Proc. Indian natn. Sci. Acad., 55, A, No. 1, 1989, pp.96–100.
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AN ELECTRON MICROSCOPIC OBSERVATION ON NUCLEASE DIGESTION OF CHICKEN ERYTHROCYTE CHROMATIN

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(*Received 28 December 1987; Accepted 5 July 1988*)

Chicken erythrocyte nuclei were digested with micrococcal nuclease. The soluble chromatin corresponding to different digestion times were examined under the electron microscope. Alongwith fragmentation of the chromatin, a change in the nucleosome conformation was observed, which indicates some sort of nucleosome sliding.

Key Words : Nucleosomes; Chromatin; Micrococcal Nuclease; Electron Microscopy

INTRODUCTION

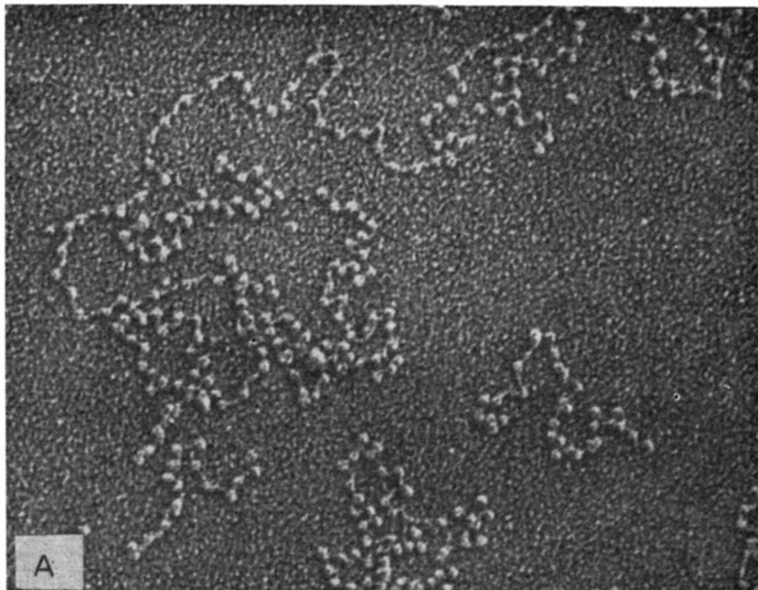
THE architecture of eukaryotic chromosome involves several levels of compaction.[1,2] Micrococcal nuclease digestion has proved to be a very useful probe for the analysis of DNA organisation inside chromatin.[3-5] The present work is an electron microscopic study of nuclease digested chromatin from chicken erythrocyte nuclei.

MATERIALS AND METHODS

Nuclei were isolated from chicken erythrocytes and progressively digested with micrococcal endonuclease as described earlier.[5] The chromatin, soluble in 0.2mM EDTA (ethylenediaminetetraacetic acid) 0.1mM PMSF (phenylmethylsulphonyl-fluoride), *p*H 7.9 was separated as usual from different fractions corresponding to four different digestion times e.g. 10 sec, 1 min, 4 min and 8 min. For electron microscopy, each chromatin fraction was diluted to a concentration of 1μg/ml in the same buffer. Specimens were prepared following Dubochett's technique[6] and they were micrographed in Siemens Elmiskop 101B at an electronic magnification of 30,000 X.

For the measurement of the lengths of the chromatin fragments and the diameter of the nucleosomes in each case, 20 electron micrographs were taken from specimens made on 5 different sets of digestion experiments. Negatives were enlarged to a final magnification of 1,50,000 X and chromatin length measurements were made as described earlier.[5] In the first two stages i.e. for digestion times 10 sec and 1 min, 50 chromatin fragments were measured in each case. In the next two stages corresponding to digestion times of 4 and 8 min, measurements were made on 100 fragments. Previously, the average length of

the soluble chromatin was determined from gel electrophoresis experiments[5] and they were found to fit nicely with electron microscopic data. The nucleosome diameters were measured with a magnifier fitted with a graticule with 0.1mm division. To estimate the correct size of the nucleosomes, the increase in dimen-



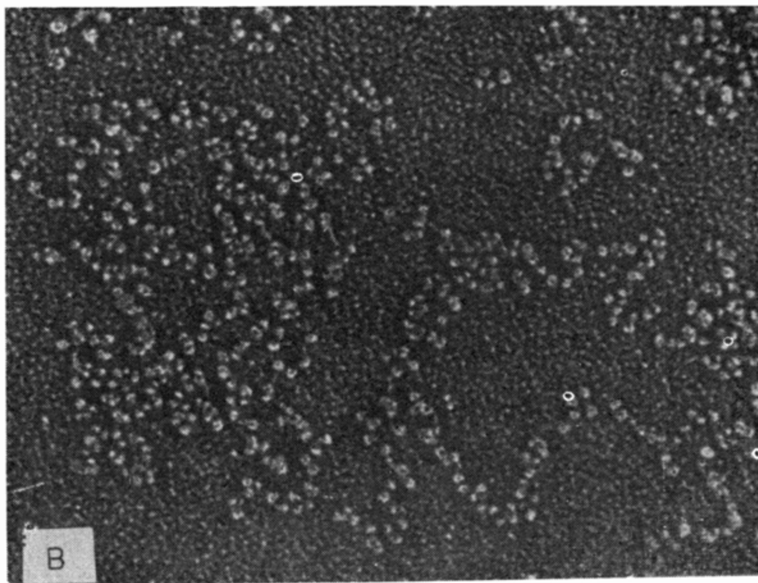


PLATE I Electron micrographs of chromatin obtained after (A) 10 sec., and (B) 1 min., digestion with micrococcal nuclease. Magnification : 1,50,000 X.

sions due to stain and shadowing material were eliminated as described earlier.[7] In each case, measurements were made on 200 nucleosomes from different micrographs.

Results and Discussion

Plate I (A, B) shows the electron micrographs of chromatin fractions corresponding to earlier periods of digestions i.e. 10 sec and 1 min respectively, while Plate II

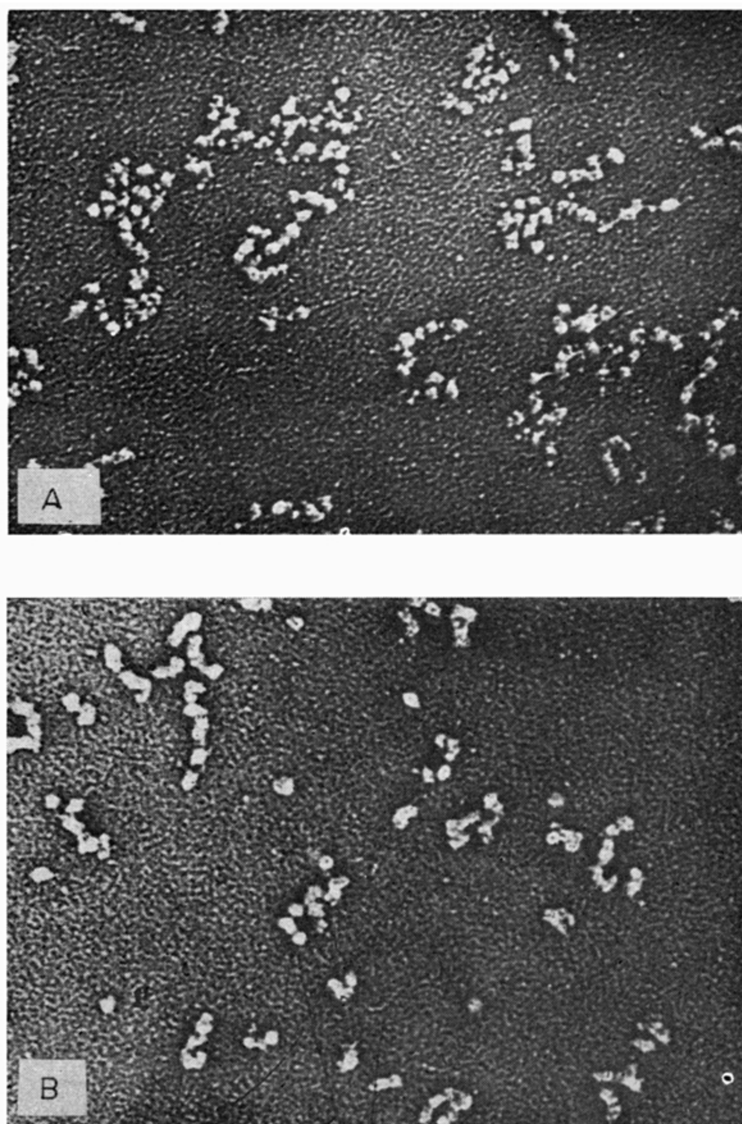


Plate II Electron micrographs of chromatin obtained after (A) 4 min., and (B) 8 min. digestion with micrococcal nuclease. Magnification : 1,50,000 X.

(A, B) represents the micrographs of chromatin fractions obtained after longer digestion times, 4 and 8 min respectively. The estimated average lengths of these chromatin fractions and the average nucleosome diameter in each fraction are listed in Table I.

TABLE I

Diameter of the nucleosomes and the length of the chromatin fractions at different digestion times

Time of digestion	Average dimeter of nucleosomes in Å	Average length of chromatin fractions in Kbp
10 sec.	100 ± 2	22
1 min.	115 ± 5	13
4 min.	115 ± 6	3
8 min.	125 ± 8	1

From the micrographs, it is seen that together with a reduction in size of the individual fragments with progress of nuclease digestion, there was also a change in the appearance of the chromatin fibre. In the early digested chromatin (Plate IA), the nucleosomes were uniform in size (100 ± 2Å). This uniformity was lost to a great extent at the next period of digestion (Plate IB). The average nucleosome diameter also increased to about 115 ± 5Å. Similar phenomenon was also observed previously in specimens prepared using BAC (Benzyldimethyl-alkylammonium chloride) diffusion technique.[5] But the structural alteration was much prominent in the present experiment since no external agent was used here for fixation or specimen preparation.

The irregularity in the nucleosome dimension was more vivid in the higher stages of digestion and the nucleosomes were found to be more closely spaced along the chromatin fibre (Plate IIA, IIB). After 4 min digestion, although the average length of the chromatin fraction decreased considerably, the nucleosome diameter remained almost the same as in the previous stage (Plate IIA). The chromatin fibre was more fractioned in the next stage of 8 min digestion (Plate IIB) and the average nucleosome size in this micrograph increased further to about 125 ± 8Å.

The prominent beads-on-a-string like appearance in the early stage of digestion revealed loss in the secondary structure of the chromatin fibre as a result of release of some of the internucleosomal histones H1 and H5.[5,8] Similar conversion of the compact chromatin fibre to the beaded structure was also observed by Losa *et al*[9] after digestion of chromatin with α-chymotrypsin. The exact role of the histone H1 in the higher order structure of chromatin is not yet clear and is being studied recently in many laboratories.[9,10] The closeness in the spacing of the nucleosomes in the subsequent stages must be due to the removal of almost all the internucleosomal histones, which is followed by sliding of the nucleosome cores along the chromatin fibre.[11] The irregularity in the nucleosome

size reflects some sort of loosening in the outer most binding of the nucleosome cores, required for nucleosome sliding. The mobility of the nucleosomes observed in the present experimemt has some relevance to both replication and transcription.

ACKNOWLEDGEMENT

Authors are thankful to Professor D L Bhattacharya for his interest and encouragement in this work.

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