Different efficiencies of the Tag and AlkA DNA glycosylases from *Escherichia coli* in the removal of 3-methyladenine from single-stranded DNA

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Abstract ***Escherichia coli*** **possesses two different DNA repair glycosylases, Tag and AlkA, which have similar ability to remove the alkylation product 3-methyladenine from double-stranded DNA. In this study we show that these enzymes have quite different activities for the excision of 3-methyladenine from single-stranded DNA, AlkA being 10–20 times more efficient than Tag. We propose that AlkA and perhaps other glycosylases as well may have an important role in the excision of base damage from single-stranded regions transiently formed in DNA during transcription and replication.**

Key words: Alkylation DNA damage; 3-Methyladenine; DNA repair; 3-Methyladenine DNA glycosylase

1. Introduction

Alkylation damage to DNA can result from nonenzymatic methylation by endogenous methyl donors like *S*-adenosylmethionine and by exposure to chemical agents in the environment [1]. One of the most important lesions induced by alkylation is 3-methyladenine (m^3A), which acts as a block to DNA replication [2,3]. *Escherichia coli* possesses two DNA glycosylases which excise m^3A from DNA with high efficiency, i.e. m^3A DNA glycosylase I (Tag) and m^3A DNA glycosylase II (AlkA). Tag (M_r 21.1 kDa) is constitutively expressed, whereas AlkA (M_r 31.4 kDa) is induced by cell exposure to sublethal doses of alkylating agents [4,5]. The only product shown to be excised efficiently from methylated DNA by Tag is m^3A, although the enzyme also exhibits some activity for removal of 3-methylguanine (m^3G) [6]. By contrast, AlkA has a broad substrate specificity and can excise a variety of methylated bases in addition to m^3A from methylated DNA [7,8].

It is now well established that the nucleotide excision repair pathway is coupled to transcription and perhaps also to replication [9]. The base excision repair pathway is generally considered not to be associated with these processes; however, some data indicate the opposite [10] and not much has been done to elucidate this question. In this study we have analyzed the ability of the alkylation repair DNA glycosylases from *E. coli* to remove alkylation damage from single-stranded DNA, in view of the likelihood that a DNA glycosylase potentially associated with transcription or DNA replication would have the ability to remove damaged bases from the template strand after strand separation. It appears that the AlkA enzyme but not the Tag DNA glycosylase will remove m^3A from single-stranded DNA. A model is proposed suggesting a role of DNA glycosylases for base removal from single-stranded DNA formed transiently during replication and transcription.

2. Materials and methods

Tag was purified to homogeneity from a strain mutated in *alkA* transformed by a multicopy *tag*$^+$ plasmid as described [11]. AlkA was purified to homogeneity from a strain mutated in *tag* transformed by a multicopy *alkA*$^+$ plasmid by a protocol similar to that used for Tag [12]. Poly(dA) (4000 pmol) was treated with 0.48 mCi *N*-[^{3}H]methyl-*N*-nitrosourea (Amersham, TRQ 5044, 9.8 Ci/mmol) in 0.25 M sodium cacodylate, pH 7.4/1 mM EDTA in a total volume of 0.4 ml at 37°C for 2.5 h in the dark. Poly(dA)/poly(dT) (2000 pmol) was treated in the same way with 0.24 mCi of this reagent in a total volume of 0.2 ml. The alkylated oligonucleotides were purified on a NAP-5 column (Pharmacia Biotech) equilibrated with 0.1 M Tris-HCl/10 mM triethylamine/1 mM EDTA, pH 7.7 followed by further purification on Nensorb 20 (DuPont NEN). The fraction of alkylated bases in the substrate was determined by acid depurination (70°C for 20 min) and HPLC [11]. Enzyme reactions and reverse phase HPLC (Spheri-5 RP-18, 220×4.6 mm, Brownlee Labs) of the alkylated bases released were performed as described [6]. Average retention times were 3.5 min for 1-methyladenine (m^1A), 7.7 min for m^3A, 23.2 min for 6-methyladenine (m^6A) and 12.6 min for 7-methyladenine (m^7A). Reference compounds: m^1A and m^3A were obtained from Fluka and m^6A was obtained from Sigma; m^7A was a gift from Dr. T. Lindahl.

3. Results

Because the two different m^3A DNA glycosylases in *E. coli*, Tag and AlkA, have been shown to excise m^3A from double-stranded DNA with similar efficiency [13], we addressed the question as to whether they are both involved in m^3A removal from single-stranded DNA. To investigate this, the activities of Tag and AlkA were tested on methylated poly(dA). The poly(dA) substrate was chosen as a model for alkylated single-stranded DNA, inasmuch as interference with methylated guanines, which are substrates for AlkA, as well as problems with secondary DNA structures could be avoided.

After treatment with *N*-[^{3}H]methyl-*N*-nitrosourea the fractions of m^1A, m^3A and m^7A in poly(dA) were quantified by acid depurination and HPLC to be 39%, 7.9% and 6.8%, respectively, of the material recovered after HPLC (Fig. 1A; Table 1). By HPLC analysis of methylated bases released by Tag and AlkA from the substrate it was observed that AlkA effected 27% and Tag 2.7% excision of m^3A (Fig. 1B; Table

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Abbreviations: AP, apurinic/apyrimidinic; HPLC, high performance liquid chromatography

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1); in another experiment 12% excision by AlkA and 0.6% excision by Tag were observed (Table 1). Neither of the enzymes excised m^1A (Fig. 1B; Table 1), the major alkylation product formed in poly(dA) (Fig. 1A [14]). The structure of poly(dA)/poly(dT) has been determined to be a B-type double helix with a narrow minor groove [15], and as a control, HPLC analysis of methylated bases released by the enzymes from alkylated poly(dA)/poly(dT) was performed (Fig. 2A,B). The results showed essentially the same excision of m^3A by Tag and AlkA, whereas m^7A was excised by AlkA but not by Tag from this substrate (Fig. 2B; Table 1). This is consistent with data previously obtained with alkylated double-stranded calf thymus DNA [7,13]. In addition, neither AlkA nor Tag excised m^1A from alkylated poly(dA)/poly(dT) (Fig. 2A,B; Table 1).

4. Discussion

Individual mutants of *E. coli* carrying *tag* or *alkA* are both sensitive to alkylation exposure suggesting individual roles of these enzymes in the repair of alkylation damage. Most, but not all of the sensitivity of the *alkA* mutant can be ascribed to a defect in the repair of m^3G which is an important substrate for AlkA [16]. In this paper we have looked at the possibility that Tag or AlkA might have distinct roles in the repair of m^3A and that one enzyme might be specifically involved in removal of m^3A from single-stranded DNA. Single-stranded regions are formed transiently during transcription and DNA replication and it may be of benefit for the cell to be capable of removing damage at the replication fork or residing in the template strand during transcription. The present results have shown that the AlkA enzyme has the capability of removing m^3A from single-stranded DNA and therefore could serve such a purpose in the cell.

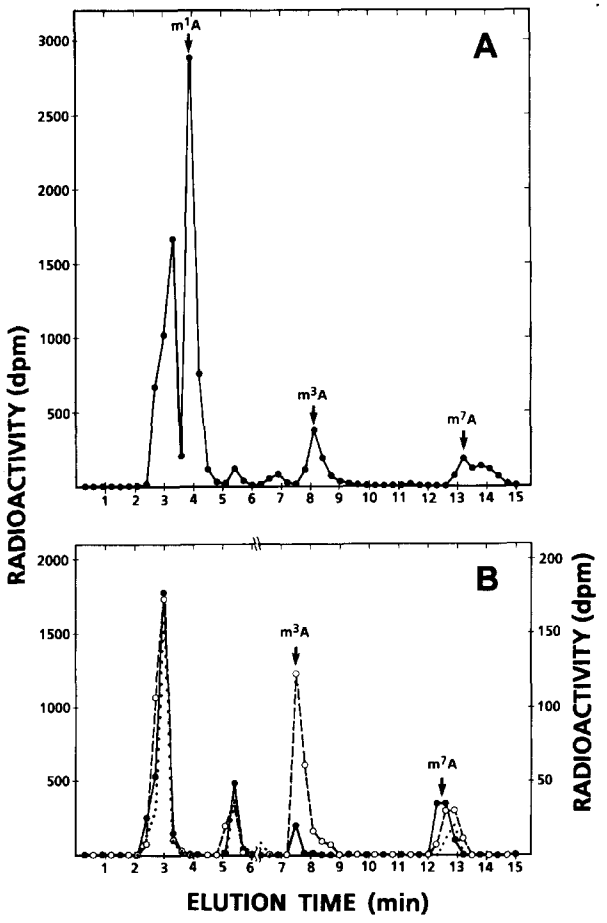

Fig. 1. A: Reverse phase HPLC of methylated bases released by acid depurination from *N*-[^{3}H]methyl-*N*-nitrosourea-treated 18-mer poly(dA). Following acid treatment the DNA was precipitated with ethanol and the supernatant analyzed by HPLC. B: HPLC of methylated bases released by Tag (●) or AlkA (○) from the same amount of substrate as used in A. DNA (1000 pmol) containing 1.65 pmol *methyl*-^{3}H (36 000 dpm) was incubated with 50 pmol enzyme in a volume of 150 μl for 30 min at 37°C. The DNA was precipitated with ethanol and the supernatants analyzed by HPLC. The dotted line represents incubation without enzyme. Note the change in scale of the Y-axis and breaks in the X-axis after 6 min elution time. Radioactivity measured in the different fractions is presented in Table 1 (Exp. 1).

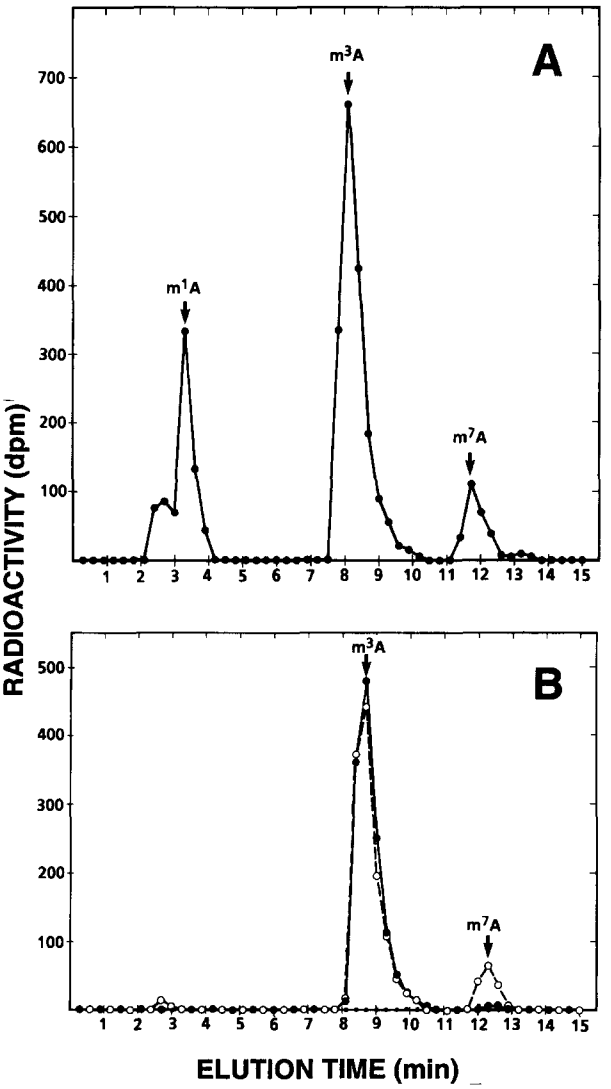

Fig. 2. A: Reverse phase HPLC of methylated bases released by acid depurination from *N*-[^{3}H]methyl-*N*-nitrosourea-treated 18-mer poly(dA)/poly(dT). B: HPLC of methylated bases released by Tag (●) or AlkA (○) from the same amount of substrate as used in A. DNA (170 pmol) containing 0.368 pmol *methyl*-^{3}H (8000 dpm) was incubated with 50 pmol enzyme in a volume of 150 μl for 30 min at 37°C. The DNA was precipitated with ethanol and the supernatants analyzed by HPLC. The dotted line represents incubation without enzyme. Radioactivity measured in the different fractions is presented in Table 1 (Exp. 1).

Table 1
Release of methylated bases from single- and double-stranded DNA by enzymatic removal with Tag or AlkA, or by acid depurination

	Exp. no.	Radioactivity							
		m^1A		m^3A			m^7A		Total
		dpm	%	dpm	%		dpm	%	dpm
Poly(dA)									
acid		4000	39[a]	807	7.9[a]	100[b]	692	6.8[a]	10258[c]
Tag	1	1		22		2.7[b]	48		3893[c]
	2	0		5		0.6[b]	30		3269[c]
AlkA	1	0		216		27[b]	46		4399[c]
	2	0		98		12[b]	47		3576[c]
no enzyme	1	0		0			32		3103[c]
	2	0		0			21		3020[c]
Poly(dA)/poly(dT)									
acid	1	580	20[a]	1786	60[a]	100[b]	258	8.7[a]	2970[c]
	2	497	14[a]	2251	64[a]	100[b]	165	4.7[a]	3519[c]
Tag	1	0		1310		73[b]	6		1356[c]
	2	0		1640		73[b]	2		2018[c]
AlkA	1	0		1215		68[b]	144		1425[c]
	2	0		1175		52[b]	43		1702[c]
no enzyme	1	0		0			5		38[c]
	2	0		12			1		354[c]

[a]In percent of total dpm.
[b]In percent of m^3A released by acid treatment.
[c]Recovered after HPLC.

Presently available data may also suggest why AlkA and not Tag exhibits significant activity towards base residues on single-stranded DNA. The major and possibly only substrate for Tag in vivo is the frequently formed *N*-alkylation product m^3A, although some activity has also been detected towards the less frequently formed m^3G in vitro [6]. The 3-methylpurines are quite similar in structure and block replication. It is not evident that excision of m^3A and m^3G at the replication fork would be of any advantage since this would only produce noncoding AP sites that also prevent strand elongation. However, the AlkA enzyme is capable of removing several different lesions from the DNA of which some are clearly promutagenic and do not pose a block to replication, e.g. O^2-methylpyrimidines [8,17]. To avoid mutations arising from replication past miscoding lesions it may be better to produce noncoding AP sites. Such sites will interrupt the progression of the replication fork and can be handled subsequently by the base or nucleotide excision repair pathways in combination with postreplication repair. The uracil DNA glycosylase has also been shown to work efficiently on single-stranded DNA, in fact with similar or even higher rate than on double-stranded DNA [18]. The hypothesis of replication-linked base removal is supported by reports on the existence of larger protein complexes including the human uracil DNA glycosylase and DNA polymerase α [19].

A possible close relation between the base and nucleotide excision repair pathways may also exist, since the AP site generated when e.g. m^3A is removed from DNA is known to be a substrate for the nucleotide excision repair complex as opposed to m^3A which is not [20]. Thus, such residues can be made available for the nucleotide excision repair pathway which is known to operate on the transcribed strand. However, whether preferential base excision at transcribed regions in DNA takes place is presently not known.

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