Linker histone-DNA complexes: enhanced stability in the presence of aluminum lactate and implications for Alzheimer's disease

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The binding of human brain linker histone proteins to a radiolabelled human Alu repetitive element was examined by mobility shift assay. Analysis of the complexes formed from protein extracts of whole neocortical nuclei, under physiological conditions in vitro revealed that linker histone H1° has the highest affinity for the Alu DNA sequence. The linker histone-DNA complexes assembled in the presence of aluminum lactate were more resistant to sodium chloride-induced dissociation than those formed in the presence of sodium lactate. The enhanced stability of deoxyribonucleoprotein (DNP) complexes in the presence of the aluminum cation may be of significance in neurodegenerative conditions such as Alzheimer's disease where aluminum preferentially associates with DNA containing structures of the nucleus.

Linker histone; Histone H1°; Alu repetitive element; Chromatin structure; Aluminum neurotoxicity; Alzheimer's disease; (Brain)

1. INTRODUCTION

Linker histones are a family of lysine-rich chromatin proteins which are considered to be partly responsible for the packing of chromatin into inactive, condensed states, and thereby act as general gene repressors [1]. There is an inverse relation between the abundance of linker histones and gene activity in normal neurons when compared to glia [2], in neuroblastoma [3] and in Alzheimer's disease [4]. The homology of linker histones to nuclear factor 1 (NF 1) suggest that the interaction of these DNA-binding proteins with specific DNA sequences may be a critical factor in the regulation of genetic processes such as transcription [5,6].

Aluminum has been shown to accumulate in Alzheimer affected neocortical nuclei [7], possibly interacting with various components of the nuclear apparatus to disrupt genetic processes [8,9]. The presence of aluminum impairs salt-induced dissociation of linker histones from control nuclei preincubated with aluminum and the elution profile closely resembles that found for nuclei extracted from Alzheimer affected cerebral cortex [10]. There is also a strong positive correlation between the amount of aluminum measured on Alzheimer dinucleosomes released by light micrococcal nuclease digestion and the quantity of linker histones associated with that chromatin fraction [11]. We have recently proposed that this neurotoxic cation may induce an increase in the stability of linker histone-DNA complexes and thereby contribute to genetic malfunctions observed during aluminum intoxication [11].

In the present paper we have examined the binding of human linker histones H1b, H1a and H1° [10,12,13] to a human 300 base pair (bp) double-stranded Alu repetitive element [14,15] utilizing a mobility shift assay [16,17]. Discrete DNP species were analyzed through integration in-

to a TGSDS-PAGE system to ascertain the type of interacting protein species [4]. DNP complexes were assembled in both the presence and absence of the aluminum cation and subsequent sodium chloride-induced dissociation of the preformed DNPs was used as a measure of their intrinsic stability [10]. Our results indicate that under conditions of 0.12 M salt in vitro, the strongly electropositive linker histone H1° [13] has the highest affinity for Alu repetitive DNA and that linker histone-DNA complexes formed in the presence of aluminum lactate are particularly resistant to sodium chloride-induced dissociation when compared to those formed in the presence of sodium lactate.

2. MATERIALS AND METHODS

2.1. Preparation of protein

Both 0.75 M sodium chloride and 5% PCA-soluble extracts were prepared from mixed glial and neuronal nuclei isolated from neurologically normal human cerebral cortex as previously described [10].

2.2. Preparation of radiolabelled Alu DNA

The plasmid pBlur8 contained the human Alu repetitive sequence originally described by Schmid and co-workers [14,15]. Briefly, the plasmid was amplified and isolated according to standard methodologies [18]. The Alu sequence (Blur8) was excised with BamHI (BRL), electropurified and end labelled using the 5′-DNA terminus labelling forward reaction (BRL) to specific activities of 10^6 cpm/pmol.

2.3. Formation of DNP complexes and gel retardation assay

The gel mobility shift protocol of Strauss and Varshavsky [16] was employed. After DNPs were assembled, heparin was used as a competitor polyanion to eliminate nonspecific complex formation [17]. The molar ratio of input protein/labelled DNA/heparin was typically 3–15:1:0.1 (fig.1, lanes 2–5). Aluminum lactate in the assembly reaction was approx. 50 atoms of aluminum per 300 bp of DNA; these are conditions which approximate those found in the human neurological condition Alzheimer's disease [10,11].

2.4. Sodium chloride-induced dissociation

Microliter volumes of 5 M sodium chloride, yielding final salt concentrations of 0.25 M, 0.30 M, 0.35 M, and 0.40 M NaCl, were added to the DNP complexes after formation to examine their stability to salt-induced dissociation (fig.2). Human brain linker histones are known to dissociate from control nuclei over this salt concentration range [10].

2.5. Gel electrophoresis and autoradiography

DNP complexes were analyzed on 10 × 14 × 0.4 cm 5% polyacrylamide TAE slab gels horizontally polymerized under argon. Gels were run for 4 h at 32 W in the cold room under continuously circulating 1 × TAE buffer. After electrophoresis,

gels were vacuum dried onto 3 mm Whatman chromatography paper and exposed to Kodak XAR film for 3 h with Dupont Quanta III intensifying screens. For protein analysis, wet slab gels were briefly autoradiographed, the appropriate band was excised, briefly soaked in TGSDS buffer, then placed in a 5% acrylamide TGSDS stacking slot over a 15% TGSDS separating gel as described in [4]. After electrophoresis, gels were stained with nickel to visualize proteins associated with specific DNPs (Kodak Kodavue process).

3. RESULTS

Fig.1A shows the relative mobilities of free 5′-end labelled Alu and primary linker protein-DNA complexes formed. The retardation of mobility is a mixed function of the increasing mass of the migrating species, changes in DNA conformation induced by protein binding and charge shielding of the DNA polyphosphates migrating toward the anode [17]. Linker histones make up the predominant proteins of both 0.75 M NaCl and 5% PCA-extracted neocortical nuclei. When resolved on a 0.5 mm thick 12% polyacrylamide TGSDS-PAGE system three major migrating species are apparent, histones H1b, H1a and H1° [10]. Nickel-stained proteins from the synthetic DNPs in lanes 2–5, fig.1A are shown in lanes 2–5, fig.1B. Note the first linker histone bound (fig.1B, lane 2) is the methionine containing histone H1° as identified by comigration with Bio-Gel P-100
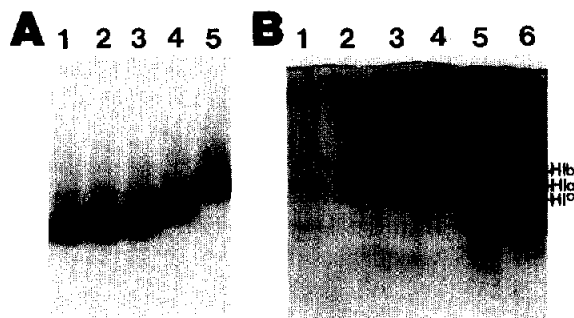

Fig.1. (Panel A) Assembly of linker histone-DNA complexes. Lanes: 1, free 5′-end labelled human Alu repetitive sequence insert from pBlur8; 2–5, linker histone-DNA complexes formed with increasing amounts of input linker protein/DNA using heparin as the competitor polyanion. (Panel B) Analysis of proteins of DNP complexes as analyzed by SDS-PAGE integration analysis [4] and stained with nickel. Lanes: 1, Biogel P100 purified human linker histone H1°; 2–5, protein complement associated with DNPs of lanes 2–5 in panel A; 6, 5% PCA extract of normal human neocortical nuclei [10]. Note the initial association of linker histone H1° with Alu DNA in lane 2 followed by linker histones H1a and H1b, respectively.
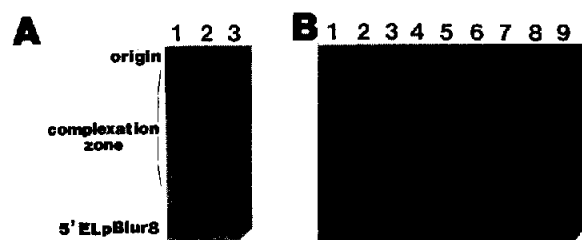
Fig.2. (Panel A) Assembly of linker histone-DNA complexes. Lanes: 1, free 5'-end labelled Alu DNA; 2, H1-Blur8 DNP complex formed in the presence of sodium lactate; 3, H1-Blur8 DNP complex formed in the presence of aluminum lactate. Molar ratios of protein/DNA = 9 in lanes 2 and 3 in the absence of heparin. (Panel B) Sodium chloride induced dissociation of H1-DNA complexes. Lanes 1–4 were formed in the presence of sodium lactate (A, lane 2) whereas lanes 6–9 were formed in the presence of aluminum lactate (A, lane 3). Salt dissociation, lanes: 1 and 6, 0.25 M NaCl; 2 and 7, 0.30 M NaCl; 3 and 8, 0.35 M NaCl; 4 and 9, 0.40 M NaCl; 5, free 5'-end labelled insert of pBLUR8. Note resistance of complexes formed in the presence of aluminum lactate (lanes 6–9) to dissociation by sodium chloride.

(BioRad) purified H1° and by cyanogen bromide fragment analyses ([19]; data not shown). Larger molecular mass DNP complexes appear to be formed by the tandem addition of H1a and then H1b (lanes 3–5) onto the H1°-DNA complex.

Fig.2A describes the formation of linker histone-Alu complexes in the presence of sodium (lane 2) and aluminum lactate (lane 3). Notably, no discrete DNP complexes were observed to form on heat denatured (single-stranded) Alu DNA or when free Alu DNA was incubated with aluminum lactate. Fig.2B shows DNP stability towards sodium chloride-induced dissociation over a 0.25–0.40 M sodium chloride gradient. Note that in complexes formed in the presence of sodium lactate, linker histones are completely dissociated from the Alu sequence at 0.30 M NaCl (fig.2B, lane 2). This is in contrast to linker histone-DNA complexes formed in the presence of aluminum lactate which do not dissociate until a 0.40 M concentration of NaCl is reached (fig.2B, lane 9).

4. DISCUSSION

The interaction of linker histones with DNA is thought to be a function of their polycationic character and amino acid structure which recognizes both specific nucleic acid sequences

[5,6] and features of DNA such as A + T content [20]. Human H1° and H1b contain respectively 33.1 and 28.4% positively charged residues which may explain H1°'s high affinity for naked Alu DNA. A 5'-TGGCA-3' sequence occurs near the center of many human Alu sequences which has been shown to be recognized by NF-1, a nuclear regulatory factor biochemically indistinguishable from H1 linker histone [6]. This 5'-TGGCA-3' sequence is flanked upstream by an A + T-rich sequence [14,15]. Unpublished observations from this laboratory indicate that linker protein H1° binding to the Alu repeat protects the DNA from restriction endonuclease attack at or near this site (unpublished). Site-specific binding of linker proteins onto the $3-5 \times 10^5$ member Alu repeat family in vivo may be of particular importance in the positioning of nucleosomes in and around the regulatory regions of genes [14,15,21].

Perelygina et al. [21] have recently described Alu-specific binding proteins derived from a 0.35 M nuclear extract of HeLa cells [20]. These extracts are enriched in LMG protein while linker histones are incompletely extracted from nuclei at this salt concentration [10]. At present it is not known if LMG proteins interact independently or cooperatively with linker histones in complex formation with Alu elements. We are currently assessing the interaction and significance of 5% PCA-soluble linker proteins and whole nuclear extracts with both Alu and DNA sequences located immediately 5' to specific class II genes.

Whatever the exact mode of linker histone-DNA binding, we propose that the presence of the aluminum cation stabilizes normal linker protein-DNA interactions and thereby contribute to genetic malfunctions observed in Alzheimer's disease [8,9]. Unique species of human brain linker histones may provide the unique targets for this aluminum interaction [11]. If linker histone release from gene regulatory regions is a necessary prerequisite for transcription initiation [22] then an aluminum-mediated enhancement of normal linker histone-DNA binding could be responsible for alterations in template-directed processes such as transcription. This could explain in part the nonrandom reduction of mRNA pool size in Alzheimer afflicted neocortex as measured by quantitative cytophotometric [23] and molecular hybridization techniques [24].

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