Nucleic Acids Symposium Series No. 42 267–268

Crystal structures of the 64M-2 and 64M-3 antibody Fabs complexed with DNA (6–4) photoproducts

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ABSTRACT

Crystal structures of the 64M-2 antibody Fab fragment complexed with DNA photoproducts of dT(6-4)T and dTT(6-4)TT, and of the 64M-3 Fab fragment complexed with dT(6-4)T were determined. The 5'-thymine base of the bound dT(6-4)T ligand is in a half-chair conformation, and its base plane is nearly perpendicular to the planar 3'-pyrimidone base. The 64M-2 and 64M-3 Fabs have a common structure suitable for accommodating the dT(6-4)T ligand. In each of the antigen binding sites of the 64M-2 and 64M-3 Fabs, basic residues of His 35H and Arg 95H are located at the bottom of the binding pocket, and are hydrogen-bonded to the base moieties of dT(6-4)T. Two water molecules are involved in the interactions that intervene between the base moieties and the binding site. Aromatic residues of Trp 33H and Tyr 100iH form a side-wall of the pocket and are in van der Waals interactions with the base moieties. The Trp 33H side-chain is placed in parallel to the 3'-pyrimidone base, and the Tyr 100iH side-chain is nearly perpendicular to the 5'-thymine base. His 27dL, Tyr 32L, Leu 93L, and Ser 58H forming another side-wall are located in the vicinity of the sugar-phosphate backbone. In the 64M-2 Fab complex with dTT(6-4)TT, 5'- and 3'-side phosphate groups are also involved in interaction with Fab residues.

INTRODUCTION

Ultraviolet light (UV) is well-known to cause DNA damages which lead to mutations, cell death, and neoplastic transformation. Among photo-damaged DNAs, (6-4) photoproducts[1,2] (Figure 1) are more mutagenic than cyclobutane pyrimidine dimers[3]. Therefore, (6-4) photoproducts might play an important role in the biological effects produced by UV-light[2].

Mouse monoclonal antibodies, 64M-2 and 64M-3, specific for (6-4) photoproducts have been established simultaneously from the same BALB/c mouse[4] and their cDNAs have been sequenced[5]. In order to elucidate mechanisms of the recognition of (6-4) photoproducts by these antibodies, we determined the crystal structures of the Fab fragments of 64M-2 and 64M-3 antibodies, both complexed with their ligands of dT(6-4)T (d2mer) and dTT(6-4)TT (d4mer) by the molecular replacement method.

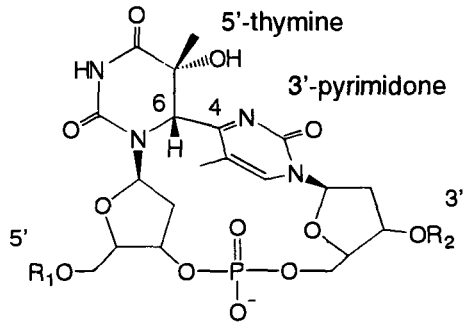
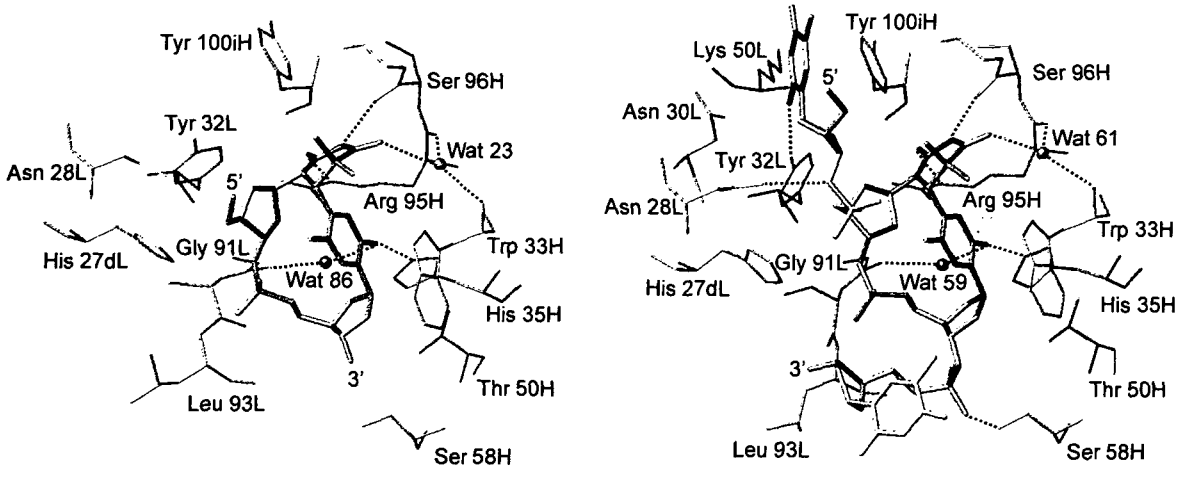


Figure 1. DNA (6-4) photoproduct

RESULTS AND DISCUSSION

The crystal structures of 64M-2 Fab-d2mer, 64M-2 Fab-d4mer, and 64M-3 Fab-d2mer complexes were determined at 2.4 Å, 2.4 Å, and 2.8 Å resolution, with crystallographic *R*-factors of 0.199, 0.209, and 0.187, respectively. The 64M-2 Fab-d2mer structure shows an rms positional difference of 0.5 Å in main-chain atoms from either of the 64M-2 Fab-d4mer and 64M-3 Fab-d2mer structures.

The dT(6-4)T segment of each ligand consists of two modified thymine bases of 5'-thymine and 3'-pyrimidone, two deoxyribose rings, and a phosphate group (Figure 1). The 5'-thymine base is in a half-chair conformation with C5 atom above the plane defined by N1, C2, N3, and C4 atoms and with C6 atom below this plane (Figure 2). Both the C5 methyl group and the 3'-pyrimidone ring are axial to the 5'-thymine chair. The 3'-pyrimidone base is nearly planar. The 5'-thymine base is nearly perpendicular to the 3'-pyrimidone base, since the



(a) complex with dT(6-4)T (b) complex with dTT(6-4)TT

Figure 2. Antigen binding pocket of the 64M-2 Fab

angle between the 5'-thymine plane defined by N1, C2, N3, C4, C5, and C6 atoms, and the 3'-pyrimidone plane similarly defined, is 97° in the 64M-2 Fab-d2mer structure. This perpendicularity is brought by steric hindrance to the covalent (6-4) bond from the C5-OH and 5'-deoxyribose groups. The 5'-thymine base is in *anti* and the 3'-pyrimidone is in *high-anti* conformation of glycosyl bond[6]. Both the phosphodiester bond angles ζ (C3'-O3'-P-O5') and α (O3'-P-O5'-C5') are in the *gauche-* range.

In the 64M-2 Fab-d2mer complex (Figure 2a), basic residues of His 35H and Arg 95H are located at the bottom of the antigen binding pocket. The d2mer ligand forms five hydrogen bonds to the Fab and water molecules. At the 5'-thymine location, hydrogen bonds are formed between the C2 carbonyl O and Arg 95H Nε atoms, and between the N3 and Ser 96H O atoms. The C4 carbonyl O atom is hydrogen-bonded to Wat 23 which is also hydrogen-bonded both to Arg 95H O and to Trp 33H N. As for the 3'-pyrimidone moiety, the C2 carbonyl O atom is hydrogen-bonded both to His 35H Nε2 and to Wat 86. Wat 86 is then hydrogen-bonded to Gly 91L O. These two water molecules of Wat 23 and Wat 86 are commonly involved in the interactions between the base moieties and the Fabs. Aromatic residues of Trp 33H and Tyr 100iH form a side-wall of the pocket. The Trp 33H side-chain is positioned parallel to the 3'-pyrimidone base with a ring-to-ring spacing of 3.5 Å. The Tyr 100iH side-chain is nearly perpendicular to the 5'-thymine base. His 27dL, Tyr 32L, Leu 93L, and Ser 58H forming another side-wall are located in the vicinity of the sugar-phosphate backbone.

The 64M-3 Fab recognizes the dT(6-4)T ligand in a manner similar to the 64M-2 Fab, although the dT(6-4)T phosphate is in interaction with the main-chain atoms of His 93L, as in the case of Leu 93L of the 64M-2 Fab complex. Therefore the 64M-2 and 64M-3 antibodies possess a common binding site structure suitable for accommodating dT(6-4)T that adopts a common circular base-normal conformation in the bound states.

In the 64M-2 Fab-d4mer complex, the central dT(6-4)T segment is recognized as in the 64M-2 Fab-d2mer complex (Figure 2b). Two water molecules placed at the binding pocket are also located. The 5'- and 3'-side phosphate groups form hydrogen bonds with the Asn 28L Nδ2 and Ser 58H Oγ atoms, respectively. The 5'-terminal thymine base is in interaction with Tyr 32L, Lys 50L, and Tyr 100iH, whereas the 3'-terminal thymine base is not in interaction with the Fab residues. The higher binding affinity of the d4mer ligand than the d2mer is attributable to these interactions additionally formed at both the terminals.

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