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STRUCTURAL STUDIES ON ALLIUM CEPA L. CHROMATIN: ENHANCED STABILITY OF INTERNUCLEOSOME INTERACTIONS IN PLANT CHROMATIN

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SUMMARY - The pattern of micrococcal nuclease digestion of chromatin from different organs of Allium cepa has been studied. The DNA from small oligonucleosomes appears to be highly degraded and heterogeneous. In solutions of intermediate ionic strength (0.15 M NaCl) histones H2A and H2B form dimers, however at high salt concentrations (2 M NaCl) they tend to form complexes of higher order, such as tetramers. It is proposed that a correlation exists between the ability of these proteins to form tetramers and the particular stability of internucleome interactions.

Little information is available on plant chromatin structure compared with the number of studies devoted to animal systems. The existence of a subunit structure in chromatin of plant cells was established in pea seedlings (1) and it has been confirmed in other systems. The nucleosomal repeats in these nuclei have values similar to those observed in animal tissues, i.e. around 200 bp. The structural requirements for the functions of chromatin in plants might well differ from those in animal cells. However little is known about such requirements and, at least in one aspect, the DNAse I sensitivity of potentially active gens, has also been found in wheat embryos (2). Plant chromatin has the same number of histone fractions as animal systems. However two of the core histones have lower electrophoretic mobility and they have been called "plant histones" (3,4). It appears that these histones are the equivalent to fractions H2A and H2B from animals. The cross-complexing pattern of histone fractions is the same in plant than in animal histones with the formation of a complex between H3 and H4 and between H2A and H2B (5). It has also been shown that the two groups H3-H4 and H2A-H2B can be interchanged between animal and plant systems to form nucleosomes in vitro (6).

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MATERIALS AND METHODS

All manipulations were carried out at 4°C unless it is otherwise stated. Bulbs and meristems of the adventitious roots of Allium cepa L. were used, dormant meristems being dissected from the unsprouted bulb. Proliferating meristems were obtained by placing the base of the bulb in filtered water which was continuously aerated in the darkness at 15ºC for 48 hours. The second 1 mm segment from the root apex was used (7). Nuclei were prepared as described (4).

The suspension of nuclei was incubated at 4°C or 37°C with micrococcal nuclease (Boehringer) at 60 u/mg DNA for different periods of time. The reaction was stopped with 3 mM EDTA. After digestion with 50 µg/ml pancreatic RNAse A (Sigma) at 20°C for 20 min, the chromatin was deproteinized and DNA was analyzed in either 1.6 or 2% agarose gels in 90 mM Tris-Borate buffer, 2.5 mM EDTA, pH 8.3. The gels were photographed in a Polaroid MP-4 camera and the negatives scanned in a Chromoscan 3 (Joyce Loebl) densitometer. For the preparation of oligonucleosomes micrococcal nuclease digested nuclei were dialyzed against 40 mM KCl, 0.2 mM EDTA, 0.5 mM PMSF, 10 mM Tris-HCl pH 7.4. After centrifugation at 3000 rpm for 10 min, the supernatant was applied to 5-20% sucrose gradients in the same buffer and centrifuged at 30,000 rpm in a Beckman SW40 rotor for 12 h.

Histones were prepared essentially as described by Johns (8) by adding to the nuclear pellet resuspended in 0.2 mM EDTA, 10 mM Tris-HCl, 20 mM NaCl, 0.5 mM PMSF an equal volume of 0.5 N HCl and stirring overnight. Partial extractions were carried out by treating with acid with short periods of time (30-60 minutes). Gel filtration chromatography was carried out in a 1 m long, 1 cm diameter column (Pharmacia). The protein concentration was 1 mg/ml. Fractions of 1 ml were collected, they were made 20% in trichloroacetic acid and after 30 minutes the absorbance at 400 nm was read. Samples were centrifuged in an Eppendorf microcentrifuge, washed with acetone-HCl 0.1%, with acetone and finally dried to be analyzed by electrophoresis in 15% polyacrylamide slab gels (9).

RESULTS AND DISCUSSION

Tissues from different organs of Allium cepa having different states of biological activity (bulb, proliferating meristem and dormant meristems), have been studied in the present work. Nuclei from the three tissues have been digested with micrococcal nuclease in order to study the pattern of nucleosome fragments produced. The different speed in digestion can be observed in the agarose gels shown on Figure 1. It appears that for an equal digestion time proliferating meristem yields nucleosomes of lower multiplicity as compared with bulb, as well as does when compared with dormant meristem. The differential accessibility of onion tissues to micrococcal nuclease has been previously shown by microdensitometry of permeabilized meristems (10). The nucleosomal repeat in these tissues in the same (204 $\pm$ 4 bp), in the three cases. It is clear from the gels that the monomer band is much broader than the other bands corresponding to oligonucleosomes of higher multiplicity and it has a lower proportional intensity. At longer times of digestion (see Figure 1 sample c from prolifera-

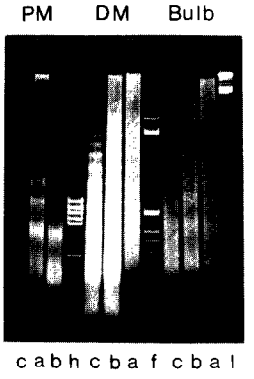

Figure 1. Agarose gel electrophoresis of a micrococcal nuclease digestion of Allium cepa nuclei from bulb (B), dormant meristem (DM) and proliferating meristem (PM) at 37°C. Samples a, b and C correspond to 5, 15 and 30 minutes digestion respectively. Samples 1, f and h are size markers.

ting meristem) the monomer band becomes prominent but it is still very broad.

Mono- di- and trinucleosomes were prepared by sucrose gradient centrifugation and the size of the DNA fragments contained in each fraction was analyzed by gel electrophoresis. The results are shown in figure 2 in the form of densitometric scans of the gels of the three fractions at two different times of micrococcal nuclease digestion. At the first time of digestion (15 min, part A) the most frequent DNA fragment found in monomer, dimer and trimer fraction correspond to the DNA size for one, two and three nucleosome, respectively. However the dimer fraction contains DNA material corresponding to monomer and the trimer fraction contains dimer DNA. This effect is more clear at longer digestion times. In the right hand-side of the figure (part B) it can be seen that the dimer and trimer fractions present broad distributions of DNA fragments ranging

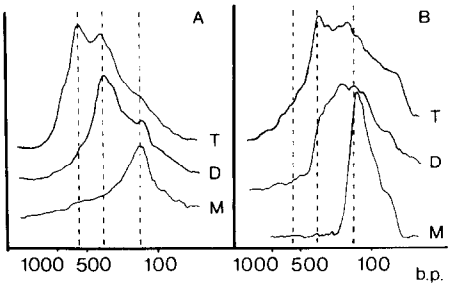

Figure 2. Densitometric scans of agarose gels of DNA extracted from sucrose gradient fractions corresponding to monomer (M), dimer (D) and trimer (T) oligonucleosomes from Allium cepa bulb nuclei. Part A corresponds to 15 min digestion and part B to 30 min digestion. Base pair calibration was done with pBR 322 DNA restricted with Hae III.

from 500 to 60 base pairs. It is interesting to note that no band at around 600 bp is observed in the trimer fraction.

These results indicate that the nuclease has cut at many places and not exclusively in the linker between nucleosomes. They also mean that the nucleosome dimer or trimer still keep their structure in spite of having its DNA fragmented to sizes lower than the corresponding dimer or trimer size. The features observed with onion chromatin agree with similar studies from tobacco leaves where a very compact chromatin structure was observed by electron microscopy (11) and with the existence of an intermediate material between monomer and dimer shown in pea embryos (12), and interpreted as the result of compact dimer formation. The sliding of nucleosomes leading to compact oligonucleosomes has been shown in 0.6M NaCl treated chromatin (13), conditions very different from those used in our experiments. If this was the case it would mean a different behaviour of plant chromatin also indicating an enhanced tendency of nucleosomes to interact one to another.

One posible explanation of the results presented in the previous paragraph is that the main difference observed in plant chromatin, the special plant histones, could play a role in this characteristic behaviour. In order to analyze the kind of complexes that can result from the interactions between histones, a chromatographic procedure such as the one employed before (14,15) was used. A partial extraction of bulb histones that contained mainly fractions H2A, H2B and H1, was chromatographed through a Bio Gel A 0.5 m column in 2 M NaCl, pH 7.4. High ionic strength (2 M NaCl) has been used in studies of histone complexes to mimic the conditions existing in chromatin and at least for octamer histones they show several features that are identical in these conditions and in chromatin (16). As the lower part of figure 3 shows, two peaks were observed: peak A which appears in the exclusion volume of the column, and corresponds to soluble aggregates, and peak B, centered at fractions that correspond to 80000 dalton. This is a molecular weight near to what should be expected for a tetramer of histones H2A-H2B. The gel electrophoresis of the peaks is also presented. It shows that peak A contains heterogeneous material while in peak B mainly H2A-H2B and H1 are found. These results contrast with what happens in similar experiments carried out with chicken erytrocyte histones (15). in this case H2A-H2B clearly elute as a dimer at high ionic strength.

In figure 4, the chromatography on histones H2A, H2B and H1 in Bio Gel P150 in 0.15 M NaCl is presented. In this case the H1 and H2A-H2B histones are found in two distinct peaks, the latter in peak C centered

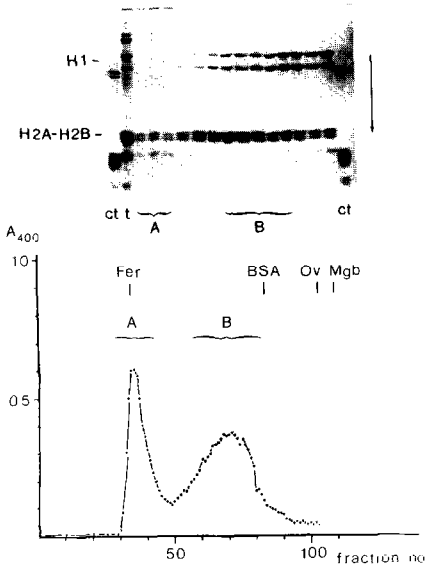

Figure 3. Gel filtration chromatography in 2 M NaCl of a partial extraction of bulb onion histones in Bio Gel A 0.5 m. Ferritin (Fer), bovine serum albumin (BSA), ovalbumin (Ov) and myoglobin (Mgb) were used as molecular weight standards. The electrophoresis of proteins in different fractions is also shown. Sample t is the total sample loaded and sample ct is calf thymus total histones.

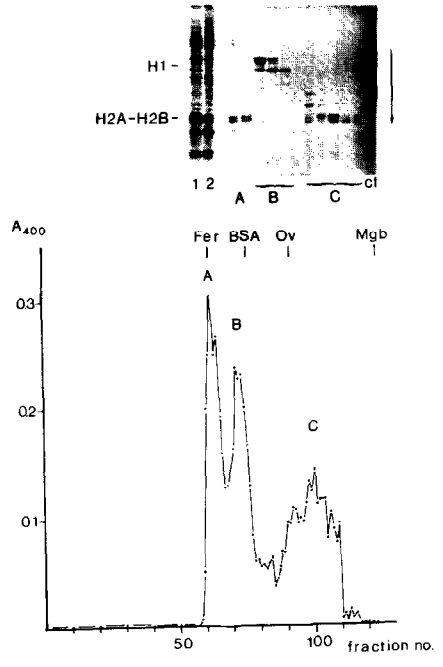

Figure 4. Gel filtration chromatography of the same sample as in figure 3 in 0.15 M NaCl. Samples 1 and 2 correspond to total proteins from bulb and proliferating meristem nuclei, respectively, after micrococcal nuclease digestion.

at molecular weights around 40000 dalton, zones expected for a dimer. Two bands intermediate between H1 and H2A-H2B in the electrophoretic gels can also be observed and they may correspond to minor components of the H2A-H2B fractions, as they have similar chromatographic behaviour. Therefore, in conditions of high salt molarity the typical plant histones, H2A-H2B appear to be able to form tetramers while in 0.15 M NaCl they behave like the corresponding fraction from animal systems. This result may be correlated to te enhanced stability of the interaction between nucleosomes. This could be achieved through H2A-H2B interactions.

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