The structure of the nucleosome core particle of chromatin in chicken erythrocytes visualized by using atomic force microscopy

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ABSTRACT

The structure of the nucleosome core particle of chromatin in chicken erythrocytes has been examined by using AFM. The 146 bp of DNA wrapped twice around the core histone octamer are clearly visualized. Both the ends of entry/exit of linker DNA are also demonstrated. The dimension of the nucleosome core particles is ~ 1-4 nm in height and ~ 13-22 nm in width. In addition, superbends (width of ~ 48-57 nm, height of ~ 2-3 nm) are occasionally revealed, two turns of DNA around the core particles are also detected.

Key words: *Nucleosome core particle, chicken erythrocytes, atomic force microscopy.*

INTRODUCTION

All eukaryotic chromosomes consists of a regularly repeating protein-DNA complex called the nucleosome (Kornberg, 1977)[1]. It is traditionally considered to be the fundamental unit of chromatin structure. The physical properties of nucleosome depend on solution condition such as ionic strength and divalent-ion concentration as well as on

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The nucleosome core particle visualized by AFM

histone-modification state. Recently, the X-ray crystal structure of the nucleosome core particle at 2.8Å resolution clearly showed how the histone protein octamer was assembled and how 146 bp base pairs of DNA were organized into a superhelix, around it both histone/histone and histone/DNA interactions depended on the histone fold domains (Luger et al 1997)[2]. Our previous studies revealed the chromatin folding patterns in chicken erythrocytes by AFM. At the first level of DNA packing, our data showed that an extended beads-on-a-string (width of ~ 15-20 nm, height of ~ 2-3 nm for each individual nucleosome) could be consistently observed. Furthermore, superbeads (width of ~ 40 nm, height of ~ 7 nm) arranged with irregular distance along the extended beads-on-a-string were demonstrated (Qian et al 1997)[3]. Here, we will show the structure of the nucleosome core particle of chromatin in chicken erythrocytes examined with tapping-mode scanning force microscopy. The nuclei prepared from chicken erythrocytes were digested with micrococcal nuclease and the samples were then applied to a sepharose 4B column. The mono-, di- and oligo-nucleosomes were prepared and the linker histone as well as nonhistone proteins were stripped from them (Lutter et al 1978)[4].

MATERIALS AND METHODS

Preparation of nucleosomes from chicken erythrocytes

Nuclei were prepared from chicken erythrocytes and processed as described by Lutter (1978) [4]. These nuclei were then digested with 40 units micrococcal nuclease/ml in the presence of 1 mM- CaCl_2 for 5 min at 37°C. The digestion was terminated by addition of Na2EDTA to 2 mM. The nuclei were then centrifuged for 10 min at 5000 g, resuspended and lysed by pipetting up and down in an equivalent volume of 0.2 mM Na2 EDTA (pH 7.0) and centrifuged again for 10 min at 5000g. The supernatant contains soluble chromatin and 0.127 vol. of 4M-NaCl was added dropwise at 0°C. The sample was then applied to a sepharose 4B column (2 cm × 80 cm) which had been equilibrated with 0.45 M-NaCl, 5 mM-Tris (pH 7.5), 0.2 mM-2-mercaptoethanol. Fractions containing A_{260} absorbing material were collected. The samples of linker histones-stripped nucleosomes were then diluted to 0.1 M NaCl with 20 mM Tris (pH 7.0) and were used for AFM analysis.

Atomic force microscopy of nucleosomes

For AFM analysis, the mono-, di- and oligo- nucleosomes were deposited on mica which was pretreated with 1% APS (3-Aminopropyl Triethoxysilane). Excess liquid was blown off with gas. Tapping mode images were obtained on a nanoscope III AFM (Digital instruments, Santa Barbara, CA), using nanoprobe silicon tips, scan rate 1-1.5 HZ.

RESULTS

AFM images of mono-nucleosomes

In order to gain further insight into the structure of the nucleosome core particle, AFM imaging of native and linker histone-stripped mono-nucleosomes from chicken erythrocytes was performed. Interestingly, the remarkable features of the nucleosome core particles displayed at different orientation can be visualized by AFM. The 146 bp of DNA

Fig 1.

AFM images of the mono-nucleosomes isolated from chicken erythrocytes:

The mono-nucleosomes are displayed at different orientations. **a.** The nucleosome core particle wrapped twice by 146 bp of DNA can be visualized. Small arrows point to both the ends of entry/exit of linker DNA. **b.** The core particle wrapped twice by DNA is also visible (arrow head indicated). Small arrow points to both the ends of entry/exit of linker DNA. **c.** The nucleosome core particle in which two turns of 146 bp DNA can't be visualized at this orientation. **d.** The amplification of Fig 1c. arrow and arrow head mark both crescent-shaped conformations respectively. The linkers between two parts can be also visualized.

The nucleosome core particle visualized by AFM

wrapped twice around the core histone octamer are clearly visible and both ends of entry/exit of linker DNA are revealed (Fig 1a). The height of the nucleosome core particle is ~ 4 nm and the width of it is ~ 21 nm.

In Fig 1b, another nucleosome core particle wrapped twice by the DNA is also observable (arrow head indicated). Its both ends of entry/exit of linker DNA can be revealed (arrow indicated). However, The height of this core particle is ~ 2 nm and its width is ~ 57 nm. It is difficult to determine whether this measurement reflects the true dimension of some core particles or if some of the cores are depressed by the force load of the AFM probe. Furthermore, Fig 1c, d. Display a nucleosome core particle in which two turns of the DNA can't be visualized owing to the different orientation of the nucleosome core particle. However, both crescent-shaped conformations, which may consist of core histones, seem to be revealed. Several linkers between the two parts can be also observed (Fig 1d). The height of this nucleosome core particle is ~ 2 nm and the width of it is ~ 20 nm.

Visualization of oligo-nucleosomes by AFM

The nucleosome core particles in oligo-nucleosomes (lacking linker histones) were also examined by using AFM. Three nucleosomes are demonstrated in Fig 2a. Two turns of the 146 bp DNA around the core histone octamer can still be visualized, with the dimension of the core particles ~ 13 -17.5 nm in width and ~ 1 -1.6 nm in height. Fig 2b shows the mono-, di- and oligo-nucleosomes. The 146 bp of DNA wrapped twice around the core histone octamer can be detected in some core particles. The width of core particles is ~ 17 -20 nm and the height is ~ 1 -2 nm. In addition, some core particles (width of ~ 43 -50 nm, height of ~ 2 -3 nm) can be also revealed (thick arrow indicated in Fig 2b). They are very similar to the superbeads as mentioned before (Qian et al 1997)[3] Two turns of DNA around these superbeads can be visualized.

DISCUSSION

For many years, biologists have been trying to understand how DNA is packaged into chromosomes. At the first level of compaction, the histones and DNA are organized in repeating units called nucleosomes, the histone octamer has been shown to be internally organized as a tripartite protein assembly (Arents et al 1991)[5]. A centrally located (H3-H4) tetramer is flanked by two H2A-H2B dimers. It has been hypothesized that the 146 bp of DNA is wrapped twice around the histone octamer. This complex of the histone octamer and 146 bp of DNA is known as the nucleosome core particle, each nucleosome core particle is associated with linker histone (H1 or H5). The nucleosome (nucleosome core particle, linker DNA and linker histone) is a fundamental unit of transcriptionally inactive chromatin in all eukaryotic chromosomes and most of its sequence are in the inactive state for most of time. The nucleosomes (lacking histone H1 and H5) are mobile in physiological condition. Moreover, the X-ray crystal structure of the complete nucleosome core particle was resolved at 2.8Å resolution (Luger et al 1997)[2]. The new structure

Fig 2.

Visualization of the mono-, tri- and oligo-nucleosomes isolated from chicken erythrocytes: a. Three nucleosomes are revealed. Arrow points to the nucleosome core particle which is wrapped twice by 146 bp of DNA. b. Thin arrows point to the nucleosome core particle (width of ~ 17 -20 nm and height of 1-2 nm) and thick arrow marks the superbead (width of ~ 48 -53 nm and height of ~ 2 -3 nm) in which two turns of DNA around the core particles can be visible.

provides a detailed view of the protein and DNA organization. The overall DNA trajectory approximates 1.65 turns of a superhelix, but the diameter and bending are not uniform.

Recently, the atomic force microscopy has been used for exploring the structure of chromatin (Allen et al 1993 Leuba et al 1998)[6-7]. As a useful tool in biological research, AFM can image biological molecules under conditions close to their native environment. So far it is the only microscope that can achieve nanometer scale resolution on biological samples under native condition. AFM provides more clearly resolved images of the structure of chromatin than that attained by electron microscopy.

In this report, the structure of nucleosome core particle of chromatin in chicken erythrocytes was analyzed by AFM. The evidence that 146 bp of DNA wrapped twice around the core histone octamer was revealed and both the ends of entry/exit of linker DNA could be clearly visualized. In addition, the oligo-nucleosomes (lacking linker histones) were also visible with AFM. The height of the nucleosome core particles is ~ 1 -4 nm and

The nucleosome core particle visualized by AFM

the width is 13-22 nm. Furthermore, super-beads were occasionally revealed. It seems that the dimension of the nucleosome core particles detected by AFM is larger than that measured by neutron scattering and x-ray diffraction (Imai et al 1986; Richmond et al 1984)[8-9]. Since the present studies were carried out with a tapping mode AFM, in which samples were absorbed to the surface of mica and examined in air at ambient humidity and very large amount of energy could be deposited into the specimen with each "tap", the nucleosome core particles might be loosed and their width might be larger than that measured by other methods. Thus, the improvement of the AFM technique and its resolution will be undoubtedly essential for the further elucidation of the structure and function of chromatin. Moreover, the results presented here may provide some new clues to the structure of the native and linker histone-depleted nucleosome core particles prepared from chicken erythrocytes.

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