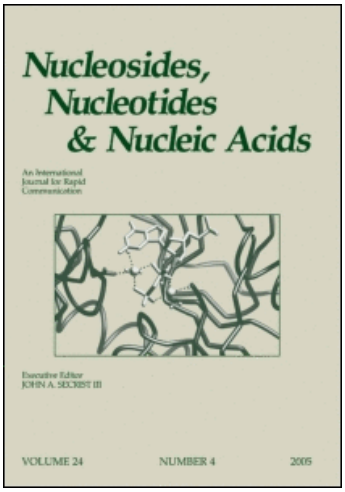

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Recognition of Oxidized Thymine Base on the Single-Stranded DNA by Replication Protein A

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RECOGNITION OF OXIDIZED THYMINE BASE ON THE SINGLE-STRANDED DNA BY REPLICATION PROTEIN A

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□ *Replication protein A (RAP) is a eukaryotic single-stranded DNA binding protein involved in DNA replication, repair, and recombination. Recent studies indicate that RPA preferentially binds the damaged sites rather than the undamaged sites. Therefore, RPA is thought to be a member of repair factories or a sensor of lesion on DNA. To obtain further information of behavior of RPA against the oxidized lesion, we studied the binding affinity of RPA for the single-stranded DNA containing 5-formyluracil, a major lesion of thymine base yielded by the oxidation, using several synthetic oligonucleotides. The affinity of RPA for oligonucleotides was determined by gel shift assay. Results suggest that the surrounding sequence of 5-formyluracil may affect the affinity for RPA, and that the 5-formyluracil on the purine stretch but not the pyrimidine stretch increases the affinity for RPA. Results of affinity labeling experiment of RPA with the oligonucleotides containing 5-formyluracil indicate that RPA1 subunit may directly recognize and bind to the 5-formyluracil on the single-stranded DNA.*

Keywords 5-Formyluracil; Affinity labeling; Recognition; Replication protein

INTRODUCTION

Several external or internal factors induce many types of lesions on the chromosomal DNA. For example, ultraviolet light forms the thymine-thymine dimer and pyrimidine(6-4)pyrimidone photoproduct, whereas active oxygen yields several oxidized bases such as 8-oxoguanine, 2-hydroxyadenine, or 5-formyluracil.[1–5] These lesions lead to mutations during DNA replication with a high frequency. To avoid these mutations, the lesions are recognized and repaired through the cellular repair pathways.

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This article is dedicated to Professor Eiko Ohtsuka on the occasion of her 70th birthday.
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The XPA or XPC, a member of the xeroderma pigmentosum gene products, is a well known protein factor that recognizes several DNA lesions, such as pyrimidine(6-4)pyrimidone photoproduct or acetylaminofluorene adduct.[6–13] Recently, it is reported that the replication protein A (RPA) also shows a high affinity for the pyrimidine(6-4)pyrimidone or cisplatin adduct.[14–20] Furthermore, Zou and Flledge have reported that RPA may recruit ATR/ATRIP complex, an essential protein kinase for the cell cycle checkpoint, to the lesion site.[21] Therefore, RPA has an important role on the cell cycle checkpoint as a sensor of lesion on DNA. RPA was firstly found as a protein factor required for the eukaryotic DNA replication by the analysis of *in vitro* human DNA replication system.[22] RPA is a single-stranded DNA-binding protein composed of three subunits, 70 kDa (RPA1), 32 kDa (RPA2), and 14 kDa (RPA3). Among them, both RPA1 and 2 subunits have DNA binding activity.[22] Although several characteristics of RPA on the recognition of DNA damages are reported, the behavior of RPA against the oxidized lesion is not clarified.

The 5-formyluracil is a major lesion yielded by the oxidation of thymine base.[4,5] Studies with the prokaryotic and eukaryotic cells have revealed that the 5-formyluracil is a mutagenic nucleotide base.[23–29] Indeed, DNA polymerase incorporates dGTP or dCTP, in addition to dATP, opposite 5-formyluracil residue on the template.[30–34] Differently from the UV-damage or cisplatin adduct, the 5-formyluracil is repaired by the base excision. Many repair enzymes for the 5-formyluracil are reported.[35–41] To elucidate the exact role of RPA on the recognition of oxidized bases, we studied the affinity of RPA for 5-formyluracil by the gel shift assay using the synthetic oligonucleotides containing 5-formyluracil at the defined site. Here, we demonstrate that RPA may directly recognize and bind to the 5-formyluracil on the single-stranded DNA with a high affinity.[42]

MATERIALS AND METHODS

Oligonucleotides

Oligonucleotides containing 5-formyluracil (18 mer, 19 mer, and 37 mer) used in this study were chemically synthesized as described.[43] Other oligonucleotides were purchased from Sigma Genosis. Sequences of each oligonucleotide are shown below. These oligonucleotides are designated as T18, FoU18, T19, FoU19, T37, and FoU37, respectively. When used as a probe for gel shift assay, the 5′-end of each oligonucleotide was labeled with ^{32}P. The 5′-end of competitor oligonucleotide was not phosphorylated.

5′-GAGA**X**GGAGCGAAAGCTG-3′ (18 mer)

X = T (T18)

X = 5-formyluracil (FoU18)

5′-GATCC**Y**CTAGAGTCGACCG-3′ (19 mer)

Y = T (T19)

Y = 5-formyluracil (FoU19)

5′-TCTCGATTAGTCCTGA**Z**CTAGAGTCGACCGTCTCAGG-3′

(37 mer)

Z = T (T37)

Z = 5-formyluracil (FoU37)

Preparation of Replication Protein A

Replication protein A (RPA) was purified from the egg extract derived from *Xenopus laevis*. The egg extract (5 ml) was applied onto a column of single-stranded DNA-cellulose (1-ml bed volume). The column was washed with 40 ml of Hepes-KOH buffer (40 mM Hepes-KOH, pH 7.5, 1 mM 2-mercaptoethanol, 1 mM EDTA and 10% glycerol) containing 1 M NaCl, then eluted with 10 ml of Hepes-KOH buffer containing 2 M NaCl. Fractions (1 ml each) were collected, and the proteins containing each fraction were detected by SDS-polyacrylamide gel electrophoresis. The fractions containing RPA were combined and dialyzed against the buffer containing 0.1 M NaCl and applied on a column of Hitrap Q (1 ml bed volume). The column was washed with 20 ml of the buffer containing 0.1 M NaCl, followed by elution with a linear gradient of NaCl from 0.1 to 0.5 M in the buffer (30 ml). Fractions (1 ml each) were collected, and the proteins containing each fraction were detected by SDS-polyacrylamide gel electrophoresis. RPA was eluted at 0.2 to 0.3 M NaCl. The fractions containing RPA were combined and dialyzed against 1 l of the buffer containing 60% glycerol and stored at −20°C until use. Purified RPA was composed of three polypeptides having a molecular weight of 70 kDa, 32 kDa, and 14 kDa, respectively, as judged by SDS-polyacrylamide gel electrophoresis.

Gel Shift Assay

Binding affinity of the purified RPA for several synthetic oligonucleotides was determined by the gel shift assay. The reaction mixture (10 μl) containing 10 ng of purified *Xenopus* RPA, 40 mM Hepes-KOH, pH 7.5, 1 mM EDTA, and 1 pmol 5′-^{32}P labeled oligonucleotide was incubated on ice for 10 min, then subjected to 8% polyacrylamide gel electrophoresis. RPA bound and unbound oligonucleotides were detected by phosphor image analyzer with Fuji BAS 1500. The amount of RPA bound oligonucleotide was determined. When the competition assay was performed, the indicated amount of competitor oligonucleotide was added to the reaction mixture.

Scatchard Plots

Gel shift assay was performed with 10 ng of purified RPA and several amounts (0.1 fmol-2 pmol) of 5′-^{32}P labeled oligonucleotide as described above. The amounts of RPA bound and unbound oligonucleotides were determined. Based on these results, Scatchard plots were made. The Kd values for each oligonucleotide was obtained from the slope of the graph.

Affinity Labeling of RPA with Oligonucleotide Containing 5-Formyluracil

The mixture (10 μl) containing 10 ng of RPA, 40 mM Hepes-KOH, pH 7.5, 1 mM EDTA and 1 pmol 5′-^{32}P labeled oligonucleotide containing 5-formyluracil (FoU18, FoU19 or FoU37) was incubated on ice for 10 min. To this mixture, 1 μl of 1 M $NaBH_4$ was added (final concentration was 100 mM). The mixture was further incubated at 37°C for 30 min, and subjected to SDS-15% polyacrylamide gel electrophoresis. The ^{32}P-oligonucleotide crosslinked with RPA was detected by phosphor image analyzer with Fuji BAS 1500.

RESULTS

Affinity of RPA for Oligonucleotide Containing 5-Formyluracil

To study the binding affinity of RPA for oligonucleotide containing 5-formyluracil, the gel shift assay was performed with the 5′-^{32}P-labeled undamaged 18 mer oligonucleotide (T18) as a probe. RPA was purified from *Xenopus* egg extract. Typical result is shown in Figure 1A. The ^{32}P-labeled oligonucleotide was retarded on polyacrylamide gel electrophoresis by the addition of RPA (lanes 1 and 2). The competition analysis with the unlabeled oligonucleotides was then performed. The amount of ^{32}P-labeled T18 and RPA complex was decreased according to the increasing amount of unlabeled T18 (lanes 3–6) or the oligonucleotide containing 5-formyluracil (FoU18) (lanes 7–10). The amount of ^{32}P-oligonucleotide-RPA complex in the absence or presence of competitors was measured. The amount of complex without competitor was taken as 100% and that with the several amount of competitor was determined. Resulting graph of the average of three independent experiments is shown in Figure 1B. As can be seen in this figure, the FoU18 oligonucleotide showed the stronger competition effect on the formation of ^{32}P-labeled T18 and RPA complex rather than T18 oligonucleotide. To confirm this result, the same experiment was carried out with the 5′-^{32}P-labeled FoU18 oligonucleotide as a probe. RPA also bound FoU18 oligonucleotide and the retarded band on polyacrylamide gel electrophoresis was appeared (Figure 1C, lane 2). Competition experiment with the unlabeled T18 and FoU18 was also performed (lanes 3–10). The average amount of

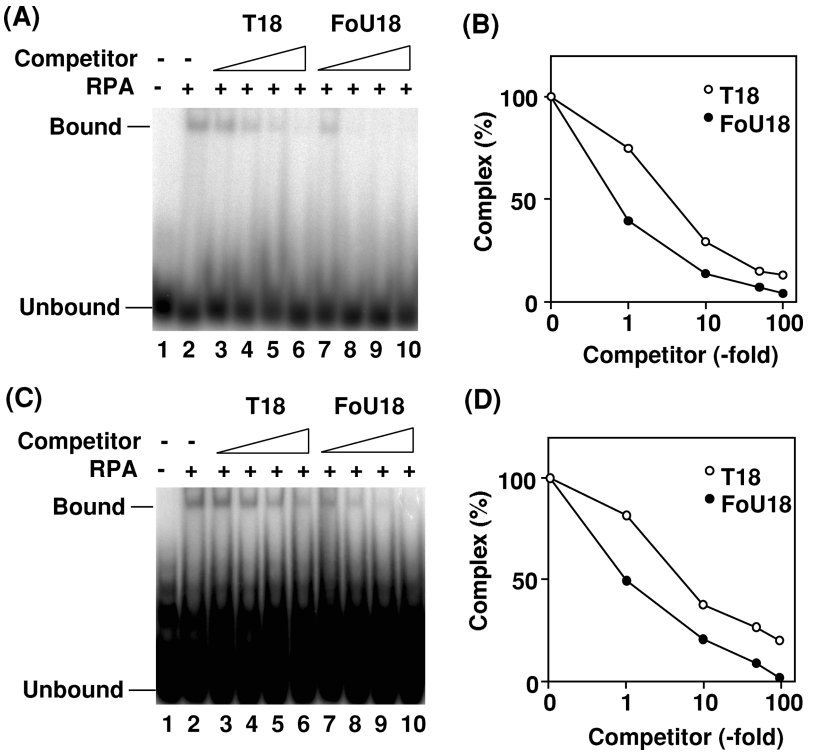

FIGURE 1 Gel shift assay of RPA binding to 18 mer oligonucleotides. Competition experiments for the formation of RPA and 1 pmol ^{32}P-labeled T18 (A) or ^{32}P-labeled FoU18 (C) was performed in the absence or presence of several competitors. The ^{32}P-labeled band was detected and analyzed with Fuji BAS 1500. Amount of competitor was 1 pmol (lanes 3 and 7), 10 pmol (lanes 4 and 8), 50 pmol (lanes 5 and 9), or 100 pmol (lanes 6 and 10). Kinds of competitors are shown above the panel. Position of bound and unbound ^{32}P-labeled probe is left of panel. The average of amount of RPA-^{32}P-labeled T18 (B) or RPA-^{32}P-labeled FoU18 (D) complex with or without the competitors from three independent experiments was determined as percent, and represented as a graph.

^{32}P-oligonucleotide-RPA complex in the absence or presence of competitors from three independent experiments was determined and shown in Figure 1D. Similar to the case of T18 probe, the FoU18 oligonucleotide had the stronger competition effect than T18 oligonucleotide. These results suggest that RPA may preferentially bind to the oligonucleotide containing 5-formyluracil than the undamaged single-stranded DNA.

To compare the affinity of RPA for these oligonucleotides, the dissociation constant (Kd value) was determined by Scatchard plots. The gel shift assay was again performed with the several concentrations of oligonucleotides and the amounts of RPA bound and unbound oligonucleotides were determined. Based on these data, Scatchard plots were made. The Kd values were obtained from the slop of graph and summarized in Table 1. The Kd value of T18 was 5.0 nM, whereas that of FoU18 was 0.45 nM. The affinity of RPA for FoU18 was 11-fold higher than for T18.

TABLE 1 RPA Binding to Oligonucleotides

Oligonucleotide	Kd (nM)	Kd for T/Kd for FoU[a]
T18	5.0	
FoU18	0.45	11
T19	0.45	
FoU19	0.45	1
T37	0.45	
FoU37	0.10	4.5

[a]Ratio of Kd value for undamaged oligonucleotide/Kd value for oligonucleotide containing 5-formyluracil.

Effects of Surrounding Sequence of 5-Formyluracil on the Affinity for RPA

To examine the effect of surrounding sequence of 5-formyluracil on the affinity for RPA, the gel shift assay was further performed with the other oligonucleotides, T19 and FoU19. The surrounding sequence of 5-formyluracil in FoU19 is pyrimidine rich, whereas that of FoU18 is purine-rich (sequences are shown in Materials and Methods). RPA also bound to the ^{32}P-labeled T19 or FoU19 and gave the retarded bands (Figures 2A and 2C). The amount of ^{32}P-labeled T19 and RPA complex was decreased according to the increasing amount of unlabeled T19 or FoU19 (Figure 2A, lanes 3–10). However, contrary to the case of 18 mer oligonucleotides, no significant difference on the competition effect was observed between T19 and FoU19 (Figure 2B). Similar result was obtained when the ^{32}P-labeled FoU19 was used as a probe (Figures 2C and D). The Kd values of T19 and FoU19 were also determined and summarized in Table 1. Both Kd values of T19 and FoU19 were almost the same. Therefore, RPA may have the same affinity for both T19 and FoU19. Combined with the results of 18 mer, it is suggested that the affinity of RPA for the oligonucleotide containing 5-formyluracil may vary according to the surrounding sequence of 5-formyluracil.

Effects of Position of 5-Formyluracil on the Affinity for RPA

We then asked the effect of position of 5-formyluracil in the oligonucleotide on the affinity to RPA using 37 mer (T37 and FoU37). The location of 5-formyluracil in FoU37 is near the center of oligonucleotide, whereas that in FoU18 or FoU19 is near the 5′-end. The results of gel shift assay showed that RPA also bound to both T37 and FoU37 (Figures 3A and C). Similar to the case of 18 mer, the competition effect of FoU37 on the formation of ^{32}P-labeled T37 and RPA complex was stronger than that of T37 (Figure 3B). The same result was obtained when the ^{32}P-labeled FoU37 was used as a probe (Figure 3D). The Kd values of T37 and FoU37 were also determined by Scatchard plots and summarized in Table 1. The Kd value of FoU37 for RPA was 4.5-fold lower than that of T37. This suggests that the position of

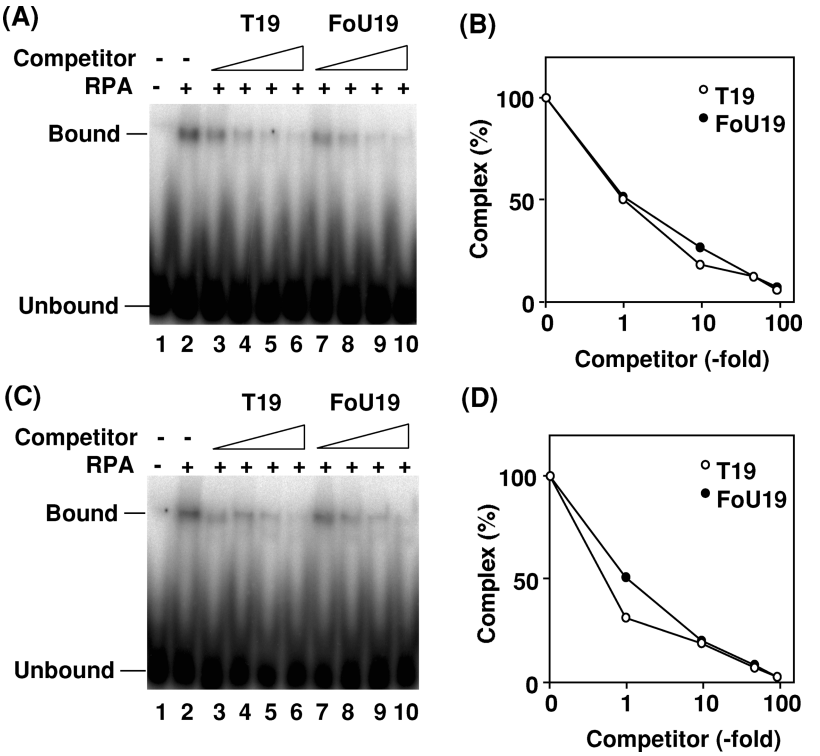

FIGURE 2 Gel shift assay of RPA binding to 19 mer oligonucleotides. Competition experiments for the formation of RPA and 1 pmol ^{32}P-labeled T19 (A) or ^{32}P-labeled FoU19 (C) was performed in the absence or presence of several competitors. The ^{32}P-labeled band was detected and analyzed with Fuji BAS 1500. Amount of competitor was 1 pmol (lanes 3 and 7), 10 pmol (lanes 4 and 8), 50 pmol (lanes 5 and 9), or 100 pmol (lanes 6 and 10). Kinds of competitors are shown above the panel. Position of bound and unbound ^{32}P-labeled probe is left of panel. The average of amount of RPA-^{32}P-labeled T19 (B) or RPA-^{32}P-labeled FoU19 (D) complex with or without the competitors from three independent experiments was determined as percent, and represented as a graph.

5-formyluracil on the oligonucleotide may have little effect on the affinity for RPA.

Affinity Labeling of RPA with Oligonucleotide Containing 5-Formyluracil

The substrate containing the formyl residue is often used as a crosslinking reagent for its target protein, since this residue is highly reactive with the primary amino residue and forms a stable C-N bonding in the presence of reducing reagent such as $NaBH_4$ through the formation of Shiff's base. To study which subunit of RPA recognizes and binds to the 5-formyluracil, we examined the crosslinking reaction with the oligonucleotides containing 5-formyluracil and RPA. The mixture of RPA and ^{32}P-labeled T18 or FoU18 was treated with $NaBH_4$, then subjected to SDS-polyacrylamide gel

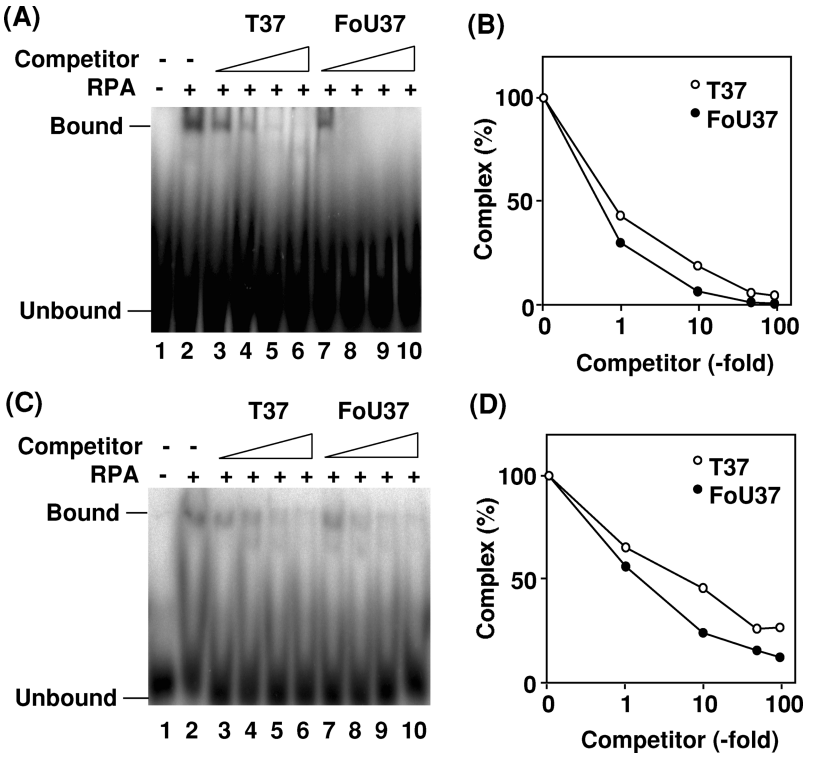

FIGURE 3 Gel shift assay of RPA binding to 37 mer oligonucleotides. Competition experiments for the formation of RPA and 1 pmol ^{32}P-labeled T37 (A) or ^{32}P-labeled FoU37 (C) was performed in the absence or presence of several competitors. The ^{32}P-labeled band was detected and analyzed with Fuji BAS 1500. Amount of competitor was 1 pmol (lanes 3 and 7), 10 pmol (lanes 4 and 8), 50 pmol (lanes 5 and 9), or 100 pmol (lanes 6 and 10). Kinds of competitors are shown above the panel. Position of bound and unbound ^{32}P-labeled probe is left of panel. The average of amount of RPA-^{32}P-labeled T37 (B) or RPA-^{32}P-labeled FoU37 (D) complex with or without the competitors from three independent experiments was determined as percent, and represented as a graph.

electrophoresis. The result is shown in Figure 4. The mixture without RPA gave no ^{32}P-labeled band on the gel even if treated with $NaBH_4$(lanes 1–4). On the other hand, when the mixture of RPA and ^{32}P-labeled FoU18 was treated with $NaBH_4$, the distinct band of 70 kDa corresponding to RPA1 subunit was appeared (lane 8). This band was not observed from the mixture with ^{32}P-labeled T18 (lanes 5 and 6) or without $NaBH_4$treatment (lane 7). Other oligonucleotides containing 5-formyluracil, FoU19, and FoU37, also reacted with RPA and the crosslinked complex of oligonucleotide-RPA1 subunit was appeared only when the mixture was treated with $NaBH_4$ (lanes 11, 12, 15, 16). Neither T19 nor T37 did not react with RPA (lanes 9, 10, 13, 14). This indicates that the formyl residue of 5-formyluracil on the oligonucleotide may efficiently crosslink with RPA1 subunit, and that the surrounding sequence or position of 5-formyluracil in the oligonucleotide may not affect the efficiency of crolinking reaction.

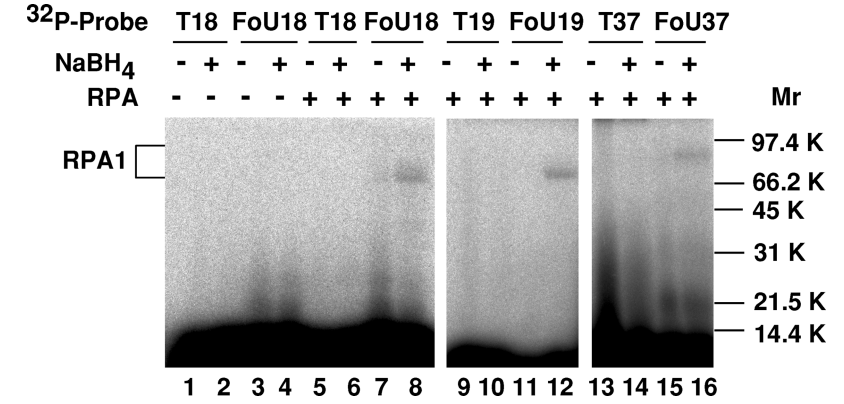

FIGURE 4 Crosslinking of RPA with oligonucleotides containing 5-formyluracil. The mixture of RPA and 1 pmol ^{32}P-labeled oligonucleotide was treated with $NaBH_4$ and subjected to SDS-polyacrylamide gel electrophoresis. The ^{32}P-labeled band was detected and analyzed with Fuji BAS 1500. Kinds of ^{32}P-labeled oligonucleotides and $NaBH_4$ treatment are shown above the panel. Position of RPA1 subunit and the standard molecular weight maker are left and right of panel, respectively.

DISCUSSION

RPA has a multiple role in the eukaryotic cells on replication, repair, and recombination. Among them, the recognition of DNA lesion is an important role for recruiting the cell cycle checkpoint kinase, ATR/ATRIP.[21] In the present article, we asked whether RPA could recognize the oxidized nucleotide base on the single-stranded DNA and studied the behavior of RPA against the 5-formyluracil, a major oxidized lesion of thymine base, using the synthetic oligonucleotides. The affinity of RPA for several oligonucleotides was studied by the gel shift assay. As shown in Figure 1, RPA showed the higher affinity for the 18 mer containing 5-formyluacil (FoU18) than for that without 5-formyluraci (T18), and the Kd value of FoU18 was 11-fold lower than that of T18 (Table 1). This indicates that RPA may have a high affinity for 5-formyluracil residue on the single-stranded DNA. However, in the case of 19 mer, no difference of affinity for RPA was seen between T19 and FoU19 (Figure 2 and Table 1). The 18 mer used in this study is purine rich (14 purine bases and 4 pyrimidine bases), whereas the 19 mer is not (9 purine bases and 10 pyrimidine bases). It has been reported that RPA prefers the pyrimidine stretch rather than the purine stretch,[19,22] and RPA displays an increased affinity as DNA length increased.[44] Indeed, the affinity of RPA for purine-rich T18 was much lower than for T19 (Table 1). Our result indicates that the 5-formyluracil residue in the purine-rich single-stranded DNA may greatly increase the affinity to RPA. On the other hand, the surrounding sequence of 5-formyluracil in the 19 mer is pyrimidine-rich such as 5′-TCCYCT-3′ (Y=5-formyluracil). Thus, the 5-formyluracil in the pyrimidine stretch may not affect the affinity for RPA since the pyrimidine stretch itself has a high affinity for RPA. The affinity of 37 mer containing 5-formyluracil

(FoU37) to RPA was 4.5-fold higher than that of T37 (Figure 3 and Table 1). The surrounding sequence of 5-formyluracil in FoU37 is neither purine-rich nor pyrimidine-rich, and the 5-formyluracil locates near the center of oligonucleotide (17th from 5′-end and 21st from 3′-end). Taken together, it is suggested that the affinity of RPA for the single-stranded DNA containing 5-formyluracil may basically higher than for the undamaged DNA, although that varies according to the surrounding sequence of 5-formyluracil but not the position in oligonucleotide.

We also performed the crosslinking reaction of RPA with the oligonucleotides. All 5′-^{32}P-labeled oligonucleotides containing 5-formyluracil were reacted with RPA, and the distinct band around 70 kDa on the SDS-polyacrylamide gel electrophoresis was appeared (Figure 4). The apparent molecular weight of labeled band with FoU37 was slightly larger than that with FoU18 or FoU19 since FoU37 was somewhat longer than FoU18 or FoU19. This indicates that RPA1 subunit but not RPA2 subunit may bind to the oligonucleotide containing 5-formyluracil. Schweizer *et al.* have also performed the crosslinking of RPA1 subunit with a cisplatin-modified oligonucleotide carrying 5-iodo-2′-deoxyuridine, and reported that RPA may have a specific positioning with respect to the platination site.[45] The RPA1 subunit has three DNA binding domains (DBD), DBD-A, -B, and -C, respectively.[46–52] Analysis of the 5-formyluracil-crosslinked product may provide the useful information for the molecular mechanism of RPA on the recognition of DNA lesion.

Mu *et al.* have reported that RPA is involved in the nucleotide excision repair factory for the UV-damaged site.[53] Many repair enzymes for 5-formyluracil are reported.[35–41] Among them, the single-strand selective monofunctional uracil-DNA glycosylase (SMUG1) excises the 5-formyluracil on the single-stranded DNA.[35–37] However, at present, the interaction between SMUG1 and RPA has not been found. RPA may work as a sensor for 5-formyluracil to recruit ATR/ATRIP rather than a member of 5-formyluracil repair factory.

REFERENCES

1. Friedberg, E.C.; Walker, G.C.; Siede, W. *DNA repair and Mutagenesis*, ASM Press, Washington, DC **1995**.
2. Kasai, H.; Nishimura, S. Hydroxylation of deoxyguanosine as the C-8 position by ascorbic acid and other reducing agents. Nucleic Acids Research **1984**, 12, 2137–2145.
3. Kamiya, H.; Kasai, H. Formation of 2-hydroxyadenosine triphosphate, an oxidatively damaged nucleotide, and its incorporation by DNA polymerases. Steady-state kinetics of the incorporation. Journal of Biological Chemistry **1995**, 270, 19446–19450.
4. Kasai, H.; Iida, A.; Yamaizumi, Z.; Nishimura, S.; Tanooka, H. 5-Formyldeoxyuridine: a new type of DNA damage induced by ionizing radiation and its mutagenicity to *Salmonella* strain TA102. Mutation Research **1990**, 243, 249–253.
5. Douki, T.; Delatour, T.; Paganon, F.; Cadet, J. Measurement of oxidative damage at pyrimidine bases in γ-irradiated DNA. Chemical Research in Toxicology **1996**, 9, 1145–1151.

6. Robins, P.; Jones, C.J.; Biggerstaff, M.; Lindahl, T.; Wood, R.D. Complementation of DNA repair in *Xeroderma pigmentosum* group A cell extracts by a protein with affinity for damaged DNA. EMBO Journal **1991**, 10, 3913–3921.
7. Venema, J.; van Hoffer, A.; Karcagi, V.; Natarajan, A.T.; van Zeeland, A.A.; Mullenders, K.H.F. *Xeroderma pigmentosum* complementation group C cells remove pyrimidine dimes selectively from the transcribed strand of active genes. Molecular Cellular and Biology **1991**, 11, 4128–4134.
8. Guzder, S.N.; Sung, P.; Prakash, L.; Prakash, S. Yeast DNA-repair gene RAD14 encodes a zinc metalloprotein with affinity for ultraviolet-damaged DNA. Proceedings of the National Academy of Sciences USA **1993**, 90, 5433–5437.
9. Jones, C.J.; Wood, R.D. Preferential binding of the *Xeroderma pigmentosum* group A complementing protein to damaged DNA. Biochemistry **1993**, 32, 12096–12104.
10. Masutani, C.; Sugasawa, K.; Yanagisawa, J.; Sonoyama, T.; Ui, M.; Enomoto, T.; Takio, K.; Tanaka, K.; van der Spek, P.J.; Bootsma, D.; Hoeijimakers, J.H.J.; Hanaoka, F. Purification and cloning of a nucleotide excision repair complex involving the *Xeroderma pigmentosum* group C protein and a human homolog of yeast RAD23. EMBO Journal **1994**, 13, 1831–1843.
11. Sugasawa, K.; Ng, J.M.Y.; Masutani, C.; Iwai, S.; van der Spek, P.J.; Eker, A.P.M.; Hanaoka, F.; Bootsma, D.; Hoeijimakers, J.H.J. *Xeroderma pigmentosum* group C protein complex is the initiator of global genome nucleotide excision repair. Molecular Cell **1998**, 2, 223–232.
12. Sugasawa, K.; Okamoto, T.; Shimizu, Y.; Masutani, C.; Iwai, S.; Hanaoka, F. A multistep damage recognition mechanism for global genomic nucleotide excision repair. Genes and Development **2001**, 15, 507–521.
13. Kusumoto, R.; Masutani, C.; Sugasawa, K.; Iwai, S.; Araki, M.; Uchida, A.; Mizukoshi, T.; Hanaoka, F. Diversity of the damage recognition step in the global genomic nucleotide repair in vitro. Mutation Research **2001**, 485, 219–227.
14. Clugston, C.K.; McLaughlin, K.; Kenny, M.K.; Brown, R. Binding of human single-stranded DNA binding protein to DNA damaged by the anticancer drug *cis*-diamminedichloroplatinum (II). Cancer Research **1992**, 52, 6375–6379.
15. He, Z.; Henricksen, L.A.; Wold, M.S.; Ingles, C.J. RPA involvement in the damage-recognition and incision steps of nucleotide excision repair. Nature (London) **1995**, 374, 566–569.
16. Burns, J.L.; Guzder, S.N.; Sung, P.; Prakash, S.; Prakash, L. An affinity of human replication protein A for ultraviolet-damaged DNA. Journal of Biological Chemistry **1996**, 271, 11607–11610.
17. Patric, S.M.; Turchi, J.J. Stopped-flow kinetic analysis of replication protein A-binding DNA. Journal of Biological Chemistry **2001**, 276, 22630–22637.
18. Lao, Y.; Gomes, X.V.; Yingiie, R.; Taylor, J.S.; Wold, M.S. Replication protein A interactions with DNA. III. Molecular basis of recognition of damaged DNA. Biochemistry **2000**, 39, 850–859.
19. Hey, T.; Lipps, G.; Krauss, G. Binding of XPA and RPA to damaged DNA investigated by fluorescence anisotropy. Biochemistry **2001**, 40, 2901–2910.
20. Patrick, S.M.; Turchi, J.J. Human replication protein A preferentially binds cisplatin-damaged duplex DNA in vitro. Biochemistry **1998**, 37, 8808–8815.
21. Zou, L.; Flledge, S.J. Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. Science **2003**, 300, 1542–1548.
22. Wold, M.S. Replication protein A: a heterotrimeric, single-stranded DNA-binding protein required for eukaryotic DNA metabolism. Annual Review of Biochemistry **1997**, 66, 61–92.
23. Privat, E.J.; Sowers, L.C. A proposed mechanism for the mutagenicity of 5-formyluracil. Mutation Research **1996**, 354, 151–156.
24. Fujikawa, K.; Kamiya, H.; Kasai, H. The mutations induced by oxidatively damaged nucleotides, 5-formyl-dUTP and 5-hydroxy-dCTP, in *Escherichia coli*. Nucleic Acids Research **1998**, 26, 4582–4587.
25. Miyabe, I.; Zhang, Q.M.; Sugiyama, H.; Kino, K.; Yonei, S. Mutagenic effects of 5-formyluracil on a plasmid vector during replication in *Escherichia coli*. International Journal of Radiation Biology **2001**, 77, 53–58.
26. Ånensen, H.; Provan, F.; Lian, A.T.; Reinertsen, S.H.; Ueno, Y.; Matsuda, A.; Seeberg, E.; Bjelland, S. Mutations induced by 5-formyl-2′-deoxyuridine in *Escherichia coli* include base substitutions that can arise from mispairs of 5-formyluracil with guanine, cytosine and thymine. Mutation Research **2001**, 476, 99–107.
27. Bjelland, S.; Ånensen, H.; Knævelsrud, I.; Seeberg, E. Cellular effects of 5-formyluracil in DNA. Mutation Research **2001**, 486, 147–154.

28. Klungland, A.; Paulsen, R.; Rolseth, V.; Yamada, Y.; Ueno, Y.; Wiik, P.; Matsuda, A.; Seeberg, E.; Bjelland, S. 5-Formyluracil and its nucleoside derivatives confer toxicity and mutagenicity to mammalian cells by interfering with normal RNA and DNA metabolism. Toxicology Letters **2001**, 119, 71–78.
29. Kamiya, H.; Murata-Kamiya, N.; Kario, N.; Ueno, Y.; Matsuda, A.; Kasai, H. Induction of T → G and T → A transversions by 5-formyluracil in mammalian cells. Mutation Research **2002**, 513, 213–222.
30. Zhang, Q.M.; Sugiyama, H.; Miyabe, I.; Matsuda, S.; Saito, I.; Yonei, S. Replication of DNA template containing 5-formyluracil, a major oxidative lesion of thymine in DNA. Nucleic Acids Research **1997**, 25, 3969–3973.
31. Zhang, Q.M.; Sugiyama, H.; Miyabe, I.; Matsuda, S.; Kino, K.; Saito, I.; Yonei, S. Replication in vitro and cleavage by restriction endonuclease of 5-formyluracil- and 5-hydroxymethyluracil-containing oligonucleotides. International Journal of Radiation Biology **1999**, 75, 3969–3973.
32. Yoshida, M.; Makino, K.; Morita, H.; Terato, H.; Ohyama, Y.; Ide, H. Substrate and mispairing properties of 5-formyl-2′-deoxyuridine 5′-triphosphate assessed by in vitro DNA polymerase reactions. Nucleic Acids Research **1997**, 25, 1570–1577.
33. Masaoka, A.; Terato, H.; Kobayashi, M.; Ohyama, Y.; Ide, H. Oxidation of thymine to 5-formyluracil in DNA promotes misincorporation of dGMP and subsequent elongation of a mismatched primer terminus by DNA polymerase. Journal of Biological Chemistry **2001**, 276, 16501–16510.
34. Miyashita, T.; Ono, A.; Izuta, S. Kinetic analysis of nucleotides incorporated opposite oxidized thymine bases on template DNA. Nucleic Acids Research, (Suppl.) **2002**, 2, 255–256.
35. Haushalter, K.A.; Todd Stukenberg, M.W.; Kirschner, M.W.; Verdine, G.L. Identification of a new uracil-DNA glycosylase family by expression cloning using synthetic inhibitors. Current Biology **1999**, 9, 174–185.
36. Masaoka, A.; Matsubara, M.; Hasegawa, R.; Tanaka, T.; Kurisu, S.; Terato, H.; Ohyama, Y.; Karino, N.; Matsuda, A.; Ide, H. Mammalian 5-formyluracil-DNA glycosylase. 2. Role of SMUG1 uracil-DNA glycosylase in repair of 5-formyluracil and other oxidized and deaminated base lesion. Biochemistry **2003**, 42, 5003–5012.
37. Matsubara, M.; Tanaka, T.; Terato, H.; Ohmae, E.; Izumi, S.; Katayanagi, K.; Ide, H. Mutational analysis of the damage-recognition and catalytic mechanism of human SMUG1 DNA glycosylase, Nucleic Acids Research **2004**, 32, 5291–5302.
38. Hazra, T.K.; Izumi, T.; Boldogh, I.; Imhoff, B.; Kow, K.W.; Jaruga, P.; Dizdaroglu, M.; Mitra, S. Identification and characterization of a human DNA glycosylase for repair of modified bases in oxidatively damaged DNA. Proceedings of the National Academy of Sciences USA **2002**, 99, 3523–3528.
39. Miyabe, I.; Zhang, Q.M.; Kino, K.; Sugiyama, H.; Takao, M.; Yasui, A.; Yonei, S. Identification of 5-formyluracil DNA glycosylase activity of human hNTH1 protein. Nucleic Acids Research **2002**, 30, 3443–3448.
40. Takao, M.; Kanno, S.; Kobayashi, K.; Zhang, Q.M.; Yonei, S.; van der Horst, G.T.; Yasui, A. A back-up glycosylase in Nth1 knock-out mice is a functional Nei (endonuclease VIII) homologue. Journal of Biological Chemistry **2002**, 277, 42205–42213.
41. Liu, P.; Burdzy, A.; Sowers, L.C. Repair of the mutagenic DNA oxidation product, 5-formyluracil. DNA Repair **2003**, 2, 199–210.
42. Kasama, T.; Ono, A.; Izuta, S. Binding activity of replication protein A tosingle-stranded DNA containing oxidized pyrimidine base. Nucleic Acids Research (Suppl.) **2001**, 1, 167–168.
43. Ono, A.; Okamoto, T.; Inada, M.; Nara, H.; Matsuda, A. Nucleosides andnucleotides. 131. Synthesis and properties of oligonucleotides containing 5-formyl-2′-deoxyuridine. Chemical and Pharmaceutical Bulletin (Tokyo) **1994**, 42, 2231–2237.
44. Kim, C.; Snyder, R.O.; Wold, M.S. Binding properties of replication protein A from human and yeast cells. Molecular and Cellular Biology **1992**, 12, 3050–3059.
45. Schweizer, U.; Hey, T.; Lipps, G.; Krauss, G. Photocrosslinking locates a binding site for the large subunit of human replication protein A to the damaged strand of cisplatin-modified DNA. Nucleic Acids Research **1999**, 27, 3183–3189.
46. Gomes, X.V.; Wold, M.S. Functional domains of the 70-kilodalton subunit of human replication protein A. Biochemistry **1996**, 35, 10558–10568.
47. Pfuetzner, R.A.; Bochkarev, A.; Frappier, L.; Edwards, A.M. Replication protein A. Characterization and crystallization of the DNA binding domain, Journal of Biological Chemistry **1997**, 272, 430–432.

48. Bochkarev, A.; Pfuetzner, R.A.; Edwards, A.M.; Frappier, L. Structure of the single-stranded-DNA-binding domain of replication protein A bound to DNA. Nature (London) **1997**, 385, 176–181.
49. Brill, S.J.; Bastin-Shanower, S. Identification and characterization of thefourth single-stranded-DNA-binding domain of replication protein A. Molecular and Cellular Biology **1998**, 18, 7225–7234.
50. Bochkareva, E.; Korolev, S.; Bochkarev, A. The role for zinc in replication protein A. Journal of Biological Chemistry **2000**, 275, 27332–27338.
51. Bochkareva, E.; Belegu, V.; Korolev, S.; Bochkarev, A. Structure of the major single-stranded DNA-binding domain of replication protein A suggests a dynamic mechanism for DNA binding. EMBO Journal **2001**, 20, 612–618.
52. Bastin-Shanower, S.A.; Brill, S.J. Functional analysis of the four DNA binding domains of replication protein A. Journal of Biological Chemistry **2001**, 276, 36446–36453.
53. Mu, D.; Wakasugi, M.; Hsu, D.S.; Sancar, A. Characterization of reaction intermediates of human excision repair nuclease. Journal of Biological Chemistry **1997**, 272, 28971–28979.