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Differential repair of etheno-DNA adducts by bacterial and human AlkB proteins

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ABSTRACT

AlkB proteins are evolutionary conserved Fe(II)/2-oxoglutarate-dependent dioxygenases, which remove alkyl and highly promutagenic etheno(ε)-DNA adducts, but their substrate specificity has not been fully determined. We developed a novel assay for the repair of ε-adducts by AlkB enzymes using oligodeoxynucleotides with a single lesion and specific DNA glycosylases and AP-endonuclease for identification of the repair products. We compared the repair of three ε-adducts, 1,N^6-ethenoadenine (εA), 3,N^4-ethenocytosine (εC) and 1,N^2-ethenoguanine (1,N^2-εG) by nine bacterial and two human AlkBs, representing four different structural groups defined on the basis of conserved amino acids in the nucleotide recognition lid, engaged in the enzyme binding to the substrate.

Two bacterial AlkB proteins, MT-2B (from *Mycobacterium tuberculosis*) and SC-2B (*Streptomyces coelicolor*) did not repair these lesions in either double-stranded (ds) or single-stranded (ss) DNA. Three proteins, RE-2A (*Rhizobium etli*), SA-2B (*Streptomyces avermitilis*), and XC-2B (*Xanthomonas campestris*) efficiently removed all three lesions from the DNA substrates. Interestingly, XC-2B and RE-2A are the first AlkB proteins shown to be specialized for ε-adducts, since they do not repair methylated bases. Three other proteins, EcAlkB (*Escherichia coli*), SA-1A, and XC-1B removed εA and εC from ds and ssDNA but were inactive toward 1,N^2-εG. SC-1A repaired only εA with the preference for dsDNA. The human enzyme ALKBH2 repaired all three ε-adducts in dsDNA, while only εA and εC in ssDNA and repair was less efficient in ssDNA. ALKBH3 repaired only εC in ssDNA. Altogether, we have shown for the first time that some AlkB proteins, namely ALKBH2, RE-2A, SA-2B and XC-2B can repair 1,N^2-εG and that ALKBH3 removes only εC from ssDNA. Our results also suggest that the nucleotide recognition lid is not the sole determinant of the substrate specificity of AlkB proteins.

1. Introduction

AlkB proteins belong to the superfamily of Fe(II)/2-oxoglutarate-dependent dioxygenases, and the first AlkB member was discovered in *Escherichia coli* (EcAlkB) [1–5]. EcAlkB is a nucleic acid repair enzyme that hydroxylates alkyl groups bound to the nitrogen atoms at position 1 of purines and 3 of pyrimidines. The resulting unstable hydroxylalkyl group is then spontaneously released as an aldehyde, which leads to the regeneration of the unmodified base (Fig. 1) [2,3]. The main substrates repaired by EcAlkB are 1-methyladenine (m^1A) and 3-methylcytosine (m^3C) in single-stranded (ssDNA) and double-stranded DNA (dsDNA) [2,3,6–8]. Intriguingly, EcAlkB is also able to remove methyl lesions from RNA, suggesting a role for AlkB proteins in RNA repair [9–12]. In addition to the methyl lesions, EcAlkB repairs larger groups, *e.g.* ethyl, hydroxyethyl, propyl,

Abbreviations: ε, etheno; εA, 1,N^6-ethenoadenine; εC, 3,N^4-ethenocytosine; 1,N^2-εG, 1,N^2-ethenoguanine; 1-meA, 1-methyladenine; 3-meC, 3-methylcytosine.

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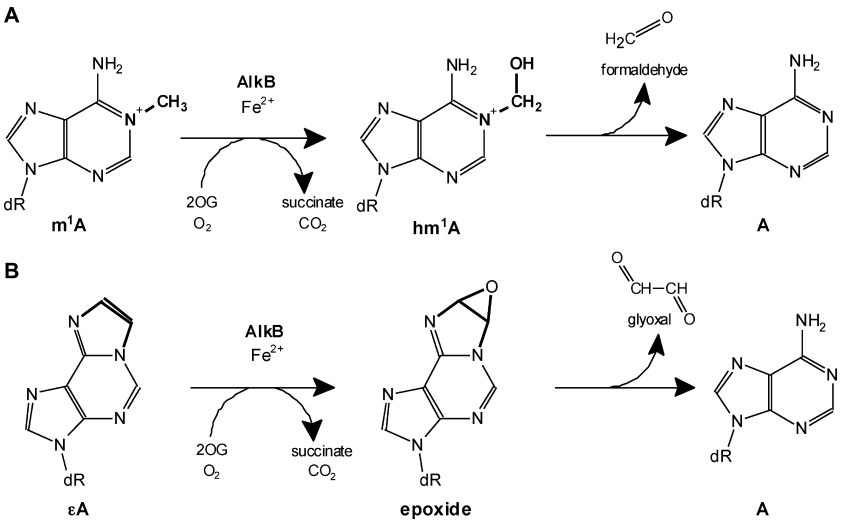

Fig. 1. The mechanism of AlkB-mediated DNA repair through oxidative dealkylation of 1-methyladenine (m^1A) (A) and 1,N^6-ethenoadenine (εA) (B).

hydroxypropyl, and some exocyclic DNA adducts (ε-adducts) [13–15].

Homologues of EcAlkB are present in numerous bacteria, eukaryotes, and certain plant RNA viruses. Nine AlkB homologues have been identified in humans: ALKBH1-ALKBH8 (AlkB homologue) and fat mass and obesity-associated protein FTO [1,16–18]. ALKBH2 and ALKBH3 are the sole human homologues that complement AlkB deficiency in *E. coli* regarding reactivation of ssDNA phages in response to methylating agents (*e.g.*, methyl methane sulfonate (MMS) [9,13]. ALKBH3 demethylates m^1A and m^3C in ssDNA and RNA but is inactive toward these lesions in dsDNA [8–10,19–21]. ALKBH2 repairs alkylated bases only in DNA, preferentially in dsDNA [9,19,20]. Among vertebrate AlkB proteins, only ALKBH2 is considered a true repair enzyme, since ALKBH2-null mice accumulate m^1A in their genomes, but ALKBH3-null mice do not [22]. Recently, the function of ALKBH proteins has been expanded beyond nucleic acid repair. FTO and ALKBH5 were found to demethylate N^6-methyladenine in mRNA [23–25], whereas ALKBH8 catalyzes the final two step modifications of wobble uridine in tRNA anticodons [26–29]. Finally, ALKBH1 and ALKBH4 have been proposed to demethylate lysine in histone H2A and actin, respectively [30,31].

AlkB proteins also repair the highly mutagenic exocyclic ε-adducts [15,32–36], *e.g.* 1,N^6-ethenoadenine (εA) and 3,N^4-ethenocytosine (εC). During the AlkB-mediated repair by oxidative dealkylation, the etheno bridge is converted to an epoxide and the product undergoes non-enzymatic hydration to a glycol which is then released as glyoxal (Fig. 1B) [32,34]. These lesions, as well as N^2,3-ethenoguanine (N^2,3-εG), and 1,N^2-ethenoguanine (1,N^2-εG) are formed in the reaction of a variety of reactive bifunctional electrophilic agents with a nitrogen atom of the DNA base, followed by dehydration and ring closure [37,38]. These ubiquitous lesions are generated in cellular DNA either by endogenous metabolic processes, *e.g.*, oxidative stress-induced lipid peroxidation, or by the reaction of the DNA with diverse chemical carcinogens, *e.g.*, bioactivated derivatives of vinyl chloride or urethane [39–42]. Since the additional exocyclic ring disrupts the Watson–Crick hydrogen bonding, the ε-lesions generate a broad spectrum of base substitutions (transitions and transversions) or frameshift mutations [41,43]. It is estimated that in mammalian DNA from 14% to 60% of ε-adducts give rise to mutations, while only 3% of 8-oxoguanine residues are pro-mutagenic in cells with functional DNA repair [44–46]. These promutagenic properties of ε-lesions strongly underlie their contribution to carcinogenesis in mammals [47–49].

It is commonly believed that the main mechanism for removal of the ε-adducts from DNA is base excision repair (BER) initiated by specific DNA glycosylases. In mammalian cells εA and 1,N^2-εG are removed by *N*-methylpurine-DNA glycosylase (ANPG) [50,51], and εC is repaired by mismatch-specific thymine-DNA glycosylase (TDG) [52,53]. In addition to TDG, a protein that binds methylated CpG islands (MBD4/MED1) and uracil-DNA glycosylase specific for ssDNA (SMUG1) are also capable of excising εC from the DNA [54,55]. In *E. coli*, N^2,3-εG is excised by alkyl-*N*-purine-DNA glycosylase AlkA, which also excises εA, but the latter with a very low efficiency [50,56]. Mismatch-specific uracil-DNA glycosylase from *E. coli*, Mug excises from the DNA εC and 1,N^2-εG [51,53,57]. Recently it has been shown that εA and εC can also be repaired *via* an alternative nucleotide incision repair (NIR) pathway, in which human AP endonuclease APE1 makes an incision 5′ to the ε-base in a DNA glycosylase-independent manner [58]. Additionally, εA and εC are removed from the DNA *in vitro* by EcAlkB and ALKBH2 [15,32–36].

AlkB proteins are widespread among bacteria, and some bacterial species possess two or three AlkBs, but their substrate specificities are poorly characterized. Based on sequence alignment and phylogeny analysis bacterial AlkB proteins have been divided into four groups 1A, 1B, 2A, and 2B each characterized by the presence of specific conserved amino acid residues in the nucleotide recognition lid, which has been postulated to specify substrate recognition, at least partially [59].

The main goal of this work was to gain insight into repair of ε-adducts by bacterial and human AlkB proteins. To reach this goal we developed a new, sensitive, and time and cost effective BER-mediated assay, and analyzed the ability of nine bacterial and two human AlkB proteins representing all four groups mentioned above (1A, 1B, 2A, and 2B) to remove from the DNA εA, εC and 1,N^2-εG *in vitro* in ss and dsDNA. To the best of our knowledge repair of 1,N^2-εG has not been presented before, and for several of the studied enzymes repair of εC was not studied as well. In this newly developed assay the DNA with a single lesion is subjected to AlkB protein, and subsequently digested with an appropriate DNA glycosylase excising the damaged base and AP-endonuclease, which cleaves the DNA at the site of the lesion if the damage was not repaired by AlkB protein.

The majority of the bacterial AlkBs tested were able to repair ε-lesions. Three of the proteins analyzed, SA-2B (*Streptomyces*