Investigation of chromatin folding patterns by atomic force microscopy

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Abstract The chromatin folding patterns in air and liquid were studied by atomic force microscopy (AFM). A gentle water-air interface method was adopted to spread chromatin from interphase nucleus of chicken erythrocyte. The chromatin was absorbed on APS-mica surface and studied with AFM. Beads-on-a-string were observed and many higher-order structures such as superbeads with dimensions 40~60 nm in diameter and 4~7 nm in height were found to string together to make chromatin fibers. When sample spreading and absorbing time were shortened, higher-order chromatin fibers with 60~120 nm in width were observed in air as well as under water environment. These chromatin structures may reflect chromatin folding patterns in the living cells.

Keywords Chromatin folding patterns, Atomic force microscopy, Chicken erythrocytes

1 Introduction

To understand the mechanisms involved in gene expression and in the structural organization of chromatin in eukaryotic nucleus, many efforts have been devoted to investigate the structural details of chromatin folding patterns at the different levels. Among all of the used methods, the electron microscopy (EM) has been proved to be very useful in revealing chromatin structural details. Some chromatin folding patterns, such as nucleosomes[1~3], 30 nm chromatin fibers[4], 60~80 nm chromatin fibers and 100~130 nm chromonema fibers[5,6] were revealed, and 200~400 nm chromatid-like regions[6] were identified by EM. Based on EM results several models[6~14] have been proposed to illustrate the chromatin structural organizations. However, it is still unclear how a long DNA fiber compacts into a thick chromosome.

Recently, a sophisticated technique, Atomic force microscopy (AFM)[15], has been applied to investigate the chromatin structural details in air [16~22] and under liquid.[21,22] Compared to EM, the AFM obviously has advantages in the following aspects: 1) it can work under air and liquid environments, and the operation conditions are near to the native situation of the biological samples; 2) owing to its peculiar imaging mechanism the AFM can obtain height information, which enables it to investigate a sample surface to obtain peculiar information with relatively high resolution.

In this work, in order to probe the chromatin folding mechanisms, a gentle method was adopted to spread the unfixed, unstained native chromatin sample onto a chemical-modified mica surface. AFM im-

Supported by the Grant of the Chinese Academy of Sciences KJ951-A1-603 and Shanghai Open Laboratory of Life Sciences
Manuscript received date: 1998-07-06

ages of several structural states and intermediate structures of chromatin from interphase nucleus of chicken erythrocyte were presented. Of course, Atomic force microscopy by itself can not prove a structure in a living cell, but the morphology seen may reflect the native chromatin structures and improve our knowledge of the chromatin folding patterns.

2 Materials and methods

Nuclei were isolated from chicken erythrocytes as described by ZHU J D *et al.*[23] A 50μL nucleus solution was suspended in 1mL PBS buffer, and centrifugalized at 5000 r/min for 1 min. The sediment was resuspended with 50μL PBS buffer. A clean Teflon cylinder (2.5 cm in depth and 2cm in diameter) was filled with water to make a water meniscus stick out the edge to form a water-air interface. Then, the 50μL sample solution was transferred quickly onto the water-air interface and allowed to spread for 2 min. Next, freshly cleaved mica sheet pretreated with 1% APS (3-aminopropyl triethoxysilane) was put onto the water-air surface for 2 min. Finally, the sample adsorbed on mica-APS surface was dried with N_2 flow and studied with AFM.

The data were collected by a Tapping-Mode AFM (Digital Instruments, Nanoscope IIIa, Santa Barbara, CA) in air and water. The tips used were Si (in air) and Si_3N_4 (under water) cantilevers (Digital Instruments). When operated in air the relative humidity was controlled at 30%~40%. Typically, the tips used have a curvature radius of less than 20nm. The Tapping-Mode AFM was operated at a scan rate of ~1.5 Hz in the air or ~1 Hz in water. Height, widths and length measurements were made with the installed Nanoscope software. In this paper all the widths were referred to the half-peak width.

3 Results and discussions

3.1 Chromatin particles

A typical AFM image of native chromatin from chicken erythrocyte according to our preparing method is shown in Fig.1. The nucleus contents had been released owing to the effects of hypotonic lysis and surface tension, and the chromatin is visible at the border region of the nucleus (see Fig.1a). When zooming in the border region the beads-on-a-string structures were identified (see Fig.1b~1c). Each nucleosome on the fibers was ~18 nm in diameter and ~2 nm in height. The results are consistent with previous researches.[16~20,22] Larger chromatin particles were also observed (see Fig.1b~1c). These so-called "superbeads" were strung together to make chromatin fibers. The dimensions of the superbeads varied from ~40 nm to ~60 nm in width and from ~7 nm to ~4 nm in height according to our AFM results. Normally, the larger the higher. And the chromatin particles had the center-to- center distance varying from ~40 nm to ~60 nm. We are still unclear if these superbeads represent intermediate structures resulted from our sample preparation process or a native structure in nucleus which might relate to some biological function, but one conclusion can be made that when we extended the spreading time from 2 min to 15 min, the large particles could not be seen, the remained were all in beads-on-a-string form (Fig.1d).

Using the EM, Hanswalter and Werner[11] have demonstrated that higher-order particles (~36 nm in diameter) from chicken erythrocyte chromatin can be obtained by brief nuclease digestion in high salt solution and their previous work[24] described the globular supranucleosomal units (~30 nm in diameter) in chromatin of whole chick erythrocyte nuclei. Fritzsche *et al.*[22] have described their AFM results about chromatin beads from chicken erythrocyte with dimensions of 25 nm in width

and 4~6 nm in height. Compared with those results, our results suggested varied dimensions of the superbeads.

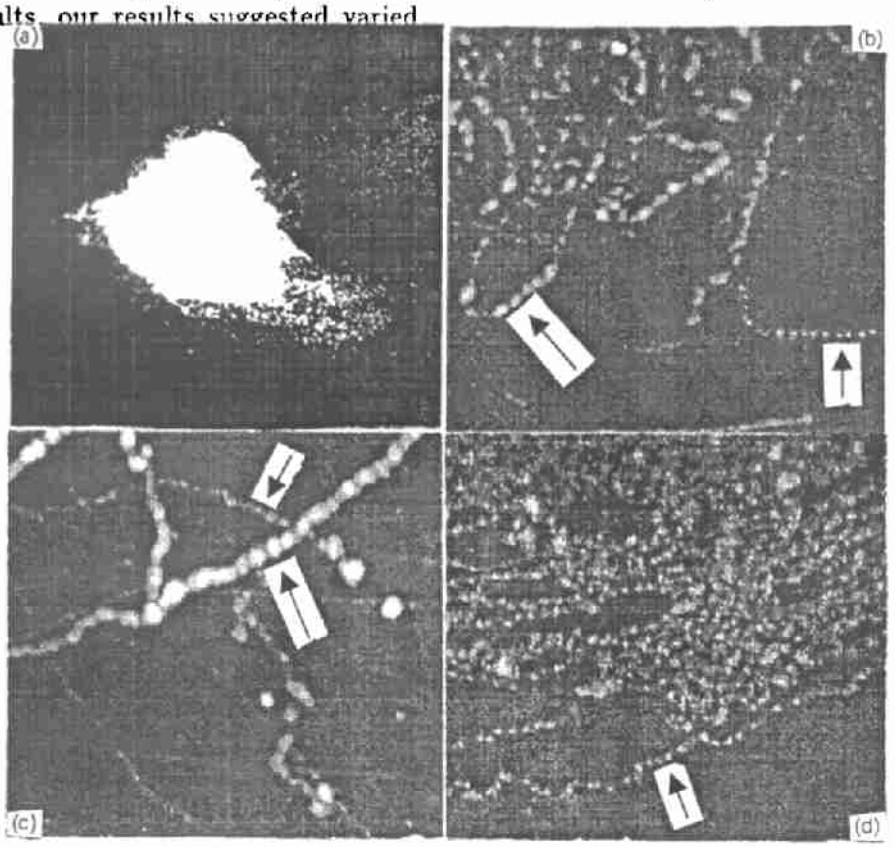

Fig.1 a) Typical AFM image of nucleus of chicken erythrocyte. Scan size was 30μm×30μm. b~c) Image obtained when zooming the border region of the nucleus. Extended beads-on-a-string (small arrows) and superbeads (large arrows) can be observed. Scan size of Fig.1b was 1.9μm×1.9μm. Scan size of Fig.1c was 1.6μm×1.6μm. d) The arrow indicated the remained beads-on-a-string form when we extended the spreading time. Scan size was 1.3μm×1.3μm

3.2 Chromatin fibers

By using a AFM, Allen *et al.*[16] have revealed 50~100 nm nodular fibers of sperm chromatin, Fritzsche *et al.*[22] have observed ~150 nm chromatin fibers from human B lymphocyte in air and ~70 nm chromatin fibers under liquid. In order to probe a higher-order chromatin structure in interphase nucleus of chicken erythrocyte, we shortened both the sample spreading and adsorbing time from 2 min to 30 s. AFM studies of these samples showed that the higher-order chromatin fibers were found on the upper regions of the nuclei (Fig.2a). These thick chromatin fibers were about 60~120 nm in width and 6~10 nm in height.

The samples were rehydrated in the microscope liquid cell by injecting pure water, and were investigated under water environment. Similar folding patterns were found (Fig.2b). The width of chromatin fibers was similar to those measured in air, but the apparent height (15~20 nm) was significantly larger.

Considering the short decondense time, we proposed that this kind of folding pattern is more close to true chromatin structure in interphase nucleus. Previously,

Belmont *et al.*[5] has found ~130 nm chromatin structural domains with an EM in interphase nuclei. Belmont's EM results as well as our AFM results suggest that there exist higher-order structures other than 30nm chromatin fiber in interphase nucleus.

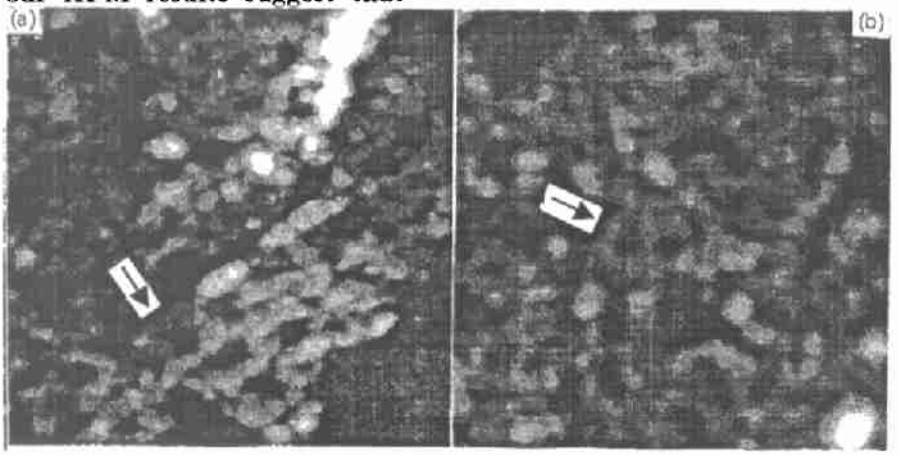

Fig.2 a) AFM image of thick chromatin fibers of the nucleus. The arrow indicated the thick chromatin fiber. Scan size was 2.0μm×2.0μm.

b) Images of thick chromatin fibers obtained with a Tapping-mode AFM under water. The arrow indicated the thick chromatin fiber. Scan size was 2.5μm×2.5μm

References

1 Olins A L, Olins D E. Science, 1974, **183**:330~332

2 Finch J T, Noll M, Kornberg R D. Proc Natl Acad Sci USA, 1975. **72**:3320

3 Ouder P M, Gross-belland, Chambon P. Cell, 1975; **4**:281

4 Brasch K. Exp Cell Res, 1976, **5**:396

5 Belmont A S, Braunfeld M B, Sedat J W *et al.* Chromosoma, 1989, **98**:129~143

6 Belmont A S, Bruce K. J Cell Biol, 1994, **127**, 2:287~302

7 Finch J F, Klug A. Natl Acad Sci USA, 1976, **73**,**6**:1897~1901

8 Woodcock C L F, L-L Y Frado, J B Rattner. J cell Biol, 1984, **99**:42~52

9 Horowitz R A, Agard D A, Sedat J W *et al.* J Cell Biol, 1994, **125**,**1**:1~10

10 Thoma F, Koller T H, Klug A. J cell Biol, 1979, **83**:403~427

11 Zentgraf H, Frake W. J Cell Biol, 1984, **999**:272~286

12 Boy de la Tour E, Laemmli U K. Cell, 1988, **55**:937~944

13 James R Paulson, U K Laemmli. Cell, 1977, **12**:817~828

14 Rattner J B, Lin C C. Cell, 1985, **42**:291~296

15 Binnig G, Quate C F, Gerber C. Phys Rev Lett, 1986, **56**:930~933

16 Allen M J, C Lee, J D Lee *et al.* Chromosoma, 1993, **102**(9):623~630

17 Allen M J, Dong X F, O'Neill T E *et al.* Biochemistry, 1993, **32**:8390~8396

18 Sanfold H L, Guoliang Y, C Robert *et al.* Proc Natl Acad Sci USA, 1994, **91**:11621~11625

19 Martin L D, Vesenka J P, Henderson E *et al.* Biochemistry, 1995, **34**:4610~4616

20 Qian R L, Liu Z X, Zhou M Y *et al.* Cell Research, 1997, **7**:143~150

21 Vesenka J, Hansma H, Siegerist C *et al.* SPIE Proc, 1992, **1639**:127

22 Fritzsche W, Schaper A, Jovin T V. Scanning, 1995, **17**:148~155

23 Zhu J D, Sun X P, Wang F. Biochim Biophys Acta, 1991, **1089**(2):158~165

24 Hanswalter Zentgraf, Ulrike Muller, Werner W Franke. Euro J Cell Biol, 1980, **23**:171~188