



Fang Zhang 張放 (ID=DB1101)

Supervisors: Profs. Akio Takénaka &amp; Yuji Kikuchi

The thesis contains two subjects. [1] A diet high in red meat (especially preserved) stimulates *N*-nitrosation of glycine, and its derivative (diazoacetate) alkylates guanine bases in DNA forming *O*<sup>6</sup>-carboxymethylguanine (*O*<sup>6</sup>-CMG). This modification induces DNA mutations which is associated with increased risk of colorectal cancer. [2] For invasion of human immunodeficiency virus (HIV-1) into human cell, high mannose-type glycan (HMTG) which covers the gp120 glycoprotein protruded from HIV-1 surface is required to contact with human CD4 protein at the beginning. A new anti-HIV lectin actinohivin (AH) can bind to HMTG and disturb HIV approaching. Therefore, AH has been expected to be a good candidate for investigation as an effective microbicide to help prevent HIV transmission.

[1] In order to obtain insights into the pairing geometry of DNA duplexes containing *O*<sup>6</sup>-CMG as such a damaged base, and to further understand its biological implications, three self-complementary DNA dodecamers with the sequences d(CGCG[*O*<sup>6</sup>-CMG]ATTCGCG) (*O*<sup>6</sup>-CMG5T), d(CGC[*O*<sup>6</sup>-CMG]AATTCGCG) (*O*<sup>6</sup>-CMG4C) and d(CGC[*O*<sup>6</sup>-CMG]AATTTGCG) (*O*<sup>6</sup>-CMG4T) were synthesized, and their crystal structures have been determined by X-ray analyses<sup>1,2</sup>. And [2] in order to obtain structural insight of AH which specifically binds to the target D1 chain of HMTG, X-ray analysis of AH in complex with  $\alpha$ (1,2)mannotriose (D1) was performed<sup>3</sup>.

In the modified DNA [1], each of the three dodecamers forms a right-handed double helix similar to the unmodified duplexes. Introduction of *O*<sup>6</sup>-CMG into the dodecamers gives no significant effects on the intrinsic B-form conformation. Because the carboxy methyl groups of the *O*<sup>6</sup>-CMG residues are protruded into the major groove of the duplex. In the *O*<sup>6</sup>-CMG5T duplex (Fig. 1a), the *O*<sup>6</sup>-CMG residue forms a pair with T in the Watson-Crick type geometry similar to the canonical Watson-Crick A:T pair, but the pair formation is followed by a large propeller twist (-18°) to release the O...O repulsion. While in the *O*<sup>6</sup>-CMG4C duplex (Fig. 1b), the *O*<sup>6</sup>-CMG and its complementary C base form a pair to each other through the two hydrogen bonds, N<sup>1</sup>(*O*<sup>6</sup>-CMG)...N<sup>4</sup>(C) and N<sup>2</sup>(*O*<sup>6</sup>-CMG)...N<sup>3</sup>(C), so that the guanine moiety of *O*<sup>6</sup>-CMG displaces toward the major groove side. This wobble direction is reversed from those found frequently in G:T wobble pairs. In addition, this pairing is further stabilized by an additional hydrogen bond between the carboxyl oxygen and the paired C amino group. In the case of *O*<sup>6</sup>-CMG4T (Fig. 1c), however, the corresponding thymine base is remarkably displaced toward the major groove to form a hydrogen bond between thymine N<sup>3</sup> and carboxyl group of *O*<sup>6</sup>-CMG. In addition, a water molecule bridges between

O<sup>2</sup>(T) and N<sup>2</sup>(*O*<sup>6</sup>-CMG) to stabilize the pair formation through the two hydrogen bonds. The methylene carbon atom of the carboxymethyl group also participates in the interaction with O<sup>2</sup> atom of thymine base.

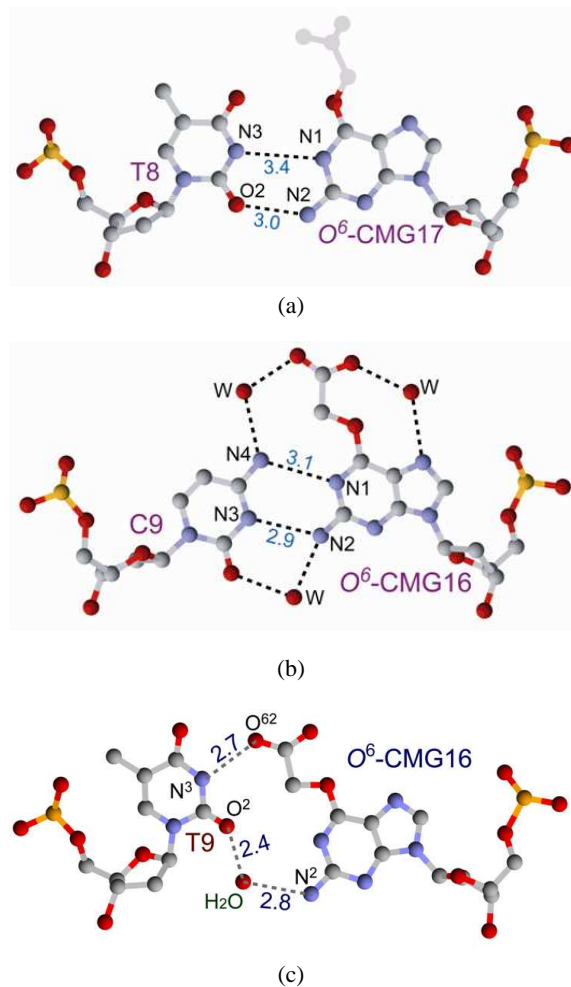


Fig. 1. Base pairs found in *O*<sup>6</sup>-CMG5T, *O*<sup>6</sup>-CMG4C and *O*<sup>6</sup>-CMG4T.

It is interesting to note that the geometries of *O*<sup>6</sup>-CMG:T pairs differ between *O*<sup>6</sup>-CMG5T and *O*<sup>6</sup>-CMG4T; the former is required to have a large propeller-twist (-25° in average). This could be ascribed to the difference of the position in the DNA sequence. In *O*<sup>6</sup>-CMG5T, the *O*<sup>6</sup>-CMG residue positions in the flexible AATT-tract, wherein the base pairs are allowed to adopt a large propeller-twist, while in *O*<sup>6</sup>-CMG4T, the *O*<sup>6</sup>-CMG residue locates in the rigid CGCG-tetrad wherein the base pair is forced to be planar. In other words, this finding means that *O*<sup>6</sup>-CMG and T can form a pair in the two modes depending on the context of neighboring sequence.

*In silico* model building of DNA polymerase in complex with  $O^6$ -CMG-containing DNA has suggested that the Watson-Crick-type  $O^6$ -CMG:T and the reversed-wobble type  $O^6$ -CMG:C pairing geometries are acceptable for incorporation of dCTP or dTTP against the opposite  $O^6$ -CMG by the polymerase. It implies that the  $O^6$ -CMG residues modified in a DNA template not only direct the incorporation of complementary dCTP but also direct the non-complementary dTTP to be incorporated into the newly synthesized DNA strand. Therefore, it can be concluded that the Watson-Crick-type  $O^6$ -CMG:T pair formation induces G:C→A:T transition mutation of genes demonstrated by *in vitro* and *in vivo* experiments, as an origin of increased risk of colorectal cancer. Although high wobble  $O^6$ -CMG:T pair is possible to occur depending on the tract context, in the DNA replication step, however, DNA is once split into two single strands and incorporated into the active site of DNA polymerase so that  $O^6$ -CMG can form a Watson-Crick type pair.

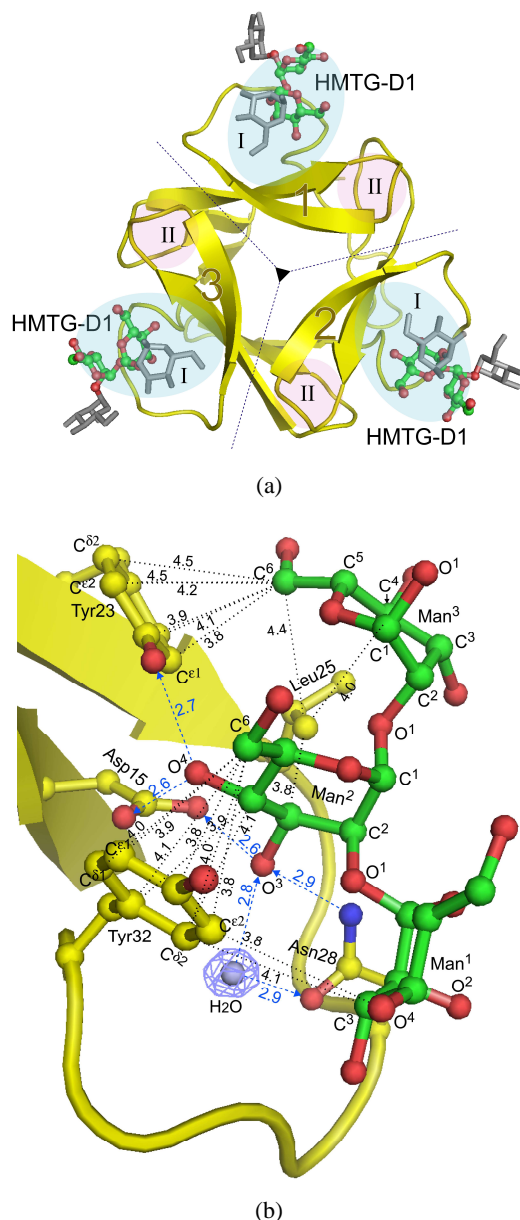


Fig. 2. AH bound to three D1 chains (a) and D1 bound in the pocket of a module (b).

In the AH:D1 complex [2], three D1 molecules are separately bound in the three pockets of AH which is composed of the three modules related by a molecular three-fold symmetry, as shown (Fig. 2a). In each pocket, the two mannose residues (MB parts) of D1s are definitely identified. However, the electron densities appear at the both ends of MB parts suggest that the crystal contains two different complexes in which a MB part (Man<sup>1</sup> and Man<sup>2</sup>) and another MB part (Man<sup>2</sup> and Man<sup>3</sup>) are bound to AH. However, the former MB part would be reasonable to choose for discussion because it just corresponds to the most sensitive end of D1 chain. Accordingly the Man<sup>3</sup> residue interacts with the hydrophobic residues of AH. In each of the three pockets, D1 adopts a double bracket-shape conformation (Fig. 2b) to help AH to be recognized specifically. In addition, an isolated water molecule is trapped inside of the pocket to stabilize the D1 binding through two hydrogen bonds. In addition, many hydrophobic interactions are involved in the Man<sup>1</sup>, Man<sup>2</sup> and Man<sup>3</sup> recognition.

From the Man<sup>3</sup> residue, the O<sup>1</sup> atom is protruded in the chain-extension direction suggesting how the remaining mannose residues can approach the D3 chain and others. A structural model of three-HMTG bound AH constructed based on this feature is useful for drug designing. However, it is still necessary to confirm this model by X-ray analyses of AH in complex with D3, HMTG and gp120.

As the Dr Thesis has been prepared based on the following three published papers<sup>1,2,3</sup>, it contains the structures of [1] damaged DNA and [2] anti-HIV active protein which are typical molecules in the life system. The results and discussions on the damaged DNA in the present studies are useful for understanding the gene mutation mechanism and for applying the chemical modification to nucleic acid drugs such as anti-gene DNA and anti-sense RNA, while those on a new lectin actinohivin are important knowledge for understanding biology of lectins as well as useful for designing more effective anti-HIV drugs for human health.

## Reference

1. Zhang, F., Suzuki, K., Tsunoda, M., Wilkinson, O., Millington, C. L., Williams, D. M. Morishita, E. C. & Takénaka, A. (2013) Structures of DNA duplexes containing  $O^6$ -carboxymethylguanine, a lesion associated with gastrointestinal cancer, reveal a mechanism for inducing transition mutations. *Nucleic Acids Res.*(IF=8.278), **41**, 5524-5532.
2. Zhang, F., Tsunoda, M., Suzuki, K., Kikuchi, Y., Wilkinson, O., Millington, C. L., Margison, G. P., Williams, D. M. & Takénaka, A. (2014)  $O^6$ -carboxy methylguanine in DNA forms a sequence context dependent wobble base pair structure with thymine. *Acta Cryst. D*(IF=14.1), **70**, 1669-1679.
3. Zhang, F., Hoque, M. M., Jiang, J., Suzuki, K., Tsunoda, M., Takeda, Y., Ito, Y., Tanaka, H. & Takénaka, A. (2014) The characteristic structure of anti-HIV actinohivin in complex with three HMTG-D1 chains of HIV-gp120. *Acta Cryst. D*(IF=14.1), submitted.