Analysis of structural change of XRCC4 by pseudo-phosphorylation using VUV-CD and SAXS

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XRCC4 is a key protein to repair severe DNA damage of double strand breaks (1). The XRCC4 dimer has been reported to form a multimer scaffold with another repair protein, the XRCC4-like factor (XLF). Enzymatically induced phosphorylation at several amino acid residues in XRCC4, such as serine (2), might cause a local change of statically electric charge, resulting conformational alteration to activate the protein. However, the correlation between the structural change and activation has not been understood yet. Full-length XRCC4 was not able to be crystallized because of its intrinsically disordered C-terminal region including several phosphorylation sites. Previously, we have applied circular dichroism (CD) spectral analysis for the full length of wild type XRCC4 (denoted as WT) in an aqueous solution and reported its characteristic secondary structure (3).

In the present study, we focused further the structural change by phosphorylation. We prepared a dimer and multimers of WT XRCC4 as well as mutated ones in which serine residues were substituted to an aspartic acid (denoted as S260D and S327D) to mimic phosphorylation in terms of statically electric charge. Spectra of XRCC4 dimer proteins were similar irrespective of phosphorylation. On the other hand, spectra of multimers were significantly different between WT and mutated proteins (**FIGURE 1**). The composition ratios of the secondary structures were calculated for each sample using a software, SELCON3 (**TABLE 1**). The results indicate that the multimerization and pseudo-phosphorylation increase β-strands. Small angle X-ray scattering (SAXS) at BL6A at the Photon Factory in KEK was also applied to further obtain structural information of XRCC4. The results suggest that the XRCC4S260D dimer has a slightly larger radius of inertia (Rg) than WT (**TABLE 2**),

which may correspond to the slight increase in β-strands shown in the CD analysis. The β-strand formation by the phosphorylation at the C-terminal might stabilize the NHEJ machinery after DSB repair completion.

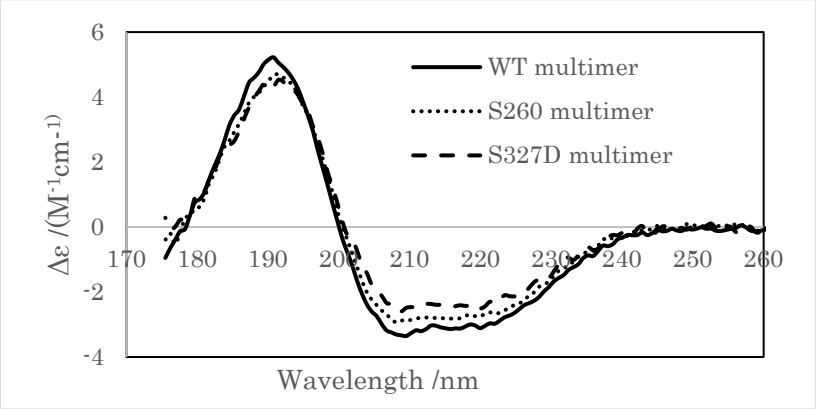

FIGURE 1. Typical CD spectra of wild type and mutated XRCC4 multimers.
The solid, dotted and broken lines represent the spectrum of the wild type, XRCC4S260D and XRCC4S327D dimers, respectively.

TABLE1. Contents of secondary structures of dimer of WT and multimers of WT and mutant of XRCC4.

Structure content (%)		α-Helix	β-Strand	Turn	Unordered
Wild type	dimer	50.8±0.9	5.8±0.8	20.1±2.2	23.2±2.1
	multimer	36.4±1.0	15.7±0.8	22.5±1.4	25.4±0.6
S260D	dimer	50.2±1.9	7.1±3.6	19.7±2.7	23.0±1.2
	multimer	34.2±0.5	17.3±0.9	23.1±0.3	25.5±1.1
S327D	dimer	47.4±2.1	11.5±4.5	18.4±4.1	22.8±1.3
	multimer	32.0±1.7	19.6±0.8	22.7±0.2	25.7±1.2

TABLE 2. Radius of inertia of dimer of WT and mutant of XRCC4.

	WT	S260D	S327D
Rg (Å)	56.25± 0.77	58.02 ± 0.07	57.75 ± 1.04

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