One-domain interaction of histone H4 with nucleosomal core DNA is restricted to a narrow DNA segment

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The interaction of histone H4 with DNA in the nucleosomal core particle has been studied by crosslinking DNA to proteins through their lysine residues. We have compared the crosslinked peptides of H4 at the detected DNA-binding sites: H4(55), H4(65), H4(88), located, respectively, at about 55, 65 and 88 nucleotides from the core DNA termini. For all these binding sites, the patterns of crosslinked peptides were shown to be very similar. This suggests the presence of a single DNA-binding domain in the H4 molecule. The H4-binding sites are located within a narrow DNA segment close to one another on the complementary strands across the DNA grooves, overlap with sites ±1 of the DNA sharp bending [(1984) Nature 311, 532–537] and with the strong binding sites for histone H3: H3(75) and H3(85).

DNA-histone binding *DNA-protein cross-linking* *Histone H4* *Lysine residue* *Nucleosome* *Peptide mapping*

1. INTRODUCTION

A nucleosomal core particle is made up of pairs of histones H2A, H2B, H3, and H4 and of DNA, about 145 bp long, wound into 1.8 turns of left-handed superhelix around histones. The current detailed models of the nucleosomal particle are based on data obtained by various experimental approaches, most notably, X-ray crystallographic studies [1,2] and DNA-histone [3,4] and histone-histone crosslinking experiments. The detailed linear map of histone arrangement along the nucleosomal DNA derived from DNA-histone crosslinking reveals multiple sites of crosslinking for each histone, e.g. for histone H4 the binding sites H4(55), H4(65) and H4(88) map to approx. 55th (site ±2), 65th (site ±1) and 88th (site ±1.5) nucleotides from the 5′-end of either DNA strand [4] (see the legend to fig.2 for notation). This may be due either to multiplicity of contacts between DNA and different regions of the histone molecule or to superposition of several structural variants (static or dynamic) of the core particle.

To gain a better insight into the structure of histone-DNA interactions, we have compared the regions in histone H4 that are involved in contacts with different segments of the core DNA by mapping according to [5] the peptides of H4 that are crosslinked to DNA within the core particle.

2. EXPERIMENTAL

Core particles isolated from H1-depleted chromatin of chicken reticulocytes were crosslinked using the dimethylsulfate methylation-partial depurination-$NaBH_4$ reduction protocol [3]. A small aliquot (1/30) of the crosslinked sample was labeled with $Na^{125}I$ via ICl, recombined with the bulk sample and electrophoresed under denaturing conditions in the presence of urea in a discontinuous SDS-polyacrylamide gel electrophoretic system. A first-dimension gel strip was excised and incubated in 70% HCOOH/2% diphenylamine/1% L-lysine·HCl/1% cysteamine·HCl at 70°C for 20 min to hydrolyse DNA. The liberated histones were resolved in the second

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dimension of gel electrophoresis in the presence of SDS [3,4] as shown in fig.1A. After autoradiography, the horizontal trace of ^{125}I-H4 was then cut into 10 slices and eluted; each eluate was freed of SDS by repeated washes with 0–0.1 N HCl, 85% acetone, treated with 5 mM *N*-bromosuccinimide in 1 M acetic acid for 5 min to remove ^{125}I label (no cleavage of peptide bonds was detected under such conditions), incubated successively with bacterial alkaline phosphatase in 0.1 M Tris-HCl, pH 8.0, and with a mixture of micrococcal nuclease/spleen phosphodiesterase in 20 mM succinic acid-NaOH, 8 mM $CaCl_2$, pH 6.0. The ^{32}P label was then introduced with [γ-^{32}P]ATP and polynucleotide kinase, H4 was purified by polyacrylamide gel electrophoresis, and the resulted samples incubated with TPCK-treated trypsin in 0.2 M NH_4HCO_3 at 37°C for 4 h. The resultant labeled nucleotide peptides were electrophoresed towards the cathode in slabs of 18% acrylamide/0.6% methylenebisacrylamide (w/v) gel buffered with 30 mM glycine/300 mM acetic acid, pH 2.7 (1 kV, 2 h in the cold) (fig.1B). The dried gel was exposed onto X-ray film with an intensifying screen at −70°C.

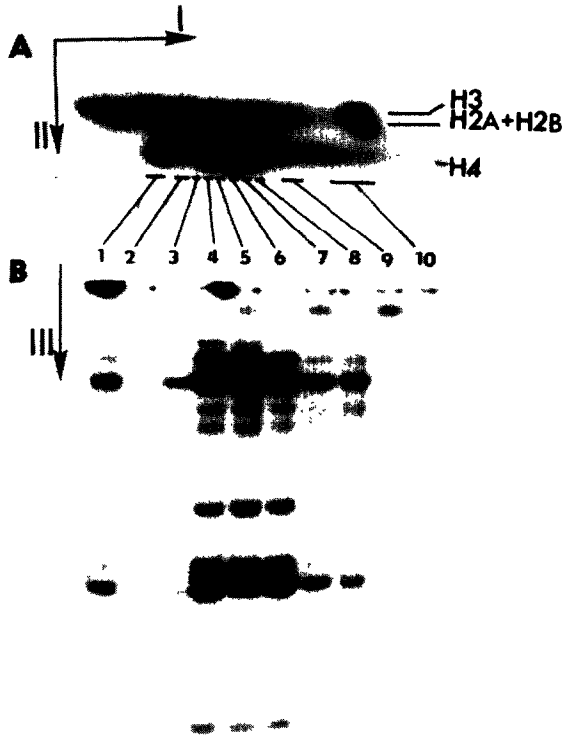

Fig.1. Mapping of H4 peptides involved in contacts with segments of core particle DNA by 3-dimensional gel electrophoresis. (A) The map of sequential arrangement of histones along nucleosomal DNA is produced by gel electrophoresis of DNA-histone crosslinked complexes in the 1st (I) direction and of histones released by DNA hydrolysis in the 2nd (II) direction [3]. (B) The gel regions corresponding to the sites of crosslinking: H4(88), lane 1; H4(65), lane 4; H4(55), lanes 5, 6, and also from other gel areas were excised from the H4 horizontal trace. H4 molecules were eluted from the gel, labeled with ^{32}P at the lysine residues crosslinked to DNA, digested with trypsin and displayed in the 3rd (III) direction of gel electrophoresis.

3. RESULTS

Our approach was a combination of the protein-DNA crosslinking method, 2-dimensional 'protein' polyacrylamide gel electrophoresis that had been developed for determination of the arrangement of histone-binding sites along nucleosomal DNA [3] and subsequent characterization of these binding sites in histone molecules by mapping the peptides labeled with ^{32}P at the site of crosslinking [5]. Briefly, histones within the core particles were crosslinked to partly depurinated DNA through the aldimine bonds between lysine ϵ-amino groups and deoxyribose aldehydes at the depurinated sites, with concomitant DNA-chain scission, 3′ to the site of crosslinking. The length of the 5′-terminal DNA fragment crosslinked to a histone molecule indicates the precise distance of the histone crosslinking site on nucleosomal DNA from its 5′-termini. To separate histone molecules crosslinked to different DNA sites and from one another, the denatured crosslinked complexes were fractionated according to the DNA size by polyacrylamide gel electrophoresis and the DNA in the complex was hydrolysed directly in gel. The released histones were further resolved in the second dimension of gel electrophoresis (fig.1A) and the spots of histone H4 crosslinked to different DNA segments were eluted for subsequent peptide mapping. Due to the mechanism of acid hydrolysis of DNA, about 50% histones liberated from DNA harbour 5′-phosphate mono- or oligopyrimidine tails attached to significantly modified deoxyribose at the site of crosslinking

[5]. Successive treatments of histones with phosphatase and micrococcal nuclease/spleen phosphodiesterase trim the heterogeneous oligonucleotide tail to homogeneous 5'-OH pyrimidine nucleotide attached to a modified depurinated residue. Thereafter, the crosslinked sites were labeled with [γ-^{32}P]ATP by polynucleotide kinase-catalysed phosphorylation, and the histones bearing the label at the sites of contact with DNA were fragmented with trypsin. The resultant radioactive nucleotide peptides were resolved by specially devised polyacrylamide gel electrophoretic systems in one or 2 dimensions [5].

The results of the experiments outlined above are summarized in fig.1. The tryptic maps of the H4 contacts at the sites H4(88)- ± 1.5 (lane 1), H4(65)- ± 1 (lane 4), and H4(55)- ± 2 (lanes 5,6) are identical in both position and relative intensity of the bands. The equivalence of peptide maps for these H4-binding sites was also preserved when we used a higher resolution 2-dimensional gel electrophoresis of nucleotide peptides or substituted staphylococcal protease for trypsin.

The maps of all nucleosomal contacts of H4 within the core particle have not revealed any new bands as compared with the maps in fig.1B (not shown). Thus, chemical crosslinking detects no other H4 lysines interacting with nucleosomal DNA besides those at sites H4(55), H4(65) and H4(88) which represent all detected DNA-binding sites for histone H4.

At present, the number of lysines crosslinked to DNA is not certain, for a number of reasons [5,6], but can be roughly estimated as 3–5 residues [5].

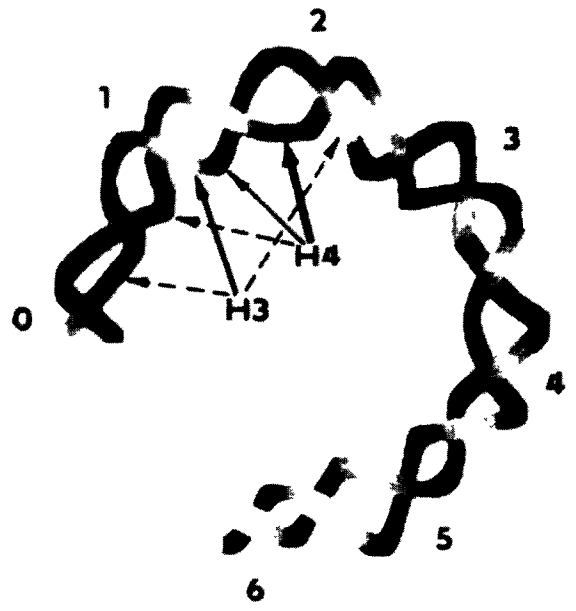

Fig.2. Scheme of contacts between histones H3 and H4 and one half of symmetrically folded nucleososmal DNA. The crosslinked sites are shown in order of decreasing intensity of crosslinking [4] by heavy, thin and broken arrows for H4(55), H4(88) and H4(65), respectively, and by an unbroken arrow for H3(85), broken short and broken long arrows for H3(75) and H3(95), respectively. For details see fig.8 in [4] and fig.1 in [13]. Since no coordinates for DNA phosphates are yet known, location of contacts on the DNA double helix is provisional and is correlated with the DNase I digestion data [9]. The course of the DNA backbone along the superhelical path is reproduced from [1] with the numbers from 0 to 6 measuring the distance along DNA starting from the point where the core particle dyad axis passes through DNA.

4. DISCUSSION

A number of conclusions can be drawn from the data reported here. The same set of lysines crosslink at sites H4(55), H4(65) and H4(88), thus behaving as if all belonged to one histone domain. Now, all the crosslinks are juxtaposed at sites ± 1, ± 1.5 and ± 2 (fig.2) in the core particle DNA double helix, according to [1]. These sites face one another across the major (55th and 88th) and minor (65th and 88th) grooves [3], although an unambiguous assignment of the sites relative to the grooves has not yet been made. A simple interpretation would be that either the DNA-binding domain of H4 tumbles between the grooves or, more likely, the space between the grooves could be swept by lysine side chains (~7 Å in length) [3]. Note that the distances between the points to be bridged are markedly diminished by a rather sharp bending of the nucleosomal DNA, especially at site ± 1 where the major and minor grooves facing inward are highly compressed to widths of 11 and 7 Å, respectively [1]. The charge-charge interactions between the DNA phosphates and lysine side chains do not require the latter to be immobilized [7]. In fact, most lysines within the core particle were shown by NMR to be unconstrained [8]. Moreover, the excision of a base should increase the local mobility of depurinated residues in the

DNA double helix and thus enhance the effect of lysine oscillation. However, the possibility of superposition of static conformational variants is not excluded as the efficiency of H4(65) crosslinking varies strongly and under certain conditions becomes virtually negligible [4]. We can therefore conclude that the strongest binding site is H4(55), followed by H4(88), less intensively crosslinked, and the third, H4(65) can be negligible.

The approach we used localized only interacting lysines and our data do not rule out some binding of H4 through arginines to other sites on the core DNA. However, it would be rather surprising if any binding domain of H4 contained exclusively arginine residues, moreover lysine residues are always present in arginine-containing regions of H4. From this it follows that, whatever the other contacts, one H4-binding domain occupies a singular, rather narrow segment on the core DNA ($\pm 1 - \pm 2$) which has some structural peculiarities. The DNA is reported to be most sharply bent at sites ± 1 [1]. Nucleotides 63–64 and 84–86 from the 5′-end at these sites are either protected against the attack of DNase I, DNase II and micrococcal nuclease or display a marked discontinuity in location of cleavage sites [9–11]. The site around the 62nd nucleotide shows an enhanced reactivity towards dimethylsulfate methylation [12]. The H4 domain spanning across the grooves at sites ± 1 may cause the DNA to bend owing to lateral neutralization of DNA phosphates [13] and this situation resembles the spermine molecule bridging across the major groove at the point of bending of a synthetic DNA dodecamer [14] as well as the anticodon stem of $tRNA^{Phe}$ [15]. The H4 binding to site ± 1 overlaps the H3 contacts at sites H3(75) and H3(85). Assuming that 'a stiff molecule like DNA need only be fixed at a few points and the bending stiffness will do the rest' [16], we suggest the overlap between the DNA binding domains of H4 and H3 to be one of the 'strategic points' to which the core particle DNA is hooked and around which it is tightly bent. It is worth mentioning here that another segment of core DNA sharp bending at positions ± 4 [1] also contains strong crosslinking sites for both H2A and H2B histones [4].

To collect structural data on other nucleosomal histones, one can make use of the above approach supplemented with a suitable procedure to identify the polypeptide regions involved in DNA-protein interactions, especially because the available X-ray data on the nucleosomal core particle [1] and histone octamer [2] seem rather controversial. Our data support the assignment of histone H4 contacts around site ± 1.5 of nucleosomal DNA but do not provide evidence for any substantial interaction of H4 with segments $\pm 3.5 - \pm 4.5$ (at least, not through lysine residues) suggested by X-ray studies [1,2]. The crosslinking methods might be also helpful in studies of other DNA-protein complexes.

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