Functional Characterization of Zinc-Finger Motif in Redox Regulation of RPA−ssDNA Interaction†

Jin-Sam You, Mu Wang, and Suk-Hee Lee*

Department of Biochemistry and Molecular Biology, Indiana University Cancer Center and Walther Oncology Center, Indiana University School of Medicine, 635 Barnhill Drive, Indianapolis, Indiana 46202

Received May 26, 2000; Revised Manuscript Received August 17, 2000

ABSTRACT: The 70-kDa subunit of eukaryotic replication protein A (RPA) contains a conserved four cysteine-type zinc-finger motif that has been implicated in regulation of DNA replication and repair. Unlike other zinc-finger proteins, RPA zinc-finger motif is not a DNA-binding component, and deletion of the zinc-finger had very little effect on its ssDNA binding activity. Recently, we described a novel function for the zinc-finger motif in regulation of RPA's ssDNA binding activity through reduction−oxidation (redox). In this study, we carried out a detailed analysis of wild-type RPA and zinc-finger mutants in redox regulation of their ssDNA binding activity. Any mutation at a zinc-finger cysteine abolished its redox role in regulation of RPA−ssDNA interaction, suggesting that all four zinc-finger cysteines are required for redox regulation. Reactivity of cysteine residues to 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) indicated that wild-type RPA contained 8.2 reactive thiols/molecule including all four cysteines in the zinc-finger motif. Zinc-finger cysteines slowly reacted to DTNB as compared to others. Zn(II) was not only essential but also uniquely qualified for redox regulation of RPA−ssDNA interaction, suggesting that Zn(II)−cysteine coordination is crucial for the zinc-finger function. Redox status significantly affected initial interaction of RPA with ssDNA but had no effect after RPA formed a stable complex with DNA. Together, our results suggest that the zinc-finger motif mediates the transition of RPA−ssDNA interaction to a stable RPA−ssDNA complex in a redox-dependent manner.

Replication protein A (RPA;[1] also known as human single-stranded DNA binding protein, HSSB) is a three-subunit complex (70-, 34-, and 11-kDa; p70, p34, and p11, respectively) essential for DNA replication, nucleotide excision repair, and genetic recombination (ref *1* and the references therein). In simian virus 40 (SV40) DNA replication, RPA is involved in unwinding of the replication origin in the presence of SV40 large tumor antigen (T-ag) and topoisomerase I/II. During replication, RPA interacts with T-ag and the DNA polymerase α-primase complex at the origin (*2*, *3*), where it participates in the initiation process (*2*, *4*, *5*). In DNA repair, RPA preferentially binds to UV- and cisplatin-damaged DNA (*6*, *7*) and interacts with a DNA-damage recognition factor, *Xeroderma pigmentosum* group A-complementing protein (XPA), which stabilizes the XPA-damaged DNA interaction (*8*−*12*). After forming an RPA−XPA complex on damaged DNA, RPA recruits XPG and XPF-excision repair cross complementation group 1 (ERCC 1) (3′- and 5′-endonucleases, respectively) (*9*, *10*, *13*).

The large subunit of RPA (p70) contains the ssDNA binding domain that resides in the middle region of p70 with two homologous subdomains in tandem positions (*14*−*17*). p70 also contains an evolutionarily conserved four cysteine-type zinc-finger domain (*14*, *16*, *18*). Deletion analysis indicated that RPA's zinc-finger domain, unlike others, is not essential for its ssDNA binding activity (*14*, *15*, *19*). However, mutations at the zinc-finger domain differentially affected its function in DNA replication and nucleotide excision repair (*19*, *20*), suggesting a possible role for the zinc-finger domain in regulation. It remains unclear whether the zinc-finger is directly involved in redox since the zinc-finger structure in most proteins is required for DNA binding, and any mutation in the zinc-finger domain would inactive the protein. In contrast, RPA's zinc-finger is not a DNA binding component and has a little or no effect on its DNA binding activity (*14*−*16*), which renders it an excellent and unique model to study the role of redox in regulation of zinc-finger proteins.

In our previous studies, we have shown that RPA's ssDNA binding activity is regulated by redox through the cysteines in a putative zinc-finger domain (*23*). The four cysteine-type zinc-finger contains Zn(II) that tetrahedrally coordinates four cysteine residues to form the structure (*24*). Under reducing conditions, the zinc-finger structure is favorably formed and Zn(II) may function to protect the cysteine residues from engaging in disulfide bond formation. Under nonreducing (or oxidized) conditions, however, oxidation of Zn(II)−thiolate bond induces the releases Zn(II) from the

† This research was supported by grants from NIH (GM52358) and the Indiana University Cancer Center. M.W. was supported by a postdoctoral fellowship from NIH (F32 GM20167-01).

* Corresponding author. Phone: (317)278-3464. Fax: (317)274-4686. E-mail: slee@iupui.edu.

[1] Abbreviations: DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); DTT, dithiothreitol; redox, reduction and oxidation; ERCC 1, excision repair cross complementation group 1; RPA, replication protein A; ssDNA, single-stranded DNA; SPR, surface plasmon resonance; SV40, simian virus 40; T-ag, SV40 large tumor antigen; XPA, *Xeroderma pigmentosum* group A complementing protein.

10.1021/bi001206f CCC: $19.00
Published on Web 09/21/2000

zinc-finger (*25*), which promotes the formation of disulfide bonds (*26*). In this study, we examined the individual cysteines for their role in redox regulation of RPA's ssDNA binding activity. We found that all four zinc-finger cysteines are required for redox regulation of RPA's DNA binding activity. Redox status of RPA significantly affected initial interaction with ssDNA but had no effect after RPA formed a stable complex with DNA, suggesting that redox regulation of the zinc-finger may be involved in mediating initial RPA−ssDNA interaction to form a stable RPA−ssDNA complex.

MATERIALS AND METHODS

Construction of RPA Zinc-Finger Mutants. Cysteine to alanine substitution mutation was carried out using the Quick Change site-directed mutagenesis kit (Stratagene, La Jolla, CA) with the following primers: 5′-CATGTACCAAGCCG-CCCCGACTCAGGAC-3′, 5′-GTCCTGAGTCGGGGCG-GCTTGGTACATG-3′ (for C481A), 5′-GGATTGTACCG-CGCTGAGAAGTG CGAC-3′, 5′-GTCGCACTTCTCAGC-GCGGTACAATCC-3′ (for C500A), and 5′-CGCTGT-GAGAAGGCCGACACCGAATTTC-3′, 5′-GAAATTCG-GTGTCGGCCTTCTCACAGCG-3′ (for C503A). The PCR reactions were carried out using a Perkin-Elmer DNA Thermal Cycler 480 with the first cycle at 95 °C for 0.5 min followed by 16 cycles of amplification (95 °C for 0.5 min, 55 °C for 1 min, and 68 °C for 24 min). *Dpn*I was used to digest the parental plasmid at 37 °C for 1 h. *Dpn*I-digested PCR product was transformed into supercompetent *Epicurean coli* XL-1 Blue cells. The plasmid DNAs were purified using the QIAGEN Midi Plasmid Kit and were examined for a proper mutation by DNA sequencing prior to baculovirus transfection.

Preparation of Wild-Type RPA and Zinc-Finger Mutants. RPA was prepared according to the procedure described previously (*27*) with slight modifications. Briefly, lysates were prepared from insect cells (Sf-9), co-infected with recombinant baculoviruses encoding wild-type p11, wild-type p34, and either wild-type or mutant p70. After adjusting salt concentration to 0.5 M NaCl, lysates were loaded onto a ssDNA cellulose column equilibrated with buffer A (25 mM Tris-HCl, pH 7.5, 10% glycerol, 0.02% Nonidet P-40, 1 mM DTT, 0.5 mM EDTA, 0.1 mM phenylmethanesulfonyl fluoride, 0.1 μg/mL leupeptin, and 0.2 μg/mL antipain) containing 0.5 M NaCl. The column was successively washed with 20 column volumes of buffer A containing 0.5 M NaCl and 0.8 M NaCl. The proteins were eluted with buffer A containing 2.0 M NaCl and 40% ethylene glycol. The eluted fractions were diluted 5-fold with buffer A and loaded onto an Affi-Gel Blue (Bio-Rad) column equilibrated with buffer A containing 0.5 M NaCl. After washing the column with buffer A containing 0.5 M NaCl and 0.8 M NaCl, proteins were eluted with buffer containing 2.5 M NaCl and 40% ethylene glycol. The RPA-containing fractions were pooled and dialyzed against buffer A containing 50 mM NaCl and further purified on a Q-Sepharose column with a linear salt gradient (50 mM−0.4 M NaCl). All purification procedures were carried out at 4 °C, and RPA was monitored by immunoblotting using anti-p70 and anti-p34 antibodies (*27*). The fractions with at least 90% purity by Coomassie staining were collected and stored at −80 °C.

RPA−ssDNA Binding Assay. Oligo(dT)$_{50}$ was labeled with [γ-^{32}P]ATP (ICN) and T4 polynucleotide kinase (Roche Molecular Biochemical) based on the manufacture's instructions. The indicated amount of wtRPA or mutant RPA was incubated with 100 fmol of 5′-^{32}P-labeled oligo(dT)$_{50}$ at room temperature for 25 min in a reaction mixture containing 50 mM Hepes−KOH (pH 7.8), poly(dI:dC) (0.2 μg), bovine serum albumin (0.2 μg/μL), 200 mM NaCl, and an indicated amount of other chemicals. The RPA−ssDNA complex was analyzed on 5% polyacrylamide gels in 0.5× TBE (acrylamide:bisacrylamide = 43.2:0.8). The gels were dried and exposed to X-ray films (Kodak). For quantitation, the bands of interest were excised from the gels and measured for radioactivity using a Beckman Scintillation Counter LS 6500. No RPA control lane was subtracted from all the other samples to determine accurate fentomoles bound to DNA.

Biomolecular Interaction Analysis. Interaction of wtRPA or mutant RPA with ssDNA was monitored using a surface plasmon resonance (SPR) biosensor instrument, BIAcore 3000 (BIAcore). For preparation of the biosensor surface with DNA, 5′-biotinylated 70-mer DNA was diluted to 1.5 nM in a buffer containing 10 mM sodium acetate, pH 4.8, and 1.0 M NaCl and manually injected onto an immobilized streptavidin surface of the BIAcore sensor chip to the desired density in different flow cells. One flow cell was left underivatized to allow for refractive index change correction. Proteins were diluted in the running buffer containing 10 mM Hepes, pH 7.4, 150 mM NaCl, 2 mM $MgCl_2$, 0.005% polysorbate-20, and 1 mM DTT. Each experiment was repeated at least twice to ensure reproducibility.

Measurement of 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB) Reactivity. DTNB (Sigma Chemical Co., St. Louis, MO) was dissolved in ethanol and freshly diluted in buffer A containing 200 mM NaCl. wtRPA or zinc-finger mutant (750 nM) were incubated in a buffer containing 25 mM Tris-HCl, pH 7.5, 10% glycerol, 0.02% NP40, 200 mM NaCl, and 50mM DTNB at 25 °C. Absorption spectra were measured with a Perkin-Elmer UV/VIS spectrophotometer Lambda 6. A molar extinction coefficient of 13 600 cm^{-1} at 412 nm (*28*) was employed to estimate the concentration of TNB anion produced.

RESULTS

DTNB Reactivity of RPA Zinc-Finger Cysteines. Earlier studies with RPA zinc-finger mutant suggested that cysteine 486 is essential for redox regulation of RPA−ssDNA interaction (*23*). To further analyze the role of cysteine(s) at the zinc-finger domain in redox regulation, we generated a series of zinc-finger mutants in which each cysteine was replaced by alanine (Figure 1A). Wild-type and mutant RPAs were purified to more than 90% purity from insect cells (Sf9) co-infected with recombinant baculoviruses encoding p34, p11, and either wild-type or mutant p70 (Figure 1B). We examined wtRPA and zinc-finger mutants for DTNB reactivity by measuring the number of free thiols under native conditions (Figure 2). wtRPA contained 8.2 reactive thiols/molecule, which represents approximately half of the total cysteines (16 cysteines) in RPA. Kinetic analysis indicated that some cysteines reacted with DTNB less than 1 min (so-called "fast-reactive" thiol group) and that the other cysteines slowly responded over a 30-min period (so-called "slow-reactive" thiol group). All four zinc-finger mutants (C481A, C486A, C500A, and C503A) demonstrated the same kinetics

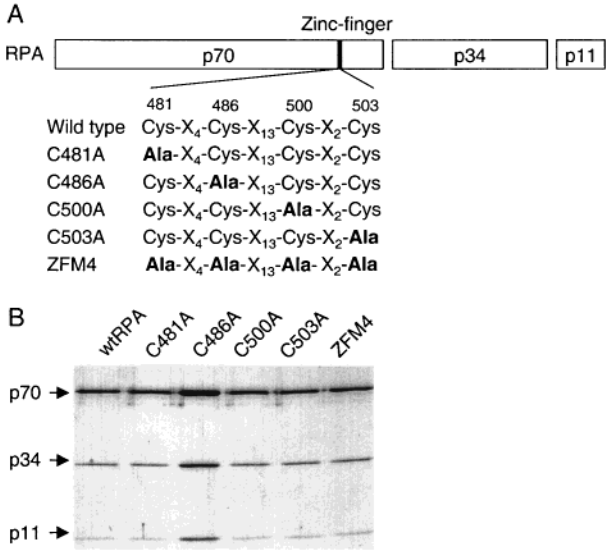

FIGURE 1: (A) Schematic presentation of wild-type RPA and four zinc-finger mutants (cysteine to alanine). (B) SDS−polyacrylamide gel electrophoresis (PAGE) of purified RPAs (see Materials and Methods for detailed purification procedure). Proteins were separated by 14% PAGE followed by Coomassie blue staining.

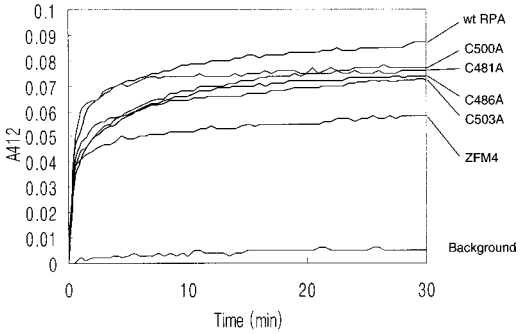

FIGURE 2: Kinetic analysis of the DTNB reactivity of wtRPA and zinc-finger mutants. wtRPA or zinc-finger mutant (750 nM) was added to a buffer [25 mM Tris-HCl (pH 7.5), 10% glycerol, 0.02% NP40, and 200 mM NaCl] containing 50 mM DTNB and incubated at 25 °C prior to kinetic analysis with UV/VIS spectrophotometer.

as that of wtRPA in early DTNB reaction (fast-reactive thiol group) but resulted with one reactive thiol less than wtRPA. ZFM4 (mutations at all four zinc-finger cysteines) also showed the same kinetics as that of wtRPA in fast-reactive thiol group reaction (Figure 2). In addition, the data from wtRPA and ZFM4 fit into double-exponential equations (Jandel Sigma Plot, Scientific Graphing Software, version 3.0) showed 4.3 fast-reactive cysteines (data not shown). These results suggest that all four zinc-finger cysteines may be the slow-reactive thiol group. Interestingly, C500A exhibited a distinct DTNB reactivity from other zinc-finger mutants in that all seven thiols responded within 5−10 min, whereas the other zinc-finger mutants (C481A, C486A, and C503A) slowly reacted over a 30-min period, suggesting that cysteine 500 may be the slowest reactive cysteine to DTNB. It is possible that a mutation at C500 could disrupt the secondary structure such that the kinetics observed in the C500 mutant are different from wtRPA or other ZFM mutants where the secondary structure may not be altered.

Role of Zinc-Finger Cysteines in Redox Regulation of RPA−ssDNA Interaction. To understand the role of individual zinc-finger cysteines, we examined all four zinc-finger

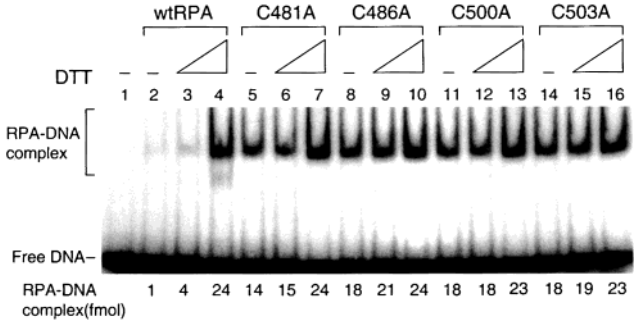

FIGURE 3: Effect of reducing agent on RPA−ssDNA binding activity. wtRPA or zinc-finger mutant (20 ng) was pretreated with 0 mM (lanes 2, 5, 8, 11, and 14), 0.2 mM (lanes 3, 6, 9, 12, and 15), and 2 mM (lanes 4, 7, 10, 13, and 16) DTT before incubation for 15 min with 100 fmol of 5′-^{32}P-labeled oligo $(dT)_{50}$. The RPA−DNA complex was analyzed by 5% polyacrylamide gel in 0.5× TBE (acrylamide:bisacrylamide = 43.2:0.8). For quantitation, regions corresponding to RPA−ssDNA complex were excised and measured for radioactivity. No RPA was included in lane 1.

mutants for ssDNA binding activity under various redox conditions. As shown previously (*23*), wtRPA poorly formed a complex with ssDNA under nonreducing conditions (in the absence of DTT) and was markedly stimulated by DTT (Figure 3). On the other hand, all four zinc-finger mutants (C481A, C486A, C500A, and C503) formed a stable complex with ssDNA in the absence of DTT, although a significant difference in ssDNA binding activity was observed among the mutants following the addition of DTT (Figure 3). To further understand the redox regulation of RPA−ssDNA interaction, wtRPA and zinc-finger mutants were examined for DNA binding affinity using surface plasmon resonance (SPR) technique, which allows macromolecular interactions to be measured in real time (*29*). wtRPA or zinc-finger mutant (2.5 nM) was injected onto a biosensor chip containing low level (33 RU) of 70-mer ssDNA for 600 s followed by 360 s of buffer injection period for dissociation. The sensorgram indicated that wtRPA exhibited very low ssDNA binding activity under nonreducing conditions, whereas all zinc-finger mutants strongly supported ssDNA binding under the same conditions (Figure 4B). Under reduced conditions, however, both wtRPA and zinc-finger mutants bound to ssDNA with high affinity (Figure 4B). These results were consistent with the data from the gel mobility shift assay (Figure 3), suggesting that (i) RPA zinc-finger domain regulates its DNA binding affinity through redox and (ii) all four cysteines at the zinc-finger domain are necessary for redox regulation of RPA's ssDNA binding activity.

Redox Regulation of Zinc-Finger Affects Initial RPA−DNA Interaction but Has No Effect after RPA Formed a Stable Complex with DNA. RPA's zinc-finger cysteines may form disulfide bond(s) under nonreducing conditions, which may consequently alter protein conformation and affect its ssDNA binding activity. Alternatively, zinc-finger cysteines engaged in disulfide bond(s) may directly interfere with RPA's DNA binding domain (*23*). If the latter is the case, we may observe an inhibition of RPA−ssDNA interaction and/or disruption of the RPA−ssDNA complex by the addition of an oxidizing agent (or removal of DTT). To address this question, wtRPA and ZFM4 were examined for ssDNA binding affinity under various redox conditions using SPR analysis. Injection of wtRPA under reducing conditions demonstrated high affinity

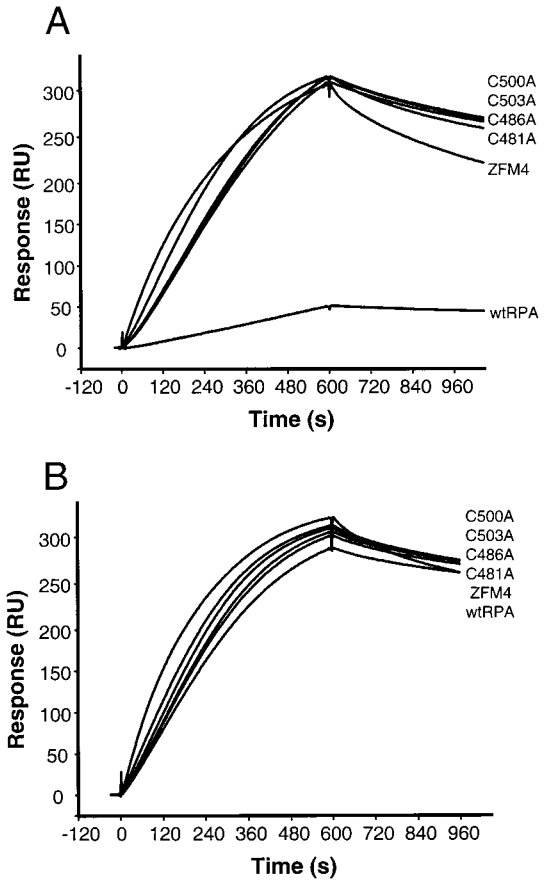

FIGURE 4: Biomolecular Interaction (BIAcore) analysis. Binding of wtRPA and zinc-finger mutant to ssDNA. wtRPA or mutant (2.5 nM) was injected into ssDNA surface (33 RU) using the KINJECT function of BIAcore 3000. In the absence (A) or presence (B) of 1 mM DTT. Association phase was allowed for 600 s followed by 360 s of buffer injection period for dissociation (see Materials and Methods for details).

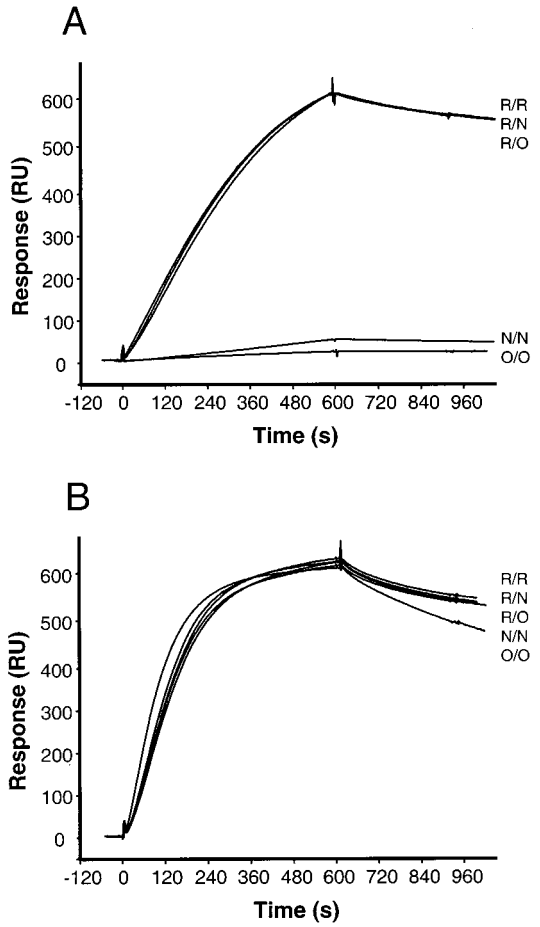

FIGURE 5: BIAcore analysis of RPA−ssDNA binding under various redox conditions. 5 nM wtRPA (A) or zinc-finger mutant, ZFM4 (B) was injected into ssDNA surface (33 RU) using the KINJECT function of BIAcore 3000. Both sample injection (association phase) and buffer injection (dissociation phase) were carried out with three different redox conditions [R, reducing conditions (1 mM DTT); N, nonreducing conditions (0 mM DTT); O, oxidized conditions (0.4 mM H_2O_2)]. Association phase was allowed for 600 s followed by 360 s of buffer injection period for dissociation.

binding to ssDNA, which was maintained even after buffer injection under oxidizing conditions (association phase/dissociation phase; R/R or R/O, Figure 5A). On the other hand, injection of RPA under nonreducing conditions (N/N) or oxidized conditions (O/O) showed a very low affinity binding to ssDNA. In accordance with the results in Figure 4, zinc-finger mutant ZFM4 demonstrated high affinity binding to ssDNA regardless of the redox status (Figure 5B). This result suggests that redox regulation of zinc-finger only affects initial RPA−ssDNA interaction but has no effect after RPA forms a stable complex with ssDNA. It is interesting to note that ZFM4 showed a faster rate of dissociation under nonreduced conditions, and this was not observed under reduced conditions (Figures 4A and 5B).

Role of Zn(II) in Redox Regulation of RPA−ssDNA Interaction. Recent studies demonstrated that inhibition of RPA's ssDNA binding activity by a strong metal chelating agent *o*-phenanthroline can be reversed by the addition of Zn(II) (*23*, *29*), suggesting that Zn(II) may be essential for RPA's ssDNA binding activity. Furthermore, *o*-phenanthroline can also alter the redox status by interacting with reducing agents such as DTT, which affects RPA−ssDNA interaction. To investigate whether the effect of *o*-phenanthroline on RPA is due to the removal of Zn(II) rather than a change in its redox status, we examined RPA for its ssDNA binding activity in the presence of either *o*- or *p*-phenanthroline (Figure 6A). The latter is a nonchelating structural isomer that retains the ability to alter the redox status. Increasing the amount of *o*-phenanthroline significantly inhibited RPA's ssDNA binding activity, whereas *p*-phenanthroline had very little effect (Figure 6A), suggesting that the inhibitory effect of *o*-phenanthroline on RPA is due to the removal of Zn(II). As predicted, *o*-phenanthroline had little or no effect on ssDNA binding activity of all zinc-finger mutants (Figure 6B), suggesting that the role of Zn(II) in RPA's ssDNA binding activity is strictly confined to coordinating the cysteine residues within the zinc-finger motif and maintaining the structure.

DISCUSSION

Zinc-finger motifs are key DNA binding components for many sequence-specific DNA binding proteins and likely play a role in regulation of DNA binding activity through redox (*23*). Nonetheless, the study of zinc-finger motifs in redox regulation has been hampered by the fact that the zinc-finger domain is essential for DNA binding and any mutation at the zinc-finger domain abolishes DNA binding activity. On the other hand, RPA's zinc-finger is not a DNA binding element and mutations at the zinc-finger motif have very

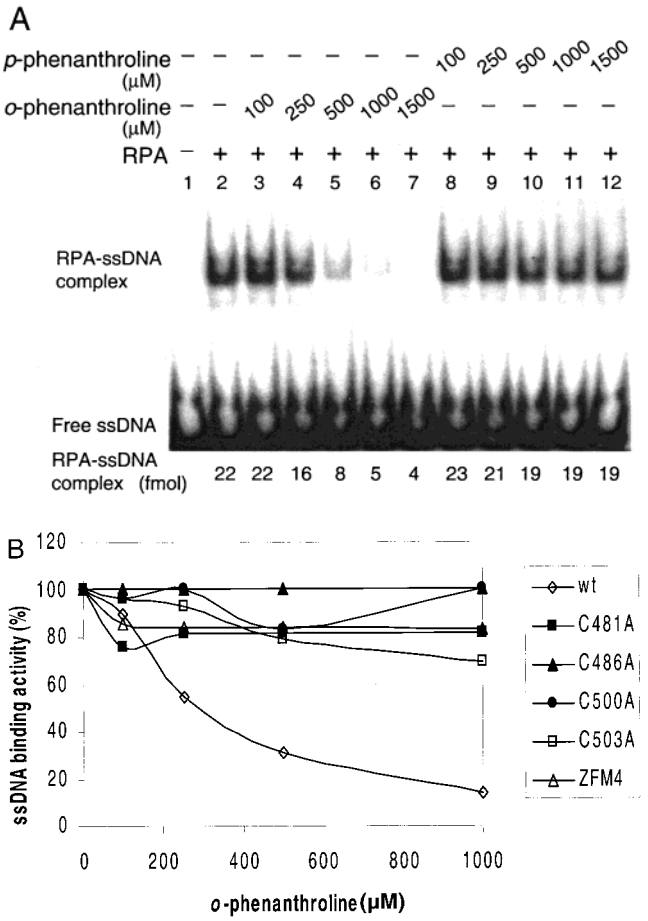

FIGURE 6: (A) Effect of metal chelating agent on RPA−ssDNA interaction. wtRPA (20 ng) was treated with increasing amounts of *o*-phenanthroline or *p*-phenanthroline before incubation for 15 min with 5′-^{32}P-labeled oligo $(dT)_{50}$. The RPA−ssDNA complex was analyzed by gel mobility shift assay as described in Figure 3. No RPA was included in lane 1. (B) Effect of metal chelating agent, *o*-phenanthroline on RPA−sDNA binding activity. wtRPA or zinc-finger mutant (20 ng) was treated with various amounts of *o*-phenanthroline before incubation for 15 min with 100 fmol of 5′-^{32}P-labeled oligo $(dT)_{50}$.

little impact on its DNA binding activity (*14*, *15*, *19*), implicating that RPA as an excellent and unique model to study the role of zinc-finger motifs in redox regulation. In this study, we used biochemical tools to analyze the role of the zinc-finger in redox regulation of RPA−ssDNA interaction.

Previously we proposed a model that oxidation of Zn(II)−thiolate bond induces the release of Zn(II) from the zinc-finger, which likely promotes disulfide bond formation between cysteine 486 and another cysteine (either within or outside the zinc-finger) (*23*). Mutation at any zinc-finger cysteine destroyed redox regulation of RPA−ssDNA interaction (Figure 3), suggesting that all four cysteines are necessary for redox regulation of RPA. In addition, DTNB reactivity with wtRPA and zinc-finger mutants suggested that all four zinc-finger cysteines responded to redox change in a slow reactive manner (Figure 2). Slow reactivity of zinc-finger cysteines with DTNB may be explained by steric hindrance caused by the coordinated interaction of cysteines with Zn(II) and is consistent with our model for the role of Zn(II)-coordinated structure in redox regulation of RPA (*23*). These findings are certainly not in favor of a model that disulfide bond formation occurs between a zinc-finger cysteine and a cysteine outside the zinc-finger (particularly one of the cysteines in DNA binding domain) (*23*). On the contrary, it supports a model that two disulfide bonds may form between the four zinc-finger cysteines. Formation of two disulfide bonds may be necessary for inhibition of RPA−ssDNA interaction under nonreducing conditions. If true, mutation at any zinc-finger cysteine (amino acids 481, 486, 500, or 503) would be sufficient to abolish redox sensitivity of RPA's ssDNA binding, as shown in Figure 3.

Redox regulation of the zinc-finger significantly affected initial RPA−ssDNA interaction but had no effect after RPA formed a stable complex with DNA (Figure 5), suggesting that the zinc-finger motif mediates the transition of RPA−ssDNA interaction to a stable RPA−ssDNA complex in a redox-dependent manner. Under reducing conditions, Zn(II)−cysteine coordination at the zinc-finger mediates the conformational change (*23*) that may facilitate formation of a stable complex of RPA with ssDNA. Under nonreducing conditions, however, release of Zn(II) promotes formation of disulfide bond(s) at the zinc-finger domain, which no longer supports a proper conformation to allow ssDNA binding and results in weak and unstable RPA−ssDNA interaction. In this respect, the lack of redox regulation in the zinc-finger mutants (Cys to Ala mutation or deletion mutation) may be explained by the fact that they are unable to form disulfide bond(s) at zinc-finger domain. Alternatively, these mutants may be able to bypass the conformational change and directly form a stable complex with ssDNA because they do not have Zn(II)−cysteines coordinated structure. On the other hand, we cannot rule out the possibility that the zinc-finger may be involved in regulation of ssDNA binding activity through an alternative mechanism such that zinc-finger domain, once forms disulfide bond(s), may function as a negative regulatory element that directly interferes with the neighboring DNA binding domain (*23*). Further structural analysis would be necessary to evaluate the detailed conformational change.

RPA's zinc-finger motif is different from other known zinc-finger proteins in that zinc-finger mutations render RPA redox-insensitive without affecting its DNA binding activity (ref *23* and Figure 3), whereas zinc-finger mutations in most other proteins results in inactivation of DNA binding activity (*30*−*32*). However, the fundamental nature of redox regulation of zinc-finger and its effect on protein−DNA interaction may be similar (*21*, *22*). For example, in many zinc-finger proteins, the binding of Zn(II) stabilizes the folded conformation of the zinc-finger domain so they can facilitate protein−DNA interaction (*33*). wtRPA−ssDNA interaction was significantly inhibited by the chelating agent *o*-phenanthroline but not by a nonchelating structural isomer *p*-phenanthroline, whereas zinc-finger mutants were not affected by either one of them (J.-S.Y. and S.-H.L., data not shown), suggesting that (i) removal of the intrinsic Zn(II) results in oxidation of the cysteines and (ii) the role of Zn(II) in RPA−ssDNA interaction is to stabilize zinc-finger motif through Zn(II)−cysteine coordination. It is conceivable from this study that RPA's zinc-finger is involved in controlling its DNA binding activity through redox change, which may be crucial for its role in regulation of DNA replication and repair in intact cells upon environmental stress.

ACKNOWLEDGMENT

We would like to thank Dr. W. Bosron for allowing us to use the spectrophotometer for DTNB study, Dr. A. Mahrenholz for the suggestions on the BIAcore analysis, and the members of the Lee laboratory for continuous discussions and critical reading of the manuscript.

REFERENCES

1. Wold, M. S. (1997) *Annu. Rev. Biochem. 66*, 61−92.
2. Dornreiter, I., Erdile, L. F., Gilbert, I. U., von Winkler, D., Kelly, T. J., and Fanning, E. (1992) *EMBO J. 11*(2), 769−776.
3. Lee, S. H., and Kim, D. K. (1995) *J. Biol. Chem. 270* (21), 12801−12807.
4. Melendy, T., and Stillman, B. (1993) *J. Biol. Chem. 268* (5), 3389−3395.
5. Collins, K. L., and. Kelly, T. J. (1991) *Mol. Cell. Biol. 11*, 2108−2115.
6. Burns, J. L., Guzder, S. N., Sung, P., Prakash, S., and Prakash, L. (1996) *J. Biol. Chem. 271* (20), 11607−11610.
7. Stigger, E., Drissi, R., and Lee, S.-H. (1998) *J. Biol. Chem. 273*, 9337−9343.
8. Patrick S. M., and Turchi, J. J. (1999) *J. Biol. Chem. 274*, 14972−14978.
9. Matsuda, T., Saijo, M., Kuraoka, I., Kobayashi, T., Nakatsu, Y., Nagai, A., Enjoji, T., Masutani, C., Sugasawa, K., Hanaoka, F., and Tanaka, K. (1995) *J. Biol. Chem. 270* (8), 4152−4157.
10. Li, L., Lu, X., Peterson, C. A., and Legerski, R. J. (1995) *Mol. Cell. Biol. 15* (10), 5396−5402.
11. He, Z., Henricksen, L. A., Wold, M. S., and Ingles, C. J. (1995) *Nature 374*, 566−569.
12. Lee, S. H., Kim, D. K., and Drissi, R. (1995) *J. Biol. Chem. 270* (37), 21800−21805.
13. Matsunaga, T., Park, C. H., Bessho, T., Mu, D., and Sancar, A. (1996) *J. Biol. Chem. 271* (19), 11047−11050.
14. Kim, D. K., Stigger, E., and Lee, S. H. (1996) *J. Biol. Chem. 271* (25), 15124−15129.
15. Gomes, X. V., and Wold, M. S. (1996) *Biochemistry 35* (32), 10558−10568.
16. Lin, Y.-L., Chen, C., Keshav, K. F., Winchester, E., and Dutta, A. (1996) *J. Biol. Chem. 271* (29), 17190−17198.
17. Bochkarev, A., Pfuetzner, R. A., Edwards, A. M., and Frappier, L. (1997) *Nature 385* (6612), 176−181.
18. Gomes, X. V., and Wold, M. S. (1995) *J. Biol. Chem. 270* (9), 4534−4543.
19. Lin, Y.-L., Shivji, M. K. K., Chen, C., Kolodner, R., Wood, R. D., and Dutta, A. (1998) *J. Biol. Chem. 273* (3), 1453−1461.
20. Dong, J., Park, J. S., and Lee, S. H. (1999) *Biochem. J. 337* (Pt 2), 311−317.
21. Nakshatri, H., Bhat-Nakshatri, P., and Currie, R. A. (1996) *J. Biol. Chem. 271* (46), 28784−28791.
22. Xanthoudakis, S., Miao, G., Wang, F., Pan, Y. C., and Curran, T. (1992) *EMBO J. 11* (9), 3323−3335.
23. Park, J. S., Wang, M., Park, S. J., and Lee, S. H. (1999) *J. Biol. Chem. 274* (41), 29075−29080.
24. Luisi, B. F., Xu, W. X., Otwinowski, Z., Freedman, L. P., Yamamoto, K. R., and Sigler, P. B. (1991) *Nature 352* (6335), 497−505.
25. Casadevall, M., and Sarkar, B. (1998) *J. Inorg. Biochem. 71* (3−4), 147−152.
26. Hathout, Y., Fabris, D., Han, M. S., Sowder, R. C., II, Henderson, L. E., and Fenselau, C. (1996) *Drug Metab. Dispos. 24* (12), 1395−1400.
27. Stigger, E., Dean, F. B., Hurwitz, J., and Lee, S. H. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91* (2), 579−583.
28. Qiu, H., Kodadek, T., and Giedroc, D. P. (1994) *J. Biol. Chem. 269* (4), 2773−2781.
29. Wang, M., Mahrenholz, A., and Lee, S.-H. (2000) *Biochemistry 39* (21), 6433−6439.
30. Pan, T., and Coleman, J. E. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87* (6), 2077−2081.
31. Narayan, V. A., Kriwacki, R. W., and Caradonna, J. P. (1997) *J. Biol. Chem. 272* (12), 7801−7809.
32. Eagle, P. A., and Klessig, D. F. (1992) *Virology 187* (2), 777−787.
33. Berg, J. M., and Shi, Y. (1996) *Science 271* (5252), 1081−1085.

BI001206F