Preferential condensation of SAR-DNA by histone H1 and its SPKK containing octapeptide repeat motif

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Abstract Linker histone H1 binds preferentially the scaffold associated region (SAR) DNA elements that contain characteristic oligo dA·dT tracts. In the present study, we have compared the condensation brought about by histone H1 of a SAR DNA fragment in the histone spacer region of *Drosophila melanogaster* with that of a random DNA (pBR322 *Eco*RI-*Sal*I) fragment by circular dichroism spectroscopy. The condensation of the SAR DNA fragment by histone H1 is 3–4-fold higher than that of the random DNA fragment. A 16-mer peptide, ATPKKSTKKTPKKAKK, the sequence that is present in the C-terminus of histone H1d, which has recently been shown to possess DNA and chromatin condensing properties, also condenses the SAR DNA fragment preferentially in a highly cooperative manner. We have proposed a model for the dynamics of chromatin structure involving histone H1-SAR DNA interaction through SPKK containing peptide motifs and its competition by AT-hook peptides present in the nonhistone chromosomal proteins like HMG-I and HMG-Y.

Key words: Scaffold associated region; Histone H1; AT-hook peptide motif

1. Introduction

Scaffold associated regions (SAR, also termed matrix associated regions, MAR) are DNA sequence elements that mediate specific binding to the nuclear scaffold forming a base of DNA loops in an eukaryotic cell [1]. SAR sequences are believed to have both a structural and a functional role in regulating gene expression in the context of chromatin organization. The consensus sequences of SAR elements reveal them to be AT rich with numerous A tracts possessing a narrow minor groove with possible bends [2]. Such an altered DNA conformation within the SAR sequence is recognized specifically by proteins like topoisomerase II [3], scaffold protein SC2 [4], XCAP-C and XCAP-E [5], high mobility group proteins HMG-I and HMG-Y [6], histone H1 [2,7] and the peptide antibiotic distamycin [8].

Histone H1 is involved in the folding of transcriptionally inert polynucleosomal fiber into higher order structures [9]. It has long been known that histone H1, in vitro, can pack DNA duplexes to form soluble aggregates often referred to as condensation of DNA, generating a 'psi'-type spectrum in circular dichroism spectroscopy [10]. Although the biological significance of this DNA packing property of histone H1 is not yet clear, we have recently shown that the testis specific histone H1t is a poor condenser of DNA and chromatin when compared to somatic histone H1bdec [11]. More recently, we have shown that an octapeptide repeat motif containing two SPKK motifs that is present in the C-terminus of histone H1 can mimic the DNA and chromatin condensing property of histone H1 [12]. Since histone H1 binds preferentially to SAR containing DNA fragment in vitro [2,7], we were interested in determining the condensation of SAR containing DNA by histone H1. Here we report that histone H1bdec isolated from rat liver does condense SAR DNA fragments preferentially over random DNA fragments. Furthermore, the 16-mer peptide (containing two SPKK motifs) also preferentially condenses SAR DNA fragments in a highly cooperative manner. The possible significance of preferential condensation in relation to chromatin dynamics is discussed.

2. Materials and methods

All the reagents used were of analytical grade. Histones H1bdec and H1t were purified by the salt extraction method as described by us recently [11]. Plasmids pBR322 and pSP64-34 containing the histone spacer from *Drosophila melanogaster* harboring the SAR sequences (a gift from Dr. U.K. Laemmli) were prepared from the individual cultures of *E. coli* DH5$^{\alpha}$ cells harboring these plasmids, by the alkaline lysis method [13]. The 660 bp SAR fragment was excised from pSP64-34 by double digestion with *Eco*RI and *Bam*HI restriction enzymes (Bangalore Genei). A control DNA fragment of 650 bp was obtained from pBR322 by double digestion with *Eco*RI and *Sal*I. Both DNA fragments were purified on a 5–15% neutral sucrose gradient centrifuged at 178 000×*g* for 6 h in a SW 41 rotor at 4°C. Fractions containing the required fragment as determined on an agarose gel were pooled and precipitated with ethanol. The synthesis of the 16-mer peptide ATPKKSTKKTPKKAKK has been described earlier [12].

Circular dichroism studies of the DNA-histone and DNA-peptide complexes were carried out in a Jasco J20 spectropolarimeter. Complexes of DNA and histone H1 subtypes or 16-mer peptide were prepared by direct mixing in 150 mM NaCl/10 mM Tris-HCl (pH 7.4)/0.1 mM EDTA of increasing aliquots of histone H1 or peptide with the respective DNA samples. The spectra were recorded 10 min after each addition. A mean residue weight of 330 for the nucleic acid was used to calculate the mean residue ellipticity θ. The absorbance of the protein and peptide nucleic acid complexes were routinely checked at 320 nm for the formation of any insoluble aggregates.

3. Results and discussion

Fig. 1 shows the nucleotide sequence of the SAR DNA fragment used in the present study. The 660 bp *Eco*RI-*Bam*HI fragment of plasmid pSP64-34 corresponding to the histone spacer of *Drosophila melanogaster* that contains the consensus SAR DNA elements is rich in oligo dA·dT tracts (74.7% AT content) (underlined). The control pBR322 DNA fragment does not contain such oligo dA·dT tracts (45.7% AT content).

Fig. 2 shows the effect of increasing concentrations of histone H1bdec (histone H1d is the major component and comprises 40% of the total H1 subtypes) and histone H1t as well

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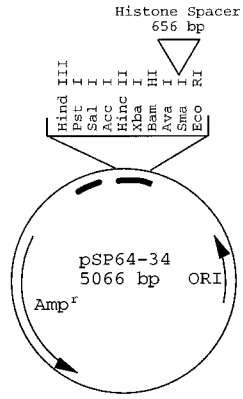

SEQUENCE OF SAR DNA

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  1 AATCATGGCA AATATTTCAC TAATTCGTAA TGTTTATTAA AATTGAAACG TTCATCCCCT
 61 AGAAGGGCTT CTGAAAGAAG CGTCTATTAT TAATTAAGAT ACTTTATTAC TTGATTTAAG
121 TTCACATTGA ACTACAGAAA AATTTGCTTT AAAGCAGCAT TCAGAAATAA TTTTCGATCG
181 TTAAAAATAC AGATACTTTC TTAAATAACA TAATATTAAT ATAATAAATA ATATTTTCTT
241 AAATTATTAA TGTGTTTGCT TGTTATTAAT GTGTTTTCAT GCATATTTTA AAATAATTTG
301 TTCTGAAATC AATCGATTTC CTCGCCACAT ATGCATTACC GTCTATGTAG TCAAATAAAT
361 AAATCAAATA AAAAATAAAA AGAAACAATT TTTGTATTAT CCTTTATTAT GTAATATATA
421 TTACATTCCG CAACAAAATT AGCCAATTTC CGTGCTGAAT TTTACATAGG TTTTATTTTT
481 TACAGACGTT TGAAGGGACA GTGTCAATTG TCCCGTACGA CCTCTTCAAT AATAACACAT
541 TCTTCAGTTA ACACATGGAA AAAATATAAA ATAGCTAGTT TTATTTTATT ATTTTCTGTT
601 ATTTTATAAA TATTGCCGTC ATCAATGTAA TTTCATTAAT TTCAAACTGT CTTACACAGA
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Fig. 1. Plasmid map of pSP64-34 harboring the 656 bp SAR from the histone spacer of *Drosophila melanogaster*. The nucleotide sequence of the SAR fragment is also given where in oligo dA·dT stretches have been underlined.

as the 16-mer peptide on the circular dichroic spectra of SAR and control DNA fragments. Addition of increasing concentrations of both histone H1bdec and H1t resulted in a progressive decrease in the positive ellipticity, θ, at 270 nm slowly generating a steep negative ellipticity characteristic of the 'psi'-type spectrum with both the SAR and control DNA fragments. However, it can be seen that at similar histone/DNA ratios, the SAR fragment was condensed much more effectively than the control DNA fragment. This is evident in Fig. 3, where we have plotted the change in the ellipticity at 270 nm termed as Δθ as a function of molar ratio of protein to DNA. The Δθ observed for SAR DNA fragment at a histone/DNA ratio of 0.06 was −93 000 with histone H1bdec against −22 000 for the control DNA fragment. A similar three-fold difference in net condensation was observed between these two DNA fragments with the testis specific histone H1t, although the Δθ observed at similar histone/DNA ratio was at least four-fold lower than with histone H1bdec. We have shown recently that a 16-mer peptide (ATPKKSTKKTPKKAKK), which is present in the C-terminus of histone H1d but absent in histone H1t, could mimic both the DNA and chromatin condensation properties of histone H1. We therefore studied its condensation effect on both the SAR and control DNA fragments and the results are presented in Fig. 2C,F. It is clear from the figure that this 16-mer peptide also condenses SAR DNA fragments much more effectively than the control DNA fragments. A more interesting observation is that the effect of the peptide is highly cooperative in nature, which is much more than that observed with the intact histone H1 molecule (Fig. 3). It is worth noting here that the binding of histone H1 to SAR DNA is also highly cooperative in nature and it is believed

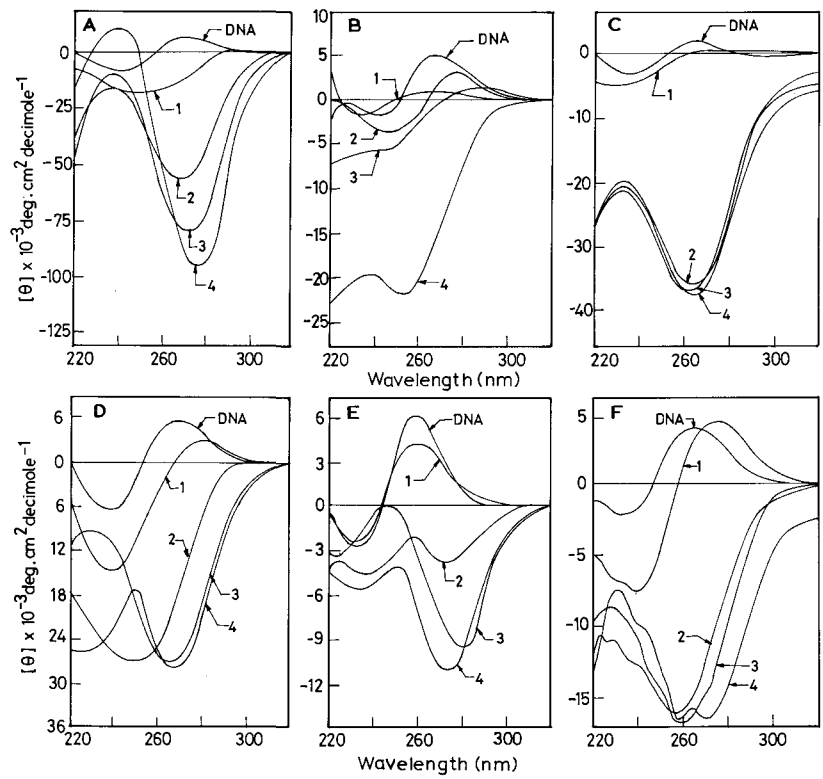

Fig. 2. Circular dichroism spectra of histone H1/16-mer peptide DNA complexes. The spectra of various protein/peptide-DNA complexes prepared in 150 mM NaCl/5 mM Tris-Cl (pH 7.4)/0.1 mM EDTA were recorded in a Jasco model J.20 spectropolarimeter. A–C: SAR DNA; D–F: control *Eco*RI-*Sal*I 650 bp fragment of pBR322; A and D: H1bdec; B and E: H1t; C and F: 16-mer peptide. Curves 1–4 represent spectra of complexes at increasing protein/peptide to DNA ratio (mol/bp) of 0.078, 0.036, 0.05 and 0.06 (A, B, D and E) and 0.18, 0.24, 0.30 and 0.40 (C and F).

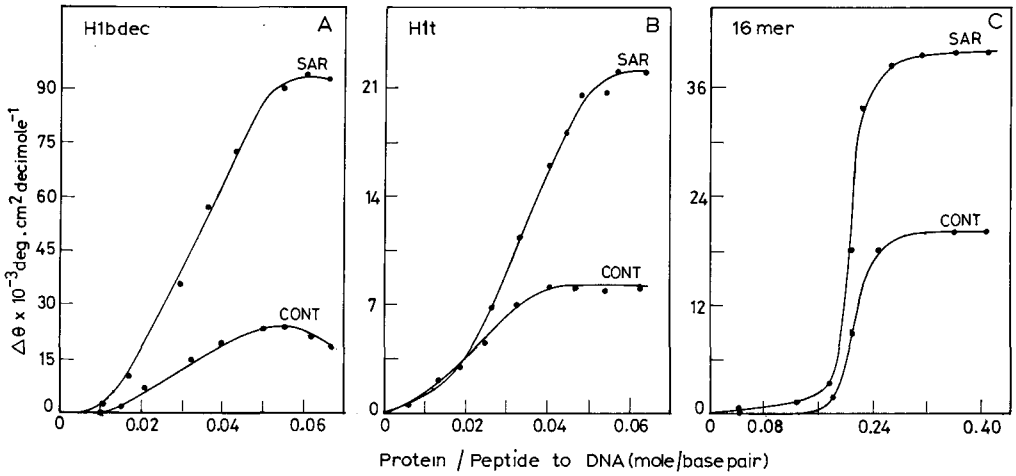

Fig. 3. Effects of histone H1bdec and H1t and 16-mer peptide on condensation of SAR and control DNA fragments. Ellipticity changes observed in Fig. 2 with the SAR and control DNA upon binding to histone H1 subtypes and 16-mer peptide termed Δθ are plotted as a function of protein/peptide to DNA ratio (mol/bp). A: H1bdec; B: H1t; C: 16-mer peptide.

that SAR DNA sequences serve as nucleation sites for deposition of histone H1 on chromatin [7].

We have discussed recently that the 16-mer peptide containing two SPKK units may be regarded as two hooks, each possibly binding to two different minor grooves of different regions of a DNA duplex thus resulting in packing of the DNA and hence condensation of chromatin [12]. The SPKK motif was originally identified by Suzuki as a DNA binding motif having similar structure as Netropsin and hence suggested to bind to the narrower minor groove of the oligo AT tracts [14]. SPKK units occur tandemly in the N-terminus of spermatogenous histones of sea urchin and are implicated in the condensation of sperm chromatin [15]. There is some controversy regarding the mode and specificity of binding of the SPKK units to the AT minor groove. More recent work of Geirstanger et al. [16] has suggested that the binding of a tandem repeat of SPKK unit may not be as specific as originally suggested by Suzuki [15]. NMR studies by Suzuki et al. [17] also suggest a more superficial binding of $(SPR/KK/R)_2$ peptides. The results presented in this communication clearly show that histone H1, in addition to binding preferentially to SAR DNA, also brings about condensation more effectively. This condensation property of the intact histone H1 molecule is also mimicked by its octapeptide repeat motif containing two SPKK DNA binding motifs. However, we would like to point out here that the 16-mer peptide that we have used contains two S/TPKK units spaced by four amino acids while all the earlier work of Suzuki et al. [17] and Bailly et al. [18] used a direct repeat of the motif. The condensation property manifested by the 16-mer peptide may probably explain the structural and functional role of histone H1-SAR DNA interactions. Structurally, histone H1 may compact the chromatin and in association with the scaffold proteins provide a basal knob like structures defining the boundaries of chromosome loops. As mentioned earlier histone H1 has also been shown to repress transcriptional activity in vitro of a DNA template containing an SAR sequence upstream of the T7-polymerase promoter sequence [6]. This can be easily explained based on the preferential condensation property of histone H1 possibly effected through its octapeptide repeat motifs present in its C-terminus [12].

Our observation that histone H1 condenses the SAR sequences preferentially is also supported by several experimental observations in the literature. Distamycin, a peptide antibiotic, has been shown to relax the chromatin structure in vivo exposing the topoisomerase II sites which are presumably occupied by histone H1 [8]. More recently, another hook peptide containing the motif PRGRP has been shown to bind to the minor groove of the oligo AT tracts much more specifically than $(SPKK)_2$ [16]. This AT-hook peptide sequence is present in the HMG-I and HMG-Y proteins. Interestingly, these HMG proteins have been shown to relieve the repression of transcriptional activity observed with histone H1 on a SAR containing DNA template described above [6], which can possibly result from a simple competition of the SAR

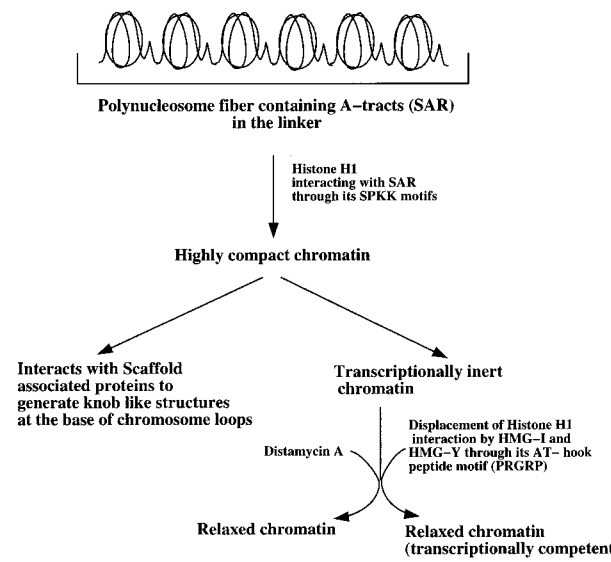

Fig. 4. A model for the dynamics of chromatin structure involving histone H1 through its SPKK motifs and its competition with Distamycin and the AT-hook peptide present in HMG-I and HMG-Y.

sequences by the AT-hook peptide of HMG-I and HMG-Y displacing the interacting SPKK motifs of histone H1.

Based on these considerations, we would like to consider an extension of the model proposed by Kas et al. [8] regarding chromosome dynamics at the SAR sites, which is presented in Fig. 4. The interaction of SAR with histones generates a highly compacted chromatin structure at the base of the chromosome loop which might be further facilitated by interaction with the scaffold associated proteins. Within a polynucleosomal fiber the SAR sequences present in the internucleosomal linker DNA also provide high affinity binding sites for histone H1 and bring about compaction presumably through the SPKK containing repeat motifs present in the C-terminus of histone H1. Such an interaction may facilitate a greater compaction than that of the bulk chromatin. Distamycin or its analogues (D-proteins) like HMG1-HMGY containing the AT-hook peptide motif PRGRP will compete out the SPKK mediated interaction of histone H1 thus causing a relaxation of the chromatin fiber. It may be pertinent to note here that multi AT-hook protein, artificially constructed, does affect chromosome dynamics in a mitotic *Xenopus* extract [19]. More recently, the AT-hook peptide motif PRGRP has been shown to be present in many of the eukaryotic transcription factors.

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