RPA Stabilizes the XPA-Damaged DNA Complex through Protein−Protein Interaction†

Mu Wang, Alan Mahrenholz, and Suk-Hee Lee*

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

Received March 1, 2000; Revised Manuscript Received March 29, 2000

ABSTRACT: The xeroderma pigmentosum group A complementing protein (XPA) and eukaryotic replication protein A (RPA) are among the major damage-recognition proteins involved in the early stage of nucleotide excision repair (NER). XPA and RPA are able to bind damaged DNA independently, although RPA interaction stimulates XPA binding to damaged DNA [Li, L., Lu, X., Peterson, C. A., and Legerski, R. J. (1995) *Mol. Cell. Biol. 15*, 5396−5402 (*1*); Stigger, E., Drissi, R., and Lee, S.-H. (1998) *J. Biol. Chem. 273*, 9337−9343 (*2*)]. In this study, we used surface plasmon resonance (SPR) analysis to investigate the interaction of XPA and RPA with two major types of UV-damaged DNA: the (6-4) photoproduct and the *cis-syn* cyclobutane dimer of thymidine. Both XPA and RPA preferentially bind to (6-4) photoproduct-containing duplex DNA over *cis-syn* cyclobutane dimer-containing DNA. The binding of XPA to (6-4) photoproduct was weak ($K_D = 2.13 \times 10^{-8}$ M), whereas RPA showed a very stable interaction with (6-4) photoproduct ($K_D = 2.02 \times 10^{-10}$ M). When XPA and RPA were incubated together, the stability of the XPA-damaged DNA interaction was significantly enhanced by wild-type RPA. On the other hand, mutant RPA (RPA:p34Δ33C) defective in its interaction with XPA failed to stabilize XPA-damaged DNA complex. Taken together, our results suggest that a role for RPA in UV-damage recognition is to stabilize XPA-damaged DNA complex through protein−protein interaction.

Nucleotide excision repair (NER)[1] is a major DNA repair pathway to remove DNA damage induced by ultraviolet (UV) light and chemical carcinogens (*3, 4*). Defects in NER proteins can cause severe genetic diseases such as xeroderma pigmentosum (XP) and Cockayne's syndrome (CS). Much progress has been made during the past decade in understanding these genetically inherited diseases, and to date, seven XP repair-deficient complementation groups (XP-A to XP-G) and a variant form have been identified (*5, 6*). Use of an in vitro repair system with XP-deficient mutants has led to the isolation and biochemical characterization of the proteins involved in NER. These protein factors include XPA, replication protein A (RPA), the p62 and p89 subunits of the basal transcription factor, TFIIH (XPB and XPD), ERCC1-XPF, XPG, XPC-hHR23B, XPE, and PCNA (*7, 8*).

The *XPA* gene product (XPA) is a multifunctional zinc-finger protein that is involved in the damage recognition step of NER (*9*−*11*). It has been shown that XPA preferentially binds to (6-4) photoproduct of UV-irradiated DNA and may also play a role in subsequent steps of NER through interaction with other repair proteins (*11*). XPA interacts with RPA, which is crucial for damage-recognition and for recruiting other DNA repair proteins to the damaged site (*12*). RPA is a heterotrimeric protein complex consisting of 70-, 34-, and 11-kDa subunits involved in DNA replication, repair, and recombination (*13*). RPA is a four-cysteine-type zinc-finger protein, and its zinc-finger motif is involved in redox regulation of its ssDNA binding activity (*14*). In NER, RPA interacts with XPA on damaged DNA and stimulates XPA−DNA interaction and also recruits other repair proteins such as XPG, ERCC1-XPF, and TFIIH to the damaged site for the subsequent incision/excision step (*12, 15*). RPA may also be involved in a later stage of NER, gap-filling, that requires PCNA, RF-C, and DNA polymerase δ (or ϵ) (*16*).

To understand the recognition of UV-damaged DNA by XPA and RPA, we carried out a real-time analysis study for the interaction of these two proteins with damaged DNA using the surface plasmon resonance (SPR) technique. Our results showed that both XPA and RPA preferentially bound to (6-4) photoproduct-containing DNA independently of each other. The XPA−DNA interaction was relatively weak and characterized by rapid dissociation, whereas RPA formed a 100-fold more stable complex with UV-damaged DNA. Wild-type RPA, but not mutant (RPA:p34Δ33C) lacking the XPA interaction domain, led to stabilization of XPA-damaged DNA complex, which implicates a role for RPA in early stage of DNA repair.

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

* Corresponding author: Department of Biochemistry & Molecular Biology, Indiana University School of Medicine, 635 Barnhill Dr., Indianapolis, IN 46202. Phone: (317) 278-3464; Fax: (317) 274-4686; Email: slee@iupui.edu.

[1] Abbreviations: DTT, dithiothreitol; ERCC, excision repair cross-complementing; hHR23, human homologue of the yeast RAD23; HPLC, high-performance liquid chromatography; NER, nucleotide excision repair; PAGE, polyacrylamide gel electrophoresis; PCNA, proliferating cell nuclear antigen; RF-C, replication factor C; RPA, replication protein A; RU, resonance unit; SPR, surface plasmon resonance; ssDNA, single-stranded DNA; TFIIH, transcription factor II H; UV, ultraviolet light; XP, xeroderma pigmentosum.

10.1021/bi000472q CCC: $19.00
Published on Web 05/02/2000

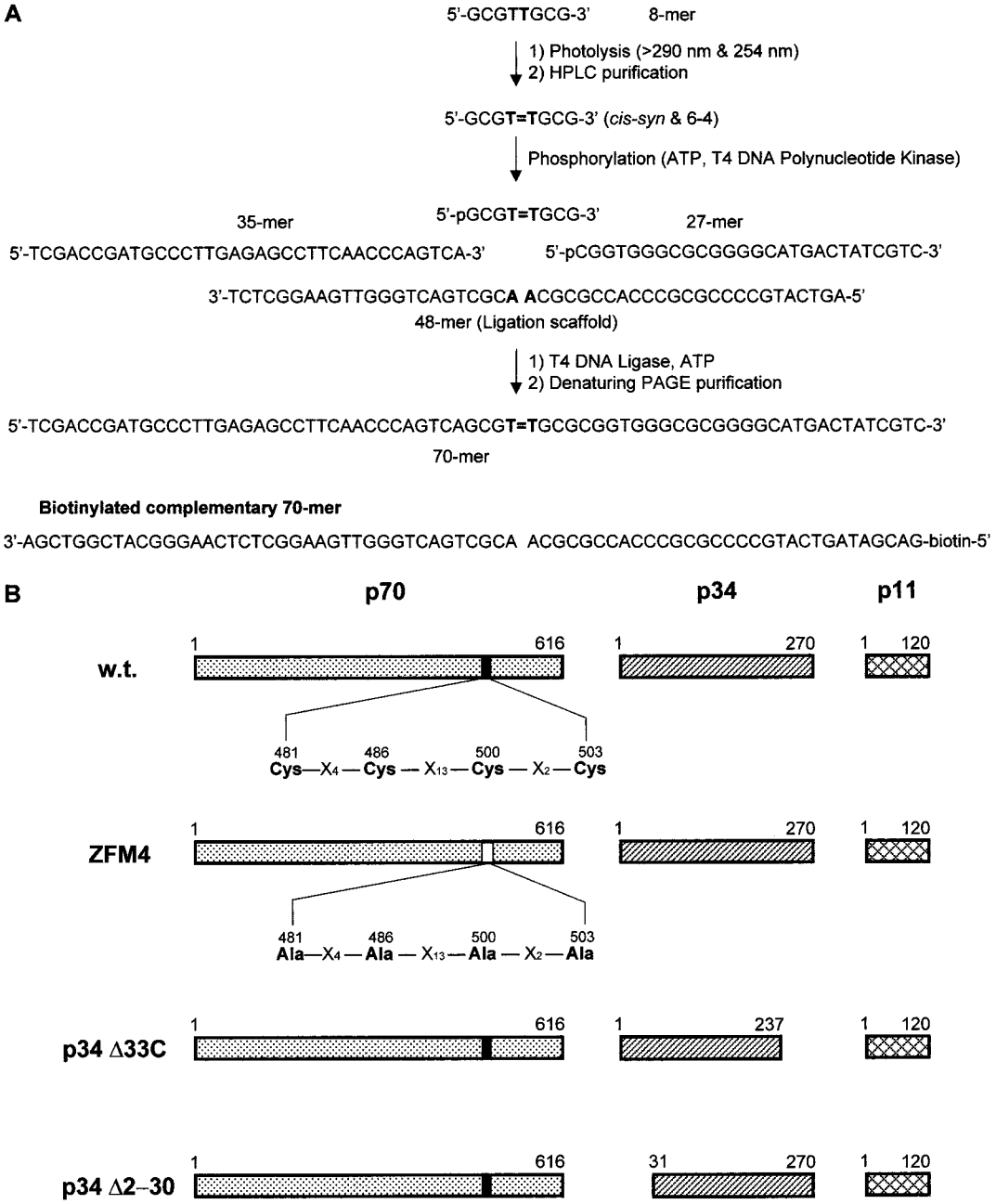

FIGURE 1: Oligonucleotides and proteins used for this study. (A) Schematic presentation of preparation of oligonucleotides containing thymidine dimer photoproducts. **T=T** refers to either a *cis-syn* cyclobutane dimer or a (6-4) photoproduct of TpT. (B) Schematic diagram of wild-type and mutant RPAs.

MATERIALS AND METHODS

Preparation of DNA and Proteins. Oligonucleotide containing either a *cis-syn* cyclobutane thymidine dimer or a (6-4) photoproduct was prepared according to previously published procedures (*17*−*19*). For *cis-syn* cyclobutane dimer preparation, an anoxic aqueous solution containing 500 μg of single-stranded 8-mer, d(GCGTTGCG), and 20 mM acetophenone was irradiated with a 100-W Sylvania black-light lamp filtered through a glass-filter. T-T *cis-syn* cyclobutane dimer containing 8-mer was then isolated by HPLC using a VYDAC-C_{18} reversed-phase column and a linear gradient of 5−11% (v/v) acetonitrile in 0.1 M triethylammonium acetate (TEAA, pH 7.0). This sensitized photolysis of d(GCGTTGCG) with >290-nm light gave the *cis-syn* cyclobutane dimer-containing 8-mer as a major photoproduct with an approximate yield of 18% after HPLC purification. Purified 8-mer containing a *cis-syn* cyclobutane dimer was then confirmed by T4 endonuclease V cleavage (*19*). For (6-4) photoproduct preparation, 500 μg of single-stranded d(GCGTTGCG) in an anoxic aqueous solution was irradiated with 90 kJ/m^2 of a 254-nm UV lamp at a fluence rate of 10 J m^{-2} s^{-1} and purified by HPLC as described for the *cis-syn* cyclobutane dimer containing 8-mer purification. This direct photolysis resulted in about 3% overall yield of the (6-4) photoproduct, as characterized by an A_{260}/A_{325} ratio of 11.8, and by cleavage with 1 M piperidine at 95 °C (*19*). The 8-mer containing either a *cis-syn* cyclobutane dimer or a (6-4) photoproduct was subsequently ligated to form a 70-mer using a scaffolding method as shown in Figure 1A and purified by 15% denaturing polyacrylamide gel electrophoresis (PAGE).

The N-terminal histidine-tagged XPA was expressed in *E. coli* and purified using Ni-NTA and conventional column chromatographic techniques as described previously (*20*). Recombinant human RPA proteins were isolated from insect cells coinfected with baculoviruses encoding wild-type and mutant RPA subunits (see Figure 1B) using procedures described previously (*21*).

Biomolecular Interaction Analysis of XPA/RPA with UV-Damaged DNA. Interactions of XPA and RPA with damaged DNA were monitored using a surface plasmon resonance (SPR) biosensor instrument, BIAcore 3000 (Biacore). For preparation of biosensor surfaces with DNA, a 5-fold molar excess of a 70-mer oligonucleotide containing a *cis-syn* cyclobutane dimer, a (6-4) photoproduct, and nondamaged DNA were annealed to a 5′-biotinylated complementary 70-mer DNA, respectively (Figure 1A), and purified by 12% native PAGE. The purified duplex DNAs containing specific damages were 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 refractive index change correction. Proteins were diluted into 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. Following RPA binding, regeneration was performed with a 30-s quick-injection of 0.25% SDS in buffer; regeneration was not required following XPA binding. Each experiment was repeated at least twice to ensure the reproducibility.

Surface Plasmon Resonance Analysis. Bia-evaluation software supplied by the vendor was used to analyze binding data using a simple Langmuir 1:1 model. Thus, for the model [A] + [B] ⇔ [AB], where [A] = concentration of analyte, total ligand on the surface $[B_0] = R_{max}$, initial response $[AB_0] = 0$, local (for each curve) numerical integration was performed using the differential equation:

$$d[AB]/dt = k_a[A][B] - k_d[AB]$$

The fit of the data was reported as an average sum of differences (χ^2) between predicted and observed data and as standard errors.

RESULTS

Preferential Binding of XPA and RPA to (6-4) Photoproduct over cis-syn Cyclobutane Dimer. Specific binding of XPA and RPA to damaged double-stranded DNA has been reported previously using either DNA pull-down or electrophoretic gel mobility shift assays (*2, 11, 22*). However, most of the studies were conducted with plasmid DNA irradiated with a high UV-dose in which the extent and types of damage were not well characterized. To study damage-specific recognition of XPA and RPA, we constructed UV damage-containing duplex DNA with a defined lesion at a specific site (Figure 1A). The binding affinity of XPA, RPA, or XPA−RPA complex to damaged DNA was examined using surface plasmon resonance (SPR), which allows macromolecular interactions to be measured in real-time. The sensorgram of XPA-damaged DNA interaction showed that XPA bound to (6-4) photoproduct-containing DNA with a higher affinity than to *cis-syn* cyclobutane dimer-containing DNA or nondamaged DNA (Figure 2A). Analysis of the association data obtained from binding of protein concentrations ranging from 0.5 to 10 nM indicated that XPA has approximately a 3-fold higher affinity for the (6-4) photoproduct compared with nondamaged DNA (Table 1). This observation was consistent with previous reports (*11, 23*). It should be pointed out that preferential binding of XPA to (6-4) photoproduct was observed only with the biosensor chip that had high levels of DNA immobilized on the surfaces (2000 RU of response corresponding to approximately 2 pg of immobilized DNA) (Figure 2A). No significant binding was observed with a biosensor chip containing low levels of DNA (33 RU) (Figure 2A). This can be attributed to XPA's low-affinity binding to duplex DNA regardless of UV-damage (Table 1).

Table 1: Equilibrium and Kinetic Binding Constants of XPA and RPA Binding to DNA, Obtained Using the Local Fitting Model[a]

XPA			
DNA	K_D (M)	k_a (s^{-1} M^{-1})	k_d (s^{-1})
6-4	$(2.13 \pm 0.23) \times 10^{-8}$	$(2.58 \pm 0.14) \times 10^{5}$	$(5.46 \pm 0.29) \times 10^{-3}$
C-S	$(4.58 \pm 0.37) \times 10^{-8}$	$(1.20 \pm 0.05) \times 10^{5}$	$(4.77 \pm 0.19) \times 10^{-3}$
ND	$(5.78 \pm 0.59) \times 10^{-8}$	$(5.00 \pm 0.26) \times 10^{4}$	$(2.62 \pm 0.13) \times 10^{-3}$
SS	$(1.30 \pm 0.12) \times 10^{-8}$	$(1.26 \pm 0.09) \times 10^{6}$	$(1.60 \pm 0.04) \times 10^{-2}$
RPA			
DNA	K_D (M)	k_a (s^{-1} M^{-1})	k_d (s^{-1})
6-4	$(2.02 \pm 0.03) \times 10^{-10}$	$(3.66 \pm 0.01) \times 10^{6}$	$(7.39 \pm 0.10) \times 10^{-4}$
C-S	$(3.84 \pm 0.09) \times 10^{-10}$	$(3.43 \pm 0.02) \times 10^{6}$	$(1.31 \pm 0.03) \times 10^{-3}$
ND	$(7.46 \pm 0.19) \times 10^{-10}$	$(2.35 \pm 0.02) \times 10^{6}$	$(1.75 \pm 0.03) \times 10^{-3}$
SS	$(7.30 \pm 0.51) \times 10^{-11}$	$(1.21 \pm 0.041) \times 10^{7}$	$(8.83 \pm 0.32) \times 10^{-4}$

[a] 6-4, C-S, and ND represent duplex DNA containing (6-4) photoproduct, *cis-syn* cyclobutane dimer, and nondamaged DNA, respectively. SS represents nondamaged single-stranded DNA. Binding constants were determined using 2000 RU surfaces for XPA, and 33 RU surfaces for RPA.

RPA bound to (6-4) photoproduct-containing DNA with nearly 2-fold higher affinity relative to *cis-syn* cyclobutane dimer and nearly 4-fold greater than nondamaged DNA (Figure 2B, Table 1). RPA bound to all DNA surfaces with 100-fold greater affinity than XPA. Thus, kinetic analysis of RPA binding required biosensor surfaces derivatized with low levels of DNA (33 RU) in order to avoid mass-transfer effects observed at higher levels of surface (2000 RU) and to approach equilibrium binding (Figure 2B). Both RPA zinc-finger mutant (RPA:ZFM4, cysteine-to-alanine mutations at C_{481}, C_{486}, C_{500}, and C_{503}) and RPA lacking 33 amino acids of p34 C-terminal (RPA:p34Δ33C) poorly supported NER activity (*2*) but still showed preferential binding to (6-4) photoproduct-containing DNA compared to those containing no damage or *cis-syn* cyclobutane dimer (Figure 3), suggesting that these mutant RPAs are not defective in interaction with damaged DNA.

Although XPA showed preferential binding to (6-4) photoproduct-containing DNA, its interaction with all DNA surfaces was relatively weak, as indicated by the derived K_D of 2.13×10^{-8} M, 1/100th of the affinity observed for RPA. In fact, it was necessary to utilize densly derivatized DNA surfaces (2000 RU) to show XPA binding at all (Figure 2A). It seems reasonable to attribute this behavior to the very rapid dissociation, seen in Figure 2A and Table 1. On the other hand, interaction of RPA with (6-4) photoproduct-containing DNA showed avid binding with slow dissociation

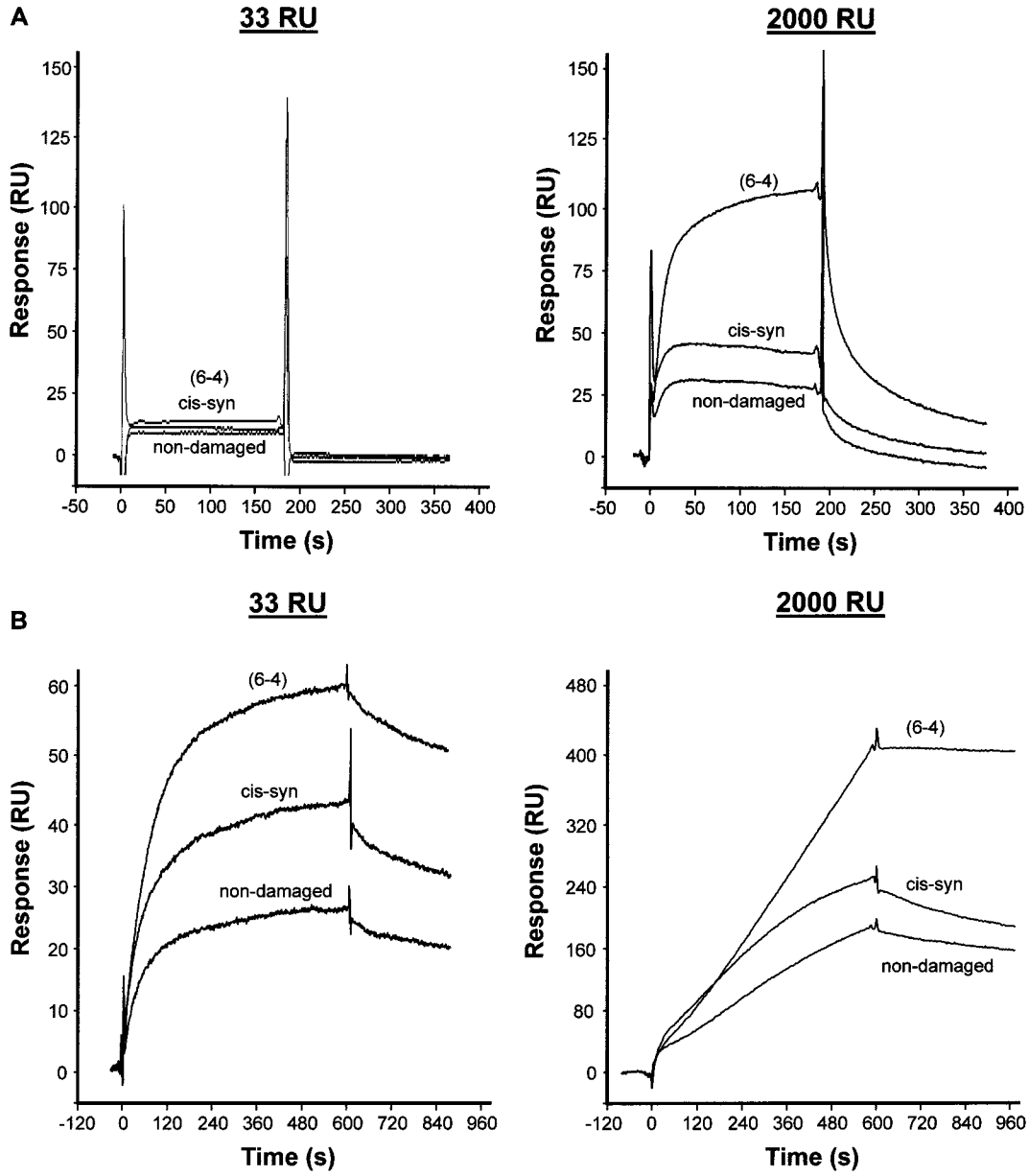

FIGURE 2: Preferential binding of XPA and RPA to (6-4) photoproduct over *cis-syn* cyclobutane dimer. (A) XPA (10 nM) was injected on either the low- (33 RU; left figure) or the high-level DNA (2000 RU; right figure) containing (6-4) photoproduct, *cis-syn* cyclobutane dimer, or nondamaged DNA using the KINJECT function of BIAcore 3000. Binding was allowed to proceed for 180 s of association phase followed by 240 s of running buffer injection period for dissociation. All curves are corrected for bulk shift using an underivatized flow cell. (B) Binding response of RPA to (6-4) photoproduct-containing DNA, *cis-syn* cyclobutane dimer-containing DNA, and nondamaged DNA. 2.5 nM RPA was injected onto 33 RU (left panel) or 2000 RU (right panel) DNA surfaces using the KINJECT function of BIAcore 3000. Binding was allowed to proceed for 600 s of association phase followed by 300 s of buffer injection period for dissociation.

(Figure 2B and Table 1). Taken together, we conclude that interaction of XPA with UV-damaged DNA is highly unstable, whereas RPA forms a stable complex with damaged DNA.

RPA Stabilizes XPA UV-Damaged DNA Complex through Protein−Protein Interaction. Previous studies suggested that RPA stimulated XPA-damaged DNA interaction, which is necessary for NER activity (*1*, *2*). To investigate this further, RPA, XPA, and a mixture of the two proteins were injected onto biosensor chip containing high levels of (6-4) photoproduct-containing DNA (2000 RU). As shown in Figure 4A, injection of 2.5 nM XPA quickly dissociated from (6-4) photoproduct-containing DNA, and the response returned to baseline within the time-course of the experiment, whereas RPA (1 nM) remained bound to the DNA surface for this time-course. Injection of a mixture of XPA and wild-type RPA resulted in a response which was slightly more than the sum of each protein binding independently to (6-4) photoproduct-containing DNA. Following the association phase, a biphasic dissociation curve was observed, characterized by an initial rapid dissociation followed by a slower dissociation which appeared similar to RPA's characteristic dissociation rate. Interestingly, during the time-course of the dissociation, the overall response remained higher than that seen with RPA alone, indicating that additional protein was bound to the DNA surface in the presence of the mixture (Figure 4A). This result suggests that low-affinity binding of XPA to damaged DNA be stabilized by RPA. The effect of RPA on stabilization of the XPA−DNA interaction was not specific to damaged DNA since it was observed with

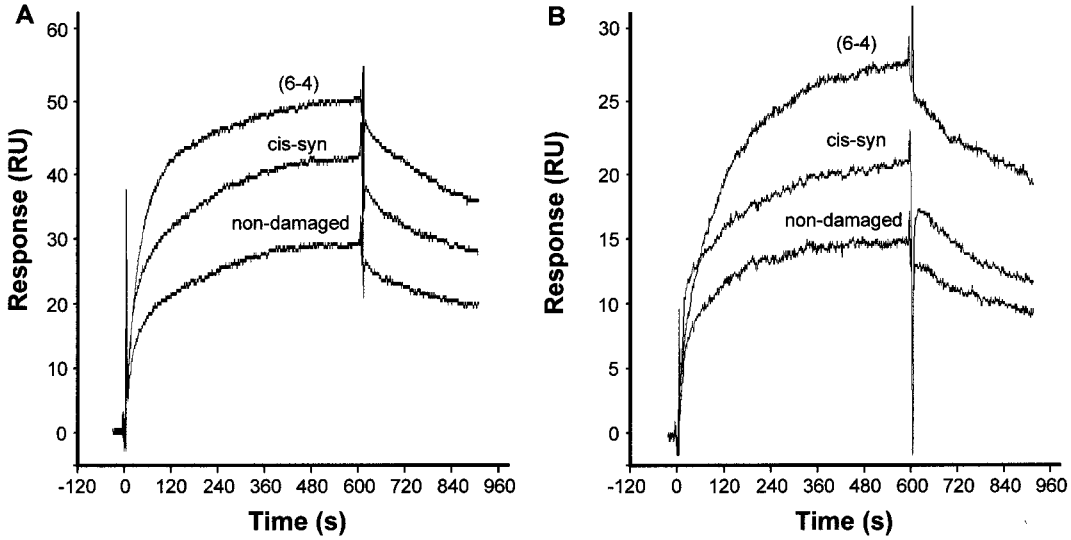

FIGURE 3: Binding of mutant RPAs to UV-damaged duplex DNA. (A) 2.5 nM zinc-finger mutant RPA (RPA:ZFM4) was injected on the low-level DNA (33 RU) containing (6-4), *cis-syn*, or nondamaged DNA. Binding was allowed for 600 s of association phase followed by 300 s of buffer injection period for dissociation. All curves are corrected for bulk shift using an underivatized flow cell. (B) Binding response of 2.5 nM mutant RPA lacking 33 amino acids at the p34 C-terminus (RPA:p34Δ33C) to (6-4) photoproduct-containing DNA, *cis-syn* cyclobutane dimer-containing DNA, or nondamaged DNA. 2.5 nM RPA was injected onto the low-level DNA (33 RU) biosensor chip.

DNA surfaces containing either a *cis-syn* cyclobutane dimer or nondamaged DNA (Figure 4E). It should be pointed out, however, that interaction of RPA with XPA on damaged DNA was much more efficient than that observed with nondamaged DNA (compare RU values between Figure 4A and 4E).

We then examined whether XPA-damaged DNA complex is stabilized through XPA−RPA interaction. For this, the RPA mutant lacking the p34 phosphorylation sites (RPA:p34Δ2−30), the RPA mutant lacking the p34 C-terminal XPA interaction domain (RPA:p34Δ33C), and the zinc-finger mutant (RPA:ZFM4) were tested. Previous pull-down assay of XPA-damaged DNA complex showed that RPA:p34Δ2−30 and RPA:ZFM4 enhanced XPA-damaged DNA interaction, but RPA:p34Δ33C did not (*2*). In our real-time SPR analysis, phosphorylation mutant (RPA:p34Δ2−30) and zinc-finger mutant (RPA:ZFM4) were able to stabilize XPA-damaged DNA interaction (Figure 4B,C), whereas RPA:p34Δ33C could not (Figure 4D). This result is in keeping with previous NER assays with these mutants (*2*) and strongly suggests that RPA's role in NER reaction may be to stabilize the interaction between XPA-damaged DNA through protein−protein interaction.

DISCUSSION

In this study, we analyzed the interaction of XPA and RPA with two major types of UV-induced DNA damage, (6−4) photoproduct and *cis-syn* cyclobutane dimer, using the SPR technique. This biosensor procedure enables us to monitor the interaction of proteins with biotinylated damaged DNA probes immobilized on a streptavidin biosensor chip in real time. This feature also allows us to examine unstable macromolecular interactions not normally detected by gel mobility shift assay. Furthermore, the SPR method may provide us more accurate kinetic parameters describing these interactions.

An earlier study with UV-damaged DNA indicated that reversal of cyclobutane pyrimidine dimers from UV-irradiated DNA by enzymatic photoreactivation did not significantly reduce binding of XPA to the irradiated DNA, suggesting that XPA preferentially binds to (6-4) photoproducts over cyclobutane dimers (*11*). In this study, XPA showed preferential binding to (6-4) photoproduct relative to *cis-syn* cyclobutane dimer or nondamaged DNA with surfaces containing high levels of duplex DNA (2000 RU), but not with low or moderately derivatized surfaces (33 or 230 RU) (Figure 2A and data not shown). This result is not surprising in view of the very rapid dissociation rate (k_d = 5.46 × 10^{-3} s^{-1}) determined for XPA, which requires increased levels of ligand in order to obtain a significant amount of complex formation (*24*). RPA also preferentially binds to UV-damaged DNA, and the interaction of RPA with UV-irradiated DNA is not affected by prior enzymatic photoreactivation of the DNA, suggesting specificity of RPA for the (6-4) photoproducts (*22*). SPR analysis confirmed this preference for (6-4) photoproduct, and demonstrated that RPA has approximately 100-fold greater affinity for (6-4) photoproduct (K_D = 2.02 × 10^{-10} M) relative to XPA. This binding affinity was comparable to that determined for ssDNA (K_D = 7.30 × 10^{-11} M). Even though the SPR method is not a solution-based measurement, the K_D value was derived from k_d/k_a using an established fitting model. Nonetheless, SPR analysis of a strong DNA binding protein such as RPA may not be able to reach a steady-state equilibrium completely (as seen in Figure 2); therefore, we will use the term approximate K_D for equilibrium constants of XPA and RPA in this study.

In SPR analysis, both XPA and RPA showed 2−3-fold preference to UV-damaged DNA over nondamaged DNA. This raises a question as to whether XPA and/or RPA are responsible for recognition of UV-damaged DNA in vivo. In NER reactions, three XP proteins (XPA, XPC, and XPE), RPA, and TFIIH are all able to interact with UV-damaged DNA. In addition, XPC-hHR23B and XPE showed a higher binding affinity to UV-damaged DNA compared with XPA (*25*−*27*). Nonetheless, XPA is essential for NER activity,

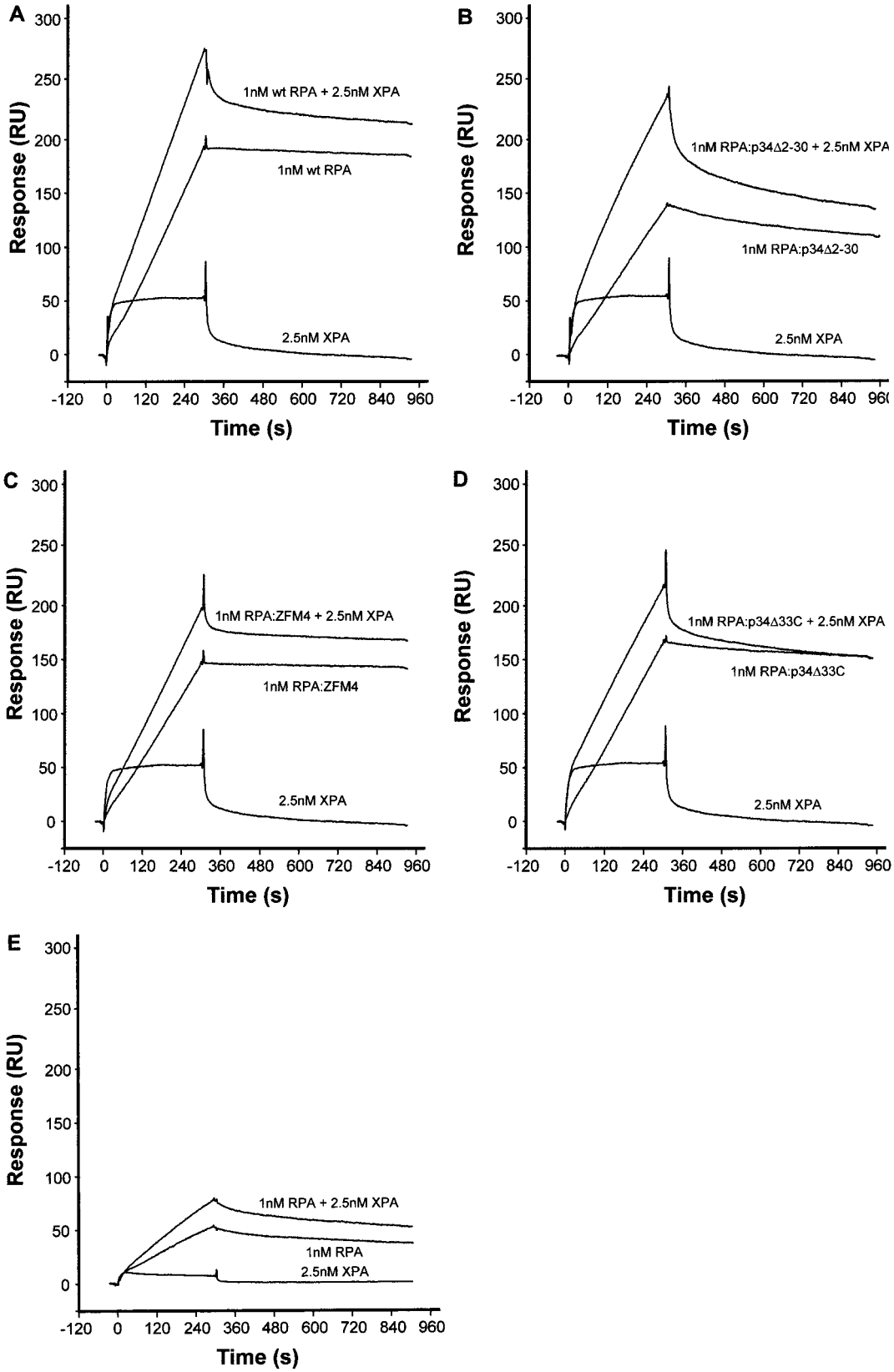

FIGURE 4: Stabilization of XPA binding to (6-4) photoproduct by RPA. Binding analysis of XPA (2.5 nM), RPA (1.0 nM), and the mixture of XPA and RPA on 2000 RU of DNA surface containing (6-4) photoproduct. Wild-type RPA (panels A and E) was replaced with various mutant RPAs in panel B (RPA:p34Δ2−30), panel C (RPA:ZFM4), and panel D (RPA:p34Δ33C). In panel E, 2000 RU of nondamaged DNA was used in place of UV-damaged DNA. Binding to (6-4) damaged DNA was performed for 300 s at 20 μL/min followed by dissociation for 600 s of buffer injection period for dissociation. All curves are corrected for bulk shift.

whereas XPC and XPE mutant cells only show moderate decrease in repair activity (*25*, *27*). This suggests that XPA may have a specific role such as recruiting other repair factor-(s) to the damaged DNA sites in the early stage of repair. In mammalian cells, the XPA, XPC-hHR23B, RPA, and TFIIH factors may all have a role in damage recognition (*28*).

The zinc-finger domain has been shown previously not to be essential for its high-affinity ssDNA binding activity (*29*, *30*), whereas recent study suggested that RPA's zinc-finger domain may be involved in recognition of ssDNA-containing (6-4) photoproduct (*31*). It is not clear what physiological role the zinc-finger domain has in recognition of ssDNA-containing UV-damage. However, our real-time SPR analysis described here strongly suggests that the zinc-finger domain is (i) not essential for recognition of double-stranded DNA regardless of UV damage and (ii) not required for stabilization of XPA-damaged duplex DNA interaction. Considering that RPA's zinc-finger is involved in redox regulation of its ssDNA binding (*14*), it may also play a role in recognition of UV-damaged DNA. In fact, our recent work strongly suggests that the binding of XPA and RPA to damaged-DNA be regulated by redox chemistry (M.W. and S.-H.L., unpublished result).

Earlier studies demonstrated that RPA stimulates interaction of XPA with UV-damaged DNA (*1*, *2*). This study also showed that coincubation of XPA and RPA prior to interaction with damaged DNA stabilized XPA-damaged DNA interaction. If XPA and RPA bound independently to damaged DNA, we would expect to observe the dissociation curve from the mixture (XPA + RPA) to return to the same level as that from RPA alone because XPA dissociates very quickly. In the case of XPA and wild-type RPA, the observed binding was the sum predicted from the two individual proteins (or slightly higher), but the dissociation phase was significantly altered. In fact, the dissociation is seen to be biphasic, consisting of a rapid dissociation of excess XPA (XPA is in 2.5-fold molar excess relative to RPA) and a slower dissociation similar to that of RPA alone. We interpret this net excess protein binding relative to that seen for RPA alone as bound complex formed between XPA and RPA. This view is based on results reported using antibodies in a gel super-shift assay to suggest the presence of both RPA and XPA in such complexes (*25*). Incubation of XPA and a mutant RPA lacking the XPA interaction domain (RPA: p34Δ33C) showed the dissociation curve from the mixture went down to the same level as that from RPA alone, suggesting a role for RPA in stabilizing XPA-damaged DNA complex through protein−protein interaction (Figure 4D). Even though XPA also interacts with RPA p70 (*3*, *4*, *13*), we can conclude that the interaction with p34 is more important for XPA-damaged DNA interaction.

In conclusion, we have used the SPR technique to demonstrate that the weak interaction between XPA and UV-damaged DNA is attributable to a very fast dissociation rate and that RPA has a 100-fold greater affinity for UV-damaged DNA, with a fast association rate and a slow dissociation rate. Importantly, we might also provide other evidence for formation of a complex between RPA and XPA on damaged DNA, which results in stabilization of the XPA-damaged DNA interaction.

ACKNOWLEDGMENT

We thank Dr. Richard Bockrath for providing us with the Sylvania blacklight lamp, Dr. John Hawes for help with HPLC purification of damaged DNA-containing oligonucleotides, and Dr. David Myszka (University of Utah) for valuable suggestions throughout the course of this study.

REFERENCES

1. Li, L., Lu, X., Peterson, C. A., and Legerski, R. J. (1995) *Mol. Cell. Biol. 15*, 5396−5402.
2. Stigger, E., Drissi, R., and Lee, S. H. (1998) *J. Biol. Chem. 273*, 9337−9343.
3. Sancar, A. (1996) *Annu. Rev. Biochem. 65*, 43−81.
4. Wood, R. D. (1996) *Annu. Rev. Biochem. 65*, 135−167.
5. Reardon, J. T., Thompson, L. H., and Sancar, A. (1993) *Cold Spring Harbor Symp. Quant. Biol. 58*, 605−617.
6. Wood, R. D., Aboussekhra, A., Biggerstaff, M., Jones, C. J., O'Donovan, A., Shivji, M. K., and Szymkowski, D. E. (1993) *Cold Spring Harbor Symp. Quant. Biol. 58*, 625−632.
7. Balajee, A. S., May, A., and Bohr, V. A. (1998) *Mutat. Res. 404*, 3−11.
8. Wood, R. D. (1999) *Biochimie 81*, 39−44.
9. Robins, P., Jones, C. J., Biggerstaff, M., Lindahl, T., and Wood, R. D. (1991) *EMBO J. 10*, 3913−3921.
10. Guzder, S. N., Sung, P., Prakash, L., and Prakash, S. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 5433−5437.
11. Jones, C. J., and Wood, R. D. (1993) *Biochemistry 32*, 12096−12104.
12. He, Z., Henricksen, L. A., Wold, M. S., and Ingles, C. J. (1995) *Nature 374*, 566−569.
13. Wold, M. S. (1997) *Annu. Rev. Biochem. 66*, 61−92.
14. Park, J. S., Wang, M., Park, S. J., and Lee, S. H. (1999) *J. Biol. Chem. 274*, 29075−29080.
15. Matsunaga, T., Park, C. H., Bessho, T., Mu, D., and Sancar, A. (1996) *J. Biol. Chem. 271*, 11047−11050.
16. Aboussekhra, A., Biggerstaff, M., Shivji, M. K., Vilpo, J. A., Moncollin, V., Podust, V. N., Protic, M., Hubscher, U., Egly, J. M., and Wood, R. D. (1995) *Cell 80*, 859−868.
17. Banerjee, S. K., Christensen, R. B., Lawrence, C. W., and LeClerc, J. E. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 8141−8145.
18. LeClerc, J. E., Borden, A., and Lawrence, C. W. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 9685−9689.
19. Smith, C. A., and Taylor, J. S. (1993) *J. Biol. Chem. 268*, 11143−11151.
20. Lee, S. H., Kim, D. K., and Drissi, R. (1995) *J. Biol. Chem. 270*, 21800−21805.
21. Stigger, E., Dean, F. B., Hurwitz, J., and Lee, S. H. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91*, 579−583.
22. Burns, J. L., Guzder, S. N., Sung, P., Prakash, S., and Prakash, L. (1996) *J. Biol. Chem. 271*, 11607−11610.
23. Cleaver, J. E., Charles, W. C., McDowell, M. L., Sadinski, W. J., and Mitchell, D. L. (1995) *Cancer Res. 55*, 6152−6160.
24. Fersht, A. (1985) *Enzyme Structure and Mechanism*, Second ed., W. H. Freeman and Company, New York.
25. Wakasugi, M., and Sancar, A. (1999) *J. Biol. Chem. 274*, 18759−18768.
26. Hwang, B. J., and Chu, G. (1993) *Biochemistry 32*, 1657−1666.
27. Hwang, B. J., Ford, J. M., Hanawalt, P. C., and Chu, G. (1999) *Proc. Natl. Acad. Sci. U.S.A. 96*, 424−428.
28. Batty, D. P., and Wood, R. D. (2000) *Gene 241*, 193−204.
29. Kim, D. K., Stigger, E., and Lee, S. H. (1996) *J. Biol. Chem. 271*, 15124−15129.
30. Gomes, X. V., and Wold, M. S. (1996) *Biochemistry 35*, 10558−10568.
31. Lao, Y., Gomes, X. V., Ren, Y., Taylor, J. S., and Wold, M. S. (2000) *Biochemistry 39*, 850−859.

BI000472Q