Zn$^{2+}$-Dependent Single-Stranded DNA Binding and DNA Replication Activities in Replication Protein A

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Replication Protein A (RPA) is a heterotrimeric (70-, 32-, and 14-kDa subunits) protein containing six oligonucleotide binding folds (OB-folds), which are referred to as DNA-binding domains (DBD-N, -A, -B, -C, -D, and -E) (Fig. 1A). Recently, we performed the PONDR (Predictors of Natural Disordered Regions) program to predict naturally disordered regions of human RPA 70 subunit. The x axis in Fig. 1B shows the residue number, whereas the y axis is the PONDR score normalized to be in the range of 0 ~ 1. Any region that exceeds a score of 0.5 is considered to be disordered. A four cysteine-type (C481-X4-C486-X13-C500-X2-C503) (arrow on the right) zinc-finger domain (ZFD) shows a most stable and structured segment in RPA70 subunit (its PONDR score is close to 0). Previous studies reveal that DBD-C, at the C terminus of RPA70, is required for the formation of the heterotrimeric core complex (with the RPA-32 and RPA-14 subunits) (Fig. 1A). and the ZFD regulates the ssDNA-binding activity of RPA through a redox change. Recently, analysis by liquid chromatography/tandem mass spectrometry revealed that two pairs of intramolecular disulfide bonds (Cys481-Cys486 and Cys500-Cys503) are formed under oxidative conditions. Interestingly, our PONDR prediction showed that the replacement of the cysteine amino acids (ZFM-RPA; Cys to Ser at 481, 486, 500, and 503) results in the ZFD being in a flexible and unstable state (right panel in Fig. 1B). Thus, the breakdown of the zinc-finger motif could influence the stabilization of DBD-C, subsequently changing the activity of the whole RPA complex.

To obtain the native RPA proteins, we expressed a constructed pET11a vector encoding human RPA cDNA in E.coli BL21 (DE3) strains using TB medium. After adding IPTG to TB medium, the RPA32 and RPA14 (but not RPA 70) subunits could be well expressed (Fig. 2, asterisk in lane 2). Considering that TB medium may not contain the enough Zn(II)-ion, 10 µM of ZnCl$_2$ was added to TB medium throughout all the RPA induction (lanes 3-4). The yield of the induced RPA70 subunit was greatly increased, when ZnCl$_2$ was added (asterisk in lane 4). We think whether Zinc-lacking results in the failure of zinc-finger motif formation in RPA70 subunit (but forming an oxidative...
state\(^{10}\) of two pairs of intramolecular disulfide bonds [Cys\(^{481}\)-Cys\(^{486}\)] and [Cys\(^{500}\)-Cys\(^{503}\)], which is a toxic form of RPA70.

The wt-RPA (Zn-), and ZFM-RPA were somewhat lower than those with wt-RPA. Furthermore, wt-RPA(Zn-) lost its DNA replication activity in the presence of DM, however wt-RPA containing the Zn(II)-ion was resistant to this oxidative stress (lanes 5-10).

In conclusion, the findings of this study support the conclusion (Fig. 5) that the RPA complex lacking the Zn(II)-ion shows lower ssDNA-binding and DNA replication activities than that containing this divalent, metallic ion. Meanwhile, the release of the Zn(II)-ion results in the activities of the RPA-complex becoming sensitive to oxidative damage.

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References