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A tandem repeat of the SPKK peptide motif induces Ψ-type DNA structures at alternating AT sequences

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The interaction between a tandem repeat of the SPKK peptide motif and calf thymus DNA or several polynucleotides has been investigated by circular dichroism. The octapeptide SPKKSPKK does not induce any important changes in the CD spectra of the polynucleotides poly(dG) · poly(dC), poly(dG-dC) poly(dG-dC) and poly(dA) · poly(dT) while the spectrum of calf thymus DNA is slightly modified. Binding of this basic peptide to the alternating copolymer poly(dA-dT) · poly(dA-dT) results in a marked Ψ-type condensation in a manner similar to that induced by the entire C-terminal domain of histone H1.

DNA condensation; Histone; Circular dichroism; DNA sequence-specificity; Basic peptide; Transcription

1. INTRODUCTION

Binding of histone proteins, more particularly histone H1, to eukaryotic DNA has been shown as being one of the primary mechanisms by which transcription is controlled. Although the multiple roles of histones in transcription processes have been recently debated [1–5], the precise nature of the molecular interactions between histones and DNA remains to be clarified. It has been proposed that the histone H1–DNA interaction implicates the DNA-binding peptide motif SPXX (X for a basic residue) [6,7]. Analogous basic peptides have been shown to be implicated in sequence-specific histone–DNA recognition processes [8,9]. Clustered S(T)-P-X-Basic motifs promote DNA–histone interactions [10] and their phosphorylation [12,13] or acetylation [14] appear as many regulatory processes involved in chromatin condensation.

The octapeptide SPRKSPRK is found at the amino and carboxyl termini of histone H1 and the amino terminus of H2B from sea urchin sperm and binds selectively to AT-rich DNA sequences [6,8,15]. We have recently shown that the covalent linkage of the related octapeptide SPKKSPKK, called S_2 peptide, to an acridine-based DNA intercalating agent leads to a conjugate able to preferentially recognize AT-rich sites in DNA [16] with potential interest as antitumor agent [17]. In order to further delineate the ability of the S_2 peptide to bind to DNA in a sequence-dependent manner, we have examined the S_2 peptide-induced DNA conformational changes using the DNA from calf thymus (i.e. a natural DNA with random sequences) and four synthetic polynucleotides with different base pair arrangements. The circular dichroism (CD) study reported here (i) provides further experimental evidence that the S_2 peptide does correspond to a sequence-selective DNA-binding motif, and (ii) shows that its binding is accompanied by a sequence-selective DNA condensation. Indeed, Ψ-type DNA condensations analogous to those described for much longer basic peptides [18,19] are detected when the peptide binds to the alternating copolymer poly(dA-dT) · poly(dA-dT).

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Abbreviations: CD, circular dichroism; CT, calf thymus; SPKK, Ser-Pro-Lys-Lys; R, Arg; T, Thr.

2. MATERIALS AND METHODS

2.1 *S_2 Peptide*

The octapeptide SPKKSPKK was purchased commercially from Néosystem SA (France). Its purity was checked by HPLC on a C_{18} column. Buffer A consisted of 0.1% aqueous trifluoroacetic acid. Buffer B contained 50% acetonitrile and 50% buffer A. The peptide was eluted with a linear gradient of 0–40% buffer B over a period of 20 min at a flow rate of 1 ml/min (detection at 210 nm; peptide elution time 7 min). The structure was analyzed by 400 MHz ^{1}H-NMR spectroscopy and the FAB mass spectrum was also fully consistent with the expected structure [FAB: 899 (M^+)]. No difference was observed with the S_2 peptide prepared by a step-wise manual liquid-phase synthesis in accordance with the previously published procedure [17].

2.2. *DNA and polynucleotides*

Calf thymus DNA (CT DNA, highly polymerized sodium salt from

Sigma Chemical Co.) was deproteinized twice with sodium dodecyl sulfate and precipitated with ethanol. The final protein content was less than 0.2%. 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 Sigma. All the nucleic acids were extensively dialyzed against the dichroism buffer (1 mM sodium cacodylate, pH 6.5) prior to measurements. Their concentrations, expressed with respect to nucleotides, were determined using extinction coefficients (ε) at 260 nm equal to 6,600, 6,600, 8,400, 6,000 and 7,400 $M^{-1} \cdot cm^{-1}$, respectively [20].

2.3. *Circular dichroism*

CD spectra of DNA, polynucleotides, peptide S_2 and peptide–DNA complexes were recorded at 20°C in the UV region (190–320 nm) on a Jobin-Yvon Mark V dichrograph interfaced to a microcomputer. Solutions of peptide and/or nucleic acids in 1 mM sodium cacodylate buffer, pH 6.5 were scanned using 1-cm pathlength quartz cuvettes. Measurements were made by progressive addition of the peptide to a pure DNA (or polynucleotide) solution at 100 μM to get the desired ligand/phosphate-DNA (L/P) ratio. The molar ellipticity [θ] was expressed in deg · cm^2 · $dmol^{-1}$ per nucleotide. The CD spectra of the different homopolymers and alternating copolymers recorded under low ionic conditions (1 mM sodium ions) are in agreement with those reported in [20] obtained at a higher ionic strength (1 mM phosphate buffer containing 10 mM NaCl).

3. RESULTS AND DISCUSSION

The CD spectrum of the S_2 peptide alone is characterized by a negative band near 200 nm (Fig. 1; spectrum 1). This feature has been found for other linear basic peptides such as the related octadecapeptide $(KTPKKAKKP)_2$ from histone H1 and could reflect a potential organization of these motifs in β-turn structures [18,21]. The S_2 peptide alone presents no CD signal in the 230–300 nm region so that the DNA structure can be characterized without interference from the peptide.

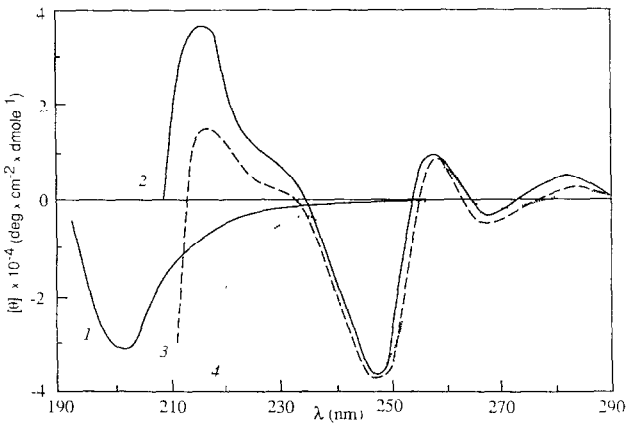

Fig. 1. Circular dichroism spectra of the complexes between the S_2 peptide SPKKSPKK and the homopolymer poly(dA) · poly(dT). Curve 1, S_2 peptide (50 μM). Curve 2, poly(dA) poly(dT) (100 μM). Curves 3 and 4, S_2 peptide–poly(dA) · poly(dT) complexes at ligand to phosphate-DNA (L/P) molar ratios (L/P) of 0.2 and 0.6, respectively. All spectra were recorded in 1 mM sodium cacodylate buffer, pH 6.5. Molar ellipticities [θ] for poly(dA) · poly(dT) and S_2 peptide are expressed in deg · cm^2 $dmol^{-1}$ per nucleotide and amino acid residue, respectively.

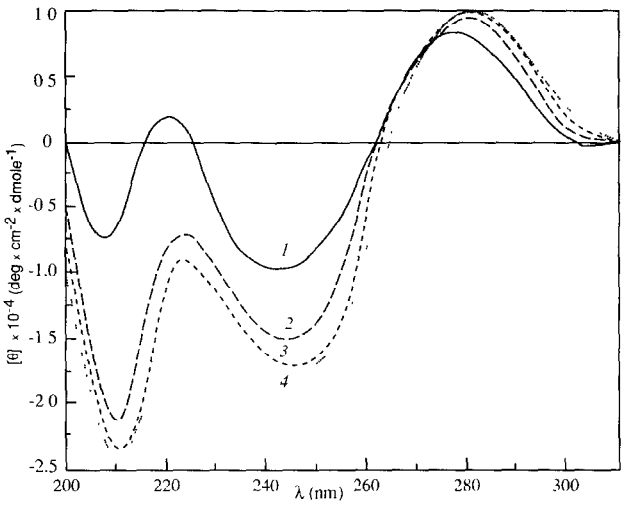

Fig. 2. Circular dichroism spectra of the complexes between the S_2 peptide and calf thymus DNA. Curve 1, CT DNA (100 μM). Curves 2, 3 and 4, S_2 peptide–calf thymus DNA complexes at ligand to phosphate-DNA molar ratios of 0.4, 0.7 and 0.9, respectively. Molar ellipticities [θ] are expressed in deg · cm^2 $dmol^{-1}$ per nucleotide.

On adding the S_2 peptide to the homopolynucleotide poly(dA) · poly(dT) (Fig. 1) only minor (if any) changes are observed within the wavelength region of 230–300 nm. This directly implies that this homopolymer does not undergo any significant structural changes upon interacting with the peptide. This lack of effect must be related to the peculiar structural characteristics of this homopolymer. Indeed, poly(dA) · poly(dT) has long been known to be structurally distinct from random DNA sequences and can adopt more rigid and bent structures [22–24]. The S_2 peptide is unable to overcome this rigidity and to alter significantly the homopolymer conformation. Consistent with our results are the observations that long poly(dA) · poly(dT) sequences cannot be reconstituted into nucleosome particles [25,26]. Similarly, the ellipticity remains unchanged when the S_2 peptide is added to a solution of the alternating copolymer poly(dG-dC) · poly(dG-dC) or the homopolymer poly(dG) · poly(dC) (not shown). This is not surprising because footprinting studies with the S_6 peptide containing six SPKK motifs [8] and the S_2 peptide–acridine conjugate [16] have revealed that the basic motif does not bind to GC-rich sequences.

Fig. 2 shows the CD spectra of CT DNA in the absence and presence of the S_2 peptide. At a peptide/DNA ratio of 0.4 the spectra of the complex is shifted by about 5 nm with respect to that of CT DNA alone and a slight increase of both the negative band near 245 nm and the positive one at 275 nm is observed (Fig. 2; spectrum 2). At higher molar ratios, the CD spectra of the S_2 peptide–CT DNA complexes are roughly similar to that obtained at a L/P ratio of 0.4; the positive and negative bands mentioned above are only slightly more

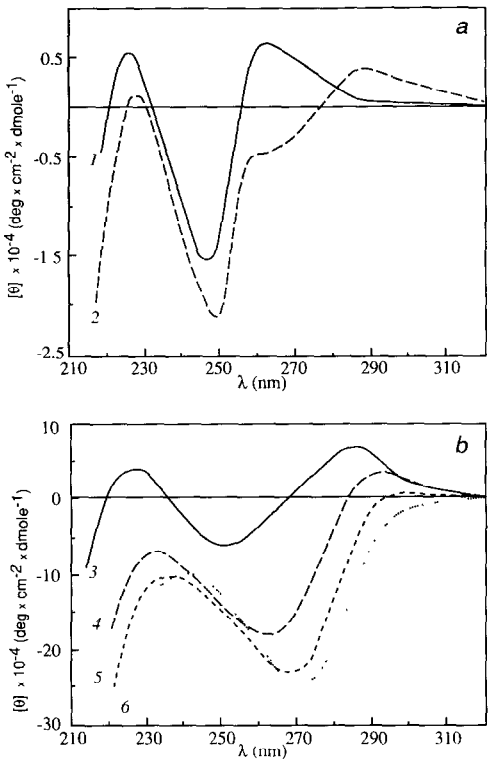

Fig. 3. Circular dichroism spectra of the complexes between the S_2 peptide and the alternating copolymer poly(dA-dT) · poly(dA-dT). Curve 1 (a), poly(dA-dT) · poly(dA-dT) (100 μM). Curves 2 (a), 3, 4, 5 and 6 (b), S_2 peptide-poly(dA-dT) · poly(dA-dT) complexes at ligand to phosphate-DNA molar ratios of 0.2, 0.4, 0 6, 0.8 and 1.0, respectively.

intense (Fig. 2; spectra 3 and 4). The shape of these spectra are on the whole comparable to that of CT DNA alone thus showing that the conformation of CT DNA, though affected by the binding of the S_2 peptide, has not been profoundly altered. From these CD spectra it is not possible to deduce any precise form adopted by the CT DNA but we can consider that the condensation into Ψ-structures does not take place. It is worth noting here that minor spectral modifications have also been observed on adding the S_2 peptide to DNAs from either *Micrococcus lysodeikticus* (72% GC) or *Clostridium perfringens* (26% GC). The octapeptide has little effect on the structure of the three natural DNAs whatever their AT/GC contents.

The CD spectra of poly(dA-dT) · poly(dA-dT) free and complexed with the octapeptide at different peptide/polynucleotide molar ratios are shown in Fig. 3. One can immediately observe that the octapeptide induces a packing arrangement of the DNA helices. At a L/P ratio of 0.2, the positive band at 265 nm disappeared and a new positive one around 288 nm is observed (Fig. 3a; spectrum 2). This spectrum is reminiscent of the one which characterizes the complex between poly(dA-dT) · poly(dA-dT) and poly-L-$(Lys_2$-Ala$)_n$ [27]. By analogy, it is plausible to believe that the peptide S_2 induces some kind of $\Psi(+)$ supercoiled structure of the polynucleotide as does the polypeptide. These Ψ-DNA phases represent a particular state of DNA condensation as found in liquid crystals [28] and exhibit characteristic CD spectra with maxima of negative or positive ellipticity corresponding to $\Psi(-)$ and $\Psi(+)$ forms, respectively. $\Psi(-)$ phases are related to left-handedness in the intermolecular DNA helical arrangement and $\Psi(+)$ to right-handedness [29,30]. At a peptide/polynucleotide ratio of 0.4, the CD spectrum of the complex is much higher in intensity than the one recorded at L/P ratio of 0.2 and displays two positive maxima around 230 and 285 nm and a negative one at 250 nm (Fig. 3b). The positive band around 290 nm is an order of magnitude higher than that observed at a L/P ratio of 0.2. This spectrum may reflect the presence of both $\Psi(-)$ and $\Psi(+)$ condensed DNA molecules. Subsequent addition of the peptide lead to a new category of CD signals attesting to the propagation of the condensation mechanism. Indeed, at higher peptide concentrations, the spectra no longer exhibit the positive CD bands at 230 and 285 nm and a single negative maximum takes place. A red-shift from 265 nm (L/P = 0.6) to 270 nm (L/P = 1.0) occurs and the amplitude of the negative CD band is three- to four-fold higher than the one found at a L/P of 0.4 and 10-fold more intense than at a L/P ratio of 0.2. Therefore, $\Psi(+)$-DNA species are progressively converted into $\Psi(-)$ structures upon further addition of the S_2 peptide. These structures are interconvertible and reversible, $\Psi(-)$ being thermodynamically favoured as compared to $\Psi(+)$ for the heteropolymer poly(dA-dT) · poly(dA-dT) [29]. At higher peptide/polynucleotide ratios (L/P > 1.0), the solutions become turbid because of the precipitation of the peptide–DNA complexes.

4. CONCLUSION

Different significant conclusions can be drawn from the present CD study. First, the S_2 peptide can induce DNA condensation in a histone-like manner. Second, the effect is confined to alternating AT sequences since such a phenomenon was not detected with random DNA and the other polynucleotides. Thus, the S_2 peptide-induced condensation is sequence-selective and this has to be related to the sequence-selectivity of the DNA-binding motif SPKK previously shown by footprinting studies [8,16]. It is striking that the octapeptide does not bring about any Ψ-type condensation of calf thymus DNA and poly(dA) · poly(dT) contrarily to the histone H_1 peptide $(KTPKKAKKP)_2$ [18,19]. This may be relevant to the larger size and positive charge density of the latter peptide. Third, the study shows that the short octapeptide is sufficient to ensure the condensation of AT-rich DNA sequences as does the octadecapeptide $(KTPKKAKKP)_2$ [19] or the entire C-terminal part of the histone H_1 [31]. This suggests that the S_2 peptide

may correspond to a determinant motif implicated in the modulation of chromatin condensation and, as a consequence, in the regulation of the transcriptional machinery. Relevant to this, it will be of particular interest to study the effect of the acetylated or phosphorylated S_2 peptides on DNA condensation. This should allow a better understanding of the molecular basis and physiological processes governing the interaction between DNA and SPXX motifs and thus chromatin condensation.

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REFERENCES

[1] Zlatanova, J. (1990) Trends Biochem. Sci. 15, 273–276.
[2] Laybourn, P.J. and Kadonaga, J.T. (1991) Science 254, 238–245.
[3] Oikarinen, J. (1991) FEBS Lett. 294, 6–10.
[4] Felsenfeld, G. (1992) Nature 355, 219–224.
[5] Hansen, J.C. and Ausio, J. (1992) Trends Biochem. Sci. 17, 187–191.
[6] Suzuki, M. (1989) EMBO J. 8, 797–804.
[7] Suzuki, M. (1989) J. Mol Biol. 207, 61–84.
[8] Churchill, M.E.A. and Suzuki, M (1989) EMBO J. 8, 4189–4195.
[9] Churchill, M.E.A. and Travers, A.A. (1991) Trends Biochem. Sci. 16, 92–97.
[10] Lindsey, G.G. and Thompson, P (1992) J. Biol. Chem. 267, 14622–14628.
[11] Suzuki, M., Sohma, H., Yazawa, M., Yagi, K. and Ebashi, S (1990) J. Biochem. 108, 356–364.
[12] Hill, C.S., Rimmer, J.M., Green, B.M., Finch, J.T. and Thomas, J.O. (1991) EMBO J. 10, 1939–1948.
[13] Poccia, D.L. and Green, G.R. (1992) Trends Biochem. Sci. 17, 223–227.
[14] Csordas, A (1990) Biochem. J. 265, 23–38.
[15] Green, G.R. and Poccia, D.L. (1985) Dev. Biol. 108, 235–245.
[16] Bailly, F., Bailly, C., Waring, M.J and Hénichart, J.P. (1992) Biochem. Biophys Res. Commun. 184, 930–937.
[17] Bailly, F., Bailly, C., Helbecque, N., Pommery, N., Colson, P., Houssier, C. and Hénichart, J.P. (1992) Anti-Cancer Drug Des. 7, 83–100.
[18] Erard, M., Lakhdar-Ghazal, F. and Amalric, F. (1990) Eur. J. Biochem. 191, 19–26.
[19] Kharrat, A., Derancourt, J., Dorée, M., Amalric, F. and Erard, M. (1991) Biochemistry 30, 10329–10336.
[20] Wells, R D., Larson, J E., Grant, R C., Shortle, B.E. and Cantor, C.R. (1970) J. Mol. Biol. 54, 465–497.
[21] Suzuki, M and Yagi, N. (1991) Proc. R. Soc. Lond. B 246, 231–235.
[22] Wells, R.D., Collier, D.A , Hanvey, J.C., Shimizu, M and Wohlrab, F. (1988) FASEB J. 2, 2939–2947.
[23] Nelson, H.C M., Finch, J.T., Luisi, B.F. and Klug, A. (1987) Nature 330, 661–664.
[24] Crothers, D.M., Haran, T.E. and Nadeau, J.G. (1990) J. Biol. Chem. 265, 7093–7096.
[25] Kunkel, G.R. and Martinson, H.G. (1981) Nucleic Acids Res. 9, 6869–6888.
[26] Prunell, A. (1982) EMBO J. 1, 173–179.
[27] Gupta, G., Sarma, M.H. and Sarma, R.H. (1984) J. Biomol. Struct. Dyn. 1, 1457–1472.
[28] Tinoco Jr., I. and Bustamante, C. (1980) Ann. Rev. Biophys. Bioenerg. 9, 107–141.
[29] Shin, Y.A and Eichhorn, G.L. (1984) Biopolymers 23, 325–335.
[30] Chaires, J.B. (1989) Biopolymers 28, 1645–1650.
[31] Allan, J., Mitchell, T., Harborne, N., Bohm, L. and Crane-Robinson, C. (1986) J. Mol Biol. 187, 591–601.