An Application of Vacuum Ultraviolet Circular Dichroism Spectroscopy to Radiation Biology: Secondary Structural Analyses of Histone Proteins

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1. Introduction

DNA wraps around core histones in eukaryotic nuclei. The core histone is an octamer of histone proteins; two H2A-H2B dimers and an H3-H4 tetramer. Histone proteins have been shown to play a significant role in DNA damage responses (DDRs), such as DNA damage repair. It is assumed that DNA repair processes involve drastic alterations in structure of chromatin (a complex of DNA, histones, and other proteins) to make DNA repair proteins more accessible to damaged sites and such structural changes basically occur via post-translational modifications of histones. However, structural changes in chromatin and histones induced by DNA lesions are scarcely reported. Recently, we observed that secondary structure alterations of H3-H4 are induced by X-ray irradiation of human cancer cells through vacuum ultraviolet circular dichroism (VUV-CD) spectroscopy [1]. We also found that secondary structure contents of methylated histone H3 differed from those of unmethylated H3 *in vitro* [2–4]. In this talk, I will present these results.

2. Materials and Methods

2.1. VUV-CD measurements of H3-H4

Human cancer cells (HeLa.S-FUCCI cells) were cultured and irradiated with X-ray at a dose of 40 Gy. After irradiation, cells were incubated for 30 min to allow for DDRs. The H3-H4 proteins were extracted from the irradiated cells. For comparison, H3-H4 proteins were also extracted from unirradiated cells. The extracted histones were dissolved in 10 mM Tris-HCl buffer supplemented with 250 mM NaF. VUV-CD spectroscopy was carried out at BL-12 of HiSOR. The contents of α-helices, β-strands, turns, and unordered structures were analyzed using SELCON3 programs.

2.2. VUV-CD measurements of methylated H3

Recombinant histone H3 trimethylated at lysine-4, -9, or -36 residues (H3K4me3, H3K9me3, and H3K36me3, respectively) and unmethylated one were purchased and used without further purification. VUV-CD spectroscopy was carried out at BL-12 of HiSOR. The contents of α-helices, β-strands, turns, and unordered structures were analyzed using SELCON3 programs.

3. Results and Discussion

Figure 1 shows VUV-CD spectra of H3-H4 proteins extracted from the unirradiated and irradiated cells. Both samples showed a positive and two negative peaks around 190 and 210–220 nm, respectively. Apparent differences were not observed in the case of negative peaks, but the CD intensities of the positive peaks were lower for irradiated sample than for unirradiated one. Since CD spectra reflect the contents of secondary structures of proteins, the change in CD spectra in the Fig. 1 shows that the structures of H3-H4 proteins extracted from unirradiated and irradiated cells differed from each other, that is, structural alterations of H3-H4 proteins were induced by irradiating the cells with X-rays. Analyzing the CD spectra, we obtained secondary structure contents (Table 1). The decrement of α-helices and increment of β-strands and unordered structures were induced by X-irradiation to cells. It suggests that some amino acid residues which formed α-helix structures before irradiation would be changed into β-strands or unordered ones during and/or after irradiating cells.

The mechanisms underlying the structural alterations of H3-H4 proteins have been unidentified yet. However, we hypothesized that post-translational (de)modifications of histones induced by DDRs changed histone structures because the (de)modifications would affect interactions between histones and DNA and/or

among histones. To confirm that, as a first step, we compared VUV-CD spectra of H3K4me3, H3K9me3, and H3K36me3, which relate to DDRs, with that of unmethylated H3. As shown in Fig. 2, the CD spectral shapes differed from each other. In particular, H3K9me3 exhibited no positive peak in the measurement region. Comparing to the secondary structure contents (Table 2), trimethylation of lysine-4 and -9 residues decreased α-helix structures, but more drastic change was induced by the latter. In contrast, the α-helix content of H3K36me3 was larger than that of unmethylated H3. Thus, trimethylation of histone H3 proteins induced various types of structural alterations depending on the methylation sites. These results would support our hypothesis, that is, post-translational (de)modifications of histones induced by DDRs changed histone structures in the X-irradiated cells.

4. Summary

We observed the structural alterations of H3-H4 in the X-irradiated cells and those of trimethylated H3 using VUV-CD spectroscopy. It is possible that the structural alterations of H3-H4 in the X-irradiated cells were caused by post-translational modifications induced by DDRs.

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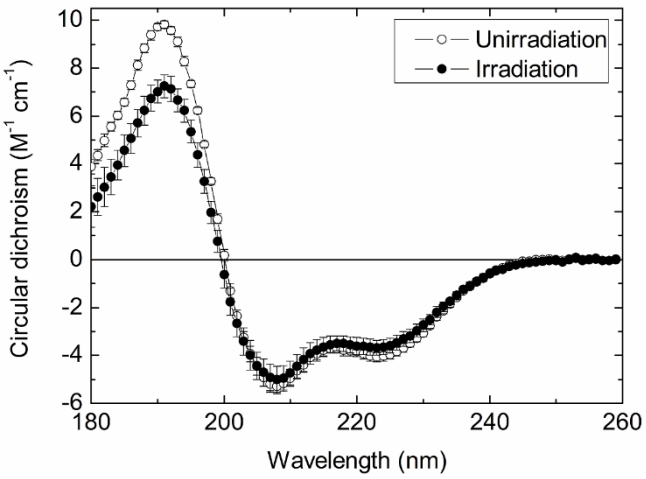

Fig. 1. VUV-CD spectra of H3-H4 extracted from unirradiated and X-irradiated cells.

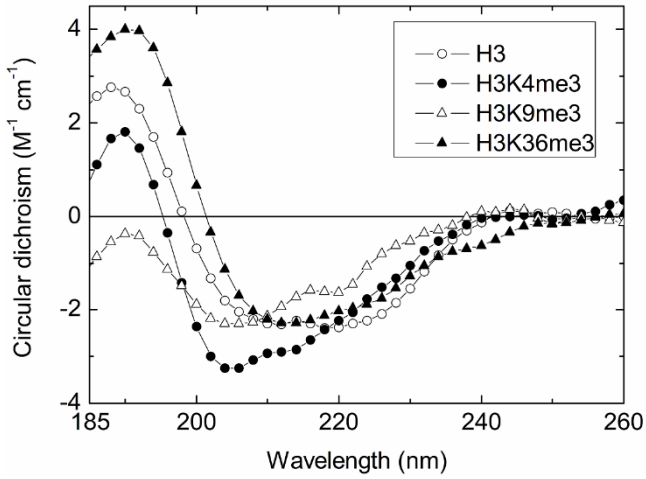

Fig. 2. VUV-CD spectra of unmethylated and trimethylated H3.

TABLE 1. Secondary structure contents of H3-H4 extracted from unirradiated and X-irradiated cells.

Secondary Structures	Unirradiation (%)	Irradiation (%)
α-Helix	61.6±0.6	48.3±0.8
β-Strand	1.9±0.7	8.0±0.3
Turn	18.2±0.6	19.1±0.4
Unordered	17.8±2.5	26.0±1.9

TABLE 2. Secondary structure contents of unmethylated and trimethylated H3.

Secondary Structures	H3 (%)	H3K4me3 (%)	H3K9me3 (%)	H3K36me3 (%)
α-Helix	25.0±1.2	21.8±0.8	13.1±0.8	35.6±1.3
β-Strand	21.3±1.5	25.1±2.0	29.6±1.9	18.7±2.5
Turn	21.1±1.0	21.4±0.7	22.7±1.2	22.3±0.9
Unordered	32.7±1.7	31.7±1.6	36.3±2.4	23.3±2.8

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