Experimental report

31/08/2015

Proposal: 8-03-824 **Council:** 4/2014

Title: Sructural Study of Functional Nucelosome with Human Varinat Histones

Research area: Biology

This proposal is a new proposal

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Samples: Canonical nucelosome
Nucleosome with one H2A.Z1
Nucleosome with two H2A.Z1s
Nucleosome with one H2A.Z2
Nucleosome with twoH2A.Z2
Nucleosome with one H2A.Z1 and one H2A.Z2

Instrument	Requested days	Allocated days	From	To
D11	2	2	07/10/2014	09/10/2014
D22	2	0		

Abstract:

Nucleosome, which consists of histone octamer and DNA, is a fundamental unit for stable storage of genomic DNA. In an activation stage, such as transcription, recombination, repair, and replication, the nucleosome has to diverge from its stable structure: for example, on the active genes, genomic DNA must detach from the histone core to allow access of the transcription factors. It is expected that the trigger of the activation could be replacement of histones by the variant histones, accompanying with the structural alternation. Recently, we have focused on the variant nucleosome with H2A.Z histone, which is predominantly localized around the promoters of active genes. In this project, we will elucidate the configuration of DNA and structural modulation of histone octamer in H2A.Z nucleosome using small-angle neutron scattering with contrast variation technique.

Experimental Report

Proposal No. 8-03-824
Experiment Team: M. Sugiyama, N. Horikoshi, K. Kurumizaka, A. Martel, L. Porcar
Title : Structural Study of Functional Nucleosome with Human Variant Histones
Instrument : D11, Date of Experiment: from : 07/10/2014 To : 09/10/2014

[Introduction]

Chromatin is composed of repeated protein-DNA complexes, referred to as nucleosomes (Fig.1). In the nucleosome, two copies of histones H2A, H2B, H3, and H4 form an octamer called the histone core, and about 150 base pairs of DNA are wrapped around this core (Fig.1). During cell life, nucleosomes are generally stable structures, but the functional regions of the genomic DNA must become accessible to the enzymes that mediate DNA metabolism, such as transcription, recombination, repair, and replication: for example, on the active genes, genomic DNA must detach from the histone core to allow access of the transcription factors (Fig.2). Therefore, the structural versatility and dynamics of the nucleosome are an important research target to understand the mechanisms of genomic DNA regulation and maintenance in eukaryotes.

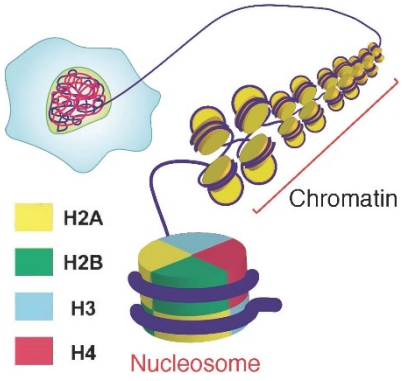

Fig.1. Structures of chromatin and nucleosome

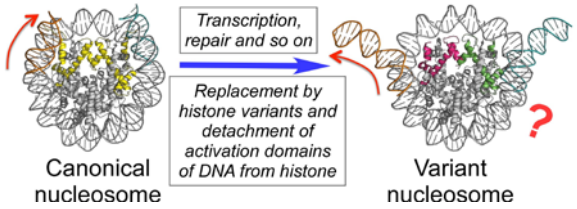

Fig.2. Possible structural alteration in variant nucleosome.

Non-allelic histone variants are considered to be genomic DNA regulators, since they induce structural alterations of the nucleosome by replacing the canonical histones. In fact, by solution scattering measurements, we previously reported that in the H2A.B nucleosome, in which the H2A histones are replaced by the H2A.B variants, the DNA was partially peeled off [1, 2]. Among H2A histone variants, H2A.Z, the major one (10% of all histone H2A species, while H2A.B is less than 0.2%) whereas H2A.B is a distant H2A variant, which shares about 50% amino acid identity with H2A. In addition, H2A.Z is predominantly localized around the promoters of active genes and could be responsible for opening a nucleosome-free region near the transcription start sites. Therefore, much interest has been focused on the structure of H2A.Z nucleosome, in which the H2A histones are replaced by their H2A.Z variant. Recently, our group reported the crystal structure of H2A.Z nucleosome and its functional analysis [3]: in vivo and in vitro studies showed that H2A.Z nucleosome is unstable as compared with the canonical nucleosome, suggesting that the histone core of H2A.Z nucleosome could be structurally unstable. However, by the crystallographic study, it was not clearly observed the structural differences in DNA configuration and /or histone octamer structure between H2A.Z nucleosome and canonical one. Therefore, the purpose of this study is to clarify the solution structure of this variant nucleosome, H2A.Z nucleosome, by employing small-angle neutron scattering with contrast variation technique.

[Experimental]

Histones H2A, H2A.Z.1, H2B, H3.1, and H4 were bacterially expressed and purified. For the nucleosome reconstitution, the histone octamers and 145 base pairs of the Widom 601 DNA were mixed in 10 mM Tris-HCl (pH 7.5) buffer, containing 2 M KCl, 1 mM EDTA, and 1 mM dithiothreitol, and the KCl concentration was gradually decreased to 0.25 M. The reconstituted nucleosomes were separated from the free histones and unbound DNA by native polyacrylamide gel electrophoresis (PAGE), using a Prep Cell apparatus (Bio-Rad).

SANS with contrast variation technique (CV-SANS) was performed with D11 instrument. The SANS intensities were measured in the q-range of 0.0085-0.13 $Å^{-1}$ at a constant temperature of 25℃. The samples were the canonical H2A nucleosome and the variant H2A.Z.1 nucleosome, at a concentration of 3.0 mg/mL in a 50 mM NaCl aqueous solution. In consideration of the contrasts between histone/DNA and solvent, four sample solutions with different H_2O/D_2O ratios were employed: 0% D_2O solution with the amount of 0.40 mL (reference), 40% D_2O solution with the amount of 0.40 mL (matching point for histone), 65% D_2O solution with the amount of 0.80 mL (matching point for DNA), and 100% D_2O solution with the amount of 0.80 mL (another reference).

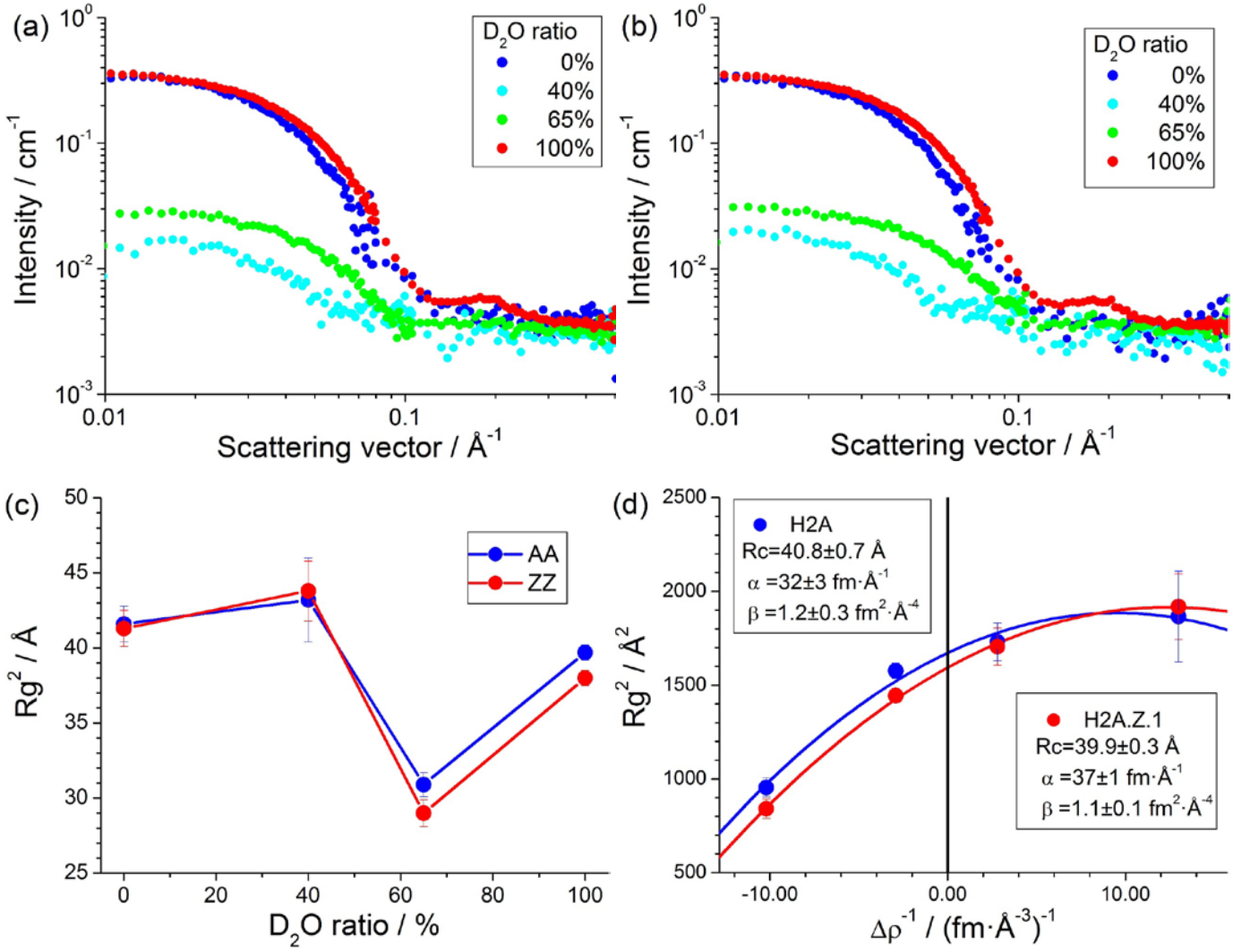

Fig.3. (a) CV-SANS profiles of canonical H2A nucleosome. (b) CV-SANS profiles of variant H2A.Z1 nucleosome. (c) Contrast dependence of gyration radius of H2A (blue) and H2A.Z1 (red) nucleosomes. (d) Stuhrmann plots of H2A (blue) and H2A.Z1 (red) nucleosomes.

[Results and Discussion]

As shown in Fig.2 (a) and (b), the SANS intensities of both nucleosomes are varied depending upon the scattering contrast. Because the outer DNA is selectively observed in the 40% D_2O solution and the inner histone octamer is also in the 65% D_2O solution, the gyration radii becomes largest in the 40% D_2O and smallest in the 65% D_2O, respectively (Fig.2(c)). In addition, the variant H2A.Z nucleosome shows the smaller gyration radius than canonical one. This feature becomes clearer by stuhrmann's analysis. As shown in Fig2 (d) (stuhrmann plot), stuhrmann's parameter, α, of H2A.Z nucleosome is smaller than that of canonical one. On the other hands, another stuhrmann's parameter, R_c, indicating the geometrical gyration radius, are also same for both nucleosome. This means that the histone of H2A.Z nucleosome is smaller than that of canonical one but the DNA configurations of both nucleosomes are almost same. Therefore, the binding between DNA and histone core in variant H2A.Z nucleosome could be looser than that in canonical one [4].

[References]

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[3] Structural polymorphism in the L1 loop regions of human H2A.Z.1 and H2A.Z.2: N.Horikoshi, H.Kurumizaka, et al., Acta Cryst., D69 (2013) 2431-2439.
[4] Solution Structure of Variant H2A.Z.1 Nucleosome Investigated by Small-Angle X-ray

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