Replication Protein A from *Saccharomyces cerevisiae* Differently Binds to Photo-damaged DNA from Normal Single-stranded DNA

Joon-Hwa Lee,† Chin-Ju Park, and Byong-Seok Choi*

*Department of Chemistry, National Creative Research Initiative Center, Korea Advanced Institute of Science and Technology, Yuseong-gu, Daejeon 305-701, Korea. *E-mail: byongseok.choi@kaist.ac.kr*
†Department of Chemistry and Research Institute of Natural Science, Gyeongsang National University, Jinju, Gyeongnam 660-701, Korea
Received August 9, 2006

Key Words : NMR, Replication protein A, Damaged DNA, Chemical shift mapping, *cis-syn* Cyclobutane pyrimidine dimer

Replication protein A (RPA) is the eukaryotic single-stranded DNA (ssDNA) binding protein and plays a crucial role in DNA metabolism such as DNA replication, nucleotide excision repair, mismatch repair and genetic recombination.[1] RPA from human as well as other eukaryotes consists of three subunits, RPA70 (70 kDa), RPA32 (32 kDa), and RPA14 (14 kDa).[1] Most of DNA-binding activity is mainly mediated by the central region of human RPA70 (hRPA70) subunit containing two DNA binding domains, RPA70A ($RPA70_{181-290}$) and RPA70B ($RPA70_{300-422}$).[2,3] NMR study of DNA binding domains of RPA with the *cis-syn* cyclobutane pyrimidine dimer (CPD)-damaged DNA duplex, in the presence of XPA showed that RPA binds preferentially to undamaged DNA strand.[4] *Saccharomyces cerevisiae* RPA (scRPA) has similar ssDNA-binding properties to hRPA.[5] Recently, NMR study suggested that the positioning of ssDNA is similar for scRPA70A and hRPA70A.[6] But, the binding property of scRPA to damaged DNA has not been studied yet. In this study, chemical shifts of amide nitrogens and protons in scRPA70A were monitored to investigate the differences between the interaction with normal and CPD-containing ssDNA.

Uniformly ^{15}N-labeled scRPA70A ($RPA70_{181-294}$) was obtained and purified as described.[6] The preparation and purification of the DNA decamer, TT-10 (5'-CGCATTAC-GC-3') was followed the previous reports.[7] Photo-damaged DNA, CPD-10 was prepared by direct 254-nm UV irradiation of a TT-10 in an aqueous solution and purified as described.[7]

The NMR data that were performed on a Varian Inova 600 MHz (KAIST, Daejeon) spectrometer were processed with NMRPipe and analyzed with the program Sparky.[8] The details of NMR experiment for the scRPA70A were reported previously.[6] Difference in average amide chemical shifts ($\Delta\delta_{avg}$) were calculated using Eq. (1),

$$\Delta\delta_{avg} = \sqrt{((\Delta\delta_N/5)^2 + (\Delta\delta_H)^2)/2} \quad (1)$$

where $\Delta\delta_N$ and $\Delta\delta_H$ are the differences in amide nitrogen and amide proton chemical shifts, respectively, between two samples.[4]

The amide backbone resonances of scRPA70A were assigned by standard triple-resonance experiments and these assignments were reported previously.[6] ^{15}N-^1H HSQC has been used to monitor the DNA binding activity of the scRPA70A. Previously, the chemical shift perturbation of the scRPA70A upon normal TT-10 was studied and this result was compared with that of hRPA70A.[6] The previous study suggested that the orientations of the residues involved in ssDNA binding are conserved in both hRPA70A and scRPA70A but the conformational changes in the two proteins are not identical.[6] The ^{15}N-^1H HSQC spectrum of the scRPA70A upon the addition of equimolar CPD-10 was acquired and then compared with those of free scRPA70A and the scRPA70A-TT-10 complex (Supporting Figure 1). The average chemical shift changes of scRPA70A upon addition of CPD-10 calculated by Eq. (1) are shown in Figure 1A.

To compare the binding properties of scRPA70A to TT-10 and CPD-10, the average chemical shift differences of scRPA70A between upon TT-10 and CPD-10 also were calculated by Eq. (1). Surprisingly, some residues showed the significant differences in the chemical shift changes of scRPA70A upon TT-10 and CPD-10 (Fig. 2A). CPD-10 is the same DNA sequence with TT-10 but contains a CPD lesion formed at the central T-T site. It was reported that CPD-10 causes similar chemical shift perturbations of hRPA70A with TT-10 does, indicating that the hRPA70A binds the same positions of the TT-10 and CPD-10 (Fig. 2B).[4] In case of hRPA70AB, the domain A prefers to bind to 5' side of ssDNA and domain B binds to the next side of ssDNA.[4]

If scRPA70A preferentially binds to 5'-side of ssDNA like hRPA70A, the chemical shift perturbation results of scRPA70A by TT-10 and CPD-10 should be similar. However, some residues showed the significant differences in the chemical shift changes of scRPA70A upon binding to TT-10 and CPD-10 (Fig. 2A). The structural study on the scRPA70A found that the positions of residues critical for ssDNA interactions were well matched in two proteins, hRPA70A and scRPA70A.[6] Although the position of K210 in scRPA70A was similar to that of the equivalent R210 in hRPA70A, the significant change in the neighboring T211 amide resonance was observed only for scRPA70A upon

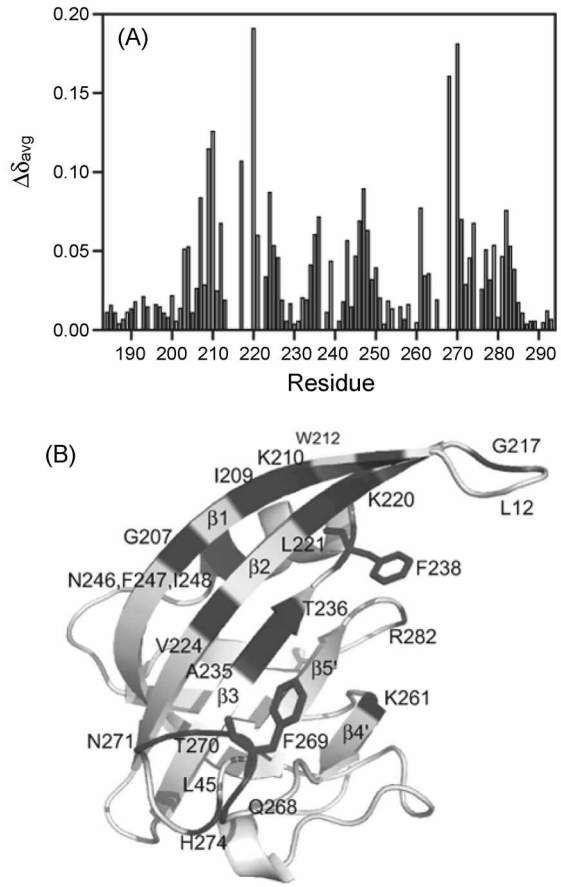

Figure 1. (A) The average chemical shift changes ($\Delta\delta_{avg}$) in the ^{1}H and ^{15}N resonances of scRPA70A between in the free and CPD-10 bound forms. (B) A ribbon diagram of scRPA70A (PDB ID 1YNX) affected by binding of CPD-10 ssDNA. Residues showed the $\Delta\delta_{avg}$ greater than 0.06 ppm by CPD-10 binding are shown in dark color.

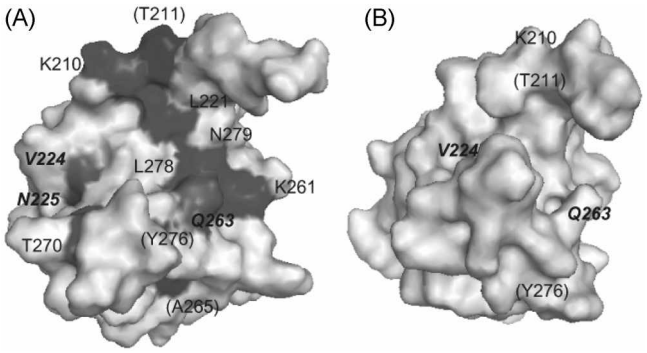

Figure 2. Different binding mode of scRPA70A (PDB ID 1YNX) to CDP-10 from normal ssDNA TT-10. (A) Residues showed the differences ($\Delta\delta_{avg}$) greater than 0.04 ppm in amide resonances between TT-10 bound and CPD-10 bound scRPA70A. Residues perturbed by only TT-10 are indicated by parenthesis and residues perturbed by only CPD-10 are indicated by italic bold letters. (B) hRPA showed no differences ($\Delta\delta_{avg}$) greater than 0.04 ppm in amide resonances between between TT-10 bound and CPD-10 bound forms.

binding to TT-10.[6] Surprisingly, this T211 amide of the scRPA70A is not perturbed by the addition of CPD-10 compared to TT-10 (Fig. 1A and 1B). The A265 in L45 and Y276 in $\beta5'$ of the scRPA70A were significantly perturbed by TT-10 like hRPA70A but showed little change in the amide resonances upon binding to CPD-10. This indicates that the DNA binding feature of the part of L45 and $\beta5'$ in the scRPA70A is changed when DNA is CPD-damaged. Some residues (K210, L221, K261, T270, L278, and N279) showed the chemical shift perturbation by both TT-10 and CPD-10 but the perturbation patterns are clearly different each other. This means that these residues are involved in the molecular interaction with ssDNA but their interacting partners of ssDNA are changed when ssDNA has the CPD damage. Contrast to hRPA70A, the three residues (V224, N225, and F226) in $\beta2$ of the scRPA70A exhibited little effects upon binding to normal TT-10 ssDNA.[6] However, when scRPA70A binds to CPD-10, these residues showed the significant or moderate chemical shift perturbation like hRPA70A (Fig. 2A). It implies that scRPA70A preferentially binds to the similar region of ssDNA with hRPA70A when the ssDNA contains CPD while it can be located any region in ssDNA without damage. In addition, the amide resonance of the Q263 residue, which has little effect in the chemical shift change by TT-10 and has different position from the equivalent K263 in hRPA,[6] is moderately perturbed by CPD-10 (Fig. 2). The differences in the chemical shifts perturbation patterns of scRPA70A upon binding to CPD-10 and TT-10 is the evidence which shows that the location of the protein in the ssDNA strand is not the same. Thus, we suggest that CPD might prevent scRPA70A from binding to the central region and drive it to seat in the 5' side of ssDNA like hRPA70A.

Acknowledgements. We thank Dr. Legerski for providing the scRPA expression vector. This work was supported by the National Creative Research Initiative Program to B.-S.C. from the Ministry of Science and Technology, Korea. This work was supported by the Research Grant Program for New Faculty to J.-H.L. from Gyeongsang National University, Korea.

References

1. Wold, M. S. *Annu. Rev. Biochem.* **1997**, *66*, 61.
2. Gomes, X. V.; Wold, M. S. *Biochemistry* **1996**, *35*, 10558.
3. Li, L.; Lu, X.; Peterson, C. A.; Legerski, R. J. *Mol. Cell. Biol.* **1995**, *15*, 5396.
4. Lee, J.-H.; Park, C.-J.; Arunkumar, A. I.; Chazin, W. J.; Choi B.-S. *Nucleic Acids Res.* **2003**, *31*, 4747.
5. Heyer, W. D.; Rao, M. R.; Erdile, L. F.; Kelly, T. J.; Kolodner, R. D. *EMBO J.* **1990**, *9*, 2321.
6. Park, C.-J.; Lee, J.-H.; Choi, B.-S. *Nucleic Acids Res.* **2005**, *33*, 4172.
7. Lee, J.-H.; Park, C.-J.; Shin, J.-S.; Ikegami, T.; Akutsu, H.; Choi B.-S. *Nucleic Acids Res.* **2004**, *32*, 2474.
8. Lee, S.; Kim, D.; Kim H., Lee, Y.; Won, H. *Bull. Korean Chem. Soc.* **2004**, *25*, 1845.