Binding activity of replication protein A to single-stranded DNA containing oxidized pyrimidine base

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ABSTRACT
To obtain the information for the role of replication protein A (RPA) on the detection of oxidized lesion in the single-stranded DNA, the binding preference of RPA purified from Xenopus egg lysate against the oligonucleotide containing one of three kinds of oxidized thymine residues, 5-formyluracil, 5-hydroxymethyluracil and 5-(1,2-dihydroxyethyl)uracil, was studied by the gel shift assay. Results of competition assay indicate that RPA preferentially binds to the oligonucleotide containing these oxidized thymine residues than the undamaged DNA.

INTRODUCTION
Replication protein A (RPA) is a single-stranded DNA binding protein which has multiple roles on replication, repair and recombination (1). It has been recently demonstrated that the human recombinant RPA preferentially binds to the single-stranded DNA which contains the pyrimidine(6-4)pyrimidone photoproduct (6-4 photoproduct) rather than the undamaged single-stranded DNA (2). This suggests that RPA may have a role on the search and detection of UV-damage on DNA. We also found that RPA purified from the egg lysate derived from Xenopus laevis showed the same binding selectivity (3). This indicate that the binding preference of RPA to 6-4 photoproduct may be a common feature among several eukaryotes. However, the behavior of RPA against other DNA lesions such as an oxidized pyrimidine base yielded by the active oxygen, or a chemically modified base by several reagents is not known. We examined the binding preference of RPA to the chemically synthesized oligonucleotide containing several oxidized pyrimidine bases. Here, we show that Xenopus RPA preferentially binds to the site containing oxidized pyrimidine bases, as well as 6-4 photoproduct, rather than the undamaged site.

MATERIALS AND METHODS
Following chemically synthesized oligonucleotides were used in this study.

Series-1: 5'-GAGAXGGAGCGAAAGCTG-3'
X = T (undamaged oligonucleotide-1)
X = 5-formyluracil (F-oligonucleotide-1)
X = 5-hydroxymethyluracil (H-oligonucleotide-1)
X = 5-(1,2-dihydroxyethyl)uracil (D-oligonucleotide-1)

Series-2: 5'-GATCCYCTAGAGTCGACCG-3'
Y = T (undamaged oligonucleotide-2)
Y = 5-formyluracil (F-oligonucleotide-2)
Y = 5-hydroxymethyluracil (H-oligonucleotide-2)
Y = 5-(1,2-dihydroxyethyl)uracil (D-oligonucleotide-2)

RPA was purified from the egg lysate derived from Xenopus laevis with a column chromatography of single-stranded DNA-cellulose (4).

Gel shift assay was performed with the 5,32P-labeled undamaged or F-oligonucleotide. The mixture (10 μl) containing 1 pmol 32P-oligonucleotide and an aliquot of RPA was incubated on ice for 10 min. RPA-bound and unbound oligonucleotides were separated by 8% polyacrylamide gel electrophoresis, and detected by phosphor image analyzer with Fuji Bass 1500.
RESULTS AND DISCUSSION

Gel shift assay was performed using the 5'-32P-labeled undamaged oligonucleotide-1 and the purified *Xenopus* RPA. As shown in Fig. 1A, the oligonucleotide and RPA complex appeared as a slower migrated band compared with that without RPA (lanes 1 and 2). In the presence of competitor oligonucleotides, the amount of RPA and 32P-undamaged nucleotide complex was decreased (lanes 3-18). When the undamaged oligonucleotide-1 was used as a competitor, this complex was gradually reduced according to the increasing amount of competitor (lanes 3-6). On the other hand, the amount of complex was rapidly reduced by the addition of F-, H- or D-oligonucleotide-1 (lanes 7-18).

To confirm this result, we also studied the binding selectivity of RPA using the 5'-32P-labeled F-oligonucleotide-1 as a probe. Similarly to the case shown in above, the formation of RPA and 32P-F-oligonucleotide complex was competed by the F-, H- or D-oligonucleotide-1 more strongly than the undamaged oligonucleotide-1 (Fig. 1B). The same results were obtained when the another oligonucleotides (series-2 shown in MATERIALS AND METHODS) which had the different sequence from series-1 oligonucleotides were used as a 32P-labeled probe and competitors (data not shown).

These results suggest that RPA may preferentially bind to the oxidized thymine residue on single-stranded DNA rather than the undamaged site, and this characteristic is not sequence-dependent.

REFERENCES