MS10-P9 **Damaged guanine residue relevant to gastrointesti-nal cancer allows thymine residue to be flexible between Watson-Crick type pairing and large wobbling.**
Fang Zhang[a], Masaru Tsunoda[b], Oliver Wilkinson[c], Christoper L. Millington[c], David M. Williams[c] and Akio Takénaka[a,b]
[a]Grad. Sch. Sci. & Eng., [b]Fclty. Pharm., Iwaki-Meisei Univ., Iwaki 970-8044, Japan, and [c]Ctr. Chem. Biol., Krebs Inst., Univ. Sheffield, Sheffield S3 7HK, UK
E-mail: atakenak@iwakimu.ac.jp

It is known that diets high in red, especially preserved meat stimulates *N*-nitrosation of glycine and that its derivative (diazoacetate) alkylates guanine bases in DNA forming O^6-carboxymethylguanine (hereafter X). This modification induces DNA mutations and is associated with increased risk of gastrointestinal cancer. In order to obtain insights into the pairing geometry of DNA duplexes containing X as such a damaged base, and to further understand its biological implications, we previously determined their crystal structures containing X which revealed that X:T base pairs adopt the Watson-Crick type, but not the well known wobble type (X5:T), and the X:C pairs adopt the reserved-wobble type but not the canonical Watson-Crick type (X4:C). To further investigate the versatility of X in base-pair formation, another damaged DNA duplex containing X at different position [d(CGCXAATTTGCG), X4:T] has been X-ray analyzed at 1.6 Å resolution in the present study. <Result> As expected, two X4:T dodecamers are associated to form a right-handed double helix with a B-form confor-mation similar to those of the unmodified duplexes, and the paired bases are all the Watson-Crick types except for X which does not form a pair with T of the partner. This is quite different from the Watson-Crick type found in the X5:T pair. The guanine moieties of two X bases are almost the same positions as guanine in the unmodified DNA and the carboxymethyl groups protrude into the major groove of the duplex, suggesting that carboxymethylation has no significant effects on the DNA conformation. In the case of X5:T, the thymine base partnered with X forms a Watson-Crick type pair through the two hydrogen bonds, N^3(T)H···N^1(X) and O^2(T)···N^2(X), which is not the case for the X4:T pair. Instead, the corresponding thymine base (in the A chain) is largely moved away (wobbled) to the major groove so that the N^3(T) atom forms a hydrogen bond with the O(X) atom of the carboxyl group at a distance 2.6~2.9Å. In addition, a water molecule is bridged by two hydrogen bonds between O^2(T) and N^2(X) to stabilize the pairing. These situations are the same as those of the complementary strand (B chain). The protruded O^4(T) atom is directly interacting with a solvent strontium cation which was used for crystallization. <Conclusion> The present result shows that X can form a pair with T in the two alternative modes, Watson-Crick type and largely wobbled type, depending on the sequence context. When the damaged DNA containing the O^6-carboxymethyl guanine is used as a template for DNA replication, X could be accommodated in the DNA polymerase active site within a Watson-Crick type base pair. This mispairing provides the basis for GC-AT transition mutations that are a consequence of such DNA damage.

Keywords: crystal structure; damaged DNA; mutagenesis

MS11-P1 **Structure-function studies on the proteolytic activity of the *Marasmius oreades* agglutinin (MOA), a cytotoxic lectin.**
Gabriele Cordara[a,b], Irwin J. Goldstein[c], Kirsten Sandvig[b,d,e] & Ute Krengel[a], *[a]Department of Chemistry, University of Oslo, Oslo, NORWAY, [b]Department of Biochemistry, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, NORWAY, [c]Department of Biological Chemistry, Medical School, University of Michigan, Ann Arbor, USA, [d]Department of Molecular Biosciences, University of Oslo, Oslo, NORWAY, [e]Centre for Cancer Biomedicine, University of Oslo, Oslo, NORWAY*
E-mail: gabriele.cordara@kjemi.uio.no

Lectins, sugar binding proteins of non-immune origin, are one of the many weapons used by plants and fungi to defend themselves against potential predators and parasites. This role is often fulfilled by the presence of an additional domain or subunit with catalytic function.

The *Marasmius oreades* agglutinin (MOA) is a Gal-a3Gal specific lectin extracted from the fruiting body of the *Marasmius oreades* mushroom. Parenterally administered MOA triggers a systemic effect in mice, which closely resembles the symptoms of the shiga toxin-induced hemolytic uremic syndrome (Stx-HUS) in humans. The structure of the MOA homodimer in complex with calcium, solved by X-ray crystallography [1], suggests an enzymatic function associated to the C-terminal dimerization domain, experimentally confirmed to be associated with proteolytic activity [2].

The enzymatically active domain of MOA shows an a/b hydrolase fold, bearing a structural resemblance to the enzymes of the papain-like cysteine peptidase family (clan CA). A notable feature in MOA is the conservation of two key residues of the catalytic triad (Cys215 and His257), while the third residue is substituted by an amino acid with similar properties (Glu274 in place of Asp). Here, we present further evidence, including crystallographic data, pointing to a key role of these residues for catalysis.

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