Conformation of DNA in chromatin reconstituted from poly [d(A-T)] and the core histones

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Received 23 July 1981

ABSTRACT

Present results provide direct evidence of the nature of a conformational change in DNA when nucleosomes are formed from core histones and poly [d(A-T)]. First, we have found some features which have characteristic aspects of the A like conformation of DNA. Thus, an increased contribution due to a sugar conformation close to C3'-endo puckering is detected in the Raman spectra. In addition, the circular dichroism (C.D.) spectra of reconstituted chromatin with poly [d(A-T)] exhibits an increased intensity at about 262 nm.

A second feature acquired by poly [d(A-T)] in nucleosome formation from core histones is related to the presence of a negative band at about 280 nm in the C.D. spectra. The nature of this change is correlated with a DNA conformation characterized by a decreased number of base pairs per turn (28,29). This indicates that these two features of reconstituted nucleosomes reflect the presence of two types of DNA conformations, which overall form is of the B type (22,36).

INTRODUCTION

Despite the considerable progress that has been achieved in establishing the basic units in the chromatin structure are the repetitive nucleosomal subunits, the detailed structure of nucleosomes and of DNA which is wrapped around the histone core is not known. (For a recent review, see ref. 1). A change in DNA periodicity, when nucleosomes are formed, was proposed in order to explain the difference in the number of superhelical turns around the nucleosome cores deduced from X-ray diffraction and electron microscopic analysis (2) and that obtained from measurements of superhelicity on circular DNA extracted from SV40 chromatin (3). In addition, mechanism concerning the recognition mechanism of functionally important regions of DNA by specific proteins and of regulation of gene expression in eukaryotes are not well understood.

It has been shown previously that differences in the conformation of the phosphate-ribose backbone, and particularly in the orientation of

the phosphate groups and in conformational transitions, characterize synthetic DNAs composed of different base sequences (4). Thus, it has been found that the alternating polynucleotide poly [d(A-T)] undergoes a structural transition to the A form, whereas the double stranded homopolymer poly [d(A)]• poly [d(T)] cannot adopt the A form (4-5). It was thus proposed that the alteration of the backbone conformation as a consequence of different base sequences may have an important role in DNA recognition and protein interaction (4-6).

The existence of a relationship between the structure of DNA of different sequences, its organization into nucleosomes and the control of gene expression may be of primary importance in eukaryotes. Thus Simian Virus 40 DNA, which in infected cells forms nucleosomes (7), was found to possess a nuclease-sensitive region localized around the origin of replication and containing a promotor region of about 300-400 pairs in length (7). This region appears to be devoid of nucleosomes as shown by electron microscopy (8-10). In vitro experiments have shown that using purified histones H2A, H2B, H3 and H4, the reconstitution of nucleosomes is possible with polydeoxynucleotides composed of alternating purine-pyrimidine bases like poly [d(A-T)] and poly [d(G-C)] but not with the complementary homopolymers such as : poly [d(A)]• poly [d(T)] and poly [d(G)]• poly [d(C)] (11,12,13). We are thus prompted to ask the question as to the structural features which allow particular DNA sequences to reconstitute nucleosomes. Reconstitution of chromatin with poly [d(A-T)] leads to nucleosomes with apparently similar periodicity (11) and similar regularity in packing as shown by electron microscopy (see below) to that of native chromatin. In addition, the use of poly [d(A-T)] has the advantage of sequence homogeneity. This allows the observation of structural features not easily detectable with DNA composed of multiple sequences leading to the variation in backbone conformation. It is thus believed that these investigations are important in understanding the structure of repetitive units of native chromatin and particularly of the fine structure of supercoiled DNA in chromatin.

MATERIAL AND METHODS

Preparation of histones :

The octamer of four histones was prepared from calf thymus by salt extraction according to Germond et al. (14). Chromatin from calf thymus was dialyzed overnight against 0.63 M NaCl, 0.1 M potassium phosphate

pH 6.7 at 4° C and then centrifuged to remove any insoluble material. About 40 mg of chromatin of DNA concentration 0.6-0.8 mg/ml was loaded on to a hydroxylapatite column (2.3 cm x 24 cm), which had been equilibrated with the same solution (0.63 M NaCl, 0.1 M phosphate buffer pH 6.7). Column chromatography was carried out according to Simon and Felsenfeld (15) and all four histones were obtained in equimolar amounts.

Reconstitution :

Reconstitution of chromatin with the octamer of core histones was performed using the general procedure of Oudet et al. (16) at Histone:DNA ratio 1:1 (wt/wt). The octamer of histones was mixed with poly [d(A-T)] in 2 M NaCl and dialyzed overnight against buffer A containing 20 mM tris pH 8.0, 5 mM dithiothreitol, 0.2 mM EDTA, 0.1 mM PMSF at 4° C. The association of the histone core with poly [d(A-T)] was carried out by stepwise dilution with buffer A and decreasing concentration of NaCl (1.6 M, 1.2 M, 1.0 M, 0.85 M, 0.75 M, 0.65 M and 0.5 M). Finally the reconstitution was completed by dialysis against 5 mM tris pH 7.3, 0.1 mM PMSF, all operations were performed at 4° C.

Poly [d(A-T)] preparation :

Poly [d(A-T)] was enzymatically synthesized using DNA-polymerase extracted from either *E. coli* or *Micrococcus luteus*. Polydeoxynucleotide was deproteinized and its purity was tested by high sensitivity melting techniques, circular dichroism and U.V. absorption.

Poly [d(A-T)] was obtained in the A conformation by the addition of 70-80 % of trifluoroethanol to an aqueous solution of poly [d(A-T)]. This procedure was based on a technique developed previously (17) where the A form was obtained in DNA solution and confirmed by X-ray diffraction studies (18-20).

Electron microscopy investigation :

The samples of reconstituted chromatin were spread onto electron microscope grids covered with a carbon film using a technique developed by Miller and Beatty (21), modified to suit our material. The electron microscopy preparations were contrasted by W-Ta rotative shadowing at an angle of about 10° and observed at 60 KV with a PHILIPS 201 electron microscope.

Raman Laser scattering measurements :

Raman spectra were measured on a Jarrell-Ash 25-400 Laser Raman spectrometer. This was interfaced to a LSI-11 microprocessor of a TRACOR TN-1710 minicomputer which allows photon counting, operational control

of the spectrometer and accumulation of the spectra. Raman-Laser scattering measurements on nucleosomes and on poly [d(A-T)] were performed as described previously (22). The precision of the measurements is $\pm 1 \text{ cm}^{-1}$.

Vacuum Ultraviolet Circular Dichroism measurements :

Vacuum ultraviolet circular dichroism spectra were measured using an apparatus constructed in the laboratory and utilizing techniques previously described (23).

RESULTS

Electron microscopy studies

Figure 1 shows electron micrographs of reconstituted chromatin from core histones, H2A, H2B, H3 and H4, and poly [d(A-T)]. It can be clearly seen that nucleosomes are well organized and almost regularly and

Figure 1 : Electron micrographs of reconstituted chromatin with poly [d(A-T)] and inner histones H2A, H2B, H3 and H4 in the ratio of 1:1 protein:DNA (x 71,091).

The samples for electron microscopy investigation were spread and shadowed as described in Material and Methods. Nucleosomes are well organized and very often closely packed along chromatin fiber, so that the axial fiber is not always clearly visible between two adjacent nucleosomes.

closely packed along chromatin fiber. The results of thermal denaturation studies (not shown) also confirm the formation of reconstituted chromatin in good agreement with previous results (11-13).

The Raman-Laser investigation

The Raman-Laser spectra of reconstituted chromatin with poly [d(A-T)] are shown in Figure 2. Figure 3 shows the high resolution spectrum of reconstituted poly [d(A-T)] with chromatin in the restricted region from 700 cm^{-1} to 850 cm^{-1} characteristic of the ribose-phosphate backbone conformation. This high resolution spectrum measured at 5°C provides information about the complexity of the backbone conformation, particularly about differences in sugar puckering. It is apparent that in reconstituted chromatin, poly [d(A-T)] exhibits three bands at 839 cm^{-1} , 820 cm^{-1} and at

Figure 2 : Raman Laser spectrum from reconstituted chromatin with poly [d(A-T)] and inner histones, ratio 1:1 DNA:protein in D₂O and 25 mM NaCl solution at neutral pD. Deuteration obtained by exchange. (In addition to the D₂O band at about 1200 cm^{-1} , one observes a broad band around 1450 cm^{-1} of HOD).

Figure 3 : Raman Laser spectra from reconstituted chromatin with poly [d(A-T)] and inner histones ratio 1:1 DNA:protein in 25 mM NaCl, D₂O solution at neutral pD, at about 5° C. This high resolution Raman spectrum is shown in the range limited to the characteristic sugar-phosphate backbone frequencies.

about 808 cm⁻¹, which leads to the following observations :

- 1) The band at 836 cm⁻¹ is always found in DNA fibers and solutions when the puckering of the sugar is in C2'-endo conformation (22,24). A slight displacement of this band to 839 cm⁻¹ is observed and its relative intensity is comparable to that of the band at 820 cm⁻¹.
- 2) A band at about 808 cm⁻¹ is observed which is characteristic of C3'-endo sugar puckering in DNA fibers in the A form (22,24) and in RNA at about 812 cm⁻¹. This band is only observed in the high resolution spectrum (Fig. 3), due to its relatively low intensity.
- 3) A band at about 820 cm⁻¹ which has only recently been observed, is apparent in poly [d(A-T)]. This band is assigned to the sugar puckering

in a conformation closely related to C3'-endo and is probably of intermediate type.

The spectrum of reconstituted chromatin with poly [d(A-T)] shown in Figure 3 at 5° C is very similar to that of double stranded base paired poly [d(A-T)] measured at 45° C. More particularly the bands at 839 cm^{-1} -820 cm^{-1} , and the shoulder at 808 cm^{-1} , are of similar intensity ; the band at 820 cm^{-1} is broad (Fig. 3). Raman scattering investigations indicate that despite reconstitution carried out at relatively low temperature (see Methods), poly [d(A-T)] adopts the conformation of the higher temperature form. This is in a very good agreement with the results of the circular dichroism investigation (see below). Thus higher temperature form of poly [d(A-T)] is characterized by a decrease in intensity of the band at about 839 cm^{-1} . This leads to an almost equal ratio of the relative intensity of the 839 cm^{-1} band to that of the band at 820 cm^{-1} plus 808 cm^{-1} shoulder.

The extended Raman spectrum shown in Figure 2 provides information about the base vibrations of poly [d(A-T)] in the reconstituted chromatin. The Raman spectrum of reconstituted chromatin was measured in D₂O solution at low salt concentration. The 1573 cm^{-1} band arising mainly from adenine vibrations exhibits a pronounced reduction in its relative intensity with respect to the free poly [d(A-T)] spectrum. Thus the intensity of the 1573 cm^{-1} band relative to the phosphate $\text{O}=\text{P}=\text{O}$ band at 1094 cm^{-1} is reduced (from 1.0 to 0.7) by about 30 % in the reconstituted chromatin spectrum. This is one of the characteristic features of the nucleosome formation which supports all our observations that the reconstitution of chromatin occurred correctly (22,36). We interpret the decrease in intensity of this adenine band as an indication of the interaction of core histones with the adenine.

Circular Dichroism investigation

The circular dichroism spectra of reconstituted chromatin and poly [d(A-T)] alone are shown in Figures 4 and 5 respectively. Each spectrum was recorded at 4° C and 22° C. There are significant changes in the spectrum of free poly [d(A-T)] at 2° C compared with that at 22° C, particularly in the region 250-295 nm. In contrast the spectrum of chromatin reconstituted with poly [d(A-T)] is only slightly modified in this temperature range. The absence of large changes in the C.D. spectra of reconstituted chromatin with temperature suggests that most of the poly [d(A-T)] possesses

Figure 4 : Circular dichroism spectrum of reconstituted chromatin with poly [d(A-T)] and inner histones ratio 1:1 DNA:protein in 2 mM cacodylate pH 8 measured at 4° C and 22° C.

a conformation which is stabilized by nucleosomes formation. In the reconstituted chromatin spectrum, the small decrease in intensity of the band in the region of 260 nm as the temperature is lowered may be due to the presence of small segments of poly [d(A-T)] which are less firmly bound in chromatin. The C.D. spectra of reconstituted chromatin and free poly [d(A-T)] at 22° C closely resemble one another. There is a decrease in intensity in the 275 nm-290 nm region whereas the intensity of the bands at 260 nm are similar. The similarity in the shape of the spectra implies that in nucleosomes poly [d(A-T)] adopts the same conformation as that of free poly [d(A-T)] at higher temperature, above 22° C (see also ref. 25). The relatively small decrease in intensity of the band at 280 nm is dependent on the ionic strength of the reconstituted chromatin solution (Fig. 6). A change of ionic strength from 2 mM cacodylate to 100 mM NaF

Figure 5 : Circular dichroism spectra of poly $[d(A-T)]$ measured at different temperatures 2°C ($\bullet\bullet\bullet\bullet\bullet$) and 22°C (—) in the ultraviolet region extended to the vacuum ultraviolet, in 20 mM NaF, D_2O solution, pD 7.5.

causes a decrease in the 275 nm-295 nm region which becomes slightly more negative whereas the band centered at 260 nm remains constant. Similar changes occur in poly $[d(A-T)]$ only at high salt concentration (26). A decrease in the intensity of the band at 275 nm for DNA exposed to concentrated salt solutions has been correlated with a decrease in the numbers of base pairs per turn of helix, i.e. an increase in the rotation angle between the base pairs (27-29).

Figure 7 shows the C.D. spectra of the A conformation of poly $[d(A-T)]$ obtained by the approach developed for DNA, i.e. by adding trifluoroethanol to an aqueous solution of poly $[d(A-T)]$ to attain 70 %-80 % (17,18). The

Figure 6 : Circular dichroism spectra of reconstituted chromatin with poly [d(A-T)] measured at different ionic strengths : (—) 0.1 M NaF and (.....) 2 mM cacodylate, pH 7.5.

occurrence of the A form in DNA was recently confirmed by X-ray diffraction measurements on fibers (19), and on precipitates which gave the A form patterns (19,20). In the poly [d(A-T)] spectrum of the A form, there is a predominant positive band at 260 nm-275 nm which is of non-conservative character, and a second characteristic band at 215 nm. In figure 7, the spectrum of reconstituted chromatin is compared with the A form of poly [d(A-T)]. Although there is a difference in the intensity between these two spectra, the overall shape are in general agreement, particularly in the 260 nm region. In the region of the far U.V., there is a strong contribution from the α -helical regions in the core histones. This comparison suggests that the increased intensity of the positive band at

Figure 7 : Comparison of circular dichroism spectra of reconstituted chromatin (—●—●—●—) with that of the A form of poly [d(A-T)] (—) in 70 % trifluoroethanol- D_2O solution in 2 mM KF, 2 mM phosphate buffer pH 7. Reconstituted chromatin with poly [d(A-T)] and inner histones in 0.1 M NaF pH 7.5. The intensity of poly [d(A-T)] in trifluoroethanol (form A) represents one half of the measured intensity.

about 260 nm of nucleosomes with respect to that of free poly [d(A-T)] at 4° C, i.e. at temperature of nucleosome formation, reflects some aspects characteristic of an A-like conformation.

Similar conformational changes have been observed in base paired poly [d(A-T)] at higher temperature (fig. 5 and ref. 25), which are interpreted as indicating an increase in the number of base pairs per turn and a decrease of the helix winding angle (27-30).

This C.D. investigation allows one to conclude that upon reconstitution of nucleosomes at low temperature (4° C), poly [d(A-T)]

gains the following conformation features :

- 1) A feature resembling some aspects of free poly [d(A-T)] the A-like conformation and also characteristic of higher temperature ordered form of poly [d(A-T)] which is also supported by the Raman scattering results (Fig. 3) and the measurements of DNA supercoiling (27,30).
- 2) The second conformational feature which is related to a decreasing number of base pairs per turn.

DISCUSSION

The present studies on reconstituted chromatin with inner histones and poly [d(A-T)] lead to two main conclusions :

- The first of these is concerned with the conformation of DNA in nucleosomes. The second point involves a more detailed analysis of DNA conformation in nucleosomes.

The present results obtained by two different methods clearly show that the conformation adopted by poly [d(A-T)] in reconstituted nucleosomes is that of the higher temperature ordered form of poly [d(A-T)]. Thus, despite the fact that all the reconstitution experiments were performed at 4° C the formation of regularly packed nucleosomes with DNA wrapped around core histones leads to a conformational change in poly [d(A-T)] which is characteristic of the higher temperature ordered form. These results are in essential agreement with the previously reported data on enzymatic digestion of reconstituted chromatin with poly [d(A-T)] nucleosomes by DNase I where there was no apparent change in the periodicity of cutting at 4° C, compared with 37° C (11). The present C.D. and Raman studies performed at different temperatures suggest that the formation of nucleosomes stabilizes poly [d(A-T)] in the higher temperature conformation.

- The second point of discussion is related to a more detailed analysis of the fine structure poly [d(A-T)] in nucleosomes which is of particular importance for the solution of the problem of supercoiled DNA in chromatin.

Previously two lines of evidence yield a different estimate of the number of DNA superhelical turns per nucleosome. On one side, circular SV40 DNA after interaction with histones and with the relaxing enzyme is found to yield a difference of about one turn per nucleosome (3). On the other side, according to the model deduced from X-ray diffraction analysis

of crystals of nucleosome cores, the DNA is wound into about two super-helical turns (2). In order to reconcile this discrepancy it was proposed that there is a change of the DNA rotation angle between bases on binding free DNA in solution on to the nucleosome core (2). Klug et al. (ref. 2 and 31) suggested that the helical periodicity of DNA free in solution might change from about 10.4-10.6 (to 1) base pairs per turn to about 10.0 in the nucleosome. Theoretical calculation of energy (33) confirmed that the helical repeat of a straight DNA in solution is expected to be different from that of the same DNA smoothly bent following the model of Finch et al. (2). It is now clearly established that the helical periodicity of free DNA in solution is of about 10.6 (± 0.1) (31-33), which is significantly different from 10.0 base pairs per turn for the B form fiber structure. However, in nucleosomes the estimate of helical periodicity is not known and the value obtained was considered by the authors as not reflecting directly the DNA structure, but rather the particularity of the environment offert to the enzyme (32).

Present results provide direct evidence that DNA conformation is changed on binding in solution on to the nucleosome core. Independent measurements by Raman spectroscopy and circular dichroism yield similar information about the nature of a conformational change in poly [d(A-T)] and particularly about two features apparent in the conformation of poly [d(A-T)] in reconstituted chromatin.

The first of these features is related to some aspects of an A-like conformation inferred from Raman Laser scattering and circular dichroism measurements. An increase of the intensity of the characteristic Raman bands closely related to C3'-endo sugar puckering is observed whereas the intensity of the band attributed to C2'-endo sugar puckering is diminished. Comparison of the C.D. spectra of reconstituted chromatin and free poly [d(A-T)] at 4° C, temperature of nucleosome formation, indicates an increase of the intensity of the positive band at 260 nm. An increase of the intensity of the positive band at 260 nm is observed in free poly [d(A-T)] with increasing temperature (Fig. 5 and ref. 25) which is correlated with a decrease of the helix winding angle is also supported by the measurements of superturns on circular DNA (27-30).

The second feature acquired by poly [d(A-T)] in nucleosome formation is related to the presence of a negative band at about 280 nm in C.D. spectra which contribution is more pronounced in the presence of slightly increasing ionic strength (Fig. 6). This assignment of this

conformational feature is made according to the similarity of the spectral changes exhibited by poly [d(A-T)] upon addition of salt. The nature of these changes can be correlated with an increase in the ionic strength of DNA solutions which promotes a decrease in the number of base pairs per turn of helix, and an increase in the average angle of rotation per base pair (28,29). Our Raman investigation (see Fig. 2) rules out any possibility of proposing a major conformational change in the DNA, e.g. corresponding to the C form geometry (22,36). Moreover, since the reconstitution of nucleosomes was made with only the inner histones H2A, H2B, H3 and H4 there is no need to evoke a tertiary structure condensed form (or ψ form) contribution as shown by Thoma, Koller and Klug (37).

Our results indicating the contribution of two conformational characters can be represented as situated in different regions of DNA in reconstituted chromatin is in agreement with the structural model of nucleosomes and is supported by numerous results obtained by different methods including X-ray diffraction, DNase digestion (2,38) and others.

Thus, we suggest that these two conformation features provide direct evidence of the nature of a conformational change when a free DNA is wound on the nucleosome core, which overall conformation is of the B-kind.

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