Exploring active structures of DNA repair protein XRCC4 using CD and SAXS

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INTRODUCTION

Ionizing radiation often produces DNA double strand breaks (DSBs). To prevent serious genetic effects from the genome damage, living cells promptly repair DSBs through several pathways. Non-homologous end joining (NHEJ) repair, which is one of the major processes, involves various proteins to rejoin separated DNA ends. XRCC4, a key player of NHEJ, constitutes a polymeric platform of the repair. Normal homodimer formation is thought to change to multimerization by post transcriptional modifications, such as phosphorylation of serine residues, by the certain kinases. So far, protein crystallography was applied to a single crystal of C-terminal deficient XRCC4. The C-terminal tail possesses five phosphorylation sites but is an intrinsically disordered region preventing it from crystallization. Our goal is to elucidate the structural character of dimer and multimer of the full length XRCC4. Two synchrotron analysis methods, namely VUC-circular dichroism (CD) and small-angle X-ray scattering (SAXS) were applied.

EXPERIMENTALS

Purification of XRCC4

To express full-length human XRCC4 (336 amino acids) linked with 6 histidine residues (HisTag), XRCC4 cDNA was obtained from human leukemia cells and inserted into the pET21d plasmid vector. The plasmid was introduced into *E. coli* cells (BL21DE3 Gold) and XRCC4 expression was induced by adding isopropyl β-D-1-thiogalactopyranoside (IPTG) to the culture medium. Using a HisTrap column, XRCC4 was extracted from harvested bacterial cells after homogenizing. The protein was eluted from the column with a buffer containing imidazole (200 mM). XRCC4 dimers were separated from its multimers by gel filtration chromatography. The agarose gel electrophoresis analysis suggested that the multimers inevitably contained a minimal DNA. We used the multimers for CD and SAXS without further purifications.

To mimic phosphorylation, we produced mutated XRCC4 proteins in which the serine residues, S260, S320, S327 or S328, were substituted with a negatively charged aspartic acid, hereinafter denoted as $XRCC4^{S260D}$, $XRCC4^{S320D}$, $XRCC4^{S327D}$ or $XRCC4^{S328D}$, respectively. The mutations were introduced in the XRCC4 coding region using a commercial kit. The results of the pseud-phosphorylated proteins were compared with wild type XRCC4 ($XRCC4^{WT}$).

CD measurements at HiSOR

CD spectroscopy was carried out at BL-12 at the Hiroshima Synchrotron Radiation Center (HiSOR). Protein sample of $XRCC4^{WT}$, $XRCC4^{S260D}$ or $XRCC4^{S327D}$ was dissolved in the sodium phosphate buffer. The sample was encapsulated in a CaF2 cell (path length, 50 μm). CD spectra were measured between 175 and 260 nm at 25°C.

SAXS measurements at the Photon Factory

SAXS was performed at BL-6A at the Photon Factory (PF) in KEK. Each XRCC4 protein sample was dissolved in HEPES buffer at 25°C. The wavelength of X-rays was 1.5 Å (8.3 keV) and the PILATUS-detector (Pixel Apparatus for the SLS) was set at 2500 mm from the sample to detect diffracted X-rays. Volume of the sample cell with a thin polyimide film window was 40 μl and the path length was 1mm.

RESULTS

Secondary structures of XRCC4 determined by CD

The obtained CD spectra of dimer and multimer of the wild type and mutated XRCC4 are shown in Fig. 1. All peak intensities at 190, 210 and 220 nm of the multimer were notably smaller than those of dimer. For

mutated proteins, we also obtained similar CD spectra of their dimer and multimer samples. The secondary structure contents of each sample were calculated using an analysis program SELCON3 and the obtained values are shown in Table 1. Comparing with a previous crystallographic study [1], α-helices and turns were significant in the intrinsically disordered C-tail. The β-strand contents were more explicit for the mutated samples. Interestingly the fraction of the β-strands was noticeably larger in multimers than in dimer.

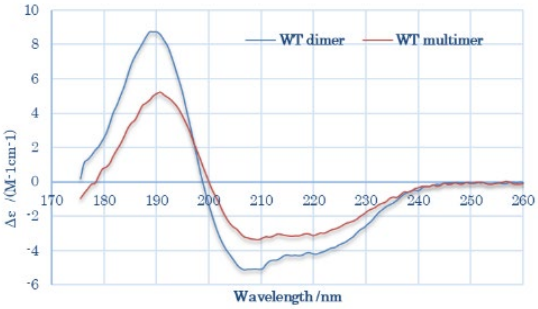

FIGURE 1. CD spectra of the dimer and multimer of wild type XRCC4.

TABLE 1. Secondary structure contents of dimer and multimer of the wild type and mutated XRCC4.

Structure content (%)			α-Helix	β-Strand	Turn	Unordered
Crystallography [1] *			38	26	8	28
CD	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

* The length of amino-acid sequence is 1th–203rd residues.

SAXS profiles of dimer and multimer of XRCC4

The scattering curve strongly depended on the state of polymerization of XRCC4 (Fig. 2). However, the pseud-phosphorylation did not noticeably affect the scattering pattern. In the low-q region for dimmers, we were able to perform Guinier plot using SAngler 2.1.3 assuming the proteins were a globular shape. For multimer, we found a q-region in which Cross-sectional Guinie plot (inset in the right panel of Fig.2) was applicable by assuming the multimers were rod shape. We obtained radii of inertia (Rg) of the dimers and cross-sectional radii of inertia (Rc) of the multimers. Rg values were about 50Å for the wild type as well as mutated ones. Rc values were slightly larger (roughly 60-70 Å) than Rg for each sample. Thus, the multimers were presumed to be filament like shape.

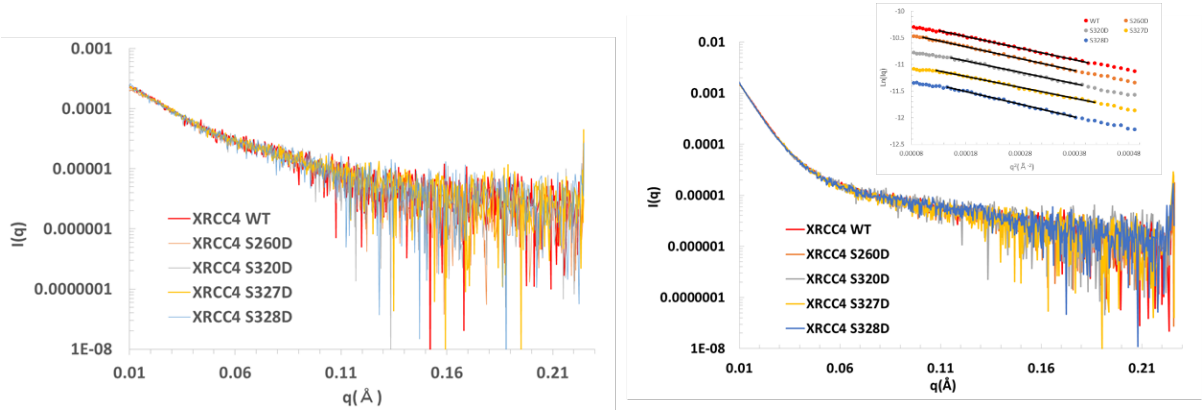

FIGURE 2. SAXS curves of dimers (left) and multimers (right) of wild type and mutated XRCC4. Inset indicates Cross-sectional Guinie plot for the scattering curves of the multimers.

DISCUSSION

The two analyses for the full-length XRCC4 in solution strongly suggest that the intrinsically disordered C-terminal tail plays an important role to induce a conformational change from inactivated homodimer to activated multimer by boosting β-strand formation as indicated by CD measurements. The phosphorylation at the C-terminal may facilitate the multimerization. Multimers formed in this way are filament-like and, presumably, include DNA molecule as a component of the super-molecular-complex of a DSB repair platform (Fig. 3).

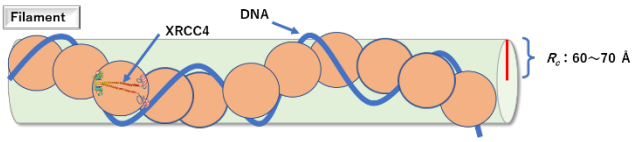

FIGURE 3. A filament model of XRCC4 multimers with DNA.

REFERENCES

1. M. S. Junop, M. Modesti, A. Guarné, R. Ghirlando, M. Gellert, and W. Yang, *EMBO J.* **19**, 5962-59 (2000).