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Electron microscopy of defined lengths of chromatin

(nuclease digestion/sucrose gradient fractionation/packing ratio)

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ABSTRACT **Defined lengths of chromatin were prepared by brief digestion with micrococcal nuclease and fractionation in a sucrose gradient. A length containing a given number of 200 base pair repeating units appeared as the same number of 100 Å beads in the electron microscope. The distance between beads within a length was small, usually less than about 20 Å.**

Several independent lines of work have led to the idea of beadlike units in chromatin, but there is disagreement over the spacing and DNA content of the beads. Two models have been put forward. One stems from a particulate appearance of chromatin fibers in the electron microscope, observed by Olins and Olins (1) and Woodcock (2). The particles were about 70 Å in diameter and connected by strands about 15 Å wide. Van Holde *et al.* (3) have identified the particles with fragments of chromatin containing 110–120 base pairs of DNA that are found after extensive micrococcal nuclease digestion. Electron micrographs of the digest showed occasional "doublet" particles with connecting strands of average length 240 Å (corresponding to 70–80 base pairs of DNA if "extended in a B-like form"). In the resulting model (Fig. 1, model I) the packing ratio (the ratio of the extended length of DNA to the corresponding length of fiber) is about 2:1.

In the other model (4) a chromatin fiber consists of a flexibly jointed chain of 100 Å repeating units that are in contact (Fig. 1, model II) and contain 200 base pairs of DNA, giving a packing ratio of about 7:1. The length of the unit in this model comes from x-ray diffraction, which shows a 100 Å repeat in the fiber direction (5, 6), and the width comes from electron micrographs showing fibers about 100 Å thick (7, 8). The amount of DNA per unit is derived from biochemical results (9, 10) and has been substantiated by studies involving brief micrococcal nuclease digestion (11). Electron micrographs of simian virus 40 (SV40) chromatin having the appearance of this model were published by Griffith (12), who compared the contour length with that of naked SV40 DNA and obtained a packing ratio of 7 ± 0.5:1. A similar packing ratio was obtained by Oudet *et al.* (13) for chromatin from other sources.

The aim of the work described here was to determine the relationship between the bead observed in the electron microscope and the 200 base pair repeating unit inferred from biochemical evidence. Counts of beads in chromatin containing defined lengths of DNA, in particular SV40 chromatin (12) and complexes formed *in vitro* between adenovirus DNA and histones (13), have suggested that the bead and the biochemical unit are the same. Chromatin containing defined lengths of DNA can also be obtained by brief digestion with micrococcal nuclease and fractionation in a sucrose gradient (11). Here we report the counts of beads in such fractions. In the course of this work we have also made measurements of inter-bead distances in an effort to decide between the extended and compact models described above.

MATERIALS AND METHODS

Chromatin (A_{260} = 46) was prepared as described (ref. 15, with the use of 110 units/ml of micrococcal nuclease for 2 min at 37° and lysis in half the stated volume of 0.2 mM NaEDTA) and 0.8 ml was layered on each of five 36.3 ml isokinetic sucrose gradients, containing 1 mM NaEDTA, pH 7, with c_t = 5%, c_r = 28.8% and V_m = 33 ml (16). The gradients were centrifuged for 22 hr at 27,000 rpm and 4° in a Beckman SW27 rotor, and analyzed with the use of a turbulence-free flow cell (Molecular Instruments Co, Evanston, Ill.). Corresponding fractions of the gradients (Fig. 2) were pooled, and dialyzed overnight against 1 mM NaEDTA, pH 7. The purity at this stage, determined as described in the *text*, was 95, 87, 78, and 67% for monomer, dimer, trimer and tetramer fractions. An aliquot (3 ml) of each fraction was further purified on a second sucrose gradient identical to the first.

For electron microscopy, the purified fractions were dialyzed against 0.5 mM NaEDTA, pH 7, adjusted to the required ionic strength by the addition of ammonium acetate, and fixed if required in 5% formaldehyde, pH 7, for 5–10 min at 30°. Drops of solutions were applied to thin carbon-coated grids and the excess solution was withdrawn with an edge of filter paper. The grids were then washed with a few drops of 1% uranyl acetate and the excess solution was again

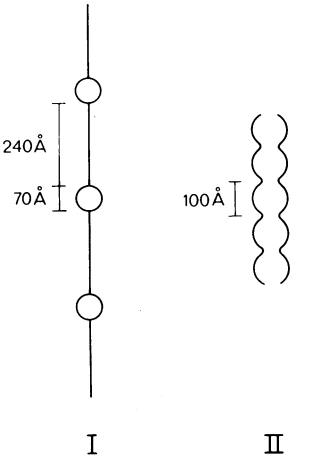

FIG. 1. Schematic drawing of models proposed for chromatin structure. Model I is from ref. 3; model II is from ref. 4.

Abbreviations: SV40, simian virus 40; EDTA, ethylenediaminetetraacetate.

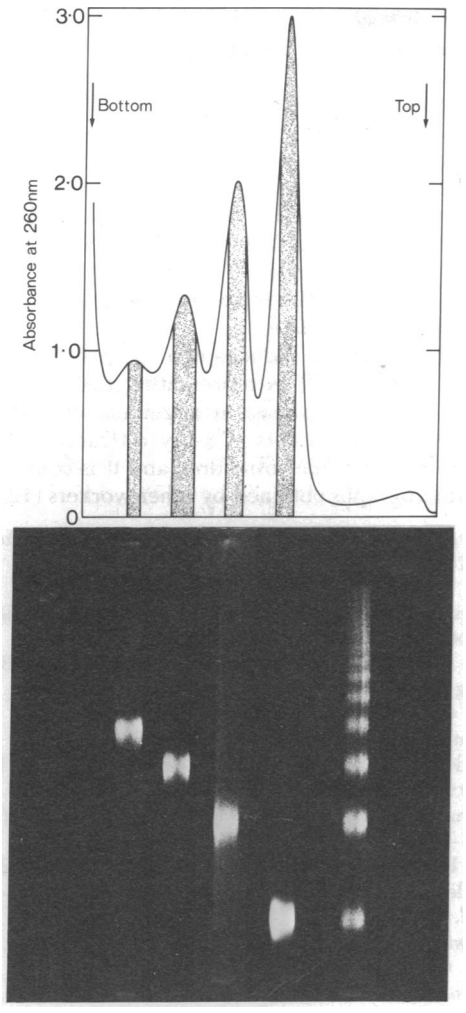

FIG. 2. Preparation of defined lengths of chromatin. *Top:* first sucrose gradient fractionation of products of micrococcal nuclease digestion; material was collected from the shaded areas for further purification. *Bottom:* polyacrylamide gel electrophoresis of DNA (15); gel at far right shows DNA from unfractionated nuclease digest, and each of the other gels shows DNA of material further purified from the sucrose gradient peak directly above.

withdrawn onto filter paper. The grids were examined in a Philips EM 300 at 40 kV and photographed at a nominal magnification of 51,000 (checked by photographing a grating replica under the same conditions).

RESULTS

Purified fractions of chromatin monomer, dimer, trimer, and tetramer (200, 400, 600, and 800 base pairs of DNA with associated histones) were obtained as follows: rat liver nuclei were briefly digested with micrococcal nuclease to cut across both DNA strands at occasional points between 200 base pair repeating units. The nuclei were than lysed in hypotonic buffer and the mixture of chromatin fragments was resolved in a sucrose gradient (Fig. 2). The material collected from each peak was further fractionated in another sucrose gradient. The purity of the final fractions was determined by electrophoresis of the DNA in polyacrylamide gels (Fig. 2). Densitometry of the gels showed less than 5% cross-contamination.

Electron micrographs of the purified fractions are shown in Fig. 3. There are exclusively single beads in the field ob-

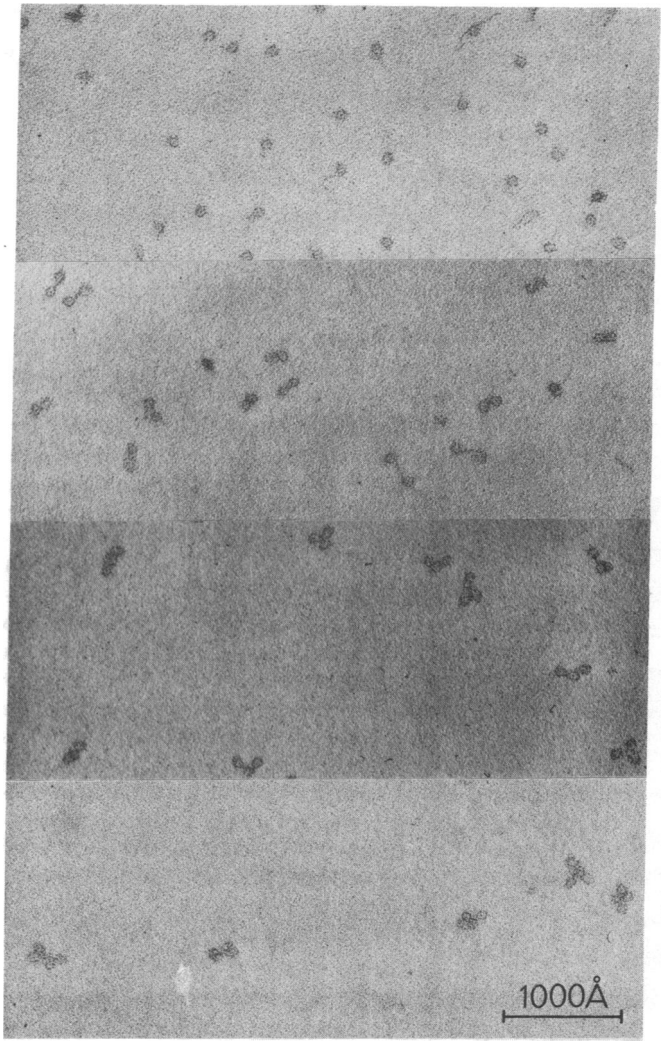

FIG. 3. Electron micrographs of fractions from sucrose gradients. From top to bottom: monomer, dimer, trimer and tetramer fractions.

tained from the monomer fraction, exclusively pairs of beads in the field obtained from dimer fraction, and so on. This proves that the bead corresponds to the unit of chromatin structure containing 200 base pairs of DNA.

In the field obtained from the monomer fraction, the images of well-preserved beads (those showing no obvious distortion) are either approximately circular or slightly elliptical. The circular images are bounded by a dark ring of outer diameter about 85 Å and often contain a central dark spot or line. The elliptical images measure about 85 × 105 Å and also show a dark perimeter but they are more variable in detail. Extensive washing after staining did not alter the images, so we attribute the main features to positive staining, presumably of the DNA. The dark perimeter would then indicate that the DNA is largely on the outside, as suggested previously (4, 14).

In the field obtained from the dimer fraction, the beads of a pair are usually close together or in contact, but occasionally they are some distance apart and connected by a strand about 20 Å in diameter. A histogram of inter-bead distances in dimers is shown in Fig. 4. The same histogram was obtained for trimers and from specimens selectively depleted in histone F1 and prepared over a range of ionic strengths (0.025–0.1). The dominant inter-bead distance is zero, but

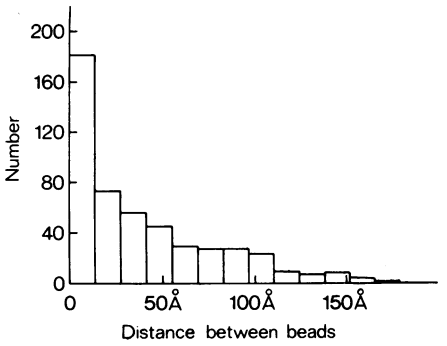

FIG. 4. Histogram of inter-bead distances in the dimer fraction. Distances were measured between edges of beads.

the breadth of the distribution would obscure a peak at a small distance of, say, 10–20 Å. The conclusion to be drawn is that the normal inter-bead distance is small; this together with the size of a bead gives a packing ratio of about 7.

The extended forms in which beads are as far apart as 150 Å (Figs. 3 and 4) may arise during the application of the specimen to the electron microscope grid. Ordinarily a drop of the specimen was formed and applied to the grid within a few seconds. However, if a drop was allowed to stand for 5–10 min before application to the grid, then most of the material was in an extended state; in extreme cases no beads were observed but only long, thin molecules of the length expected for the DNA. Thus it would appear that some denaturation occurs in the surface of a drop before its application to the grid. This denaturation phenomenon was slowed down but not stopped by prior fixation with formaldehyde.

DISCUSSION

The model proposed by Van Holde *et al.* (3) and described in the Introduction (Fig. 1, model I) was partly based on a histogram of spacings between beads, which rises from a few instances of zero spacing to a peak at 240 Å. In contrast, our histogram (Fig. 4) suggests that the inter-bead spacing is small, compatible with the idea of a compact structure (ref. 4 and Fig. 1, model II). The 240 Å spacing reported by Van Holde *et al.* could arise from a compact structure by a denaturation phenomenon such as that described above. On the other hand, it may be argued that the compact forms observed by us result from aggregation.

Griffith (12) observed a transition from a compact form of SV40 chromatin with a packing ratio of 7:1 to an extended form with a packing ratio of about 3:1 upon lowering the ionic strength from 0.15 to 0.015. In contrast, we find no dependence of the inter-bead distance upon ionic strength. The difference between the two results does not appear to be due to the absence of histone F1 from SV40 chromatin, since our result is the same with or without F1. The difference could, however, arise from some peculiarity such as the supertwisting of SV40 DNA.

On balance, we favor the idea of a compact structure (ref. 4 and Fig. 1, model II) as representing the native state of chromatin. It is the simplest arrangement of 100 Å beads compatible with the results of x-ray diffraction showing a 100 Å repeat in the fiber direction, and it is consistent with electron micrographs obtained by other workers (12, 13).

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