Methods xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Methods

journal homepage: www.elsevier.com/locate/ymeth

Chromatin structure revealed by X-ray scattering analysis and computational modeling

Kazuhiro Maeshima [a,b,c,*], Ryosuke Imai [a,b], Takaaki Hikima [c], Yasumasa Joti [c,d]

[a] Biological Macromolecules Laboratory, Structural Biology Center, National Institute of Genetics, Mishima, Shizuoka 411-8540, Japan
[b] Department of Genetics, School of Life Science, Graduate University for Advanced Studies (Sokendai), Mishima, Shizuoka 411-8540, Japan
[c] RIKEN SPring-8 Center, 1-1-1 Kouto, Sayo-cho, Sayo-gun, Hyogo 679-5148, Japan
[d] XFEL Utilization Division, Japan Synchrotron Radiation Research Institute (JASRI), 1-1-1 Kouto, Sayo-cho, Sayo-gun, Hyogo 679-5198, Japan

ARTICLE INFO

Article history:
Received 3 May 2014
Revised 23 July 2014
Accepted 18 August 2014
Available online xxxx

Keywords:
Chromatin
Chromosomes
30-nm chromatin fiber
Small-angle X-ray scattering
Cryo-electron microscopy
Computational modeling

ABSTRACT

It remains unclear how the 2 m of human genomic DNA is organized in each cell. The textbook model has long assumed that the 11-nm-diameter nucleosome fiber (beads-on-a-string), in which DNA is wrapped around core histones, is folded into a 30-nm chromatin fiber. One of the classical models assumes that the 30-nm chromatin fiber is further folded helically to form a larger fiber. Small-angle X-ray scattering (SAXS) is a powerful method for investigating the bulk structure of interphase chromatin and mitotic chromosomes. SAXS can detect periodic structures in biological materials in solution. In our SAXS results, no structural feature larger than 11 nm was detected. Combining this with a computational analysis of "*in silico* condensed chromatin" made it possible to understand more about the X-ray scattering profiles and suggested that the chromatin in interphase nuclei and mitotic chromosomes essentially consists of irregularly folded nucleosome fibers lacking the 30-nm chromatin structure. In this article, we describe the experimental details of our SAXS and modeling systems. We also discuss other methods for investigating the chromatin structure in cells.

1. Introduction

How the 2 m of genomic DNA is organized in cells remains one of the basic questions in cell biology. In the typical textbook view of chromatin, the 2-nm-diameter DNA molecule is wrapped around histones and forms a "nucleosome" (10-nm fiber) structure [1–3]. This nucleosome has been assumed to become folded into a regular "30-nm chromatin fiber" (see Fig. 3A) [4,5] and further higher-order structures.

In terms of higher-order structures, the well-established "hierarchical helical folding model" assumes that the 30-nm chromatin fiber is folded progressively into larger fibers, including 100-nm and then 200-nm fibers, to form the final mitotic chromosomes or large chromatin fibers (chromonema fibers) in the interphase cell [6–8]. Another model, the "radial loop model", suggests that the 30-nm chromatin fiber folds into radially oriented loops [9–11]. What does chromatin actually look like in a cell?

Small-angle X-ray scattering (SAXS; Fig. 1A) is a suitable technique for investigating bulky chromatin structures. When X-rays are exposed to non-crystalline materials, the small-angle scattering patterns generally reveal local periodic structures (Fig. 1B) (*e.g.*, [12]). SAXS has been used widely to analyze the structures of isolated nucleosomes and chromatin in solution (*e.g.*, [13–18]). In the case of interphase nuclei and mitotic chromosomes, using SAXS analysis, Langmore and Paulson detected a 30-nm structure [19,20], which has long been regarded as evidence for the existence of the 30-nm fiber in the interphase nuclei and mitotic chromosomes.

In this method review paper, we describe in detail a SAXS method for isolated nuclei and chromosomes [21–23]. To further understand the X-ray scattering profiles, we introduced computational modeling of condensed chromatin using three-dimensional atomic coordinates (*i.e.*, "*in silico* condensed chromatin"). Our findings support a model in which chromatin consists essentially of irregularly folded nucleosome fibers lacking the 30-nm chromatin structure [21–23]. We also discuss other strategies for studying chromatin structures in cells, including X-ray- and electron-microscope (EM)-based methods.

2. Methods

2.1. Isolation of human nuclei and chromosomes

In SAXS analysis, membranous structures, such as small vesicles, can induce very strong scattering that can hide or mask

* Corresponding author at: Biological Macromolecules Laboratory, Structural Biology Center, National Institute of Genetics, Mishima, Shizuoka 411-8540, Japan.
E-mail address: kmaeshim@nig.ac.jp (K. Maeshima).

http://dx.doi.org/10.1016/j.ymeth.2014.08.008
1046-2023/

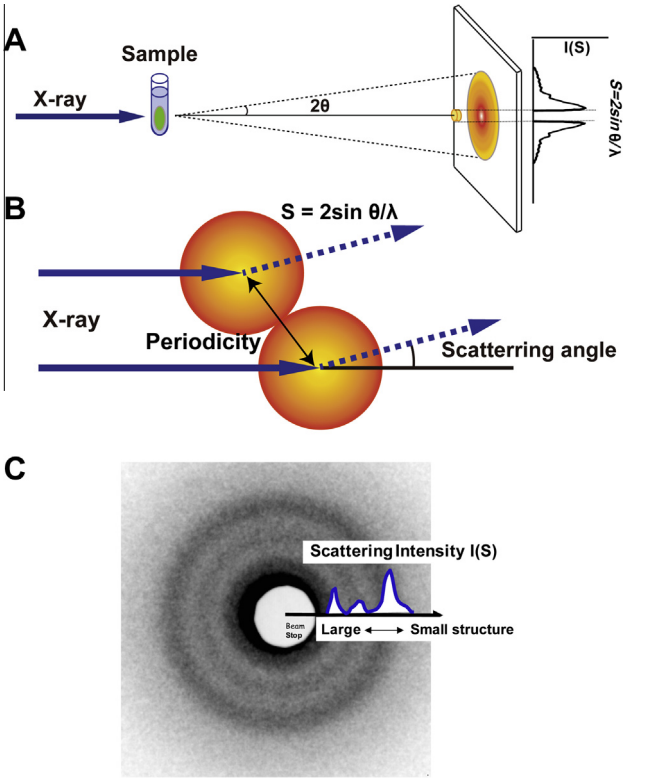

Fig. 1. Small-angle X-ray scattering (SAXS). (A) Nuclei or chromosomal pellets in quartz capillary tubes were exposed to a synchrotron X-ray beam and scattering patterns were recorded on imaging plates. (B) When X-rays irradiate non-crystalline materials, small-angle scattering generally reflects the periodicities of internal structures. (C) A typical scattering pattern features concentric rings and is reminiscent of a doughnut. Signals at smaller scattering angles (evidencing smaller scattering vectors [*S* values] with respect to the center) are indicative of larger periodic structures, and vice versa. The image was reproduced from [22], with modifications.

scattering from chromatin. Therefore, human nuclei and chromosomes were purified from HeLa cells, essentially as described previously [24,25]: HeLa cells ($\sim 2 \times 10^7$ cells) were gently resuspended in 50 mL of isotonic buffer IB [10 mM HEPES–KOH (pH 7.5), 50 mM NaCl, 5 mM $MgCl_2$, 0.1 mM PMSF, 0.1% Trasylol], and then incubated for 10 min on ice. After centrifugation, the cell pellet was suspended in 12 mL of buffer IB containing 0.5 M sucrose. After a 2-min incubation on ice, a solution of 10% NP40 and 5% sodium deoxycholate (DOC) was diluted 100-fold into the cell suspension, and the cells were disrupted immediately with 15 vigorous strokes of a small type A pestle in a Dounce homogenizer. The lysates were layered immediately onto 30 mL of a sucrose cushion containing IB and 40% sucrose, 0.1% NP40, and 0.05% DOC. Centrifugation was carried out for 30 min at 2500*g* and 4 °C. The chromosome pellets were resuspended in 0.2 mL of the same buffer.

When preparing mitotic chromosomes, we sought to avoid prolonged mitotic arrest by briefly (2 h) treating mitotic HeLa cells with 0.1 μg/mL nocodazole followed by mitotic shake-off. First, the experimental conditions of Langmore and Paulson were reproduced precisely [19,20]. Isolated nuclei and chromosomes were suspended in IB with 0.1% (v/v) NP40 and examined using SAXS. Isolated nuclei and chromosomes maintained under more physiological conditions, *i.e.*, in IB2 and IB3 [10 mM HEPES–KOH (pH 7.5), 50 mM (IB2), or 100 mM (IB3) NaCl, 5 mM $MgCl_2$, 0.1 mM PMSF, and 0.1% (v/v) NP40], yielded similar scattering profiles, each with the same three peaks [21]. To remove ribosomal aggregates from the surfaces of nuclei or chromosomes, isolated nuclei and chromosomes were suspended briefly in Buffer A [15 mM Tris–HCl (pH 7.5), 80 mM KCl, 2 mM EDTA, 2 mM spermine, 5 mM spermidine, 0.1 mM PMSF, and 0.05% (w/v) digitonin]. After centrifugation at 1000*g* for 5 min, nuclei or chromosomes were recovered as pellets and resuspended in IB for analysis.

As a control, chicken erythrocyte nuclei were prepared and evaluated, as described by Langmore and Schutt [26]: first, 1 mL of fresh chicken blood was lysed with10 mL of MLB [60 mM KCl, 15 mM NaCl, 15 mM HEPES, pH 7.3, 2 mM $MgCl_2$, 0.1% Nonidet P-40, and 1 mM PMSF] for 10 min on ice. After centrifugation at 4 °C and 1000*g* for 5 min, the supernatant was removed and resuspended in 10 mL of MLB. This step was repeated twice. The nuclei were centrifuged into glass capillaries at 1000*g* and 4 °C for 5 min.

2.2. SAXS measurements at the SPring-8 BL45XU beamline (Figs. 1 and 2)

SAXS can be performed using a laboratory X-ray generator or the beamline at a synchrotron radiation facility (*e.g.*, [12]). A beamline has the advantages of greater parallelism and a higher photon flux, compared with the beam afforded by an X-ray generator. A beam that is more parallel can be used to measure smaller scattering angles, facilitating structural analysis of larger objects. A higher photon flux allows detailed scattering data to be collected in a shorter time.

The SAXS experiments were performed at the RIKEN Structure Biology Beamline I (BL45XU) at SPring-8; this is a third-generation synchrotron facility in Japan [27]. The BL45XU beam was configured for the SAXS experiments as follows (Fig. 1A). The X-ray wavelength and sample-to-detector distance were 1.0 Å (12.4 keV) and 2.1 m, respectively. Scattering data from chromosomal samples and buffer were collected at room temperature using an imaging plate system (R-AXIS IV++; Rigaku, Tokyo, Japan). A cooled charge-coupled device (CCD) equipped with a two-dimensional (2D) X-ray image intensifier can also be used to this end. The precise distance between the sample and detector was determined using diffraction data from powdered silver behenate. Our set-up was capable of observing up to ~90- or ~120-nm range (lowest scattering vector).

A typical SAXS scattering pattern composed of concentric rings is shown in Fig. 1C. The signals at smaller angles (*i.e.*, closer to the center) reflect larger structures, and vice versa [12]. The radial average of the scattering pattern, the so-called "SAXS profile", is used for the analysis to increase the signal-to-noise ratio. In Fig. 2, the scattering signals on the concentric rings were averaged using the program Fit2d (http://www.esrf.eu/computing/scientific/FIT2D/) and are drawn as one-dimensional (1D) plots.

For SAXS measurements, concentrated nuclear or chromosomal suspensions were loaded into the top portions of quartz glass X-ray capillaries (2 mm in diameter; Tohso, Tokyo, Japan) pre-filled with a buffer such as IB. Nuclei or chromosomes in glass capillaries were next sedimented, to form pellets, at 4 °C, in a swinging bucket centrifuge running at 1000*g* for 5 min. The nuclei or chromosomes typically formed a 0.5–1.0 mm-deep pellet in a capillary, which was otherwise filled with buffer. A sample must be carefully injected to avoid bubbles and impurities that cause scattering noise. Nuclear and chromosomal samples were maintained at 0–4 °C at all times. Notably, we did not fix samples in aldehydes or osmium tetroxide, and eschewed alcohol dehydration. The use of such reagents is common during conventional electron microscopy sample processing, and might produce artifacts [28].

The pellets were exposed to the synchrotron radiation beam for 0.1–60 s, depending on the signal intensity obtained. As five sequential exposures to the X-ray beam did not change the scattering profiles (data not shown), we believe that the radiation delivered did not significantly damage the chromatin structure.