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Repair of alkylated DNA: Recent advances

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ABSTRACT

Cytotoxic and mutagenic methylated bases in DNA can be generated by endogenous and environmental alkylating agents. Such damaged bases are removed by three distinct strategies. The abundant toxic lesion 3-methyladenine (3-alkyladenine) is excised by a specific DNA glycosylase that initiates a base excision-repair process. The toxic lesions 1-methyladenine and 3-methylcytosine are corrected by oxidative DNA demethylation catalyzed by DNA dioxygenases. These enzymes release the methyl moiety as formaldehyde, directly reversing the base damage. The third strategy involves the mutagenic and cytotoxic lesion O^6-methylguanine which is also repaired by direct reversal but uses a different mechanism. Here, the methyl group is transferred from the lesion to a specific cysteine residue within the methyltransferase itself. In this review, we briefly describe endogenous alkylating agents and the extensively investigated DNA repair enzymes, mammalian 3-methyladenine-DNA glycosylase and O^6-methylguanine-DNA methyltransferase. We provide a more detailed description of the structures and biochemical properties of the recently discovered DNA dioxygenases.

1. Introduction

Environmental causes of cancer, such as cigarette smoke, ionizing radiation, sunlight, aflatoxin and certain viral infections are well documented, however, individuals who avoid exposure to these agents remain susceptible to cancer. Despite extensive epidemiological investigations, no other major environmental genotoxins have been identified. An alternative source of DNA damage must therefore exist and interest has, in recent years, turned towards endogenous DNA damage, "the enemy within".

Over 50 years ago the great biochemist Otto Warburg proposed that oxygen metabolism was a key factor in cancer induction. Indeed, experimental evidence that mutagenic and toxic DNA lesions are generated by reactive oxygen species was subsequently reported. However, in widely quoted studies from the last decade, the level of endogenous oxidative damage had been overestimated as it included oxidised bases formed during the isolation of DNA. These reports so influenced the field that many accounts of endogenous DNA damage have failed to consider other causes [1,2]. Current estimates suggest that oxidative DNA damage due to reactive oxygen species and lipid peroxidation products occurs at levels similar to other deleterious events, such as hydrolytic depurination and deamination; incorporation of damaged deoxynucleoside triphosphates into DNA; and reaction with endogenous alkylating agents [3–5].

Except for S-adenosylmethionine (SAM, Fig. 1), sources of endogenous DNA alkylation are not well defined. Other possible sources include nitroso compounds related to the well known mutagen methylnitrosourea which are generated *in vitro* by nitrosation of cellular amines including amino acids, proteins and polyamines [6].

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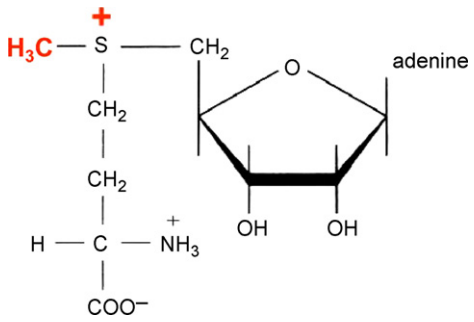

Fig. 1 – Structure of S-adenosylmethionine (SAM), the major endogenous DNA alkylating agent. The positive charge on the sulfur residue serves to activate the methyl group of the methionine which is readily donated in both enzymatic and nonenzymatic transfer reactions.

2. Endogenous DNA alkylation by SAM

SAM is the donor of methyl groups in the majority of *in vivo* enzymatic methylation events involving a wide variety of acceptor molecules. The high transfer potential of SAM however means that it will also spontaneously methylate cellular nucleic acids and proteins at a low but significant rate. SAM acts by an S_N2 (bimolecular nucleophilic substitution) mechanism and yields the same products on alkylation of DNA as the experimental alkylating agent, methyl methanesulfonate (MMS). However, the reactivity of SAM is 2000-fold weaker than MMS [7]. SAM is present in various tissues at a concentration of 25 to 50 μM [8].

The products detected on treatment of double-stranded DNA with SAM are 7-methylguanine and 3-methyladenine (3meA). Whilst 7-methylguanine is relatively innocuous, 3meA has a strong toxic effect. The methyl group of 3meA does not perturb interaction with the complementary DNA strand, rather it protrudes into the minor groove of the double helix which is normally free of methyl groups (the methyl groups of thymine and 5-methylcytosine are in the major groove). Here, 3meA efficiently blocks RNA- and most DNA polymerases resulting in a strong cytotoxic but feeble mutagenic effect. In these early studies, SAM-induced methylation of single-stranded DNA was not examined. However, other S_N2 methylating agents generate substantial amounts of 1-methyladenine (1meA) and 3-methylcytosine (3meC) in single-stranded DNA [9,10]. These lesions are unable to base pair and block DNA replication resulting in a strong toxic but weak mutagenic effect similar to 3meA. The low mutagenicity observed may be due to translesion synthesis by non-replicative DNA polymerases.

3. Mammalian 3-alkyladenine-DNA glycosylase (MAG or AAG)

Whilst some bacteria contain two or more distinct DNA glycosylases that catalyze the excision of 3meA from DNA, mammalian cells have only one (MAG, also called AAG for 3-alkyladenine-DNA glycosylase) (Fig. 2). Interestingly, mammalian AAG shows regional similarities to *Bacillus subtilis* and *Arabidopsis thaliana* 3meA-DNA glycosylases but is entirely different from *E. coli*, *S. cerevisiae* and *S. pombe* enzymes. In contrast, *E.coli*, yeast and human uracil-DNA glycosylases (UNG) show strong sequence homology [11]. This apparent difference in conservation between the UNG enzymes and the 3-methyladenine-DNA glycosylases can be explained by considering the relative stability of the glycosyl bonds of their substrates. A dUMP residue in DNA is as stable as the four common nucleotides. An efficient and highly specialized enzyme is therefore required to release uracil by promoting cleavage of the uracil-deoxyribose bond by more than 10^7-fold. By contrast, a 3meA-deoxyribose bond is intrinsically unstable with a half-life of about 26 h at 37 °C, pH 7 [12]. As a result, a glycosylase that can detect and flip out this residue in DNA and then promote cleavage of the glycosyl bond by a mere 1000-fold could succeed in excising 3meA; apparently this goal can be achieved by glycosylases using several different strategies.

Resolution of the three-dimensional structure of human AAG revealed a unique fold and provided substantial information on the base excision mechanism [13]. AAG binds and slightly widens the minor groove of DNA. The enzyme then inserts an aromatic residue into the helical stack as a probe for the altered base. This probably occurs during rapid sliding of AAG along the DNA by diffusion as was recently established for the Fpg/MutM glycosylase and its eukaryotic counterpart Ogg1 [14,15]. After recognition of a site of damage, AAG may trigger flipping-out of the altered deoxynucleoside sandwiching it between two aromatic amino acid residues in the active site of the enzyme. The positive charge of the 3meA residue will encourage these stacking interactions, whilst a strategically bound water molecule may then promote hydrolysis of the glycosyl bond [13,16].

AAG has a broad substrate specificity and besides 3-methyladenine can excise other altered purine residues, such as the minor lesions hypoxanthine and 1, N^6-ethenoadenine from DNA. AAG initially activates these neutral base lesions by protonation of the base to allow for general acid catalysis [16]. Of these three lesions, 3meA in DNA is the preferred AAG substrate and is released more rapidly than either hypoxanthine or 1, N^6-ethenoadenine [17]. Studies using oligonucleotides containing a labile 3-methyldeoxyadenosine residue have not been performed due to technical problems in preparing such a substrate. Of the multiple DNA glycosylases that occur in certain bacteria, some share the broad substrate specificity of human AAG whilst others are absolutely specific for 3meA [18]. A DNA glycosylase that releases hypoxanthine or 1, N^6-ethenoadenine but not 3meA from DNA has not been detected in bacteria or eukaryotic cells.

AAG discriminates against normal purines due to unfavourable interactions with their exocyclic amino groups [19]. Nevertheless, AAG can catalyze slow excision of undamaged adenine and guanine from DNA and highlights the broad substrate range of AAG [20]. This reaction rate is so slow that it is of little relevance *in vivo* when compared with the continuous loss of adenine and guanine from DNA by nonenzymatic hydrolytic depurination at 37 °C. However, overexpression of human AAG in *S. cerevisiae* results in a small increase in mutagenesis which may be due to the enhanced excision of normal bases and generation of mutagenic abasic sites [21].