Volume 12 Number 6 1984 Nucleic Acids Research

Chromatin superstructure: synchrotron radiation X-ray scattering study on solutions and gels

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Received 19 December 1983; Revised and Accepted 22 February 1984

Abstract

X-ray small angle scattering patterns of solutions and gels of native chicken erythrocyte chromatin and chromatin depleted of H5 histones have been measured under several ionic conditions using synchrotron radiation. Features of the patterns are interpreted as resulting from a superstructure with an outer diameter of about 300Å which is already present in uncondensed nucleofilaments. This superstructure which is shown to be maintained by the H5 histones also explains the rapid condensation of the nucleofilaments in higher ionic strengths.

INTRODUCTION

A variety of biochemical and physico chemical studies have shown that the chromatin fibre consists of a complex of basic proteins (histones) with DNA forming a highly conserved structural entity called the nucleosome. In the chromatin fibres the nucleosomes are joined together by stretchers of linker DNA (1-3).

A variety of structural techniques such as electron microscopy, neutron and X-ray scattering, but particularly crystallography have yielded the structure of the nucleosome (4-6). The overall morphology of which resembles a flattened disc or wedge of about 11nm in diameter and 5.7nm in height, with about 1.8 turns of DNA wrapped around its periphery and the protein components filling the inside.

While the structure of the nucleosome has been determined to a high degree of accuracy, the details of the arrangement of nucleosomes in the chromatin fibres remains largely undetermined although many studies have been reported in the literature (7).

We have conducted a study of the superstructure of chicken erythrocyte chromatin by synchrotron radiation low angle X-ray scattering. Given the maximum dimensions of the nucleosomes one might expect that features in the X-ray scattering vector regions between 0.01 and 0.1nm**-1 (where the scattering vector is defined as $s = 2 \sin\theta/\lambda$, 2θ scattering angle and λ the

wavelength) must be principally associated with the superstructure of the chromatin fibres.

This range of scattering angles is normally inaccessible using conventional X-ray generators but suitable X-ray optics and data acquisition systems (8) on synchrotron radiation sources, which have a considerably higher brilliance, enable this type of measurements.

The results presented below provide evidence of the existence of a well-defined superstructure in the nucleofilaments in low salt conditions in the presence of EDTA. This superstructure already displays some of the structural features exhibited by the condensed "300Å" fibres which are known to exist in the presence of cations. Further, experiments on H5-depleted material indicate that this three dimensional organization of the nucleofilament is maintained by the lysine rich histones.

MATERIALS AND METHODS

Preparation of chromatin fragments

Long chromatin fragments were prepared by mild microccocal nuclease (Sigma) digestion of chicken erythrocyte nuclei (9,10). The soluble chromatin fragments were extensively dialysed against 5mM Tris, 1mM EDTA, 0.2mM PMSF pH7.5. This material was diluted to 5mg DNA/ml in dialysis buffer and used as the starting sample for the X-ray scattering measurements.

Depletions of H5 histone

Chromatin fragments were depleted of H5 histone using Bio Rad AG50W x2 cation exchange resin in 0.65 M NaCl, 10mM Tris, 1mM EDTA, 0.2mM PMSF pH7.5 (11). After extensive dialysis of the resulting H5 depleted chromatin against 5mM Tris, 1mM EDTA, 0.2mM PMSF pH7.5 this material was concentrated to 3-5mg DNA/ml by forced dialysis against the same low ionic strength buffer using a Sartorius membrane device.

Preparation of chromatin and H5 depleted chromatin gels

Chromatin gels were prepared by concentrating chromatin solutions by forced dialysis using Sartorius membranes. This process was thought to be milder than centrifugation for the preservation of loose structures.

Analysis of the protein and DNA content of the chromatin fragments

The protein content of the samples used for X-ray scattering measurements was analysed in 18% polyacrylamide: SDS gels (12). The results obtained (not shown) indicate that the histone content of the chromatin fragments is identical, in all respects, to that found in isolated nuclei, and that the removal of H5 histones does not affect the relative amounts of the core

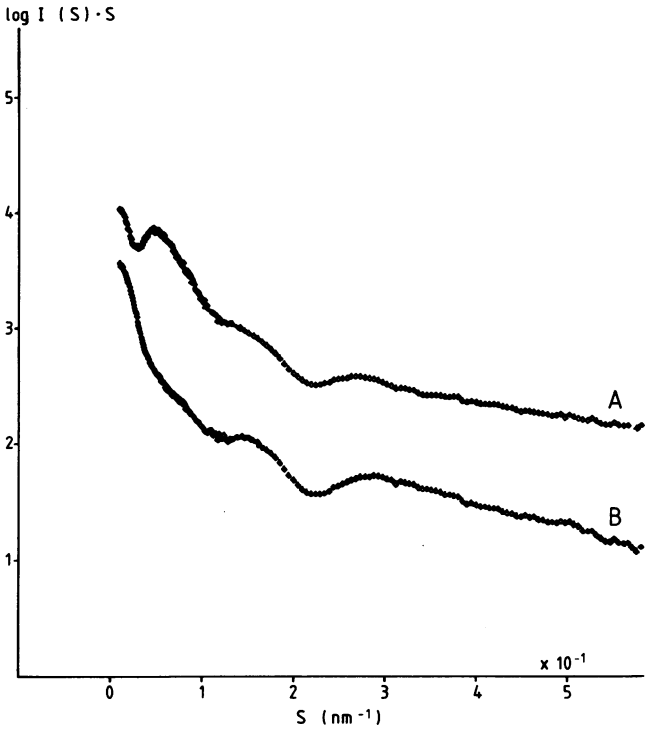

Figure 1: Bottom: A: X-ray solution scattering pattern of chromatin fragments (3mg DNA/ml) in a buffer containing 1mM EDTA, 5mM Tris, 0.2mM PMSF (phenyl) methyl sulphonyl fluoride), pH 7.5.
B: similar pattern in an identical buffer containing 100mM NaCl.

histones.

The size distribution of the chromatin fragements was determined by analysis of their DNA in 18% agarose gels (13) calibrated with a mixture of lambda DNA, lambda Hind III and lambda Hind III + Eco RI restriction fragments (14). The DNA from the "native" and from the chromatin depleted of the very lysine rich histone showed a size distribution peaking at 120 nucleosome equivalents, with dispersivity ranging between 10 to 250 nucleosome equivalents.

RESULTS AND DISCUSSION

Figure 1A illustrates the X-ray solution scattering pattern characteristic of dilute solutions of chicken erythrocyte chromatin in the presence of EDTA (i.e. when all the residual divalent cations are chelated) and figure 1B shows the pattern obtained in a buffer containing 100mM NaCl. Similar

patterns, not shown, were obtained in the presence of $MgCl_2$.

The main features of the solution scattering patterns from preparations in low ionic strength conditions are a prominent diffraction ring at $s=0.05nm^{-1}$, a shoulder at $s=0.15nm^{-1}$ and a well resolved broad maximum at $0.27nm^{-1}$. These last two features closely correspond to the so-called 5.5nm and 3.7nm reflections often observed with nuclehistone gels and fibres (15-18). In the present case these maxima appear at $0.15nm^{-1}$ and $0.27nm^{-1}$ respectively in good agreement with the observations reported, among others, by Sperling and Klug (19).

When either NaCl or $MgCl_2$ are added to the same material at identical chromatin concentrations a different scattering pattern is obtained, as illustrated by the example in figure 1B. The most prominent change occurring upon addition of salt - which is known to induce condensation of the nucleofilaments (20-22) - is the disappearance of the peak at $s=0.05nm^{-1}$ and the appearance of a small shoulder, with an edge at around $s=0.09nm^{-1}$. Another feature consistently observed after condensation is the better definition of the diffraction ring near $s=0.15nm^{-1}$ and of the peak at $s=0.27nm^{-1}$, the latter peak being also slightly displaced to higher scattering angles. These features of the small angle scattering pattern of condensed chromatin fragments are consistent with those reported for nuclei and chromosomes "in vivo" (23,24).

Since dilute solutions were used to record the scattering patterns and the maximum dimensions of the nucleosome are 11.0x11.0x5.7nm (25,26) the possibility that the maximum at $s=0.05nm^{-1}$ is due to the internal structure of the nucleosome or to an interference between nucleofilaments can be ruled out. This has been verified in a series of experiments performed at different chromatin concentrations at low ionic strength. We conclude that the diffraction ring at s=0.05nm-1 is due to a persistent internucleosomal spacing in the uncondensed nucleofilament distributed around an average value of approximately 20nm. This internucleosomal spacing disappears upon condensation.

In an attempt to confirm the origin of this reflection a variety of experiments was performed among which two proved more revealing, namely the study of the evolution of the scattering patterns at increasing chromatin concentrations and of the scattering patterns from chromatin preparations depleted of the lysine-rich histones.

An example of results for the first type of experiment is illustrated in figure 2. When the concentration of chromatin in low ionic strength solu-

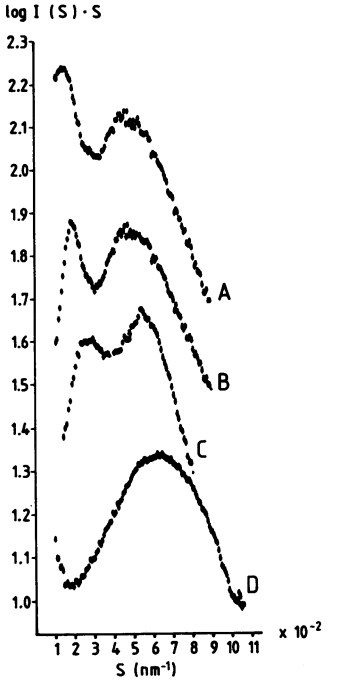

Figure 2: Solutions of chromatin fragments in 1mM EDTA, 5mM Tris pH 7.5, 0.2mM PMSF wer concentrated against the same buffer using a Sartorius membrane device. Fractions of the concentrate were removed at different times and examined by X-ray solution scattering.
Interference effects can be detected at DNA concentrations above 14mg/ml [A]. The interference maximum moves towards larger scattering angles with increasing concentration [B: 250mg DNA/ml] and reaches a position at $s=0.0285nm^{-1}$ which is not altered by further concentration [C: 300mg DNA/ml]. The pattern of a gel of chromatin fragments selectively depleted of lysine-rich histones [D], shows that the interference maximum is shifted to larger angles indicating that the superstructure of the nucleofilament is lost upon depletion of the lysine-rich histones. The maximum centred at $1/15nm^{-1}$, is due to interference between extended nucleosomal chains.

tions was increased by means of a Sartorius membrane device, which allows one to maintain fixed ionic conditions throughout the concentration process, one observes the appearance of an interference-like maximum at values of $s<0.03nm^{-1}$. This interference maximum appears at increasing s-values for increasing concentrations as illustrated in figure 2(A), (B) and (C). However, even in the most concentrated samples, this value does not exceed $s=0.033nm^{-1}$. The peak observed in solution at $s=0.05nm^{-1}$ is also clearly visible and remains, in first approximation, at the same position, although a

comparison of figures 2(A), (B) an (C) shows that small changes occur in its mean position and relative intensity. A straightforward explanation for the observations described above is provided by interfilament interference effects which are responsible for the appearance of the moving diffraction peak. The much less noticeable changes in the shape and position of the maxima at $s=0.05nm^{-1}$, are due to a contribution from the second order of the interference maximum. The fact that the interfilament distance never decreased below 30nm ($s=0.033nm^{-1}$) at low ionic strength, indicate that the chromatin fibre already has an effective outer diameter of the order of 30nm or more, rather than 10nm. The effective diameter should not be strictly regarded as a physical parameter since the minimal distance between fibres may be determined by repulsion effects.

The packing of nucleosomes in the nucleofilament is looser than in the condensed state (under high salt conditions) with an average internucleosomal spacing around 20nm. Measurements on chromatin solutions to which NaCl was added before concentrating to gels also yielded an interference peak at $s=0.03nm^{-1}$. The maximum at $s=0.05nm^{-1}$ was, however, absent in the patterns of these gels as well as of the solutions as illustrated in figure 1. These results (not shown) indicate a value of the outer diameter of chromatin in the condensed state of around 30nm, close to that obtained by electron microscopy. They also indicate that the overall diameter of the uncondensed nucleofilament and of the condensed fibres are similar. Interference effects were also observed in the patterns from pellets of EDTA swollen nuclei.

The disappearance of the maximum at $s=0.05nm^{-1}$ upon addition of salt would indicate according to the interpretation given above, that although uncondensed chromatin already has an outer diameter of about 30nm, the internucleosomal spacing decreases upon condensation. Measurements of radii of gyration of the cross-section and of changes of mass per unit length from the X-ray solution scattering patterns at very low angles which will be reported elsewhere, are also compatible with this interpretation. It should also be mentioned in this connection, that a similar feature at $s=0.05nm^{-1}$ was observed previously in neutron scattering experiments (27) on chromatin at low ionic strength, although no detailed interpretation was given.

Scattering patterns from solutions and gels of chromatin fragments depleted of their complement of lysine rich histone (H5) give further information concerning the origin of the characteristic features of the pattern of native chromatin.

In low ionic strength solutions these preparations yield a scattering pattern

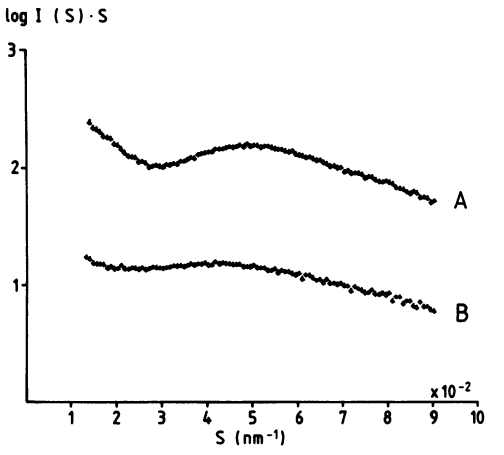

Figure 3: Solution scattering pattern of chromatin from chicken erythrocytes at 3mg DNA/ml in 5mM Tris pH 7.5, 1mM EDTA, 0.2mM PMSF [A]. The maximum at $0.05nm^{-1}$ is attributed to a diffraction band arising from the regular distribution of nucleosomes along the filaments. This maximum is still present in the pattern for H5 depleted chromatin fragments [B] but its intensity is three times smaller than in the pattern of native chromatin and has moved to $0.042nm^{-1}$. This decrease in intensity can be interpreted as resulting from the disappearance of two thirds of the scattering vectors contributing to the scattering in this region upon removal of the lysine-rich histones and the movement to smaller s-values arising from the extension of the uncondensed chromatin fibre.

which, as illustrated in figure 3B, displays a maximum at low angles at somewhat lower s-values ($s=0.045nm^{-1}$) than in native chromatin and for identical chromatin concentrations and ionic conditions the intensity of this diffraction ring is only about one-third of that found in native samples. Moreover, the interference maximum in the patterns of gels, obtained by the same concentration method as above, is located around $s=0.065nm^{-1}$. This can be interpreted assuming that the average number of internucleosomal distances in preparations containing a full complement of lysine-rich histones is about two thirds higher and that the three dimensional organisation leading to a maximal outer dimension in excess of 3.0nm in uncondensed native nucleofilaments results from the presence of the very lysine-rich histone. The latter keep a well defined spatial relationship between three or more neighbouring nucleosomes at any position along the chromatin fibre and their removal destroys this structural scaffolding, allowing the fibre to become an extended nucleosomal chain. The interference maximum then appears at a larger scattering angle corresponding to the reduced diameter of the fibres,

while the reflection at $s=0.05nm^{-1}$ may disappear altogether or alternatively be hidden by the interference maximum.

Assuming that the peak at $s=0.05nm^{-1}$ is due to an internucleosomal spacing in the uncondensed chromatin fibre and that the removal of the very lysine rich histone allows the "10nm" nucleofilament to become more extended, with the characteristic internucleosomal maximum appearing at $s=0.042nm^{-1}$ (figure 3B), then, given the dimensions of the nucleosome (25,26), the average length of the linker DNA must be of the order of 18nm or around 50 base pairs of relaxed DNA, in reasonable agreement with the data from biochemical studies (28).

Analysis of the very low angle part of the scattering curves for solutions of native nucleofilaments and of nucleofilaments depleted of the lysine-rich histones, yields values for the radii of gyration of the cross-section corresponding to equivaleng solid cylinders diameters of 28.2+1nm and 16.+1nm respectively, in good qualitative agreement with the values obtained from the position of the interference peaks in the gels.

Additional measurements, to be published elsewhere, of the radii of gyration of the cross-section for different states of condensation of the chromatin fibre, time course of the condensation kinetics, the effects induced by intercalating dyes, changes of mass per unit length upon condensation as well as of the scattering patterns obtained from whole nuclei have all yielded results fully compatible with the interpretation given above.

Data suggesting the existence of a superstructure for the nucleofilament has already been reported in the case of calf thymus chromatin (29,30) but had been interpreted differently. Electron microscopy studies of the nucleofilament in low salt conditions, whether showing the zig-zag appearance or not (21,22,31), are compatible with the existence of a superstructure with an outer diameter of the order of 30nm. The regions in the electron micrographs showing the so-called "10nm" nucleofilament can easily be due to preparative artifacts.

It is also relevant, in the present context, that the condensation process is very fast not only in chromatin solutions (half time less than or equal 50msec) but also in thick gels where the rate of the process is limited by salt diffusion (half-time around 1s), suggesting that the topological arrangement required for condensation already exists in the uncondensed nucleofilaments at low ionic strength.

The proposed model has implications for the condensation mechanism of chromatin. If one assumes that a preformed superstructure, say a loose

superhelix, exists in the uncondensed state, in which the nucleosomes are exposed to the solvent but nevertheless have a well-defined spatial relationship, the condensation process can take place in a manner similar to the folding of an accordeon. Only a minimal change in the superstructure is needed to tightly pack the nucleosomal core, a process which would be helped by the wedge shape of the nucleosomes. The structural changes which enables the nucleosomes to pack tightly and collapses the superstructure could be due to a reduction of a large helical pitch, and/or a rotation of the nucleosomes around the linker DNA or relative to each other, possibly induced by a conformational change of the lysine rich histones, by neutralization of the DNA charges or both. Moreover, in vivo, in the nuclei, where the folding and unfolding process must take place, the model suggested above provides a mechanism which minimizes the posibilities of tangling and allows a rapid unfolding of specific stretches along the chromatin fibre without the need to disrupt the whole superstructure.

One of us L.P.G. acknowledges the receipts of financial support from Commissio Interdepartmental de Recerca i Innovacio Technologica (CIRIT) of the Generalitat de Catalunya, the European Molecular Biology Organisation (EMBO) and the European Molecular Biology Laboratory (EMBL).

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