Nucleic Acids Research

Nucleosome cores reconstituted from poly (dA-dT) and the octamer of histones

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ABSTRACT

        In this paper we describe a detailed investigation of the reconstitution
of nucleosome cores from poly (dA-dT) and the octamer of histones.   We also
attempted the reconstitution from the copolymers poly dA·poly dT, poly dG·poly
dC and poly (dG-dC).   The repeat of the reconstituted chromatin fibre is dis-
cussed.   The micrococcal nuclease released poly (dA-dT) core particle is
found to contain a considerably narrower DNA size distribution that that of
the native random DNA nucleosome core (12).   In addition we have succeeded in
obtaining small crystals of the poly (dA-dT) nucleosome core.   The DNAase I
digestion pattern of the poly (dA-dT) containing nucleosome core is presented.
The periodicity of DNAase I cutting sites is found to be about 10.5 bases and
is similar to that of the native nucleosome core (12, 13).

INTRODUCTION

        It is now well established that the basic repeating unit in the chromatin

fibre, the nucleosome, consists of an octamer of histones $(H2A)_2(H2B)_2(H3)_2$

$(H4)_2$, around which are wrapped approximately 200 base pairs of DNA (see

reviews by Kornberg (1) and Thomas (2)).   A fifth histone, H1 is also assoc-

iated with the nucleosome.   When the chromatin fibre is digested with micro-

coccal nuclease it yields as a stable limit a nucleosome "core" particle which

contains approximately 140 base pairs of DNA (31).

        In order to gain some understanding into the architecture of chromatin,

several laboratories have investigated the reconstitution of chromatin using

random DNA and the purified histones H2A, H2B, H3 and H4 (4-8).   Generally,

the reconstitution by decreasing salt yields a chromatin fibre with a repeat

of approximately 140 base pairs of DNA (4, 6).   Only the reconstitution by

Laskey et al. (9) under physiological conditions produces a 200 base pair

repeat.   However, one should note that the DNA in the monomer particle

produced by both Spadafora et al. (4) and Steinmetz et al. (6) is longer than

140 base pairs of DNA.

        DNAase I has been widely used in studies of the internal organisation of

DNA in the nucleosome.   DNAase I digestion of random DNA reconstituted
nucleosomes has been studied (6) and to the first approximation the fragment
pattern is the same, as for the native nucleosome core.

    We were particularly interested in answering two questions: (1) was it
possible to reconstitute nucleosome cores from either of the copolymers poly
dA·poly dT, poly dG·poly dC, poly (dA-dT) and poly (dG-dC) and how similar
would the characteristics of such a core particle be compared to the ones of
the native random DNA nucleosome core?   (2) If nucleosome cores containing a
homopolymer could be produced, could one then obtain crystals?   The hope
would be that, becuase of the homogeneity of the DNA, the crystals would be
more highly ordered than the ones so far obtained with the native random DNA
nucleosome core (10, 11).

MATERIALS AND METHODS

    Copolymers.   The copolymers poly dA·poly dT, poly dG·poly dC, poly
(dA-dT) and poly (dG-dC) were purchased from BCL The Boehringer Corporation.
They were all dissolved in 0.1 M NaCl, 10 mM Tris-HCl pH 7.0 at + 4°C.

    Preparation of the octamer of histones.   The octamer of histone was
prepared from chicken erythrocytes following the procedure of Thomas and
Butler (14) or by the following method: long chromatin was passed through a
Sepharose 4B column equilibrated in 0.65 M NaCl, potassium cecodylate pH 6.0
0.25 mM PhMeSO$_3$F, 0.2 mM EDTA to remove H1 and H5 (3).   The H1, H5-stripped
chromatin was concentrated at least 10 times and made 2 M NaCl and 50 mM
potassium phosphate pH 7.0.   The sample was then passed through a hydroxyl-
apatite column equilibrated in 2 M NaCl, 50 mM potassium phosphate pH 7.0
0.25 mM PhMeSO$_3$F.   In 2 M NaCl the DNA is dissociated from the octamer of
histone and binds to the hydroxylapatite.   The histones elute off in the
exclusion volume as a single peak.   The yields are at least 80% of the histone
content of stripped chromatin.   The peak was analysed by SDS gel electro-
phoresis (15) and found to contain only the four small histones H2A, H2B, H3
and H4 in   equimolar   amounts (Figure 3).

    Reconstitution.   The octamer of histones was associated to the four
copolymers at histone:DNA ratios of 0.6 - 1.1 mg:1 mg against buffers contain-
ing 20 mM Tris-HCl pH 8.0 0.2 mM EDTA, 0.25 mM PhMeSO$_3$F and decreasing con-
centrations of NaCl at + 4°C: 2.0 M NaCl (4 h), 0.85 M (6 h), 0.65 M (6 h),
0.5 M (12 h) and finally 15 mM Tris-HCl pH 7.5 (12 h).   The concentration
of the copolymers in the dialysis bag was 125 µg/ml (5).

    Production and crystallisation of the poly (dA-dT) nucleosome core.   The

microccoccal nuclease released nucleosome cores were fractionated on isokinetic sucrose gradients (16). The peak containing the core was collected and dialysed versus 15 mM potassium cacodylate pH 6.0. Small crystals were grown using conditions very similar to those used to crystallise the native nucleosome core (11).

Nuclease digestion. Micrococcal nuclease (BCL The Boehringer Corporation) was used in 15 mM Tris-HCl pH 7.5, 0.5 mM $CaCl_2$ at 37^oC. DNAase I (Worthington Biochemical Corp.) in 15 mM Tris-HCl pH 7.4, 1 mM $MgCl_2$, 0.5 mM $CaCl_2$ at 4^oC and 37^oC.

Polyacrylamide gel electrophoresis of DNA. Single stranded DNA fragments were separated on polyacrylamide gel electrophoreses containing 7 M Urea (17) using Tris/borate/EDTA buffer (18). The high resolution gel electrophoresis system developed by Lutter (13) was used to determine the DNA size distribution in nucleosome cores.

RESULTS

Reconstitution from four copolymers and the octamer of histones. Reconstitution from the four copolymers poly dA·poly dT, poly dG·poly dC, poly (dA-dT) and poly (dG-dC) and the octamer of histones was investigated using various ratios of histones to polymer. Reconstitutes with a nucleosome like structure were obtained only with the alternating copolymers poly (dA-dT) and poly (dG-dC) and the octamer of histones. Micrococcal nuclease digestion of these complexes produces a discrete series of DNA fragments (Figure 1). No data is shown for poly (dG-dC). The inability of the homopolymers poly dA· poly dT and poly dG·poly dC to arrange into nucleosomes is probably due to poly dA·poly dT forming triple strands (19) in the high salt used in the reconstitution, and poly dG·poly dC can form quartets of poly dG, and also favours the A form of DNA (20, 21).

When reconstituted complexes (histone:DNA ratios of 1 mg:1 mg) containing poly (dA-dT) poly (dG-dC) were examined by electron microscopy it was found that all of the DNA appeared to be organised into nucleosomes which are closely packed along the chromatin fibre. Similar results have recently been published by Bryon et al. (22).

Micrococcal nuclease digestion of the reconstituted poly (dA-dT) chromatin. Digestion by micrococcal nuclease for increasing times was carried out at both + 4^oC and 37^oC. It was found that 37^oC gives the more distinct fragment pattern. Figure 1 shows a time course of micrococcal nuclease digestion of poly (dA-dT) chromatin at 37^oC (histone:DNA ratios 1 mg:1 mg in a) no NaCl;
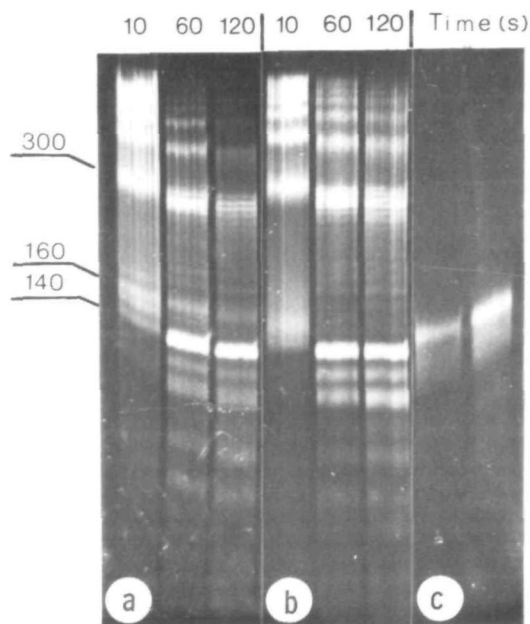
Figure 1: Polyacrylamide gel electrophoresis of the DNA from micrococcal nuclease digestion time courses of the same sample of reconstituted poly (dA-dT) chromatin: (a) in 0 NaCl, (b) in 50 mM NaCl. (c) is a marker native nucleosome core from beef kidney at two different loadings. The DNA was electrophoresed in a 6% polyacrylamide gel under denaturing conditions. The gel was stained with ethidium bromide.

b) 50 mM NaCl. At the earliest time point 10s in both (a) and (b) the main monomer band is at 160 bases of DNA. In other reconstitution experiments the 160 bases band persisted even in the later stages of digestion. If it is assumed that micrococcal nuclease cuts every ten bases as in native chromatin (23) and one counts the faint bands between the 140 monomer band up to and including the dimer band one arrives at 300 bases for the later digestion time of 60s. At 10s the dimer band is larger than 300 bases of DNA and the trimer band is also larger at digestion time 10s than at 60s. It seems reasonable to conclude that part of the population of the reconstituted cores can bind approximately 160 base pairs of DNA.

From the gel in Figure 1 it can also be seen that in 50 mM NaCl the structure of the chromatin fibre is more compact resulting in the persistance of multimer bands and a greater extent of overdigestion. The internal cuts

in the nucleosome core particle seem to be dependent on how closely the cores
are packed.   When reconstitution experiments were carried out at lower ratios
of histone:DNA, 0.6 - 0.8 mg:1 mg, less overdigestion was produced and no
greater multimer than dimers or trimers were observed (data not shown).   If
the weight of histones:DNA was increased to over 1.1 mg:1 mg the reconstitute
became insoluble.

   Production and crystallisation of the poly (dA-dT) nucleosome core.
Careful timing of the micrococcal nuclease digestion of the poly (dA-dT) re-
constituted long chromatin enabled us to produce a nucleosome core containing
a clean 140 base pair band.   This core particle was fractionated from residual
dimers and trimers on sucrose gradients (16).   The poly (dA-dT) nucleosome
core contains a slightly higher proportion of DNA smaller than 140 base pairs
than the native random DNA nucleosome core (beef kidney).   Both the native
140 base pairs core particle and the poly (dA-dT) core were analysed by high
resolution gel electrophoresis which suppresses the effect of sequence on
mobility (13).   Figure 2 shows that the average length of poly (dA-dT) from
the reconstituted core is the same as that of the native random DNA nucleosome
core recently accurately sized to contain 146 base pairs of DNA (13).   The
densitometer tracing of the gel shows that the width of the size distribution
of core poly (dA-dT) is about half of that of random DNA from the native core.

   The histones found in the isolated poly (dA-dT) nucleosome core are
shown in Figure 3.   H2A, H2B, H3 and H4 are present in equimolar amounts.
The sedimentation coefficient for the reconstituted poly (dA-dT) core is
10.3 S (measured in 10 mM Tris-HCl pH 7.0), a value very close to that of the
native core (11).

   We have succeeded in obtaining small single crystals of the poly (dA-dT)
nucleosome core, but so far they have been too small for analysis by X-ray
diffraction.   Figure 4 shows electron micrographs of two different crystal
forms.   In Figure 4a the nucleosome cores are packing in straight columns of
about 110 Å in diameter whilst in Figure 4b the cores form wavy columns which
are also 110 Å apart.   These two modes of packing are similar to those found
in crystals of native nucleosome cores (10, 11).   In both electron micrographs,
in some areas, spacings of about 60 Å and 30 Å can be seen, corresponding
respectively to the height of the platysome and the bipartite division in the
nucleosome structure (10, 11).

   Characterisation of the poly (dA-dT) nucleosome core with DNAase I.   In
order to gain some understanding of the organisation of the copolymer in the
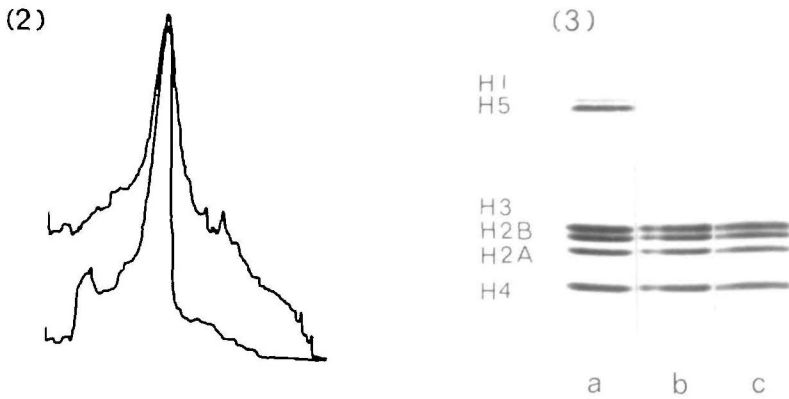poly (dA-dT) nucleosome core, DNAase I digestions were carried out at 4° C

Figure 2: Densitometer tracings of the electrophoretic separation of the DNA from reconstituted poly (dA-dT) nucleosome cores (narrow peak) and the DNA from native nucleosome cores from beef kidney (wide peak with high background). The DNA was separated in a high resolution gel electrophoresis, 6% acrylamide 1% bis-acrylamide, under denaturing conditions. The gel was stained with ethidium bromide.

Figure 3: SDS-polyacrylamide gel electrophoresis of chicken erythrocyte histones (a) in long chromatin, (b) the histones present in the octamer of histones, (c) the histones present in the purified poly (dA-dT) nucleosome core.

and 37°C. Figure 5 shows the pattern of single stranded fragments from such an experiment. The densitometer tracings clarify the similarities and
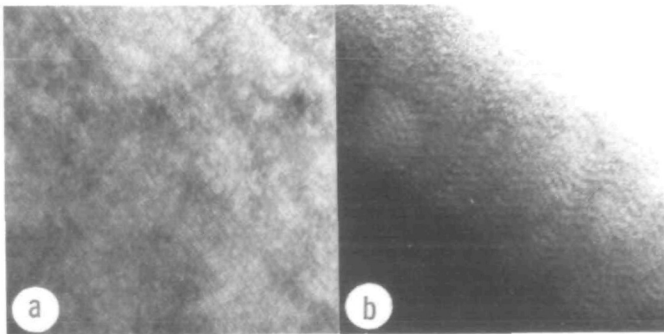

Figure 4: Electron micrographs of two different crystal forms: (a) the straight columns, (b) the wavy columns at the edge of a crystal. The distance between the straight and wavy columns is 110 Å. (X 150000) The crystals are negatively stained with uranyl acetate.
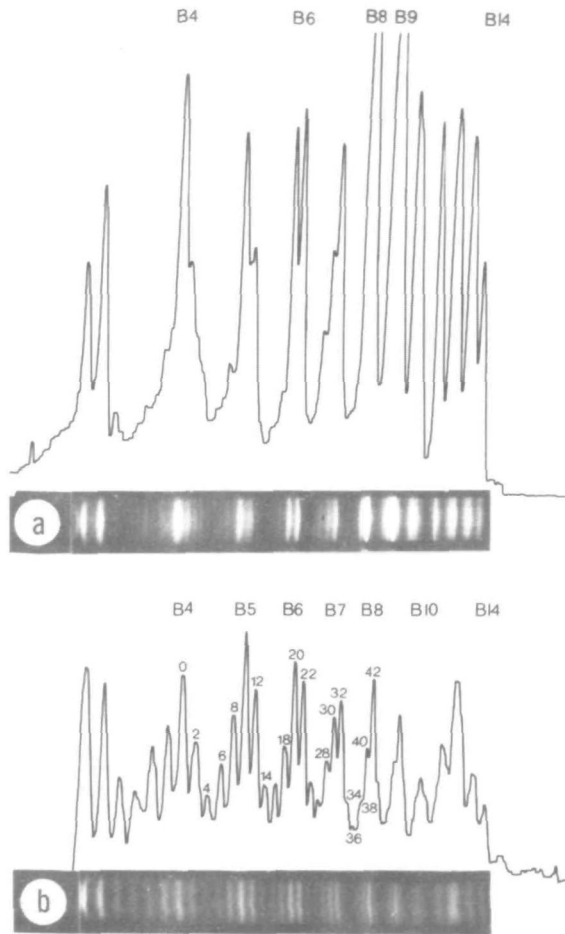
Figure 5: Polyacrylamide gel electrophoresis and densitometer tracings of the same sample of DNAase I digested poly (dA-dT) nucleosome cores: (a) at 4°C, (b) at 37°C. Under the envelopes of the bands (B) there are fine peaks which are produced by fragments differing in size by two bases. These peaks have been numbered in the region of B4 to B8 in (b). The DNA was electrophoresed in a 10% polyacrylamide gel under denaturing conditions. The gel was stained with ethidium bromide.

differences of the DNAase I produced cuts at 4°C and 37°C. At the two temperatures the strong cleavage sites are in the same positions but the cutting at 4°C is much more precise since the peaks are high and the background exceptionally low. The relative intensities of the bands is in reasonable agreement with those of the native core where B9, B8 and B4 are the strongest

bands (3) (Figure 5a).   At 37°C the peaks are broadened out and the back-
ground increases since sites between the major sites become more accessible
(Figure 5b).

It is known that when naked poly (dA-dT) is digested with DNAase I the
largest proportion of oligonucleotides produced have T at their 5' ends and
that successive oligomers differ in length by two nucleotides (24).   We find,
that when DNAase I cuts poly (dA-dT) which is bound to the octamer of histones,
the same specificity of cutting is retained producing fragments which differ
in size by two bases.   Preliminary analysis of DNAase I fragments from poly
(dA-dT) nucleosome cores show that the majority of products have Ts at their
5' ends.   The "two base" ladder is strongly modulated by the protected
regions of poly (dA-dT) in the nucleosome core.

The effect of DNAase I on reconstituted cores, as in native chromatin,
is to produce a series of bands corresponding to DNA fragments with band
maxima differing in size by about 10 bases.   The width of these bands
(labelled B in Figure 5) is broad corresponding to a distribution of fragments
which can be seen to consist of a number of fine peaks under the envelope of
the band.

The densitometer tracing of the single stranded DNAase I fragments
produced at 37°C (Figure 5b) clearly show that the distance between DNAase I
cutting sites on poly (dA-dT) is distinctly different from the 10 bases
previously suggested (25).   The pattern of sharp peaks produced by DNAase I
fragments goes out of phase with respect of the envelope of the band pattern
when comparing one part of the densitometer tracing with the other.   At B4
there is one major single fragment in the centre of the band, at B6 two frag-
ments are present, more or less equally, and by B8 or B9 the cutting produces
one major fragment again.   The peaks signifying fragments differing by two
bases have been numbered in the region of B4 to B8.   B8 is the first band
that has approximately the same fragment distribution within the envelope as
B4.   There are 21 fragments between B4 and B8 which differ in size by two
bases, making the distance between B4 and B8 42 bases.   If this number is
divided by the number of bands crossed, which is four, we arrive at a figure
of 10.5 bases.   10.5 bases is the average distance between DNAase I cutting
sites, in good agreement with the distance found for the native nucleosome
core (12, 13).

To further characterise the reconstituted poly (dA-dT) nucleosome core,
the digestion by DNAase II has been investigated.   We find that the pattern
of fragments produced in 0.5 mM $MgCl_2$ is very similar to that of native

nucleosome cores.   (Data not shown). (23)   However, DNAase II cuts at both
As and Ts producing fragments that only differ in size by one base.

DISCUSSION
     The alternating copolymers poly (dA-dT) and poly (dG-dC) assemble with
the octamer of histones (chicken erythrocyte) in the presence of decreasing
concentration of NaCl to form nucleosome-like structures.   Digestion of poly
(dA-dT) chromatin (histone:DNA ratio, 1 mg:1 mg) for increasing length of time
produces an interesting result.   At the earliest time point 10s, the shortest
predominant band is at 160 bases of DNA.   The dimer can be estimated to have
an average length of at least 300 bases of DNA at 10s (Figure 1).
     Similar results have been obtained with random DNA reconstituted
chromatin (4, 6).   It is suggested by both Steinmetz et al. (6) and Spadafora
et al. (4) that there are two populations of particles in reconstituted
chromatin.   The nucleosomes that are spaced apart can bind more than 140
base pairs of DNA, but when these nucleosomes are packed together in tight
clusters only 140 base pairs of DNA is bound.   Our results suggest that well
spaced nucleosomes can bind 160 base pairs of DNA which corresponds to two full
superhelical turns of DNA per nucleosome core (11).
     The action of micrococcal nuclease is to cut between nucleosomes and then
trim in from the ends produced.   This effect is illustrated in Figure 1 where
the band at 160 bases moves down to 140 bases at 60s, accumulating at 140 bases
as the digestion continues.   The size of the dimer at 60s can also be ex-
plained by this trimming effect.
     Careful timing of the micrococcal nuclease digestion of poly (dA-dT)
chromatin produced a nucleosome core which in homogeneity is comparable to
native nucleosome cores which have been crystallised in our laboratory (3, 10,
11).   The native nucleosome core has been accurately sized to contain an
average length of 146 base pairs DNA with a half-height width of about 3 base
pairs on either side (11).   The poly (dA-dT) helix on the reconstituted
cores is cut more precisely by micrococcal nuclease with a width at half height
of about 1.5 base pairs, but has the same average length of 146 base pairs DNA
(Figure 2).
     Recently Lomonossoff and Butler (26) have studied the base specificity of
micrococcal nuclease.   For naked random DNA they find that As and Ts are cut
much more preferentially than Gs and Cs.   This suggests that the spread of DNA
sizes, in native nucleosomes cores, produced by micrococcal nuclease, is at
least in part due to the sequence heterogeneity of random DNA, hence the

smaller size distribution found for the more homogeneous poly (dA-dT).

The results from DNAase I digestion of poly (dA-dT) nucleosome cores are very striking. At 4° C the fragment pattern (Figure 5) indicates that the copolymer is highly organised in the core particle, since the background is very low against very high peaks of the densitometer tracing of the gel. Bands B9, B8 and B4 are strong in agreement with DNAase I digestion fragment pattern of native nucleosome cores (3). At 37^OC sites located between the major cutting sites become more accessible to DNAase I. These sites are located two bases apart since in poly (dA-dT) DNAase I cuts preferentially at Ts. By counting the number of peaks and dividing by the number of bands crossed, we were able to determine that the average distance between DNAase I cutting sites is 10.5 bases. This is in good agreement with Prunell et al. (12) and Lutter (13), who have measured the periodicity of DNAase I cutting, in the native random DNA nucleosome core, and found it to be 10.4 bases. For a detailed discussion on whether the periodicity of DNAase I cutting sites reflects the screw of DNA directly see references 12 and 13.

The question arises as to why the accessibility of poly (dA-dT) to DNAase I on the nucleosome core increases from 4^OC to 37^OC. No differences of DNAase I cutting of either the native nucleosome core, or the reconstituted nucleosome core from random sequence DNA (Thomas, personal communication), have been reported. Double stranded poly (dA-dT) is a much less stable structure than random sequence DNA (27). The change in accessibility of the copolymer could reflect "breathing" of the two strands or a loosening of the binding of poly (dA-dT) to the core histones at 37^OC. However, the periodicity of DNAase I cutting does not appear to change at the two different temperatures.

A further puzzling point is as to why DNAase I cuts both naked and histone bound poly (dA-dT) at predominently Ts when the enzyme has been reported not to have any strong base specificity (28, 29). A possible explanation for this phenomena has been put forward by Klug et al. (30), who suggest that every second phosphate of the naked poly (dA-dT) backbone has a different conformation from that of B form DNA. Whatever the explanation, the fact remains, that the DNAase I cutting at every two bases is maintained in the poly (dA-dT) reconstituted into nucleosome cores.

The crystals obtained from poly (dA-dT) nucleosome cores have so far been too small to be analysed by X-ray diffraction. Our hope is, that since the DNA in these nucleosome cores is much more homogeneous, large crystals will prove to be more highly ordered, thus enabling a more accurate solution of the three-dimensional structure of nucleosome cores (10, 11).

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