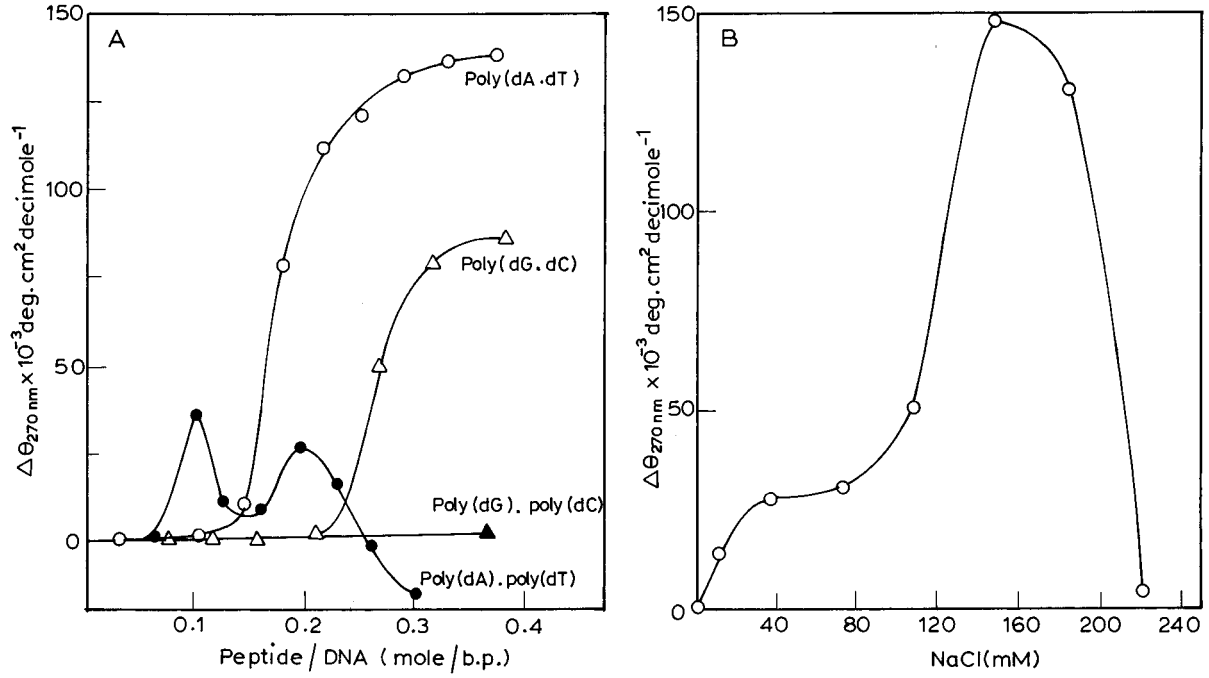

FIGURE 6: (A) Effect of the 16 mer peptide on the condensation of different synthetic polynucleotides. Ellipticity changes observed on interaction of the 16 mer (TPKK) peptide with different synthetic oligonucleotides, termed as $\Delta\theta_{270nm}$, are plotted as a function of peptide to DNA ratio (mol/bp). (B) Effect of NaCl concentration on the condensation of poly(dA-dT)·poly(dA-dT) by the 16 mer peptide. The 16 mer peptide and poly(dA-dT)·poly(dA-dT) complexes at a ratio of 0.36 (mol/base pair) were incubated in different NaCl concentrations for 12 h at 4 °C, after which the CD spectra were recorded.

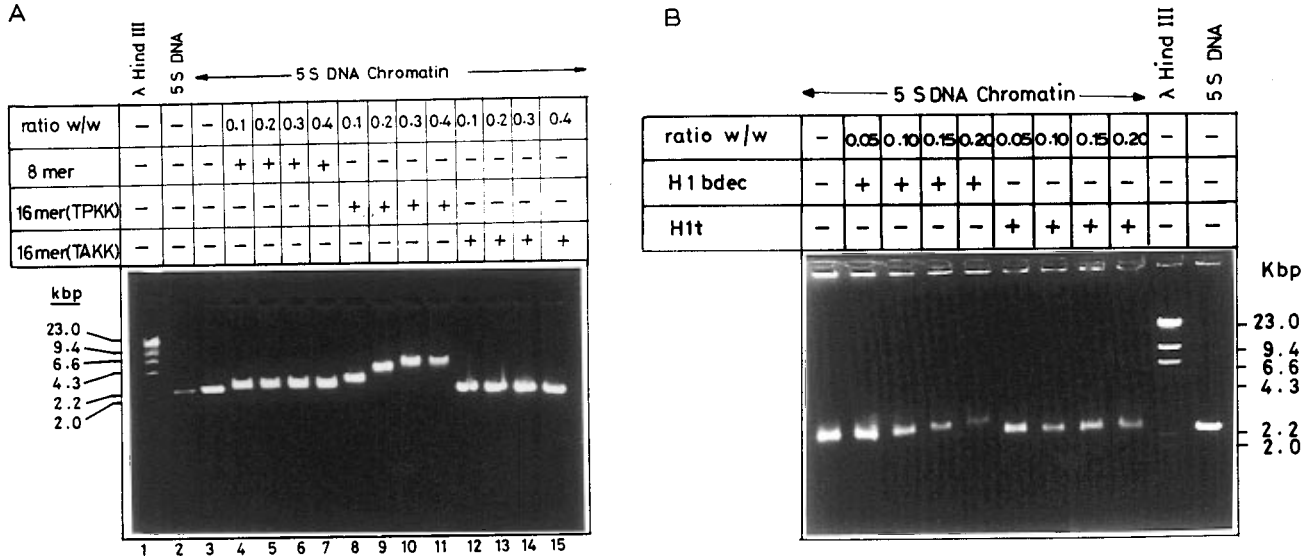

FIGURE 7: Effect of the 8 mer and 16 mer peptides and histones H1bdec and H1t on the mobility of 5S DNA chromatin. (A) The 2.5 kbp DNA fragment containing 12 tandem repeats of 5S DNA (isolated after *Hha*I digestion of plasmid p5S 207-12) was reconstituted with liver core histones according to the method of Hansen *et al*. (1989). This chromatin template was mixed with increasing concentrations of the 8 mer, the 16 mer (TPKK), and 16 mer (TAKK) peptides in 80 mM NaCl, 10 mM Tris-HCl, pH 7.5, 1 mM PMSF, and incubated for 10 min at 25 °C. The complexes were analyzed on a 1.4% agarose gel as described by Simpson *et al*. (1985) using 20 mM Tris acetate, pH 7.4, and 0.5 mM EDTA. The DNA was visualized after staining with 1 μg/mL of ethidium bromide in the presence of 0.1% SDS. (B) The 5S chromatin template was mixed with increasing concentrations of the histones H1bdec and H1t in 80 mM NaCl/10 mM Tris-HCl, pH 7.5, 1 mM PMSF, and incubated for 10 min at 25 °C. The complexes were analyzed as for the peptides.

crococcal nuclease and the DNA pattern analyzed on an agarose gel (data not shown). The 5S polynucleosome core was then mixed with increasing concentrations of the three peptides and the complexes were analyzed on an agarose gel (Figure 7A). The mobility of 5S chromatin is slightly lower than that of naked 5S DNA (lanes 2 and 3). Although the 5S chromatin has approximately twice the molecular size because of the core histones, the negative charges on the DNA are partially neutralized upon interaction with basic proteins and hence 5S chromatin moves with a mobility close to that of naked 5S DNA. Similar observations have been made by Garcia-Ramirez *et al*. (1992). It is clearly evident from the figure that the 8 mer peptide and the mutant 16 mer (TAKK) peptide had no effect on the mobility of the polynucleosome core. The 16 mer (TPKK) peptide on the other hand, generated chromatin−peptide complexes with retarded mobility. There was a progressive decrease in the mobility with increasing peptide/DNA ratio. Figure 7B

presents the gel mobility of the polynucleosome core template upon interaction with rat liver histones H1bdec and histone H1t. Histones H1bdec retarded the mobility of the polynucleosome core in a concentration dependent manner (lanes 2−5). However, the extent of retardation with histone H1bdec is less than that observed with the 16 mer (TPKK) peptide. This also correlates with their relative extent of condensation observed with histone H1-depleted chromatin in our circular dichroism experiments (Figure 5). It can also be seen from the figure that histone H1t at similar *r*-values did not bring about retardation in the mobility of polynucleosome core template. This observation is in conformity with our earlier conclusion that histone H1t is a poor condenser of chromatin. Neither histone H1bdec nor the 16 mer (TPKK) peptide retarded the mobility of naked 5S DNA (data not shown) at the concentrations used in these experiments.

DISCUSSION

Much of the early work on the nature of interaction of histone H1 with naked DNA had shown that it brings about close packing of DNA duplexes (Clark & Thomas, 1986, 1988; Draves *et al*. 1992) resulting in the formation of soluble aggregates which can be easily monitored by circular dichroism spectroscopy. One normally observes conversion of the positive ellipticity at 270 nm, contributed by the B-DNA helix, to steep negative value upon interaction with histone H1 (Fasman, 1970; Jordan *et al*., 1972; Liao & Cole, 1981). There have been some reservations as to the biological significance of such a condensation phenomenon observed in vitro. However, the recent classical experiment of Shen *et al*. (1995) has provided more direct evidence demonstrating that histone H1 is indeed involved in chromatin condensation of an interphase nucleus in vivo. Reconstitution of H1-depleted chromatin with an H1 peptide which comprises the entire C-terminal domain has been found to fully restore the sedimentation behavior and the solenoidal ultrastructure. The authors therefore concluded that folding of polynucleosomes into solenoidal fibers is primarily controlled by the C-terminal domain (Allan *et al*., 1986). CD experiments of Moran using naked DNA furthermore demonstrated that the C-terminal domain of H1 alone can confer the ordered aggregation of DNA fibers, thus suggesting a condensing function (Moran et al., 1985). In our previous communication, we have demonstrated that the testis specific histone H1t is a poor condensder of DNA and histone H1-depleted chromatin (Khadake & Rao, 1995) and we had also speculated that the absence of an octapeptide repeat motif in histone H1t, which is present in somatic histone H1 subtypes, might be responsible for the poor condensing property of histone H1t. The results presented in this communication have provided direct evidence for this hypothesis. Several control experiments have defined the basic sequence requirement to be the octapeptide repeat motif for manifestation of DNA condensing property. Firstly, an 8 mer peptide containing a single S/TPKK motif does not have any DNA condensing property, indicating that two S/TPKK motifs are required for DNA and chromatin condensation. Secondly, when the two proline residues in the 16 mer (TPKK) peptide are replaced by alanine residues in the 16 mer (TAKK) peptide, the peptide lost both DNA binding and condensation properties. This control experiment clearly demonstrates that the β-turn structure generated in the 16 mer (TPKK) peptide is of critical importance in bringing about DNA and chromatin condensation. As is evident both the 16 mer (TPKK) and 16 mer (TAKK) peptides contain the same number of lysine residues and hence the condensation observed is not just an attribute of the basic amino acids alone. As mentioned earlier, SPKK which is also present as tandem repeats in sea urchin spermatogenous histone H1 and H2B (von Holt *et al*., 1984; Poccia, 1987) was identified as a new DNA binding motif by Suzuki (1989). On the basis of competition studies with Hoechst 33258, Suzuki (1989) determined the binding constant for $(SPKK)_6$ to be 1.67×10^{10} M^{-1}. In a subsequent study, Churchill and Suzuki (1989) showed that an octapeptide SPRKSPRK interacted with A6 sites as revealed by hydroxyl radical footprinting technique. On the basis of these experiments, it was initially proposed that SPKK binding motif might recognize AT rich sequences. However, more recent experiments have suggested that it is not the AT rich sequence itself but the narrow minor groove which is recognized by the SPKK motif. Bailey *et al*. (1993) showed that the octapeptide SPKKSPKK can induce a psi-type spectrum upon binding to the alternating copolymer poly(dA-dT)·poly(dA-dT) but not with copolymer poly(dG-dC)·poly(dG-dC) or homopolymers poly(dA)·poly(dT) and poly(dG)·poly(dC) and calf thymus DNA. Most of these experiments have been carried out with tandem repeats of SPKK and such repeats are present only within the sea urchin spermatogenous histone H1 and H2B but not in mammalian histone H1 subtypes. Therefore, it may not be appropriate to extrapolate these observations to interpret the condensation properties of mammalian histone H1 subtypes. Erard *et al*. (1990) have done some preliminary studies on DNA condensation with a peptide $(KTPKKAKKP)_2$. They observed that this nonapeptide tandem repeat did bring about condensation of salmon sperm DNA at peptide to DNA molar ratios of 0.66. However, we would like to point out again that the nonapeptide sequences do not occur as tandem repeats in the C-terminal of histone H1 but are spaced by several amino acids. Our present study differs from all these reports in that we have used two peptides as they occur in the C-terminus of histone H1. One is a single-consensus octapeptide of the type KSPKKAKK and the other is a 16 mer peptide containing two SPKK motifs separated by four amino acids. These two peptide sequences are themselves separated by 10 amino acids in the rat histone H1d sequence (see Figure 8). We have also observed that the 16 mer (TPKK) peptide brings about condensation of DNA at a much lower molar ratio of peptide to DNA (0.225 and 0.325) as compared to the nonapeptide tandem repeated reported by Erard *et al*. (1990).

More recent NMR experiments have suggested SPKK may adapt a turn structure with a dynamic equilibrium between a β-turn and a σ-turn (Suzuki *et al*., 1993). The CD spectral characteristics of the 8 mer as well as the 16 mer (TPKK) peptides reported here also suggest the probable presence of β-turn structure in these two peptides. One can visualize the 16 mer (TPKK) peptide as having two DNA binding S/TPKK motifs in the form of two hooks separated by four amino acids. Such a structure can be utilized to crosslink two regions of DNA duplexes resulting in packing of the duplexes, altering the optical properties of the nucleic acid, generating a psi-type spectrum. The 8 mer peptide has only one such DNA binding motif, and therefore a single octamer

MAMMALS

hum	H1A	113	AASGEAKPKAKKAGAAKAKK PAGATPKKAKKAAGAKKAVK KTPKKAKKPAAAGVKKVAKS PKKAKAAAKPKKATKSPAKP KAVKPKAAKPKAAKPKAAKP KAKKAAAKKK	222
hum	H1B	109	AASGEAKPKAKKAGAAKAKK PAGAAKKPKKATGAATPKKS AKKTPKKAKKPAAAAGAKKA KSPKKAKAAKPKKAPKSPAK AKAVKPKAAKPKTAKPKAAK PKKAAAKKK	218
hum	H1C	111	AASGEGKPKAKKAGAAKPRK PAGAAKKPKKVAGAATPKKS IKKTPKKVKKPATAAGTKKV AKSAKKVKTPQPKKAAKSPA KAKAPKPKAAKPKSGKPKVT KAKKAAPKKK	220
hum	H1D	109	AASGEAKPKVKKAGGTKPKK PVGAAKKPKKAAGGATPKKS AKKTPKKAKKPAAATVTKKV AKSPKKAKVAKPKKAAKSAA KAVKPKAAKPKVVKPKKAAP KKK	212
hum	H1.0	112	EIKKVATPKKASKPKKAASK APTKKPKATPVKKAKKKLAA TPKKAKKPKTVKAKPVKASK PKKAKPVKPKAKSSAKRAGK KK	193
rab	H1.3	112	AASGEAKPKPKKAGAAKPKK PAGATPKKPKKAAGAKKAVK KTPKKAPKPKAAAKPKVAKP KSPAKVAKSPKKAKAVKPKA AKPKAPKPKAAKAKKTAAKK KK	213
rat	H1D	109	AASGEAKPKAKKAGAAKAKK PAGAAKKPKKATGTATPKKS TKKTPKKAKKPAAAAGAKKA KSPKKAKATKAKKAPKSPAK ARAVKPKAAKPKTSKPKAAK PKKTAAKKK	218
rat	H1.0	96	EVKKVATPKKAAKPKKAASK APSKKPKATPVKKAKKKPAA TPKKAKKPKIVKVKPVKASK PKKAKPVKPKAKSSAKRASK KK	177
rat	H1T	111	AASGNDKGKGKKSASAKAKK LGLSRASRSPKSSKTKVVKK PKATPTKGSGSRRKTKGAKG LQQRKSPAKARATNSNSGKS KMVMQKTDLRKAAGRK	207
mse	H1.0	112	EVKKVATPKKAAKPKKAASK APSKKPKATPVKKAKKKPAA TPKKAKKPKVVKVKPVKASK PKKAKTVKPKAKSSAKRGSK KK	193
mse	H1.1	100	AASGEAKPQAKKAGAAKAKK PAGAAKKPKKATGAATPKKA AKKTPKKAKKPAAAAVTKKV AKSPKKAKVTKPKKVKSASK AVKPKAAKPKVAKAKKVAAK KK	211
mse	H1E	110	AASGEAKPKAKRAGAAKAKK PAGAAKKPKKAAGTATAKKS TKKTPKKAKKPAAAAGAKKA KSPKKAKATKAKKAPKSPAK AKTVKPKAAKPKTSKPKAAK PKKTAAKKK	219

AVES

chk	H1.01	111	PGEVKEKAPRKRATAAKPKK PAAKKPAAAAKKPKKAAAVK KSPKKAKKPAAAATKKAAKS PKKAAKAGRPKKAAKSPAKA KAVKPKAAKPKATKPKAAKA KKTAAKKK	218
chk	H1.03	114	SGDVKKAPKKKTPAAKPKKP AAKKPAAAAKKPKKAVAVKK SPKKAKKPAAAATKKAAKSP KKVTKAAKPKKAVAVKSPAK AKAVKPKAAKPKATKPKAAK AKKAAPKKK	223
chk	H1	111	PGETKAKATKKKPAAKPKKP AAKKPAAAAKKPKKAAAVKK SPKKAKKPAAAATKKAAKSP KKATKAGRPKKTAKSPAKAK AVKPKAAKSKAAKPKAAKAK KAATKKK	217
chk	H5	98	SDKAKRSPGKKKKAVRRSTS PKKAARPRKARSPAKKPKAT ARKARKKSRASPKKAKKPKT VKAKSRKASKAKKVKRSKPR AKSGARKSPKKK	190
chk	H1.10	111	PGEVKEKAPRKRTPAAKPKK PAAKKPASAAKKPKKAAAAK KSPKKAKKPAAAATKKAAKS PKKATKAAKPKKAATAKSPA KAKAVKPKAAKPKAAKPKAA KAKKAAAKK	210
chk	H1.11L	115	PGEVKEKAPKKKASAAKPKK PAAKKPAAAAKKPKKAVAVK KSPKKAKKPAASATKKSAKS PKKVTKAVKPKKAVAAKSPA KAKAVKPKAAKPKAAKPKAA KAKKAAAKKK	224
chk	H1.11R	112	PGEGLEKAPKKKASAAKPKK AAAKKPAAAAKKPKKAVAVK KSPKKAKKPAASATKKSVKS PKKAAKPKKAVAAKSPAKAK AVKPKAAKPKAAKPKAAKAK KAAAKKK	218
apl	H1	112	PGETKEKATKKKPAAKPKKP AAKKPASAAKKPKKAAAVKK SPKKAKKPAAAATKKAAKSP KKAAKAGRPKKAAKSPAKAK AVKPKAAKPKAAKPKAAKAK KAAPKKK	218
dck	H5	110	DKAKKSPAGRKKKKKAARRS TSPRKAARPRKARSPAKKPK AAARKARKKSRASPKKAKKP KTVKAKSLKTSKVKKAKRSK PRAKSGARKSPKKK	193
gse	H5	110	DKAKRSPAGRKKKKKAARKS TSPKKAARPRKARSPAKKPK AAARKARKKSRASPKKAKKP KTVKAKSLKTSKPKKARRSK PRAKSGARKSPKKK	193

AMPHIBIANS

xla	H1A	114	QLQSKDKAAAKKKAPLAAEA KKPAAAAKKTAKSPKKPKKV SAAAKSPKKLKKPAKAAKSP AKKPKVAAKSPAKAKAAKPK VAKAKKAAPKKK	209
xla	H1B	114	QLETKDKAAKKKPAAPKAKK TAAGAKKAPKSPKKPKKVSA AAKSPKKVKKLAKAAKSPKK PKAVKAKKVAKSPAKKATKP KTAKSPAKAKVAKPKAAKAK KPAPKK	219
xla	H1C	114	QLETKVKAVAKKKLVAPKAK KPVTAKKKPKSPKKPKKVSA AAAKSPKKAKKPVKAAKSPK KPKAVKSKKVTKSPAKKATK PKAAKAKIAKAKAAKGKKAA AKK	216
xla	H1C-	113	QLETKVKAVAKKKLVAPKAK KPVAAKKKPKSPKKPKKVSA AAAKSPKKAKKPVKAAKSPK KPKAVKPKKVTKSPAKKATK PKAAKAKIAKPKIAKAKAAK GKKAAAKK	220
xla	H5A	99	DEGKKPAKKPKKEIKKAVSP KKVAKPKKAAKSPAKAKKPK VAEKKVKKVAKKKPAPSPKK AKKTKTVKAKPVRASKVKKA KPSKPKAKASPKKSGRKK	190
xla	H5B	97	DEVKKPAKKPKKEIKKAVSP KKAAKPKKAAKSPAKAKKPK VAEKKVKKAPKKKPAPSPRK AKKTKTVRAKPVWASKAKKA KPSKPKAKASPKKSGRKK	194

PISCES

rtr	H1	102	AVEAKKPAKKAAAPKAKKVA AKKPAAAKKPKKVAAKKAVA AKKSPKKAKKPATPKKAAKS PKKVKKPAAAAKKAAKSPKK ATKAAKPKAAKPKAAKAKKA APKKK	206
brt	H1	102	AVEAKKPAKKAAAPKAKKVA AKKPAAAKKPKKVAAKKAVA AKKSPKKAKKPATPKKAAKS PKKATKAAKPKAAKPKKAAK SPKKVKKPAAAKK	194

ECHINODERMS

spu	H1-s	107	TKTPKKAAAKKATPKKKPAA KKTKKPAAKKATKKPAKKPA AKKKVAKPAAKKAAKPVAKK ATPKKKVVKKAAKGKGKKK	185
spu	H1e	93	AAKAQASEKAKKEKEKAKLL AQREKAKEKGCSEEGETAEG SRPKKVKAAPKKAKKPVKKT TEKKEKKKTPKKAPKKPAAK KSTPKKTPKKAAAKKPKTAK PKKPAXKKAAKSK	205
pan	H1g	127	AAAKAKKAKAAAAKKARKAK AAAKRKAALAKKKAAAAKRK AAAKAKKAKKPKKKAAKKAK KPAKKSPKKAKKPAKKSPKK KKAKRSPKKAKKAAGKRKPA AKKARRSPRKAGKRRSPKKA RK	248

NEMATODES

cel	H1.1	114	AAAAKKPAAAKKPAAAKKPA AAKKATGEKKAKKPAAAKPK KAATGDKKVKKAKSPKKVAK PAAKKVAKSPAKKAAPKKIA KPAAKKAAKPAAKA	207
cel	H1.2	114	AAATKKPVAKKPVAKKAATG EKKAKKTTVAKKTGDKVKKA KSPKKIAKPAAKKVAKSPAK KAAPKKAPAKKAAAPKA	190

PLANTS

wht	H1	118	KGSYKLAKAPAAVKPKTATK KKPAAKPKAKAPAKKTAAKS PAKKAAAKPKAKAPAKAKAV AKPKAAAKKAPKPKAKAAAK KGAGAATPKKPAARKPPTKA ATPVKKAARKKPAAKKAKK	236

FIGURE 8: Amino acid sequences of the C-terminal tails of different histone H1s containing the KS/TPKKAKK motif. The K/STPKKAKK motif has been underlined. The three-letter code used is as per http://www.ncbi.nlm.nih.gov/Baxevani/Histones/database.html, and gse is a three-letter code assigned to goose.

peptide cannot interact simultaneously with two DNA duplexes. The mutant 16 mer (TAKK) peptide does not have the β-turn S/TPKK DNA binding motif and hence will not possess the two-hook type structure to interact with DNA at two different regions and therefore will not result in condensation of DNA. Our experimental observations correlate very well with these theoretical presumptions. It is worth mentioning here that SPKK tetrapeptide has been shown by Raman spectroscopy to destabilize the hydrogen bonding between the two strands of the DNA helix at the point of contact (Takeuchi & Sasamori, 1995). The significance of this observation in light of the present discussion cannot be ascertained at present.

The results presented in this paper have clearly demonstrated that the 16 mer (TPKK) peptide mimics the DNA condensing properties of histone H1 in that (a) it is salt dependent, (b) it shows a preference for AT rich sequence, (c) its interaction is cooperative in nature, and (d) it condenses histone H1-depleted chromatin. However, we would like to point out that the condensation of DNA by histone H1 is observed at a much lower mole of protein/base pair ratio (Khadake & Rao, 1995) than with the 16 mer (TPKK) peptide reported in the present study. It is possible that additional DNA binding motifs present in the C-terminal region of histone H1 (Churchill & Travers, 1994; Khadake & Rao, 1995) might contribute to the higher efficiency of DNA condensation by histone H1. The C-terminus of histone H1 is also believed to attain a stable α-helical structure when bound to DNA (Clark *et al*., 1989; Maeder & Bohm, 1991), indicating that the spatial disposition of the DNA interacting motifs like the S/TPKK containing units described here may determine the chromatin condensation/folding property of histone H1.

In the present communication, we have also shown that the 16 mer (TPKK) peptide can generate complexes with the 5S DNA polynucleosome core template that have similar retarded mobility as that of histone H1-containing complexes. At present we cannot comment on the nature of the complexes formed. Agarose gel electrophoresis for the analysis of chromatin folding is being exploited only recently (Fletcher *et al*., 1994a,b; Bartolome *et al*., 1995). The mobility of nucleoprotein complexes on an agarose gels depends on (a) average surface electrical charge density, (b) effective radius, and (c) particle deformity (Fletcher *et al*., 1994a). It may be pertinent to point out here that Bartolome *et al*. (1995) have also observed that chromatin fragments after incubation with 0.6 M NaCl (a concentration at which histone H1 is dissociated from chromatin), moves faster than fragments incubated with lower concentration of NaCl, which is similar to the observation made in the present study. We are in the process of analyzing these complexes by electron microscopy for better visualization of the complexes formed. We would also like to stress here that the testis specific histone H1t, which does not have the octapeptide repeat motif (Khadake & Rao, 1995), did not result in complexes having retarded mobility. Although the parameter measured by the agarose gel retardation assay is entirely different from that measured by CD spectroscopy it is interesting to note that the octapeptide repeat motif mimics the properties of histone H1 in bringing about both condensation of DNA as measured by CD spectroscopy and retardation of polynucleosome templates in an agarose gel.

If indeed this octapeptide repeat motif of the type 16 mer (TPKK) peptide containing two S/TPKK motifs is the DNA condensing motif of histone H1, we were curious to know how general is the occurrence of such motifs in other histone H1s whose sequences have been reported in literature. We, therefore, examined the C-terminal amino acid sequences of all the histones H1 deposited in the databank (Figure 8). It is evident that all of these histones H1 have a minimum of two octapeptide motifs of the type KS/TPKKAKK or closely related sequences containing S/TPKK within it which are separated by four to 13 amino acid residues. It is interesting to note that these spacer amino acids are rich in lysine and alanine residues. We also find that in addition to the testis specific histone H1t which does not condense chromatin (Khadake & Rao, 1995; Delucia *et al*., 1994) some other histone H1 subtypes, like histone H1 from fruit fly, histones H1B and H1g of sea urchin, histones H1Asp and H1Bsp of clam worm, histone H1 from maize and pea, also do not contain any SPKK motifs in their C-terminal tails. Our prediction would be that these histones H1 may not condense DNA or histone H1-depleted chromatin efficiently. Chromatin domains containing these histones H1, perhaps, would attain a more open conformation. A notable histone H1 subtype which does not have any S/TPKK motif is the histone H1 variant B4 that appears during the early embryonic stage of *Xenopus laevis* development and which has been suggested to be associated with loosened chromatin structure (Dimitrov *et al*., 1993). Histone H1t from other species also does not have these motifs.

What would be the physiological relevance of interaction of SPKK-containing motifs of histone H1 with chromatin? There has been considerable discussion in the literature on the nature of linker DNA accessibility to mild micrococcal nuclease digestion in the 30 nm fiber. Earlier studies have attributed the inaccesibility to internalization of the linker DNA toward the center of the fiber (van Holde, 1988; Widom, 1989). However, recently Leuba *et al*. (1994) from their studies with immobilized micrococcal nuclease have ascribed the inaccessibility of linker DNA to high compaction. This predicted compaction of the linker DNA may be the result of packing of the linker DNA by the octapeptide repeat motifs as shown in the present study. As mentioned earlier, the higher-order structure of chromatin, even at the 30 nm fiber level, is poorly understood (von Holde & Zlatanova, 1995). At a more fundamental level the conformation of the linker DNA itself in the 30 nm fiber is not clearly understood. It is quite likely that the DNA condensing property of the octapeptide repeat motif present in the C-terminus of histone H1 might contribute to the conformation of the linker DNA which in turn can influence the higher-order structure of chromatin. Our preliminary experiments reported here (Figure 7) do indicate that the 16 mer (TPKK) peptide can alter the organization of the 5S DNA chromatin so as to retard its mobility similar to that by histone H1bdec in an non-denaturing agarose gel. It would be interesting to compare the 5S chromatin complexes formed with intact histones H1 and the 16 mer (TPKK) peptide under the electron microscope. Currently, these studies are in progress. We have also begun to address this question by specifically mutating the proline residues in the C-terminus of histone H1d and using these mutant histone H1 molecules in our chromatin folding studies.

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