Nucleic Acids Symposium Series No. 44 151-152

Complex formation of double-stranded DNA (6-4) photoproducts and anti-(6-4) photoproduct antibody Fabs

Hideshi Yokoyama, Ryuta Mizutani and Yoshinori Satow
Graduate School of Pharmaceutical Sciences, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

ABSTRACT

DNA (6-4) photoproducts are major constituents of ultraviolet-damaged DNAs. We prepared double-stranded (ds) (6-4) DNA photoproducts and analyzed formation of their complexes with anti-(6-4) photoproduct antibody Fabs. Elution profiles of the mixtures of ds-(6-4) DNAs and Fabs from anion-exchange and gel-filtration columns indicate that Fab 64M-2 deprives 14mer ds-(6-4) DNA of single-stranded (ss) (6-4) DNA and shows no interaction with 18mer ds-(6-4) DNA (A18). Fab 64M-5 with an approximately 100-fold higher affinity than Fab 64M-2 forms a complex with the ds-(6-4) DNA (A18), but partly dissociates another 18mer ds-(6-4) DNA (A18-3), with a lowered G-C content, into ss-DNAs. From these results, antibody 64M-5 possibly accommodates the T(6-4)T photolesion moiety of the ds-(6-4) DNA (A18) by flipping out the moiety from its neighboring segments.

INTRODUCTION

DNA damages caused by ultraviolet (UV) light induce mutations, cellular transformation, and cell death. DNA (6-4) photoproducts[1] are major constituents of the photo-damaged DNAs, and are more mutagenic than cyclobutane pyrimidine dimers[2].

Mouse monoclonal antibodies 64M-2 and 64M-5 specific for (6-4) photoproducts have been established simultaneously from the BALB/c mice[3]. We have determined the crystal structure of the Fab fragment of antibody 64M-2 in complex with the ligand dT(6-4)T[4]. It was reported that antibody 64M-5 highly homologous to the 64M-2 shows a higher affinity to double-stranded (ds) DNAs containing (6-4) photolesion[5]. In order to elucidate recognition mechanisms of ds-(6-4) DNAs by these antibodies, we prepared ds-(6-4) DNA and analyzed the formation of their complexes with these Fabs.

MATERIALS AND METHODS

Single-stranded (ss) DNAs with various sequences were irradiated with UV light of 254 nm using UV cross-linker (Funakoshi) so as to prepare ss-(6-4) DNAs (Fig. 1). UV-irradiated ss-(6-4) DNAs were mixed with the Fab, and ss-(6-4) DNA-Fab complexes were eluted on a DEAE-5PW anion-exchange column (Tosoh). The Fab was then separated from urea-denaturated complexes on the DEAE-5PW column. Thus prepared ss-(6-4) DNAs were mixed each with their complementary ss-DNAs and were subjected to ds-(6-4) DNA formation by annealing at 90°C and then cooling down to 4°C. Resultant ds-(6-4) DNAs were mixed with the Fab, and analyzed on the DEAE-5PW and Superdex-75 (Pharmacia) gel-filtration columns.

RESULTS AND DISCUSSION

The mixture of A14 (14mer) ds-(6-4) DNA and Fab 64M-2 eluted a ss-(6-4) DNA complex with the Fab, followed by the fraction of its complementary ss-DNA (Fig. 2a). The gel-filtration profile (Fig. 2b) also showed the separation into the ss-(6-4) DNA-Fab complex and ss-DNA. These results indicate that A14 ds-(6-4) DNA was dissociated to ss-DNAs by the addition of Fab 64M-2.

In the case of the mixture of A18 (18mer) ds-(6-4) DNA and Fab 64M-2, the ds-(6-4) DNA and Fab were separately eluted as two major fractions on the DEAE-5PW column. Similar elution profiles were also obtained for the A21 (21mer) DNA (data not shown). These results indicate that the 64M-2 Fab has low affinities toward stable duplex DNAs such as A18 and A21 ds-(6-4) DNAs.

Antibody 64M-5 has an approximately 100-fold higher binding affinity to the (6-4) photoproducts as compared to antibody 64M-2. By addition of Fab 64M-5 to A18 ds-(6-4) DNA, the area for the eluted

Name	Sequence
A14	5' GCGTGAT(6-4)TATGGAC 3'
A18	GCGAGTGAT(6-4)TATGGACGG
A18-3	GCGAATAAT(6-4)TATAAACGG
A21	AGGCGAGTGAT(6-4)TATGGACGGG

Fig 1. Sequences of (6-4) DNA photoproducts.

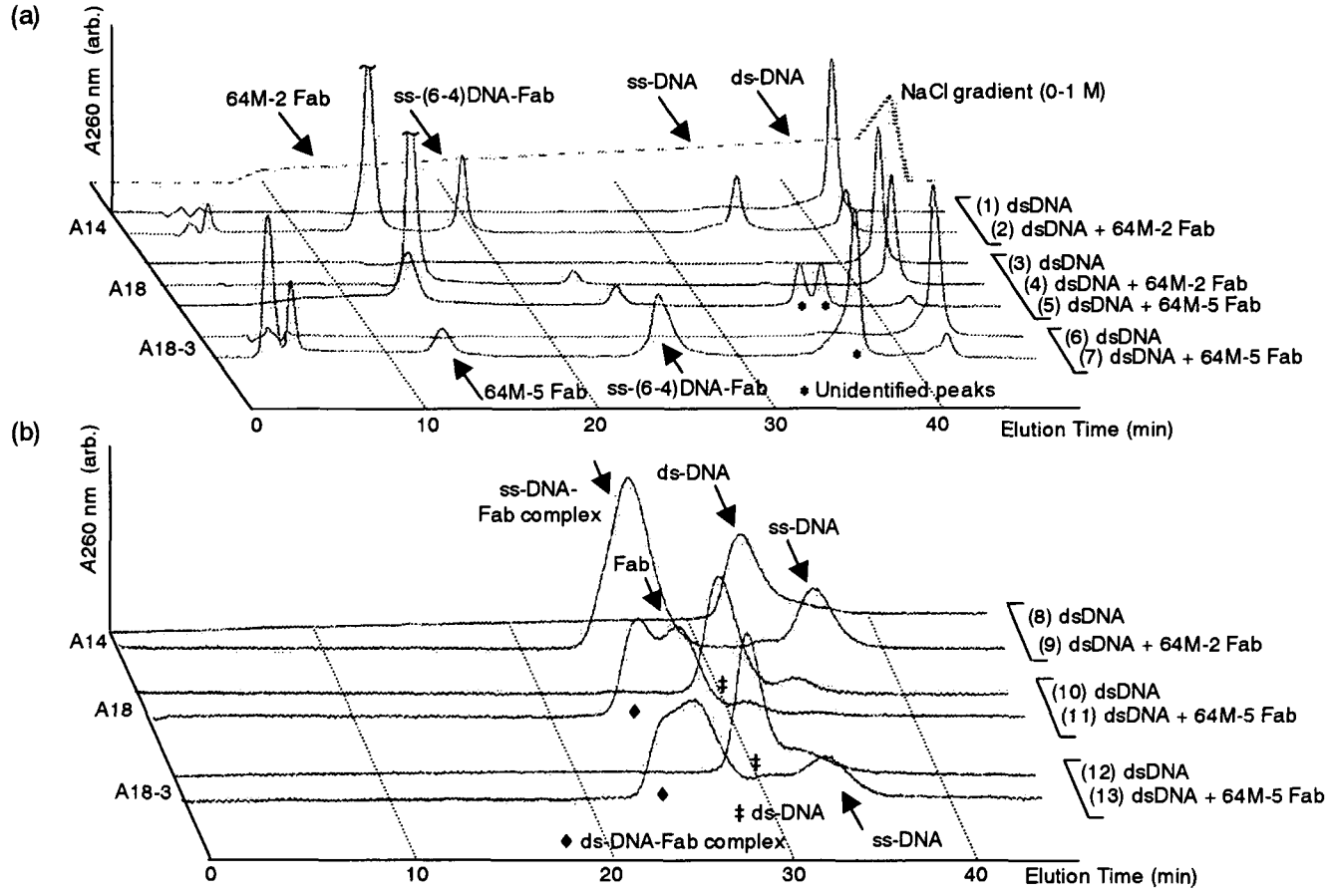

Fig 2. Elution profiles from (a) DEAE-5PW and (b) Superdex-75 columns.

peak of A18 ds-(6-4) DNA decreased, and two new peaks appeared in the DEAE-5PW profile. In the corresponding gel-filtration analysis, peaks both for the ds-(6-4) DNA-Fab complex and for the excess Fab were observed. These results indicate that a complex is formed between A18 ds-(6-4) DNA and Fab 64M-5.

A18-3 ds-(6-4) DNA is expected to form fewer base-pair hydrogen-bonds than A18 ds-(6-4) DNA. When A18-3 ds-(6-4) DNA is added to Fab 64M-5, the peak area of the ds-(6-4) DNA decreased as is observed for A18 ds-(6-4) DNA, and peaks both for the ds-(6-4) DNA-Fab complex and for the ss-DNA appeared in the gel-filtration profiles. These results indicate that some portion of A18-3 ds-(6-4) DNA is dissociated to ss-DNAs by the addition of Fab 64M-5.

A14 ds-(6-4) DNA with a shorter chain-length as well as A18-3 ds-(6-4) DNA forms fewer base-pair hydrogen-bonds than the A18, and hence tends to dissociate itself into ss-DNAs by addition of the Fabs. Complex formation is not observed for Fab 64M-2 mixed with A18 ds-(6-4) DNA as well as with A21 ds-(6-4) DNA. On the other hands, the formation of the complex between A18 ds-(6-4) DNA and Fab 64M-5 was clearly observed. In the crystal structure of Fab 64M-2[4], the dT(6-4)T moiety is fully accommodated in the binding pocket of the Fab. Therefore, the homologous antibody 64M-5 is likely to recognize A18 ds-(6-4) DNA by accommodating the T(6-4)T moiety in the similar manner. When this binding is attained, the T(6-4)T moiety and adjacent nucleotides would be flipped out from the neighboring segments still kept in the double-stranded state.

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