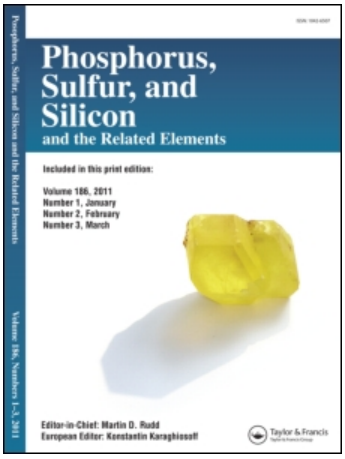

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DNA Binding Mode of Antibody Fragments Specific for TT Photo-Dimers

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DNA BINDING MODE OF ANTIBODY FRAGMENTS SPECIFIC FOR TT PHOTO-DIMERS

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A single-chain antibody specific for the pyrimidine(6-4)pyrimidone photoproduct in DNA was constructed and its binding properties to the cognate oligonucleotides were investigated. The fluorescent property of 2-aminopurine was used to study the binding mode of the antibody fragment to the pyrimidine(6-4)pyrimidone photoproduct in double-stranded DNA. The results indicated that the single-chain antibody recognizes the lesion in the single-stranded state.

Keywords: Antibodies; fluorescence; oligodeoxyribonucleotide; pyrimidine(6-4)pyrimidone photoproduct; surface plasmon resonance

INTRODUCTION

UV-induced photoproducts, such as cyclobutane pyrimidine dimers (CPD) and pyrimidine(6-4)pyrimidone photoproducts, are known to be mutagenic, and the cellular mechanisms to repair these lesions have been investigated by various approaches. We have cloned the genes for monoclonal antibodies for these photo-lesions,[1] and the amino acid sequences of the variable domains for the binding of the (6-4) photoproduct have been determined.[2] The interactions between the chemically synthesized DNA fragments containing T(6-4)T and the antigen-binding fragments (Fab) or single-chain antibodies (scFv) have been studied by surface plasmon resonance,[3,4] X-ray crystallography,[5] and ^{31}P NMR.[6,7] The roles of surface lysines[8] and tryptophan H33[9] in the binding have been investigated by engineering the scFv.

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To investigate the binding mode of the scFv to the pyrimidine(6-4)pyrimidone photoproduct in double-stranded DNA, the fluorescent properties of oligodeoxyribonucleotides containing 2-aminopurine were used in the present work.

RESULTS

The Binding Properties Measured by Surface Plasmon Resonance

The binding properties of the high-affinity scFv[3] to the cognate double-stranded oligodeoxyribonucleotides were measured. A single-stranded oligomer containing the pyrimidine(6-4)pyrimidone photoproduct, dGAAAGTAGAACAAXYAAGCAAATGGTAA (d28mer-6-4), was prepared. The duplex complexed with the complementary oligonucleotide (d32mer) was shown to bind to the scFv (KD, 4.0×10^{-9} M), as measured by the surface plasmon resonance using the procedure described previously.[3,4] An oligodeoxyribonucleotide containing a 2-aminopurine dCTCTTTCATCTTGTTA*TTCGTTTACCATTTT (3′-5′, d32mer-2AP) was prepared as the analog of the complementary oligonucleotide. Since the 2-aminopurine deoxyribonucleotide has the intrinsic fluorescence (excitation, 310 nm; emission, 370 nm), which diminishes with duplex formation, the analog was thought to be useful for this study. The amino group of 2-aminopurine is located at suitable distance for hydrogen bonding to the pyrimidone of pyrimidine(6-4)pyrimidone (Figure 1) in the complementary strand. The affinity of the analog duplex to the scFv was smaller (KD, 1.5×10^{-3} M), indicating less

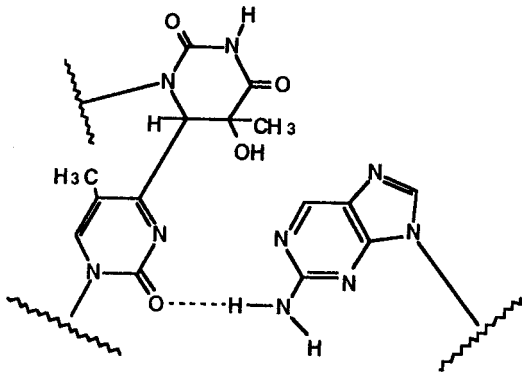

FIGURE 1 Hydrogen bond between the 3′-pyrimidone of pyrimidine(6-4)-pyrimidone and 2-aminopurine.

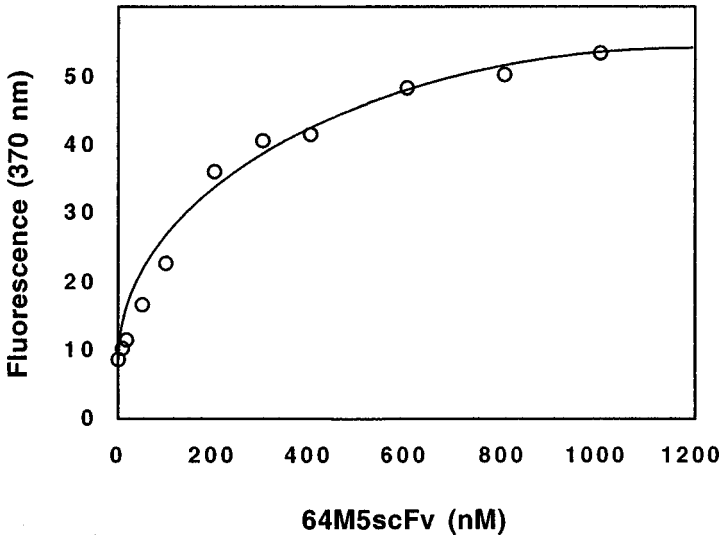

FIGURE 2 Fluorescence intensity of the 2-aminopurine with increasing amounts of the single chain antibody fragment.

availability to the lesion. This maybe explained by fluorescence measurements of the analog duplex.

The Fluorescence Measurements in the Absence and Presence of the scFv

The fluorescence intensities of 2-aminopurine in the double-stranded 32mer were recovered after the addition of the scFv. The intensity of the duplex analog increased with increasing amounts of the antibody fragment, as shown in Figure 2. These results indicated that the scFv bound the photo-lesion in the single-stranded DNA.

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