Conformational changes in the chromatin of the brain of developing rats and its modulation by zinc chloride

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Abstract

Conformational changes in the chromatin of the brain were studied during the development of the rat (3-, 14- and 30-day old) using micrococcal nuclease (MCN) and DNase I. The rate and extent of digestion of chromatin by MCN is not altered during development. However, pre-incubation of slices of the cerebral cortex with $ZnCl_2$ increases the initial rate of digestion by MCN by 2–3-fold, and also enhances the production of monomer DNA. The rate and extent of digestion of chromatin by DNase I is greater in an early stage of development. The initial rate of digestion by DNase I is stimulated by 3–4-fold after $ZnCl_2$ treatment. These data show that changes occur in the conformation of chromatin, particularly in the internucleosomal region of brain cells as they pass from dividing to the non-dividing state.

Introduction

The nucleosomes in the chromatin are linked by linker DNA which is associated with H1 histone. The remaining DNA is wrapped around an octamer of core histones, H2A, H2B, H3 and H4. Micrococcal nuclease (MCN) (1, 2) digests the linker DNA to give DNA fragments which are multiples of monomers (200 bp). The 146 bp nucleosome core has been shown to be a constant and ubiquitous level of eukaryotic chromatin structure (3, 4). Deoxyribonuclease (DNase I) cleaves inside the nucleosome at intervals of 10.4 bp and their multiples (5, 6). It preferentially digests actively transcribing genes (7, 8). $ZnCl_2$ is reported to cause hyper-physiological levels of H1 phosphohistone in hepatoma HTC cells (9). Ord and Stocken (10) have shown that the transcriptional activity of isolated rat liver nucleosomes is increased after phosphorylation. We have recently reported that butyrate modulates the conformation of chromatin of brain of rats during development by causing hyperacetylation of histones (11).

Significant conformational changes are expected to occur in the chromatin of brain cells as they pass from the proliferative to the non-proliferative state during post-natal development. We have chosen 3-, 14- and 30-day old rats as our earlier studies have shown that there is a sharp decline in [^{3}H]-thymidine incorporation into DNA of brain cells up to the 14th day of post-natal development (12) after which no significant incorporation occurs. The effect of $ZnCl_2$ on chromatin conformation was studied by incubating cerebral cortex slices with $ZnCl_2$ and digesting the purified nuclei with MCN and DNase I.

Materials and methods

Incubation of cerebral cortex slices with $ZnCl_2$

Brain from 3-, 14- and 30-day old developing rats of Wistar strain was rapidly excised at 0–2 °C and

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the cerebral cortex was dissected out and cut into slices of 0.2–0.4 mm thickness. The slices were incubated in duplicate flasks containing KRB-buffer (pH 7.4) and $ZnCl_2$ (10 mM) and bubbled with 95% O_2 and 5% CO_2 mixture for 90 min at 37 °C with constant shaking. The control set, in duplicate, did not contain $ZnCl_2$ and was run parallel with each experiment. At the end of incubation the flasks were cooled to 2 °C and the slices were taken out and washed thrice with ice-cold buffer.

Purification of nuclei

Nuclei were purified according to Hewish and Burgoyne (13) with slight modifications. Buffer-A (15 mM Tris-HCl (pH 7.4), 60 mM KCl, 15 mM NaCl, 15 mM β-mercaptoethanol, 0.5 mM spermidine, 0.15 mM spermine) and varying concentrations of chelating agents were used. 0.5 mM phenylmethylsulphonyl fluoride (PMSF) was used to inhibit proteolysis. 0.5% (v/v) Triton X-100 was used to remove cytoplasmic contamination (14).

MCN and DNase I digestion

Nuclei were suspended in digestion buffer (0.34 M sucrose, buffer-A, 0.5 mM PMSF) to a concentration of 500 μg DNA/ml and kept at 37 °C for 5 min. For MCN digestion, 2 mM $CaCl_2$ and MCN (100 units/mg DNA, Worthington) were added. DNase I digestion was carried out by adding 10 mM $MgCl_2$ and DNase I (150 units/mg DNA, Sigma) to the nuclear suspension. Aliquots were removed at different time intervals to study the kinetics of digestion. The reaction was stopped by the addition of EDTA (10 mM) and chilling on ice. The percentage A_{260} was determined in the acid-soluble fraction of DNA in 0.5 M perchloric acid –0.5 M NaCl.

Extraction of DNA and gel electrophoresis

The incubation mixture was made 1% SDS and 1 M NaCl and extracted twice with chloroform: isoamyl alcohol (24:1). Precipitation of DNA was done by adding two volumes of ethanol to the aqueous phase and keeping it overnight at –20 °C. DNase I digests were incubated for 3 min at 100 °C in 98% formamide and chilled on ice.

1.7% agarose slab gel in Tris-acetate-EDTA buffer (15) was used for electrophoresis of MCN digests. DNase I digests were electrophoresed in 12% polyacrylamide slab gels containing 7 M urea in Tris-borate-EDTA buffer (16). Gels were stained in 0.005% 'Stains-all' (Eastman) in 50% formamide (17), destained in water and scanned at 550 nm.

Results and discussion

Analysis of MCN digests on agarose gel (Fig. 1) shows that the mobilities and intensities of bands corresponding to monomers and their multiples are the same for 3-, 14- and 30-day old developing rats. The mobilities of the bands are not altered by $ZnCl_2$ treatment. $ZnCl_2$, however, increases the digestion of DNA which is evident from the enhanced intensity of monomer DNA. This effect, however, does not change during development. Hence the sizes of DNA fragments produced by MCN are the same throughout development. The kinetics of digestion

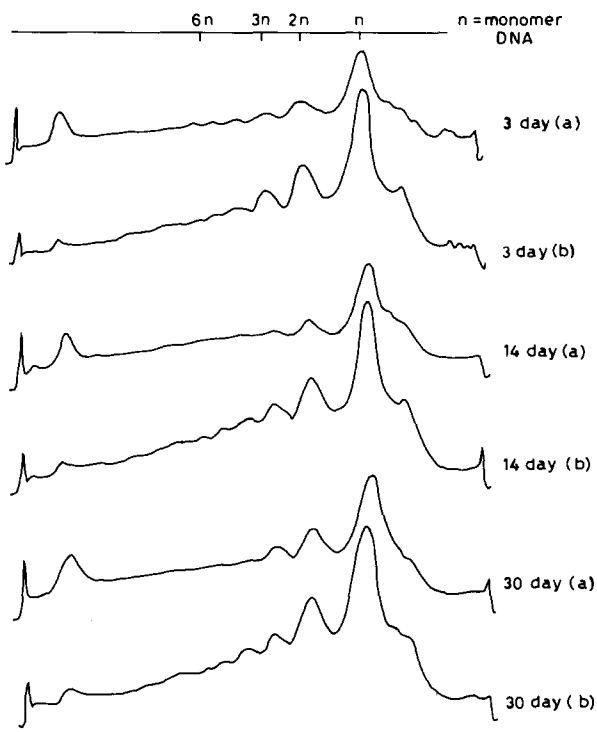

Fig. 1. Densitometric scans of DNA fragments as resolved by agarose gel electrophoresis. Nuclei of cerebral cortex of developing rats were digested by micrococcal nuclease. (a) control nuclei, (b) $ZnCl_2$-treated nuclei.

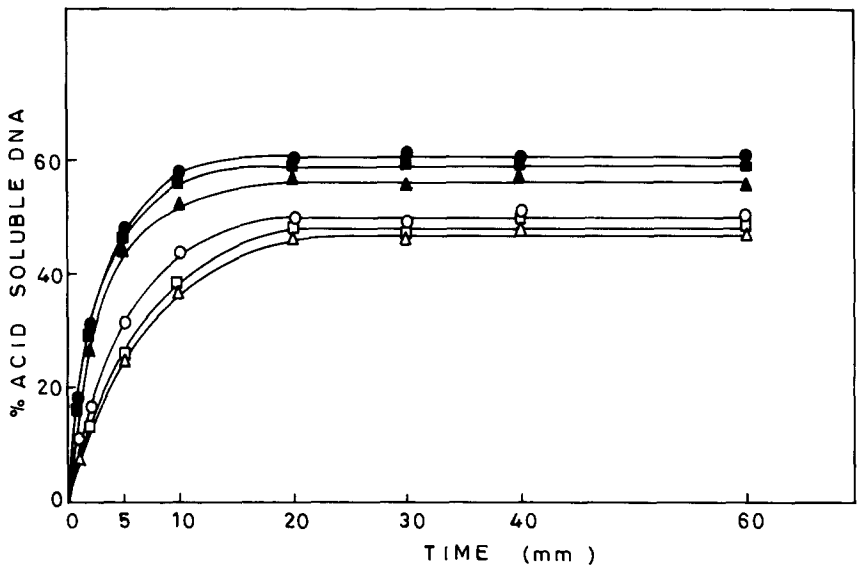

Fig. 2. Kinetics of digestion of nuclei of cerebral cortex of 3- (○, ●), 14 (△, ▲) and 30- (□, ■) day old rats by micrococcal nuclease. Control nuclei (open symbols), $ZnCl_2$-treated nuclei (closed symbols).

by MCN (Fig. 2) are nearly the same for all ages of development. This is in agreement with various reports which show that both active genes and bulk chromatin are digested by MCN at the same rate (18–20).

The initial rate of digestion of $ZnCl_2$-treated nuclei by MCN is approximately 2.5-fold faster than that of control nuclei (Fig. 2). The digestion plateaus at about 60% in $ZnCl_2$-treated nuclei, whereas it is about 50% in control nuclei. $ZnCl_2$ causes hyper-phosphorylation of histones, especially of Hl, in hepatoma HTC cells, by inhibiting histone phosphatase (9, 21). Histone Hl is reported to be more phosphorylated than other histones in HTC cells (22). Extensive phosphorylation of other histones is also observed in many other systems (23, 24). Studies of Ajiro *et al.* (25) and Wilkinson *et al.* (26) revealed that the structure of chromatin can be manipulated by phosphorylating Hl histone subtypes to specific levels. Histone Hl is bound to the linker DNA which is preferentially cleaved by MCN. Thus, our data indicate that hyper-phosphorylation of histone Hl may dissociate it from DNA at the linker region and produce conformational changes that may facilitate MCN digestion. Dolby *et al.* (27) have shown that phosphorylation of histone Hl may produce relaxed conformation of chromatin. The slight decrease in the initial rate of digestion of $ZnCl_2$-treated nuclei by MCN with progressive development may be due to conformational changes in the chromatin as development proceeds.

Figure 3 shows that the mobilities and intensities

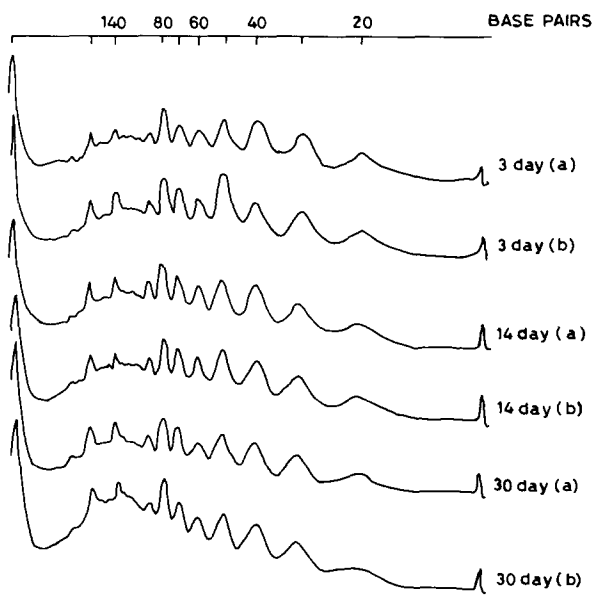

Fig. 3. Densitometric scans of DNase I digests resolved by polyacrylamide gel electrophoresis after digestion of nuclei of cerebral cortex of developing rats. (a) control nuclei, (b) $ZnCl_2$-treated nuclei.

of different DNA fragments produced by DNase I are similar in the control and $ZnCl_2$-treated nuclei in all ages of development studied. However, the initial rate of digestion of control nuclei by DNase I at 3-days is about 2-fold faster than those of 14- and 30-day old rats (Fig. 4). The percentage digestion is greater for 3-days rats. This may be due to a higher degree of acetylation of histones at 3-days (28). This is consistent with earlier reports which show that DNase digests preferentially actively transcribing and hyperacetylated DNA sequences (8, 29, 30). Although $ZnCl_2$ does not alter the size of DNA fragments produced by DNase I, it significantly enhances the initial rate of digestion of DNA (Fig. 4). The initial rate of digestion is 3–4-fold faster in $ZnCl_2$-treated nuclei.

The changes in the digestion of chromatin by MCN and DNase I, both before and after treatment with $ZnCl_2$, may be due to alterations in the conformation of nucleosomes as the brain cells pass from the dividing to the non-dividing state. The present data, together with our earlier reports (11, 12, 28) suggest that conformational changes in chromatin occurring during development may influence differential gene expression (31, 32).

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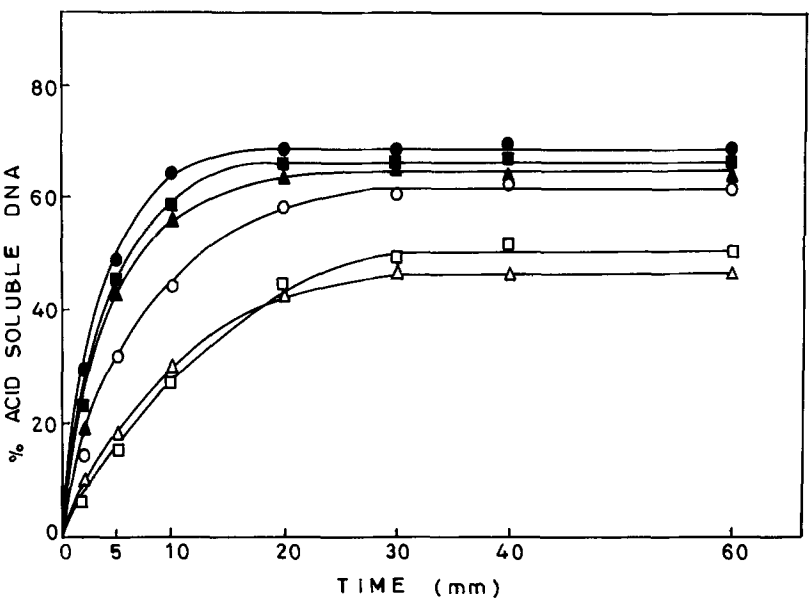

Fig. 4. Kinetics of digestion of nuclei of cerebral cortex of 3-(○, ●), 14-(△, ▲) and 30-(□, ■) day old rats by DNase I. Control nuclei (open symbols), $ZnCl_2$-treated nuclei (closed symbols).

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