Folding of the SPKK Rich Peptide in the Presence of the Octa-Oligonucleotide

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The nucleosome contains of 200 base pairs of DNA complexed with four core histone complex: H2A, H2B, H3, and H4. The fifth histone species, the H1 histone, interacts with linker DNA connecting neighbouring nucleosomes. We have studied the influence of the phosphorylation on the interactions of a repeating unit 15 residues long, containing the SPKK motif, the motif thought to induce turn along peptides sequences, enclosed within the trout testis H1 C-terminal domain with octanucleotide by means of the thermal denaturation and CD technique. The results indicate that the peptide preferentially binds to a single stranded oligonucleotide. It has been shown further that there is no β structure present but a distorted helical structure has been detected.

Introduction

Eukaryotic chromatin consists of a DNA associated with basic nuclear proteins, histones, which form a repeating subunits called nucleosomes. Nucleosome is a particle which consists of an octameric complex of the four core histones H2A, H2B, H3, and H4 and two superhelical folds of DNA, 200 bp long (Kornberg *et al.,* 1974; Oudet *et al.,* 1975). Nucleosomes are connected by a DNA, termed linker DNA, associated with histone H1 which is not present in the nucleosome. The association of H1 histone with DNA is intricate and reflects the partition of H1 histone into the three domains N, G, and C (Hartman *et al.,* 1977).

It has been established that the G domain bridges the entry and exit part of superhelical fold of the DNA on the particle (Allan *et al.,* 1970) thereby delineating a 165 bp structure (chromatosome) whereas, the $2(H2A, H2B)_2(H3, H4)_2$ octamer associates with 146 bp in order to generate the "core particle" (Clark *et al.,* 1974). The 35 bp stretch of DNA, between 165 bp and 200 bp, not associated with G-H1 is labelled as true linker although the additional 20 bp liberated when G-H1 dissociates from the particle may also be included into the linker DNA.

The third and major domain of H1 histone is the C-H1 domain and comprises a large C-terminal tail of approximately 100 residues. It has been shown that this domain is required for chromatin condensation (Hartman *et al.,* 1977) and the ordered folding of the nucleosomes into a higher order structure, solenoid (Finch and Klug, 1976). It is thought that the association between the C-H1 residues and the DNA neutralise the negative charges of a linker DNA thereby eliminates charge repelling and facilitates close approach of linker DNA in tightly packed solenoid structure (Crane-Robinson, 1979; Finch and Klug, 1976)

In this study we would like to investigate interaction of a C-terminal domain of H1 histone with a linker DNA and the influence of phosphorylation on the structural properties of C-H1 domain in the presence of DNA.

Materials and Methods

The peptides comprising 15 residues H1 (156–170) of salmon sperm (Macleod *et al.,* 1977) KKAAKSPKKATKAAK and KKAAKS*PKKATKAAK ("*" shows the phosphorylation place) were synthesised using Bmoc-chemistry. The octa-oligonucleotides 5'-ATCTTCTA-3' and 5'-TAGAAGAT-3' were synthesised using β-cyanoethyl phosphoramidite DNA sythesis cycle.

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Single-stranded octa-oligonucleotides were annealed by heating of the mixture of both strands (1:1 molar ratio) up to 70 °C and allowing slow cooling to the room temperature, approximately 20 °C. The quality of annealing was checked through melting experiments performed at 260 nm on the Varian Cary 3 spectrophotometer using Cary 13 software.

Circular Dichroism (CD) spectra were recorded at the following solution conditions: 70% (v/v)-2,2,2-trifluoroethanol (TFE) at 20 °C; 70% (v/v) TFE, 5 mM phosphate buffer at 20 °C; 20 mM NaCl, 1 mM phosphate buffer, pH 7.57 at 10 °C and 20 °C. A complex of peptide-DNA (1:1 molar ratio) was prepared by adding peptide aliquots from the stock solution, to the final concentration of 7 μg/ml, to the DNA solution of the concentration of 20 μg/ml. For complexes peptide-single stranded DNA, the 5'-TAGAAGAT-3' strand was used.

All experiments were performed using a Jasco-710 spectropolarimeter with the following acquisition parameters: band width 1.0 nm, sensitivity 2 to 10, response 4 sec, scan speed 50 nm/min, step resolution 0.5 and 20 scans per experiment. The signal to noise (S/N) ratio was equal to 9. All experiments were carried in a quartz cuvette (d=2 mm). The CD data are expressed in the following units : mdeg cm^2 $dmol^{-1}$.

Results

The thermal stability of a double stranded octa-oligonucleotide with and without the two forms of peptides, nonphosphorylated and phosphorylated was examined in 20 mM NaCl, 1 mM phosphate buffer at pH 7.6 with results as presented in Fig. 1. The influence of peptides on the melting temperature of DNA was monitored by changes of the melting temperature T_m. The T_m observed for a double stranded octa-oligonucleotide is 35 °C whereas with peptides present the T_m is 15 °C and 13 °C, for nonphosphorylated and phosphorylated forms of peptide, respectively. The CD spectrum of a double- and single-stranded oligonucleotide was recorded at the temperature of 20 °C in the following buffer: 20 mM NaCl, 1 mM phosphate buffer, pH 7.6 with results presented in Fig. 2. For double-stranded oligonucleotide a typical ψ curve is observed with (Φ_1) equal to 275 nm and (Φ_2) equal to 250 nm which are characteristic for the B-form of DNA (Fasman *et al.*, 1970). The single-stranded oligonucleotide is characterised by (Φ_1) equal to 272 nm and (Φ_2) equal to 258 nm. CD spectra of phosphorylated and nonphosphorylated peptides were recorded under the same experimental conditions as for the oligonucleotide in Fig. 3, and show a strong positive Cotton effect with (Φ_2) equal to 198 nm. This result indicates

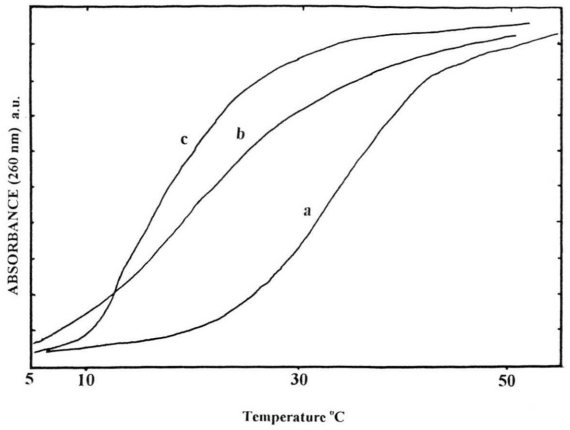

Fig. 1. Melting curves of: a) a double stranded oligonucleotide : b) nonphosphorylated peptide-DNA mixture (1:1 molar ratio); c) phosphorylated peptide-DNA mixture (1:1 molar ratio).

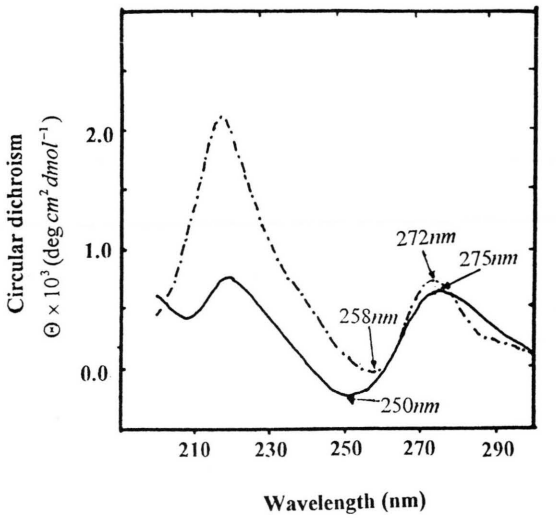

Fig. 2. CD spectra of an oligonucleotide in a buffer 20 mM NaCl, 1 mM phosphate buffer pH 7.6 20 °C; the oligo concentration is 20μg/ml; single stranded oligonucleotide (-·-·-·-); double stranded oligonucleotide (——).

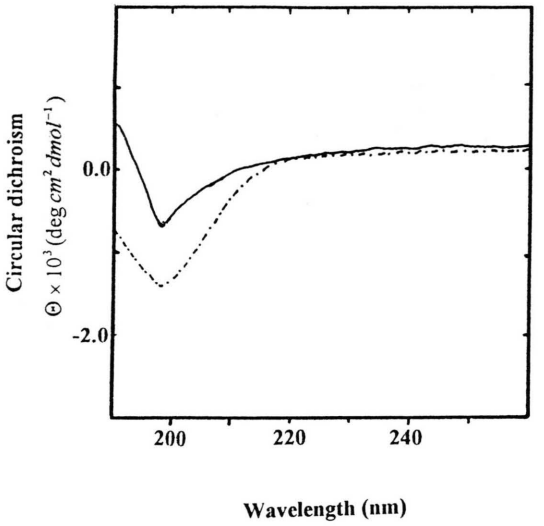

Fig. 3. CD spectra of free nonphosphorylated (——), and phosphorylated peptide (-·-·-·-); 7 μg/ml in 20 mM NaCl, 1 mM phosphate buffer, pH 7.6, 20 °C.

that under these experimental conditions both peptides adopt a random coil structure.

The influence of TFE and phosphate ions (helix-inducing factors) on the secondary structure of both peptides was also investigated. The set of spectra in 70% (v/v)TFE and in 70% (v/v)TFE, 1 mM phosphate buffer were recorded and are presented in Fig. 4A-B. The spectra of peptides recorded in 70% (v/v)TFE are characterised by a negative Cotton effect, (Φ_1) equal to 200 nm, observable for both forms of peptides. This result corresponds well with previously reported results (Clark *et al.*, 1988).

The addition of phosphate ions does not influence (Φ_1) although there is a distinctive decrease in ellipticity observed for phosphorylated peptide which is not detected for a nonphosphorylated one.

To investigate the influence of a double-stranded and single -stranded oligonucleotide on the secondary structure of peptides, CD spectra at the temperature of 20 °C and 10 °C (1:1 molar ratio peptide/DNA) were recorded and are presented in Fig. 5A-C. The temperature of 10 °C was maintained in order stabilise the double stranded form of DNA. At higher temperature, 20 °C, a double-stranded oligonucleotide is expected to be completely melted thus allowing for the interaction between the peptide and the single -stranded oligonucleotide. The CD curves recorded at 20 °C, Fig. 5A, show a negative Cotton effect present for both forms of peptide. The maximum and minimum of ellipticity for the oligonucleotide are observed at 272 nm and 250 nm. The shift of (Φ_2) in respect to the result presented in Fig. 2 indicates that binding of a peptide to a single stranded DNA occurred (Bohm and Creemers, 1993). In the region covering the absorbance of peptide the spectra recorded at 10 °C and 20 °C are characterised by a negative Cotton effect. The CD curve for the octa-oligonulceotide recorded at 10 °C, Fig. 5C, characterised is by the maximum of ellip-

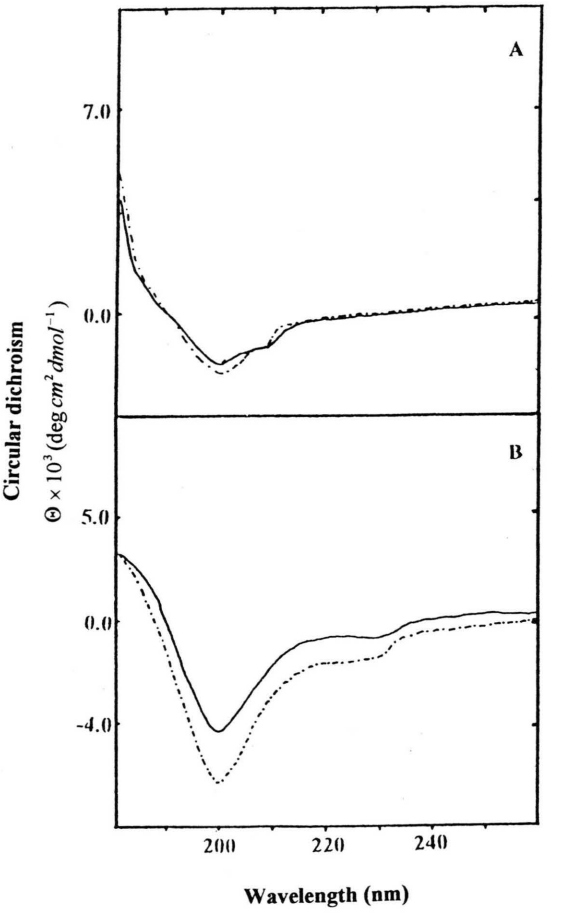

Fig. 4. CD spectra of (A): free nonphosphorylated peptide in 70% TFE (v/v) (——), 70% TFE (v/v), 5 mM phosphate buffer (-·-·-·-); (B) free phosphrylated peptide in 70% TFE (v/v) (——), 70% TFE (v/v), 5 mM phosphate buffer (-·-·-·-),pH 7.6, 20 °C.

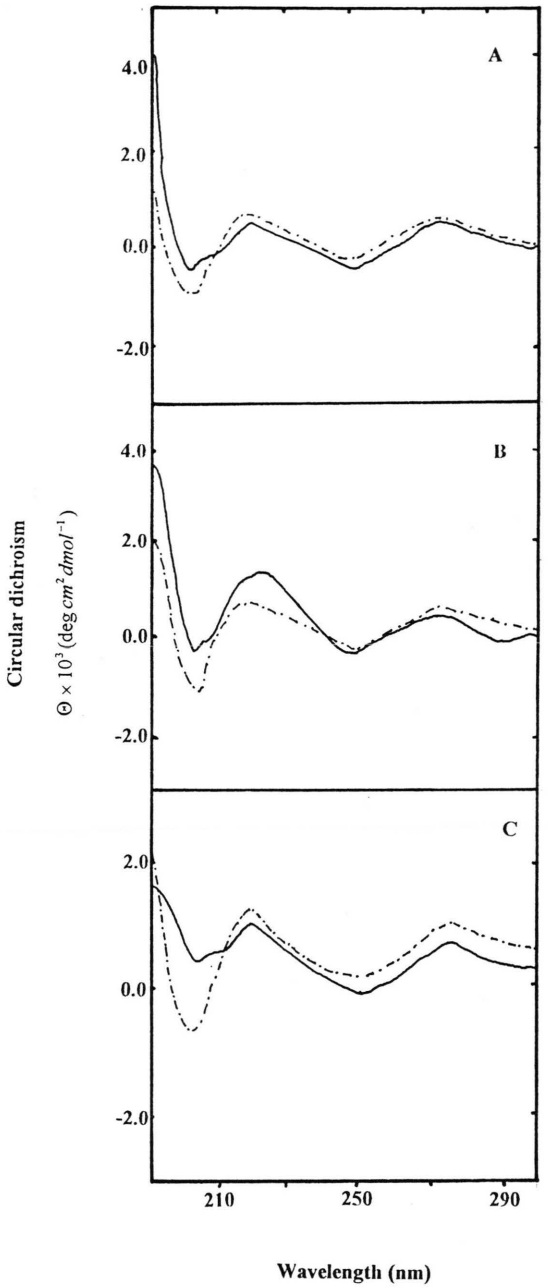

Fig. 5. CD spectra of (A): nonphosphorylated peptide+double stranded oligonucleotide (——), phosphorylated peptide +double stranded oligonucleotide (-·-·-·-) in 20 mM NaCl, 1mM phosphate buffer, pH 7.6, 20 °C; (B) nonphosphorylated peptide + single stranded oligonucleotide (——), phosphorylated peptide +single stranded oligonucleotide (-·-·-·-) in 20 mM NaCl, 1mM phosphate buffer, pH 7.6, 20 °C; (C) nonphosphorylated peptide +double stranded oligonucleotide (——), phosphorylated peptide +double stranded oligonucleotide (-·-·-·-) in 20 mM NaCl, 1 mM phosphate buffer, pH 7.6, 10 °C.

ticity at 275 nm and the minimum at 250 nm corresponding to the spectrum of a double-stranded oligonucleotide recorded without the peptide (Fig. 2). To test whether the binding between single stranded oligonucleotide and the peptide really occurred and whether the results observed in Fig. 5A are no artefacts of some sort of undefined interactions, a single-stranded oligonucleotide, with the sequence given in Materials and Methods, was mixed with peptide at the temperature of 20 °C and the CD spectrum was recorded with the result presented in Fig. 5B. The spectrum obtained is characterised by a negative Cotton effect in the spectral region of 190 nm to 220 nm, observable for both forms of peptide. The minimum and the maximum of ψ curve appeared at 272 nm and 250 nm. These results are in agreement with those presented in Fig. 5A.

Discussion

The comparison of the melting curves obtained for a double-stranded octa-oligonucleotide without and in presence of the two forms of peptides, Fig. 1 shows that both peptides decrease the thermal stability of the DNA. The difference in the thermal stability of a DNA in presence of nonphosphorylated peptide is 20 °C, while in the presence of phosphorylated peptide it is 22 °C. The greater decrease of the melting temperature caused by the phosphorylated peptide in respect to the nonphosphorylated one indicates that phosphorylation of a residue causes decrease of stability of a short DNA. The observation of structural changes induced by addition of structural factors such as TFE or DNA into the peptide solution yields results presented in Fig. 3. The CD spectra of peptides recorded in 20 mM NaCl, 1 mM phosphate buffer pH 7.57 are characterised by a positive Cotton effect characteristic for a random coil structure and in presence of TFE and phosphate ions both peptides undergo structural changes monitored by the appearance of a negative Cotton effect Fig. 4A-B. The influence of a double stranded oligonucleotide on the structure of a peptide is showed in Fig. 5A-B. It can be seen that at 20 °C the complex between the peptide and the DNA is formed and this result is confirmed by the shift of the (Φ_2) value from 258 to 250 nm. Such phenomenon was not observed at the tem-

perature of 10 °C, Fig. 5C, at which the DNA molecule is in double-stranded form and does not bind to peptide. It can also be observed that interactions between DNA and peptide not only induce structural changes in DNA but also in peptides. It is thought that this behaviour is caused by neutralisation of positive charges belonging to lysines through negative phosphate charges of a DNA.

The comparison of the results presented in Fig. 5A, B, and C allows for the conclusion that the peptide binds to single-stranded oligonucleotide.

At higher concentration of DNA and peptide an aggregation process was observed (result not shown). The changes observed for a phosphorylated peptide in the presence of a DNA, single- and double -stranded (Fig. 5A-B) indicates that phosphorylation process increases binding of a peptide to a DNA and thus allows for greater structuralisation of the peptide

It has been previously shown that the Ser-Pro-X-X sequence may adopt a β structure and connects regions without defined secondary structure (Hill *et al.*, 1989). Results presented here show that peptides containing such sequence tend to adopt a helical structure, distorted helix (Manning *et al.*, 1988), which is in agreement with previously presented results (Verdaguer *et al.*, 1993). Moreover, such sequence destabilise the double-stranded structure of a DNA. The phenomenon described here will definitely have a strong influence on the behaviour of the linker DNA and the chromatin condensation process. The phosphorylation of a residue within the stretch of amino acids causes further destabilisation of a DNA structure and structuralisation of a peptide with very important biological influence.

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