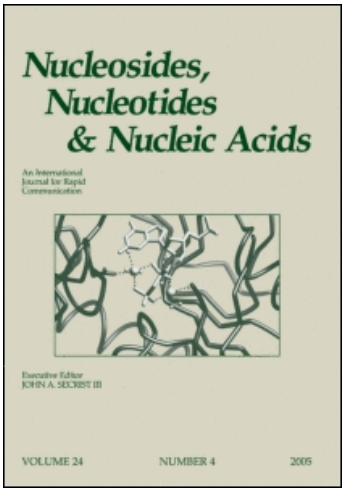

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INTERACTIONS OF HIGH AFFINITY ANTI (6-4) PHOTO-PRODUCT ANTIBODY FRAGMENTS WITH DAMAGED DNA

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ABSTRACT: The interactions between chemically synthesized DNA fragments containing a T(6-4)T and antigen binding fragments (Fab) or single-chain antibodies (scFv) were investigated by X-ray crystallography, NMR, and surface plasmon resonance. The high affinity scFv protein was found to bind to the template DNA near the (6-4) photoproduct site and to interfere with DNA polymerase reactions in vitro.

Pyrimidine dimers are the major products in photo-damaged DNA and cause cellular mutation and death.[1-3] To construct potentially therapeutic single-chain antibodies (scFv) against pyrimidine (6-4) pyrimidone photoproducts, the genes for the monoclonal antibodies (64M2, 64M3, 64M4 and 64M5)[4] have been cloned and their sequences were compared.[5] The interactions between chemically synthesized DNA fragments containing a T(6-4)T and antigen binding fragments (Fab) and a scFv were investigated by surface plasmon resonance, and the scFv was shown to have almost the same binding rates against the T(6-4)T-containing octamer as the Fab.[6] Site-directed mutagenesis was used to investigate the origin of the binding affinity differences exhibited by a series of antibodies that recognize pyrimidine (6-4)pyrimidone photoproducts. Results obtained from the chimeric scFv's from 64M3 and 64M5 suggested that the high affinity of the 64M5 was mainly due to its VL region and alanine scanning mutagenesis suggested the importance of the three VL CDR loops in the DNA binding with 64M5.[7] The role of the surface lysines of VH in the 64M5 scFv was investigated by replacement by alanine, and the results from the binding kinetics revealed that these lysine residues help to guide the DNA polyanion to the antigen binding pocket by electrostatic effects.[8] The high affinity scFv from 64M5 was found

to bind to a template DNA and to interfere with DNA polymerase reactions in vitro, and therefor it was used in binding studies with damaged double stranded DNA containing various pairings.

The ^{31}P chemical shift data indicated that the backbone conformation of the T(6-4)Tphotoproduct is affected by the presence of flanking oligodeooxynucleotides and that different backbone conformations are accommodated in the antigen binding sites of the anti-(6-4)photoproduct antibodies 64M3 and 64M5.[9]

The three-dimensional structures of the Fab fragments of 64M2, 64M3, and 64M5 have been analyzed by the use of X-ray crystallography with the molecular replacement method. Among these Fab, the 64M2 Fab was obtained as a form complexed with a T(6-4)T ligand and clearly elucidated the structure of the bound ligand as well as the ligand recognition mode. The structures of the free forms of 64M2, 64M3, and 64M5 were also analyzed and compared with the liganded structure of 64M2. These results suggest that the 6-4 phootporducts have a common three-dimensional structure that is recognized by the cognate antibodies with very similar structures.

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