A circular dichroism study of DNA-basic peptides associations in the absence or in the presence of Ca^{++}

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ABSTRACT

The interaction of DNA with basic peptides (Lys methyl ester^{*}, Lys_2 , $(\text{Lys})_2$ methyl ester) has been studied by circular dichroism.

The changes of the DNA CD spectra in the presence of peptides are interpreted as a transconformation from the B form to the C form of DNA. The presence of Ca^{++} in the mixture induces a supplementary transconformation.

These observations suggest Ca^{++} -basic peptides-DNA complexes as a structural model for chromatin.

INTRODUCTION

It was shown^(1,2,3,4) that DNA in chromatin adopts a secondary structure, which probably differs from the native DNA structure in solution. As it was suggested that a relationship exists between DNA structure and function^{5,6)}, then it must be of interest to study simple models in order to simulate structural changes such as occur in chromatin.

Changes of DNA secondary structure in chromatin may be caused by histones^(3,7), cations^(2,8) or non histone proteins⁽⁹⁾.

Histones have a singular property, that is the presence of basic amino-acids clusters⁽¹⁰⁾ which are found especially at the two ends of the molecule^(11,12). This local condensation, which is not random, may be supposed to have a functional role especially since it is precisely the two ends of histones (H2A, H2B, H3, H4), positively charged at physiological pH, which in the chromatin interact with the DNA, negatively charged⁽¹³⁾. In order to establish the roles of the clusters and cations on the DNA secondary structure, we have studied, using circular dichroism, models such as DNA plus basic peptides or DNA plus basic peptides plus divalent cations.

Our results on DNA-Lys methyl ester, DNA- $(\text{Lys})_2$ and DNA- $(\text{Lys})_2$ methyl ester complexes in the absence or in the presence of calcium are described below.

MATERIALS AND METHODS

Calf thymus was prepared in the laboratory using Schwander and Signer⁽¹⁴⁾ preparation. DNA solution preparation was previously described⁽¹⁵⁾.

The initial DNA solution was 600 $\mu\text{g/ml}$ and 0.002 M NaCl concentration. The DNA concentration was determined by 260 nm absorbance⁽¹⁶⁾.

Lys methyl ester was purchased from Sigma, $(\text{Lys})_2$ and $(\text{Lys})_2$ methyl ester were purchased from Fox Chemical Company. Peptides solution was prepared in bidistilled water. The initial peptide solution was generally 100 $\mu\text{g/ml}$. The ninhydrin method was used for determination of the concentration of peptide. All salts were "reagent grade".

DNA mixtures were prepared by direct mixing on Vortex at room temperature in order to obtain a constant final DNA concentration of 100 μM and varying concentration for the peptides, at pH 7 and phosphate buffer 0.001 M or TES buffer 0.001 M + 0.002 M NaCl. No turbidity was detected at 320 nm by spectrophotometry measurement.

CD spectra was recorded in the 230 nm-300 nm range using a CD 185 Jouan dichrograph, at room temperature, in quartz cell Hellma 1 cm or 2 cm large. Results are reported using molar ellipticity $[\theta]$ expressed in $\text{deg.cm}^2/\text{dmol}$.

RESULTS

In the range 230 nm-300 nm, the peptides show no circular dichroism. Without any doubt, all spectral modification in the mixtures DNA-peptides and DNA-peptides-cations may be attributed to DNA.

DNA spectra in the presence of 10^{-5} M EDTA are very similar to the results in the literature^(17,18) : in particular, $[\theta]_{275} = 8.4 \cdot 10^3 \text{ deg.cm}^2/\text{dmole}$ and $[\theta]_{245} = -9.6 \cdot 10^3 \text{ deg.cm}^2/\text{dmole}$.

DNA + peptide

We have reported (Fig 1 A) the CD spectrum of DNA alone and those of complexes DNA- $(\text{Lys})_2$ methyl ester for several concentrations of peptides. The spectral shapes are identical with the other peptides. These spectra are characterized by a decrease in the amplitude of the positive CD band at 275-276 nm upon addition of Lys_2 methyl ester, the amplitude of the negative CD band remaining unchanged.

The decrease of the intensity of the CD signal of DNA in the presence of basic peptides depends on the peptide concentration, and tends toward a limit which depends on the kind of peptide. Such curves are reported on Fig 1 B in terms of relative decrease $[\theta]/[\theta]_0$ at 275 nm ; $[\theta]$ represents

Fig. 1 : A - CD spectra of DNA (100 μM)-Lys₂ methyl ester complexes in TES buffer 0.001 M + 0.002 M NaCl, pH 7. 1, DNA alone ; 2, DNA + (Lys)₂ methyl ester 10 μM ; 3, DNA + (Lys)₂ methyl ester 20 μM ; 4, DNA + Lys₂ methyl ester 30 μM .

B - Concentration dependance of the CD amplitude at 275 nm of DNA (100 μM) for various peptides. C_i is the initial concentration of peptides ($\blacktriangle$ $\blacktriangle$ $\blacktriangle$) Lys methyl ester, ($\blacksquare$ $\blacksquare$ $\blacksquare$) (Lys)₂, ($\bullet$ $\bullet$ $\bullet$) (Lys)₂ methyl ester. Same conditions as in figure 1 A.

the molar ellipticity at 275 nm of the DNA in the complex and the molar ellipticity of the DNA alone in the presence of 10^{-5} M EDTA, ($[\theta_0] = 8.4 \cdot 10^3$ deg.cm²/dmole).

DNA + calcium

The addition of calcium to DNA also induces a decrease in the positive band at 275 nm without altering the negative band (fig 2 A). The initial maximum value without EDTA, $[\theta]_{275} = 8.3 \cdot 10^3$ deg.cm²/dmole, is changed to reach $[\theta]_{275} = 5.1 \cdot 10^3$ deg.cm²/dmole and corresponds to a decrease of the signal of about 40%, as was observed by Wagner and Vandecriff⁽¹⁷⁾.

In the case of calcium addition, the lower limit to which the dichroic signal tends at 275 nm is obtained from 1000 μM of Ca⁺⁺ and this value is lower than that obtained with peptides (fig 2 B).

When EDTA is added to DNA in the presence of calcium, the effect of the latter on the CD is of course suppressed.

The effect of EDTA on the DNA spectrum in presence of variable concentration of calcium allowed us to interpret the slight increase of the positive band of DNA in the presence of EDTA as being able to correspond only to an eventual concentration of calcium lower than 10^{-5} M in the solution of DNA alone.

DNA + peptides + calcium

Calcium addition to DNA-(Lys)₂ methyl ester and DNA-(Lys)₂ complexes induces a supplementary decrease of the positive 275 nm DNA band (fig 3 A).

The relative decrease of dichroic signal in the presence of calcium

Fig. 2 : A - CD spectra of DNA (100 μM)- Ca^{++} complexes in TES buffer 0.001 M + 0.002 M NaCl, pH 7 ; 1, DNA alone ; 2, DNA + Ca^{++} 10 μM ; 3, DNA + Ca^{++} 50 μM ; 4, DNA + Ca^{++} 100 μM ; 5, DNA + Ca^{++} 1000 μM .
B - Effect of the concentration of Ca^{++} on the relative decrease of DNA CD signal at 275 nm. Same conditions as in 2 A.

Fig. 3 : A - CD spectrum of DNA (100 μM) alone (1) ; of DNA (100 μM) + (Lys)₂ methyl ester (30 μM) (2) ; of DNA (100 μM) + (Lys)₂ methyl ester (30 μM) + Ca^{++} (100 μM) (3). TES buffer 0.001 M + 0.002 M NaCl, pH 7.

B - Effect of the initial concentration of (Lys)₂ methyl ester (C_i) on the relative decrease of DNA CD signal at 275 nm for various Ca^{++} concentration. (●●●) $\text{Ca}^{++} = 0$; (○○○) $\text{Ca}^{++} = 10$ μM ; (▲▲▲) $\text{Ca}^{++} = 100$ μM ; (■ ■ ■) $\text{Ca}^{++} = 1000$ μM .

tends towards a limit lower than that of DNA-(Lys)₂ methyl ester complex but equal to that of DNA-calcium complex (fig 3 B).

DISCUSSION AND CONCLUSION

The DNA-basic polypeptides associations have been extensively studied, including by circular dichroism⁽¹⁹⁻²⁴⁾. However, beside the fact that these complexes are often muddy or precipitate^(19,23,25), the dichroic spectra are considerably different from that of the DNA spectra alone, or from the DNA in chromatin^(19,21,23) and indicate a higher degree of organisation of the DNA⁽²⁶⁾. Consequently, the alterations of the secondary structure of

DNA⁽²⁶⁾ can no longer be detected by this technique ; and for this reason the small basic peptides are better suited for the study of alterations of the secondary structure of the DNA by the histones and the effect of divalent cations on chromatin.

Hanlon et al⁽¹⁸⁾ recently computed the dichroic spectra of calf thymus DNA A, B, C forms in solution and made the assumption, common to all spectra analyses, that the observed dichroic signal at a given wavelength λ_j for a calf thymus DNA solution represents a linear combination of the contribution of the three components present in the solution. This theory is only applicable in the case where tertiary structure alterations do not change the DNA circular dichroism spectra. It is supposed that the C form regroups all secondary structures other than the A and B forms, in particular the B* form⁽²⁷⁾.

In the present case, the observed molar ellipticity in solution at the wavelength of λ_j is the sum :

$$[\theta]_{\lambda_j}^{\text{obs}} = f_A [\theta]_{\lambda_j}^A + f_B [\theta]_{\lambda_j}^B + f_C [\theta]_{\lambda_j}^C \quad (1)$$

where f_A (resp. f_B , resp. f_C) is the mole fraction of A form (resp. B, resp. C) which reference spectrum (for f_A (resp. B, resp. C) = 100) has an ellipticity of $[\theta]_{\lambda_j}^A$ (resp. $[\theta]_{\lambda_j}^B$, resp. $[\theta]_{\lambda_j}^C$) at wavelength λ_j .

In the presence of 10^{-5} M EDTA, our spectrum of DNA is identical to that computed by Hanlon and co-workers⁽¹⁸⁾ for a DNA completely in the B form ($f_B = 1$, $f_A = f_C = 0$). The addition of basic peptides and/or Ca^{++} , which leave unchanged the amplitude of the negative peak, imply a trans-conformation of the B form to the C form⁽¹⁸⁾ of DNA without apparition of the A form ($f_A = 0$).

Since $f_A = 0$ and $f_B + f_C = 1$, the relation (1) allows f_B to be computed :

$$f_B = ([\theta]_{\lambda_j}^{\text{obs}} - [\theta]_{\lambda_j}^C) / ([\theta]_{\lambda_j}^B - [\theta]_{\lambda_j}^C) \quad (2)$$

The values of f_B and f_C allow us to determine the respective proportions of B form and C form for a given spectrum.

In the DNA-peptide complexes, the alteration of the spectra are not due to divalent ions contaminating the peptidic solutions, indeed the addition of 10^{-5} M EDTA alters the spectrum amplitude by less than 3%. Nor may these effects be attributed to a denaturation, or base unstacking, as the extinction coefficient of DNA at 260 nm in the complexes does not vary. We can then interpret the changes of the DNA spectra in the presence

Fig. 4 : A - Quantity of (Lys)₂ methyl ester bound to DNA (100 μM) versus initial cation concentration. TES buffer 0.001 M + 0.002 M NaCl, pH 7.

B - Effect of the initial concentration of Ca⁺⁺ on the quantity of (Lys)₂ methyl ester bound to DNA (100 μM), for various initial (Lys)₂ methyl ester concentration : (○ ○ ○) 50 μM (maximum of the association), (● ● ●) 30 μM, (■ ■ ■) 20 μM, (▲ ▲ ▲) 10 μM. Same conditions as in 4 A.

of peptides and/or divalent cations in terms of transconformation of the B form to the C form of DNA. This is what we have represented on the right hand scales of fig. 1B, 2B, 3B.

The three peptides studied, when associated with calf thymus DNA, tend to induce a transconformation of the B form to the C form. The percentage of the C form is equal at most to 23% for the Lys methyl ester and to 27% for the (Lys)₂ and the (Lys)₂ methyl ester (fig 1 B).

The alterations of the DNA structure seem the fact of bound peptides. As it is seen in the case of (Lys)₂ methyl ester, by comparing the curves 1 B and 4 A, the maximum variation of $[\theta] / [\theta_0]$ has been reached when the DNA has fixed the utmost possible quantity of peptides. Hence, it seems that there is a relation between the amount of bound peptides and the alterations of the circular dichroism spectra. However, the variations $[\theta] / [\theta_0]$ versus the amount of each of the three bound peptides (fig. 5) are not linear. According to Chang et al.⁽²⁸⁾ we may assert that the structure alterations lie probably not only at the bound bases. The most likely hypothesis is therefore that the association of one peptide with the DNA involves a local conformational change of the chain, which is not strictly limited to the association site. The percentages of structures given previously represent the global effect of these conformational changes.

The addition of Ca⁺⁺ to the DNA increases progressively the percentage of the C form to the detriment of the B form between Ca⁺⁺ 0.01 mM and 1 mM. In the presence of 0.1 mM Ca⁺⁺, we obtain 73% of B form and 27% of C form. From 1 mM Ca⁺⁺, we are in the presence of 63% of B form and 37% of C form.

Fig. 5 : Effect of the bound peptide on the relative decrease of DNA circular dichroism signal at 275 nm for various peptides : (—) Lys methyl ester, (-----) $(\text{Lys})_2$, (-·-·-) $(\text{Lys})_2$ methyl ester. TES buffer 0.001 M + 0.002 M NaCl, pH 7.

When to a mixture of DNA (100 μM) and of $(\text{Lys})_2$ methyl ester (30 μM), for which we have 76% of B form and 24% of C form, we add 100 μM of Ca^{++} , the calculations show that the amplitude of the 275 nm peak corresponds to the presence of 66% of B form for 34% of C form. We see, therefore, that the influence of the presence of Ca^{++} on the associations DNA- $(\text{Lys})_2$ methyl ester is expressed by an important conformational change, since we have an increase of 10% of the C form.

The presence of calcium always results in an increase in percentage of the C form DNA, with regard to the percentage of C form DNA in binary mixing DNA- $(\text{Lys})_2$ methyl ester, even for lower concentrations of Ca^{++} . But from 1 mM of Ca^{++} , it seems that the composition of the ternary mixtures containing 63% of B form and 37% of C form is a limiting value beyond which, even by increasing considerably the concentration of Ca^{++} , we cannot go.

We can link these results with the DNA-peptide association results in the presence of Ca^{++} : the quantity of bound $(\text{Lys})_2$ methyl ester is decreasing from 10 μM Ca^{++} until reaching a very low value beyond 1 mM (fig 4 B). The same results are obtained with the other peptides. The calcium enters into competition with the peptide for interaction with the negative charges of the DNA; and at a sufficiently high calcium concentration ($> 1 \text{ mM}$), the DNA-peptide complexes dissociate.

Hence, from this value (1 mM) the dichroic signal expresses the effect of calcium alone and we obtain indeed the same limiting value 0.61 for $[\theta] / [\theta_0]$. For intermediate value (between 10 μM et 1 mM) the signal expresses the combined effect of the peptide and the calcium.

The conformational transition due to calcium is also observed for

other peptides : Lys methyl ester, (Lys)₂. The experiments made in the presence of Mg⁺⁺ show much smaller variations⁽²⁹⁾.

The role of cations in chromatin may then be to facilitate some changes of local conformation of DNA at the nucleohistone level. This local alteration may be related to the functional role of histones. In nucleohistones^(30,31) and in chromatin^(1,7,32), the dichroic signal is broadly changed with regard to DNA alone and corresponds to the coexistence of 65% of C form and 35% of B form⁽³⁰⁾. This great decrease may be explained by the presence of an important quantity of divalent cations (Ca⁺⁺, Mg⁺⁺) whose effect on DNA would add to that of the basic histone residues.

The presence of calcium brings about a broad change in the spectra ; the variety of nucleohistone and chromatin spectra obtained in the literature may be linked with the different extraction processes yielding varying contents in divalent ions in the preparations.

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★ Abbreviations used are : Lys methyl ester : L-Lysine methyl ester ; (Lys)₂ : L-Lysyl-L- α -Lysine ; (Lys)₂ methyl ester : L-Lysyl-L- α -Lysine methyl ester ; CD : circular dichroism.

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