Biophysical Chemistry 22 (1985) 125–129
Elsevier

BPC 01001

CONDENSATION OF DNA BY THE C-TERMINAL DOMAIN OF HISTONE H1

A CIRCULAR DICHROISM STUDY

Federico MORÁN [a], Francisco MONTERO [a], Fernando AZORÍN [b] and Pedro SUAU [c,*]

[a] *Departamento de Bioquímica, Facultad de Ciencias Químicas, Universidad Complutense, Madrid,* [b] *Unidad de Química Macromolecular del Instituto de Química Bioorgánica del CSIC, ETSII, Barcelona, and* [c] *Departamento de Bioquímica, Facultad de Ciencias, Universidad Autónoma de Barcelona, Bellaterra, Barcelona, Spain*

Received 21st September 1984
Revised manuscript received 1st March 1985
Accepted 1st March 1985

Key words: DNA condensation; Histone H1; H1 domain; Circular dichroism

The condensation of DNA by the C-terminal domain of histone H1 has been studied by circular dichroism in physiological salt concentration (0.14 M NaF). As the intact H1 molecule, its C-terminal domain induces the so-called ψ state of DNA that is characterized by a nonconservative circular dichroism spectrum which is currently attributed to ordered aggregation of the DNA molecules. On a molar basis, intact H1 and its C-terminal domain give spectra of similar intensity. Neither the globular domain of H1 nor an N-terminal fragment, that includes both the globular and N-terminal domains, has any effect on the conservative circular dichroism of DNA. From these results it is concluded that the condensation of DNA mediated by histone H1 is mainly due to its C-terminal domain. The effect of the salt concentration and the size of DNA molecules on the circular dichroism of the complexes are also examined.

1. Introduction

Histone H1 is necessary for the integrity of the higher-order structure of chromatin and is also involved in the salt-induced condensation of chromatin [1–3]. It is generally admitted that H1 binds in nucleosomes at the point of DNA entry and exit, stabilizing two complete turns of DNA around the histone core [4,5]. Histone H1 contains three structural domains: a globular central region flanked by unstructured highly basic and hydrophilic C- and N-terminal tails [6,7]. Evidence has been accumulated supporting the view that the structural domains of H1 are involved in multifunctional roles in chromatin structure as also seems to be the case for core histones.

In order to contribute to the understanding of the role of histone H1 in chromatin condensation, we have studied the interaction of its structural domains with DNA by circular dichroism. Peptides that correspond to the structural domains of the H1 molecule can be prepared by enzymatic cleavage. Digestion with thrombin gives two fragments: the C-terminal fragment (CTB, residues 123–C-terminus), that matches precisely the C-terminal tail of the molecule; and the N-terminal fragment (NTB, residues 1–122), that includes both the central globular domain, possessing the globular structure of the intact molecule, and the N-terminal tail [8]. The globular domain (GH1, residues 36–121) is obtained by controlled trypsin digestion [9]. Treatment with *N*-bromosuccinimide yields a C-terminal fragment (residues 72–C-terminus) [10] that contains 50 residues more than the C-terminal domain. These extra residues be-

* To whom correspondence should be addressed.

long to the globular domain and become unfolded upon cleavage.

In this study we have used circular dichroism as a probe of DNA condensation. Uncomplexed DNA in solution shows the conservative circular dichroism spectrum characteristic of the B form of DNA with a positive band at 275 nm and a negative band at 245 nm. The interaction of H1 with DNA at physiological salt concentrations (0.14 M NaCl) has been shown [11] to result in large distortions of the DNA circular dichroism spectrum; the positive ellipticity band at 275 nm disappears completely and a large negative band appears at about 265 nm. These spectra were called PSI or ψ spectra by Jordan et al. [12] and are thought to be caused by the condensation of DNA molecules into ordered aggregates as discussed by Fasman et al. [11] and Jordan et al. [12]. We have taken advantage of the fact that the C-terminal domain of H1 also induces the ψ state of DNA to follow its binding to DNA by circular dichroism.

Before the domain structure of histone H1 was known, Fasman et al. [13] studied the interaction of its N – bromosuccinimide peptides with DNA and concluded that the C-terminal fragment was responsible for the circular dichroism changes observed in DNA upon binding of H1. In this paper we report the interaction of DNA with peptides that precisely match structural domains of H1. The experiments presented here show that the condensation of DNA by histone H1 is mainly due to the C-terminal domain of the molecule.

2. Materials and methods

2.1. Preparation of histone H1

Histone H1 was prepared from calf thymus by the method of Johns and Butler [14]. It was further purified by carboxymethylcellulose chromatography [15] as previously described [16].

2.2. Preparation of histone H1 fragments

Fragments CTB (residues 123–C-terminus) and NTB (residues 1–122) were obtained by cleavage at Lys-122 with thrombin following the method of Chapman et al. [8]. The trypsin-resistant core was prepared by a modification of the procedure of Hartman et al. [9] as previously described [17].

2.3. Protein concentrations

Protein concentrations were measured spectrophotometrically in 0.01 N HCl at 205 and 210 nm using $E^{1\%}_{205} = 310$ and $E^{1\%}_{210} = 205$. The concentrations of calf thymus H1 and its N-bromosuccinimide peptide used in the calculation of these values were determined with an amino acid analyzer; runs were in triplicate and norleucine was used as an internal standard.

2.4. DNA preparation

DNA from Sigma (type I) was further purified by chloroform/isoamyl alcohol extractions after digestion with proteinase K (0.1 mg enzyme/mg DNA) in the presence of 0.5% SDS. DNA was sonicated at a concentration of 0.1 mg/ml in 2×10^{-3} M phosphate buffer, pH 7.0, for four bursts, whilst keeping the samples cold on ice. This reduces the molecular weight down to approx. 7×10^5 as estimated by electrophoresis in 1% agarose gels. DNA of 146 base-pairs was obtained from chromatin core particles prepared as described elsewhere [18]. Core particles were further purified by precipitation of the remaining fraction of H1 containing monomers with 0.14 M KCl.

2.5. Protein-DNA complexes

Complexes were prepared by the slow addition of various concentrations of protein solution in 0.14 M NaF, 2×10^{-3} M phosphate buffer, pH 7.0, into an equal volume of DNA solution in the same buffer, under continuous gentle stirring. Unless otherwise stated, complexes were prepared with sonicated DNA and measured at a concentration of 0.018 mg DNA/ml. Complexes were neither filtered nor centrifuged as this would have resulted in the loss of a substantial fraction of the $A_{260\text{nm}}$ from solution. Spectra were recorded 1 h after complex formation at 20°C.

2.6. Circular dichroism spectra

Circular dichroism spectra were recorded in a Mark III dichrograph (Jobin-Yvon). Cells of 1 cm optical path were used to record spectra in the near-ultraviolet region (320–245 nm) and the instrument operated at a sensitivity of 1×10^{-6} ΔA mm^{-1}. Cells of 0.05 optical path were used and the sensitivity set at 5×10^{-6} ΔA mm^{-1} to obtain spectra in the far-ultraviolet region (250–190 nm). Results are expressed in molar ellipticities with the dimensions of degree cm^2 (dmol nucleotide)$^{-1}$.

The complexes of higher protein/DNA ratios in 0.14 M NaF were slightly opalescent. Light scattering was measured as $A_{320\,nm}/A_{260\,nm}$ ratios.

3. Results

3.1. Circular dichroism of complexes of H1 with DNA

It has been shown that the binding of H1 to DNA at moderate salt concentrations (0.14 M) distorts the DNA circular dichroism spectrum giving rise to the so-called ψ spectrum [11].

We examine here the circular dichroism spectra that are obtained when sonicated DNA (average M_r 7×10^5 as estimated by gel electrophoresis) is used in the formation of the complexes with H1 in 0.14 M NaF. It can be observed in fig. 1 that the positive band at 275 nm has already disappeared at $r = 0.1$ (r, weight ratio of protein/DNA); at $r = 0.4$ the ellipticity at 275 nm, $|\theta|_{275}$, reaches $-230\,000$. When complexes are formed with unsonicated DNA (average M_r $4\text{–}5 \times 10^6$, as estimated by sedimentation) much smaller ellipticities are observed as shown in fig. 1 for the complex at $r = 0.4$ that gives $|\theta|_{265} = -25\,000$. With high molecular weight DNA other authors obtained similar values under equivalent ionic conditions [11,19]. Thus, the decreased size of DNA molecules leads to an approx. 9-fold increase in the intensity of the ψ spectrum.

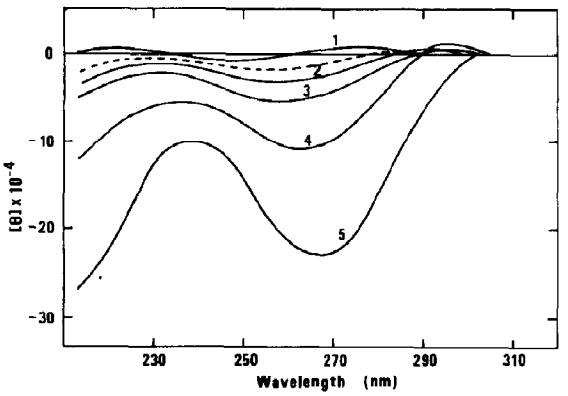

Fig. 1. Circular dichroism spectra of H1-DNA complexes as a function of r, the weight ratio of histone H1/DNA, at 0.018 mg/ml DNA, in 0.14 M NaF, 2 mM phosphate buffer, pH 7. Complexes with sonicated DNA (M_r 7×10^5): curve 1, native DNA; 2, $r = 0.1$; 3, $r = 0.2$; 4, $r = 0.3$; 5, $r = 0.4$. Complex with high molecular weight DNA (M_r $4\text{–}5 \times 10^6$): (— — —), $r = 0.4$.

To see whether a further decrease in the size of DNA molecules could be effective in the induction of even more intense ψ spectra, we prepared complexes with DNA fragments of 146 base-pairs. In this case, spectra of similar intensity were obtained (data not shown).

3.2. Circular dichroism of complexes of CTB with DNA

Complexes with CTB could only be prepared with sonicated DNA as precipitation occurred at very low protein/DNA ratios with high molecular weight DNA. This fragment causes a large distortion of the DNA spectrum. Fig. 2 shows how the circular dichroism spectra become more negative as r increases. The peak at 275 nm has already disappeared at $r = 0.1$. The negative ellipticity band at 245 nm increases in magnitude with increasing ratios and shifts to 270 nm at $r = 0.4$. The spectra become more negative at low wavelength and a large negative band is seen at 297 nm. The effect of the C-terminal domain on the circular dichroism spectrum is very intense: for example, at $r = 0.4$ the value of $|\theta|_{270}$ reaches $-350\,000$.

The decreased effect of H1 on the circular dichroism spectrum compared to the C-terminal domain at the same r value can be roughly accounted for by assuming that only the C-terminal domain is capable of distorting the circular dichroism spectrum. In fact, complexes with whole

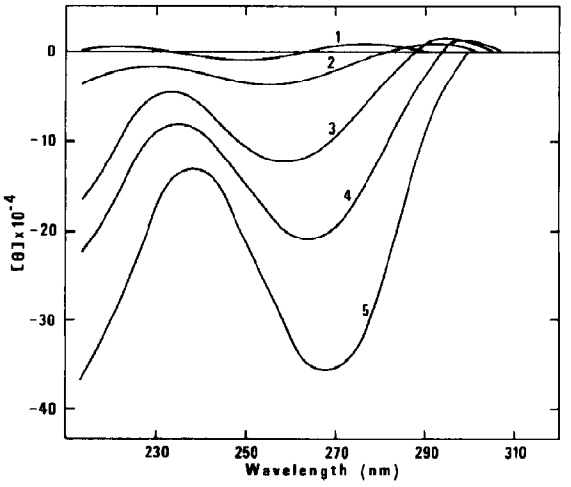

Fig. 2. Circular dichroism spectra of CTB-sonicated DNA as a function of r, the weight ratio of peptide/DNA, at 0.018 mg/ml, in 0.14 M NaF, 2 mM phosphate buffer, pH 7: curve 1, native DNA; 2, $r = 0.1$; 3, $r = 0.3$; 4, $r = 0.3$; 5, $r = 0.4$.

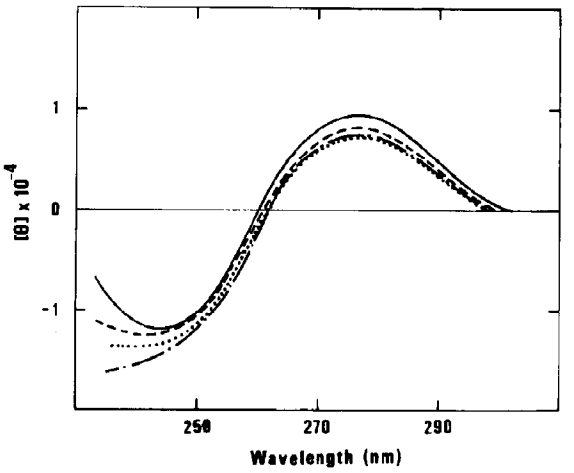

Fig. 3. Circular dichroism of GH1-sonicated DNA and NTB-sonicated DNA complexes, at 0.018 mg/ml DNA in 0.14 M NaF, 2 mM phosphate buffer, pH 7: (— — —) native DNA; (·–·–·) GH1-DNA complex, $r = 4.0$; (·····) NTB-DNA complex, $r = 2.0$; (·–··–·) NTB-DNA complex; $r = 4.0$. r, weight ratio of peptide/DNA.

H1 show $|\theta|_{270}$ values which are 15% higher than they should be if only the C-terminal domain contributed to the observed spectral change. Unfortunately, we cannot take this difference as significant as this value is similar to the standard deviation associated with our circular dichroism measurements.

As in the case of whole H1, spectra with similar values were obtained when complexes were prepared with DNA of 146 base-pairs (data not shown).

3.3. Complexes of GH1 and NTB with DNA

It was important to analyze the circular dichroism of complexes with the globular domain and the NTB fragment, that includes both the globular and the N-terminal domains, to establish whether these fragments were capable of distorting the DNA circular dichroism. As seen in fig. 3, the conservative spectrum of DNA is not significantly altered upon interaction with either GH1 or NTB even at r values as high as 4.0, the negative ellipticities at short wavelength being contributed by the protein itself.

3.4. Effect of salt on the circular dichroism of complexes of DNA with CTB formed at low salt

It has been shown [11,19] that at low ionic strength (0.01) histone H1 has little effect on the

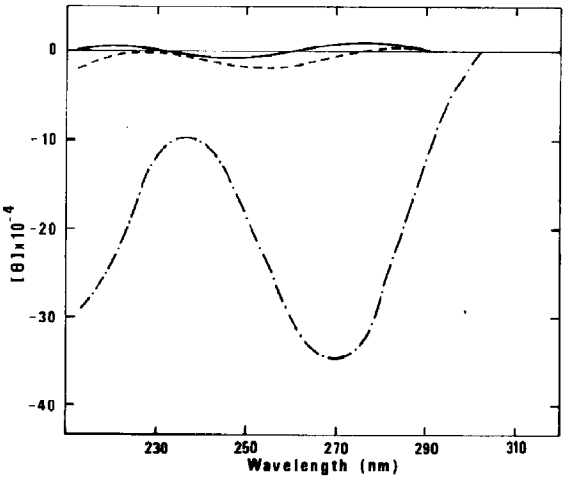

Fig. 4. Circular dichroism spectra of CTB-sonicated DNA complexes at 0.018 mg/ml: (— — —) native DNA; (— — —) $r = 0.4$ in 2 mM phosphate buffer, pH 7; (— · —) complex formed at $r = 0.4$ in 2 mM phosphate buffer, pH 7, after addition of NaF up to 0.14 M. r, weight ratio of peptide/DNA.

DNA circular dichroism. In the case of CTB the effect is more pronounced, although much less intense than in 0.14 M NaF. Fig. 4 shows the spectrum of a complex of sonicated DNA with CTB in 2 mM phosphate buffer at $r = 0.4$. The positive peak at 275 nm decreases and becomes red-shifted and a negative band appears at 250 nm with $|\theta|_{250} = -1500$. When salt is added up to 0.14 M NaF the spectrum obtained has the same $|\theta|_{270}$ value as that observed when the complex is formed directly in 0.14 M NaF. However, in this case, in contrast to what is found in complexes formed in 0.14 M NaF, the peak at low wavelength (223 nm) appears to be less intense than that at higher wavelength (274 nm).

4. Discussion

In the present investigation we have studied the interaction of intact histone H1 and its structural domains with DNA by circular dichroism.

The nonconservative spectra shown by complexes of DNA with H1 and its C-terminal domain can be attributed to ordered aggregation as discussed by Fasman et al. [11], Jordan et al. [12] and Sipski and Wagner [20]. The fact that larger negative ellipticities are observed with sonicated DNA than with higher molecular weight DNA is consistent with the view that the spectrum depends on a characteristic pattern of interhelix interactions. It would appear that shorter DNA fragments experience less steric hindrance in the transition towards the ψ state.

Since on a molar basis intact H1 and its C-terminal domain are roughly equally efficient in inducing the ψ spectrum in DNA, it is likely that the condensation of DNA by the intact molecule is mainly due to the C-terminal domain. This conclusion is reinforced by the fact that either the globular domain or the NTB fragment, that includes both the globular and the N-terminal domains, are by themselves unable to alter the DNA circular dichroism. Fasman et al. [13] studied the interaction of the *N*-bromosuccinimide H1 C-terminal fragment, that contains the C-terminal domain and about two-thirds of the globular domain, with DNA and concluded that this region was primarily responsible for the DNA ψ spectrum observed upon interaction with the intact molecule. Our results show that the residues belonging to the globular domain do not contribute significantly to the observed effect, that can be thus attributed to the well-defined C-terminal domain.

The predominant role played by the C-terminal domain of H1 in the condensation of DNA by the whole molecule suggests that this domain is also involved in the condensation of the chromatin fiber.

Acknowledgements

We thank Professor A.M. Municio for fruitful discussions. This work was supported by grants from the Comisión Asesora de Investigación Científica y Técnica (Spain).

References

1 E.M. Bradbury, S.E. Danby, H.W.E. Rattle and V. Giancotti, Eur. J. Biochem. 57 (1975) 97.
2 M. Noll and R.D. Kornberg, J. Mol. Biol. 109 (1977) 393.
3 F. Thoma, T. Koeler and A. Klug, J. Cell Biol. 83 (1979) 403.
4 R.T. Simpson, Biochemistry 17 (1978) 5524.
5 J. Allan, P.G. Hartman, C. Crane-Robinson and F.X. Avilés, Nature 288 (1980) 675.
6 E.M. Bradbury, P.D. Cary, G.E. Chapman, C. Crane-Robinson, S.E. Damby, H.W.E. Rattle, M. Boublik, J. Palau and F.X. Avilés, Eur. J. Biochem. 52 (1975) 605.
7 I. Isenberg, Annu. Rev. Biochem. 48 (1979) 159.
8 G.E. Chapman, P.G. Hartman and E.M. Bradbury, Eur. J. Biochem. 61 (1976) 69.
9 P.G. Hartman, G.E. Chapman, T. Moss and E.M. Bradbury, Eur. J. Biochem. 77 (1977) 45.
10 M. Bustin and R.D. Cole, J. Biol. Chem. 244 (1969) 5291.
11 G.D. Fasman, B. Schaffhausen, L. Goldsmith and A. Adler, Biochemistry 9 (1970) 2814.
12 L.F. Jordan, L.S. Lerman and J.A. Venable, Jr, Nat. New Biol. 236 (1972) 67.
13 G.D. Fasman, M.S. Valenzuela and A.J. Adler, Biochemistry 10 (1971) 3795.
14 E.W. Johns and J.A.V. Butler, Biochem. J. 82 (1962) 15.
15 E.W. Johns, Biochem. J. 92 (1964) 55.
16 L. Franco, F. Montero and J.J. Rodriguez-Molina, FEBS Lett. 78 (1977) 317.
17 J.L. Barbero, L. Franco, F. Montero and F. Morán, Biochemistry 19 (1980) 4080.
18 P. Suau, G.G. Kneale, G.W. Braddock, J.P. Baldwin and E.M. Bradbury, Nucleic Acids Res. 4 (1977) 3769.
19 S.L. Welch and R.D. Cole, J. Biol. Chem. 254 (1979) 662.
20 M.L. Sipski and T.E. Wagner, Biopolymers 16 (1977) 573.