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Communication

Endogenous and exogenous DNA lesions recognized by *N*-alkylpurine-DNA glycosylases*°

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The combined action of glycosylases and abasic site-specific endonucleases on damaged bases in DNA results in single strand breaks.In plasmid DNA, as a consequence, the covalently closed circular *(ccc)* form is converted to the open circular *(oc)* form, and this can be quantitated by agarose gel electrophoresis. We studied DNA lesions sensitive to *E. coli* 3-methyladenine-DNA glycosylase II (AlkA) and cloned human *N*-alkylpurine-DNA glycosylase (ANPG-40) which are known to excise alkylated bases and etheno adducts. pBR322 and pAlk10 plasmids not pretreated with mutagens were cleaved by both glycosylases in the presence of enzymes possessing endonucleolytic activity, which indicates that plasmids contain unknown, endogenously formed adducts. Plasmids pretreated with chloroacetaldehyde, a mutagen forming etheno adducts, exhibited enhanced sensitivity to both glycosylases. Adducts formed by acrolein and croton aldehyde were excised by AlkA, but not by ANPG-40, whereas malondialdehyde adducts were not excised by either glycosylase. Bulky *p*-benzochinone adducts were not excised by AlkA, however, the plasmid pretreated with this mutagen was incised by endonucleases, possibly without prior generation of an abasic site. These examples show that examination of conformational changes of plasmid DNA can be taken advantage of to study the specificity of *N*-alkylpurine-DNA-glycosylases.

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Abbreviations: ACR, acrolein; ALK, 3-methyladenine-DNA glycosylase II, AlkA protein; ANPG-40, *N*-alkylpurine-DNA glycosylase; CAA, chloroacetaldehyde; CRA, crotonaldehyde; EXO, exonuclease III; FPG, formamidopyrimidine glycosylase, MutM protein; MDA, malondialdehyde; NTH, thymine glycol glycosylase, endonuclease III; pBQ, *para*-benzoquinone.

Most of the work on the chemistry and biology of DNA damage has focused on adducts derived from exogenous chemicals, related to environmental or occupational exposure of humans. However, DNA in cells undergoes continuous damage by both, exogenous as well as endogenous factors. Hydrolytic reactions, exposure to active oxygen species formed during normal metabolism and non-enzymatic methylation by *S*-adenosylmethionine are well known endogenous sources of DNA damage [1]. Recently, the occurrence of cyclic adducts, which are most likely endogenously formed in the reaction of lipid peroxidation products (LPO) with DNA bases, was ascertained in tissues of mammals not exposed to exogenous chemicals. These include adducts of malondialdehyde (MDA) [2], the most abundant carbonyl compound generated by LPO, and adducts of acrolein (ACR) and crotonaldehyde (CRA) [3]. The identity of some I (indigenous)-compounds [4] with MDA-adducts was also suggested [5]. Etheno adducts which are markers of industrial exposure to the carcinogen vinyl chloride, were found in liver DNA of rodents and humans who were not exposed, and the possibility of formation of these aducts *via* LPO was suggested [6]. Since LPO generate a great diversity of reactive substances, it is very likely that the above mentioned examples are just the tip of the iceberg.

Both hydrolytic and oxidative DNA lesions are repaired in cells and a number of enzymes participating in their repair are known and characterized. However, there is much less information on the repair of cyclic adducts, either of endogenous or exogenous origin (see [7, 8] for recent reviews). Nevertheless, the excision by human glycosylases of etheno adducts has been well documented and enzyme excising ethenoA appears to be identical with *N*-alkylpurine-DNA glycosylase [8–11]. In our studies we have found that induction of adaptive response to alkylating agents increases survival and decreases mutagenesis of chloroacetaldehyde (CAA)-treated *E. coli* cells and phages, and this phenomenon is *alkA* dependent. This indicates that bacterial 3-methyladenine-DNA glycosylase II encoded by *alkA* excises the CAA-generated etheno adducts [12, 13]. The repair of other cyclic adducts, such as adducts of MDA, ACR, or CRA, the presence of which in DNA of mammals is well documented, remains rather obscure.

The aim of our study was to establish a method for screening the *N*-alkylpurine-DNA glycosylases from various sources for mutagen specificity. The combined action of glycosylases and abasic site-specific endonucleases on damaged bases in DNA results in formation of single strand breaks. In plasmid DNA, as a consequence, the covalently closed circular (*ccc*) form is converted to the open circular (*oc*) form and this can be quantitated by agarose gel electrophoresis. This relatively sensitive method allowing to detect one lesion in one plasmid molecule (about $1/10^4$ bases) was recently employed to study oxidative [14–16] and alkylation [17] damage in DNA. Here, we studied DNA lesions sensitive to bacterial AlkA and cloned human ANPG-40 glycosylases in mutagen treated and non-treated pBR322 and pAlk10 plasmids. Both glycosylases were tested in the presence of NTH or FPG glycosylase/endonuclease or exonuclease III (EXO) which also acts as an abasic site-specific endonuclease.

MATERIALS AND METHODS

Bacterial 3-methyladenine-DNA glycosylase II (AlkA protein, ALK) was purified from *E. coli* strain JM105 harboring pAlk10 plasmid containing *alkA* gene using the modified procedure described in [18]. The preparation of ALK (1400 units/mg, 1000 units/ml) was essentially homogeneous, as judged by SDS-polyacrylamide gel electrophoresis/PAGE. Cloned human truncated *N*-alkylpurine-DNA glycosylase (ANPG-40, 40 units/ml) was a generous gift from Dr. Timothy R. O'Connor [19]. One glycosylase unit releases 1 pmol of

methylated bases from [^{3}H]methylnitroso urea-treated DNA in 5 min at 37°C under limited enzyme conditions [18]. FPG and NTH were provided by Dr. Barbara Tudek of our Institute, whereas *E. coli* exonuclease III (175000 units/ml, defined by supplier as nmole units of exonucleolytic activity) was from Promega.

pBR322 and pAlk10 plasmids were isolated from DH5α and JM105 *E. coli* strains, respectively, following known procedures [20]. The ratio of *ccc* to *oc* forms varied from one isolation to an other, and plasmids from the same batch were used in particular experiments. The plasmid DNA (140–200 μg) was resuspended in 70 mM cacodylate buffer, pH 7.5 (total volume 100 μl) and treated with mutagens as follows: CAA (0–100 mM) 18 h, pBQ (0–50 mM) for 3 h, ACR (0–50 mM) for 3 h, CRA (0–160 mM) for 3 h, MDA (0–100 mM) for 18 h. All reactions were performed at room temperature and plasmid DNA was purified from reagents by ethanol precipitation.

To the ice-chilled solution of 20–40 μg of plasmid DNA in 17 μl of buffer appropriate for the glycosylase tested, the enzyme solutions were added and samples were incubated for 10 min at 37°C. For AlkA 10 mM Hepes-Na/HCl, pH 7.6, 2 mM EDTA, 5 mM mercaptoethanol and 5% glycerol was used, whereas for ANPG-40 this buffer was supplemented with 100 mM KCl. The amounts of glycosylases added are specified in figure legends and in Table 1. Samples were again ice-chilled, endonucleases were added and samples were incubated for another 10 min at 37°C. FPG was used in the amount of 2.7 μg/assay (this preparation had low activity because of long storage), NTH – 1.4 ng, whereas EXO – 1 enzyme unit, in all assays. The samples containing endonucleases were supplemented with 1 μl of 1 M KCl for FPG and NTH, and 0.4 μl 45 mM $MgCl_2$ for EXO.

After incubation, samples were again ice-chilled and loaded on 0.8% agarose gel containing ethidium bromide (0.5 μg/ml) and electrophoresed at 80 V for 2 h. The gel slabs were photographed in UV light using Polaroid 665 films. In order to obtain quantitative data, films were scanned and peaks corresponding to bands of *ccc*, *oc* or *linear* forms were integrated. The number of endonuclease sensitive sites (*ess*) per plasmid molecule were calculated from the equation: $ess = -\ln(1.4 \times I/1.4 \times I+II)$, where I is the intensity of *ccc* band and II is the intensity of *oc* band [14]. The intensity of *linear* form band, usually not exceeding 10 % intensity of all three bands, was added to the intensity of *oc* band.

Table 1. Number of endonuclease sensitive sites (*ess*) per plasmid molecule formed in CAA-pretreated plasmid DNA upon action of AlkA and ANPG-40 glycosylases (0.08 and 0.016 unit/assay, respectively)

CAA (mM)	ALK/ NTH[a]	ALK/ EXO[a]	ANPG/ NTH[a]	ANPG/ NTH[b]
0[c]	0.45	0.63	0.34	0.31
0[d]	0.82	0.80	0.49	0.51
2	0.75	0.70	0.45	0.65
20	0.92	0.87	0.56	–
50	1.16	0.99	0.60	–
100	1.35	1.39	0.71	1.05

(a) pBR322 plasmid; (b) pAlk10 plasmid; (c) control without enzymes; (d) control with enzymes.

RESULT AND DISCUSSION

Endogenous lesions

It appears that plasmids isolated from bacteria and non-treated with external mutagens, contain lesions which are recognized by ANPGs. Figure 1 shows that significant transformation of *ccc* to *oc* form of pBR322 plasmid occurs upon combined action of AlkA and each of the endonucleases tested. Each of the enzymes tested alone gives results similar to the control. An increase of concentration of AlkA and ANPG-40 glycosylases at a constant concentration of NTH results in an increase of the *ess* number (Fig. 2). It is interesting to note that AlkA and ANPG-40 alone gives some

Figure 1. Action of AlkA glycosylase (0.5 unit/assay) in the presence of various endonucleases on pBR322 plasmid.

transformation of *ccc* to *oc* form. This would indicate that the preparations of glycosylases are contaminated by an endonuclease or that endonucleolytic activity is an integral part of these enzymes.

FGP and NTH are repair enzymes which possess glycosylase and endonuclease activities. They recognize various oxidative lesions and abasic sites. EXO can act as abasic site specific endonuclease [7]. Our results have shown that the plasmid isolated from bacteria does not contain a significant amount of lesions of this type. In contrast, it seems that lesions which are recognizable by AlkA and ANPG are present in plasmid DNA at a higher proportion than are oxidized bases or abasic sites. The nature of these lesions is not known, however.

Etheno adducts

Chloroacetaldehyde (CAA, $ClCH_2$-CHO), a vinyl chloride metabolite, is routinely used for formation of etheno adducts under laboratory conditions. Figure 3 shows the effect of action of ANPG-40 and AlkA in the presence of NTH on CAA-pretreated pAlk10: the *ccc* form undergoes transformation into *oc* form. Treat-

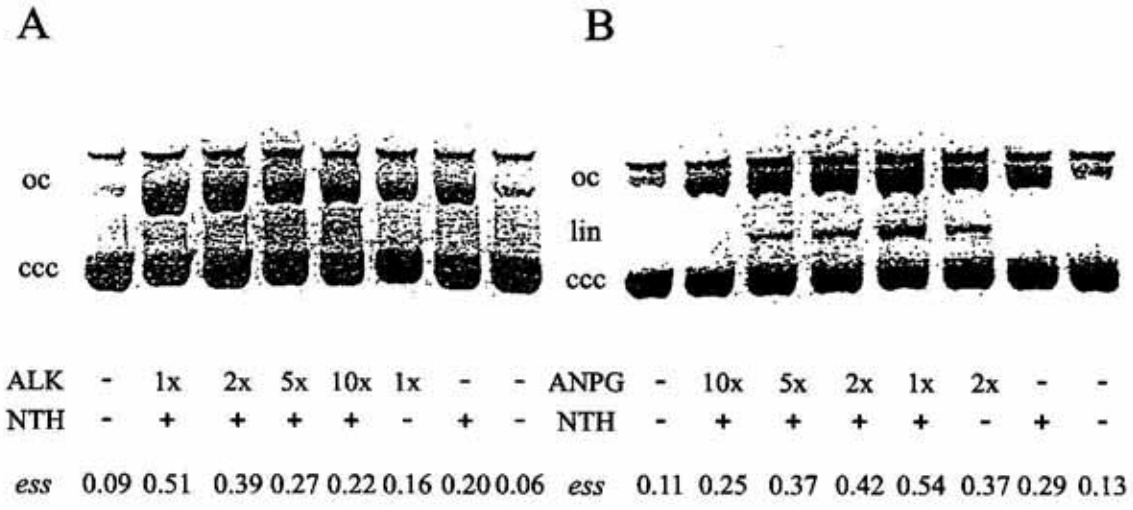

Figure 2. Comparison of action of AlkA (panel A) and ANPG-40 (panel B) glycosylases at different concentrations in the presence of NTH endonuclease on pBR322 plasmid.

Dilution factor of a glycosylase (dilution factor 1 corresponds to 0.4 unit of AlkA and to 0.08 unit of ANPG-40), presence (+) or absence (-) of an enzyme and calculated *ess* number are indicated at each lane.

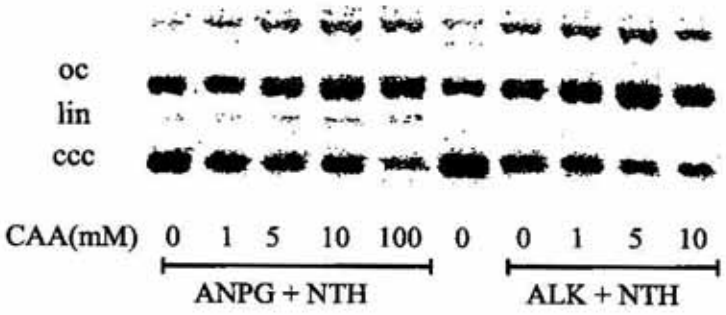

Figure 3. Action of ANPG-40 (left part, 0.016 unit/assay) and AlkA (right part, 0.08 unit/assay) in the presence of NTH on pAlk10 plasmid pretreated with CAA.

ment of plasmid by CAA itself (without subsequent glycosylase/endonuclease treatment), as well as the action of NTH or EXO alone (without AlkA or ANPG-40) on the CAA-modified plasmid, does not induce conformational changes (not shown). In Table 1 the quantitative data from four experiments are gathered. It is seen clearly that the number of *ess*, formed *via* excision of etheno adducts, is dependent on concentration of CAA used for modification of plasmids.

Our results are consistent with the results obtained by other methods [8, 12, 13, 21] and all this leads to the general conclusion that etheno adducts are repaired by ANPGs of bacterial and human origin. It should be emphasized, however, that the method used in this study does not provide information about repairability of particular adducts. The recognition of a certain adduct by homologous ANPGs from different organisms can differ considerably. For example, it has been shown that the release of ethenoA by AlkA occurs about 1000-fold, and by yeast enzyme about 100-fold slower than by rat or human enzymes [22]. On the other hand, in the same organism the particular enzymes can recognize different adducts belonging to the same category. This is exemplified by the fact that ethenoA and ethenoC are excised by different human glycosylases and there is no overlap in specificity for these two substrates [11].

Adducts of α,β-unsaturated aldehydes

Acrolein (ACR, CH_2=CH–CHO) and croton aldehyde (CRA, CH_3CH=CH–CHO) are α,β-unsaturated aldehydes and they form cyclic hydroxypropano and methylhydroxypropano adducts, respectively, with DNA bases [3].The action of AlkA in the presence of NTH on pBR322 plasmid pretreated with ACR results in transformation of *ccc* to *oc* form and the extent of this transformation depends on concentration of mutagen (Fig. 4). Virtually identical results were obtained with CRA-

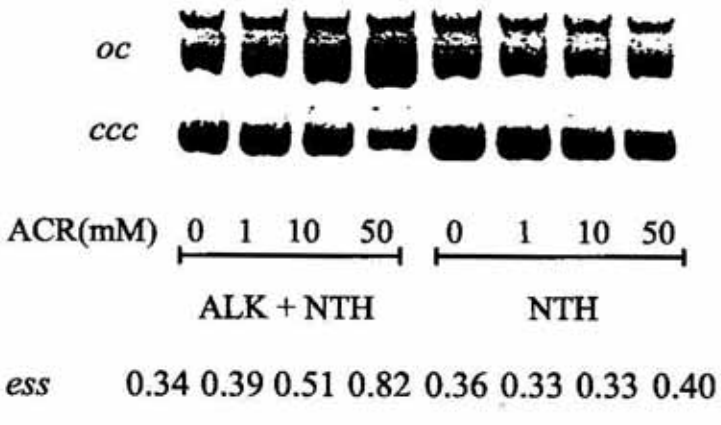

Figure 4. Action of AlkA (0.02 unit/assay) in the presence of NTH (left part) or only NTH (right part) on pBR322 plasmid pretreated with ACR.

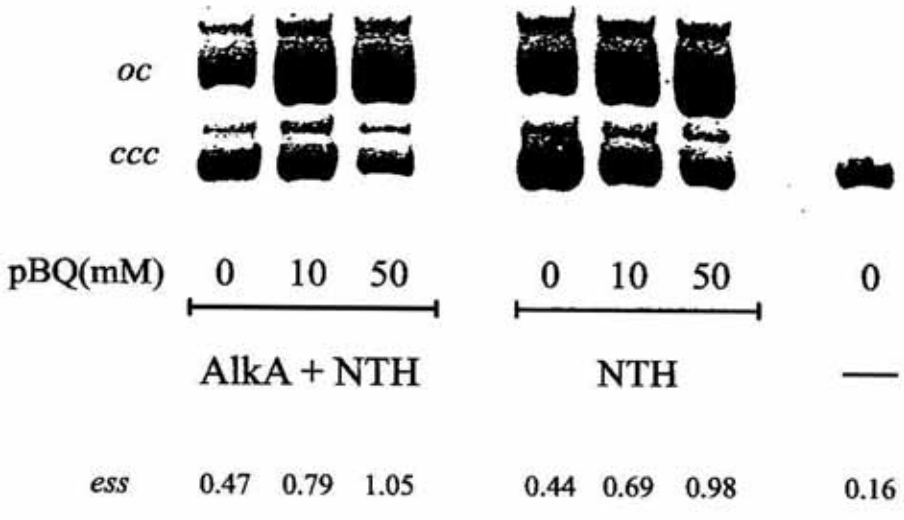

Figure 5. Action of AlkA (0.02 unit/assay) in the presence of NTH (left part) or only NTH (right part) on pBR322 plasmid pretreated with pBQ.

pretreated plasmid (not shown). The analogous experiments, in which ACR- and CRA-pretreated plasmids were reacted with ANPG-40 and NTH, gave, however, negative results (not shown). Here, ANPG-40 was tested at higher concentrations (0.02 and 0.04 unit/assay) than in experiments with excision of etheno adducts (0.016 units/assay, see above).

The existing reports suggest that ACR and CRA adducts are repaired *via* the nucleotide excision repair (NER) pathway. It has been shown that mutagenicity of these α,β-unsaturated aldehydes in *S. typhimurium* strains is *uvrB* dependent [23]. ACR is more mutagenic and toxic for *Xeroderma pigmentosum* fibroblasts than for normal human fibroblasts what implies that NER is involved in repair of its adducts also in higher organisms [24]. On the other hand, our results indicate that ACR and CRA adducts can be repaired also *via* the base excision repair (BER) pathway, at least they are excised by *E. coli* AlkA glycosylase.

Malondialdehyde (MDA, $CH_2(CHO)_2$) in solution exists in tautomeric form of β-hydroxyacrolein (HO-CH=CH-CHO). MDA, similarly to ACR and CRA, forms three-carbon bridges between exocyclic and ring nitrogen atoms of DNA bases [2]. In contrast to saturated propano bridges formed by ACR and CRA, the MDA bridges contain two double bonds which are conjugated with the double bond system of nucleobase. The rigid and planar structure of MDA adducts resembles rather structures of etheno adducts than structures of ACR or CRA adducts. However, despite structural similarity to etheno adducts, MDA adducts are not excised either by AlkA or by ANPG-40 glycosylases. In experiments with MDA modified plasmids, under conditions identical with those for excision of etheno adducts, and also at higher concentrations of glycosylases, we did not notice any significant increase of the *ess* numbers over control values (not shown).

para-Benzoquinone adducts

para-Benzoquinone (pBQ) is one of metabolites of benzene, a human carcinogen, and it forms bulky cyclic hydroxybenzeteno adducts with DNA bases [25]. Figure 5 shows the effect of AlkA action in the presence of NTH, and of NTH alone on pBQ-pretreated pBR322 plasmid. NTH alone produces essentialy the same *ess* number as the combination of AlkA and NTH, which indicates that in this case NTH but not AlkA is responsible for *ccc* to *oc* conversion. Similarly, EXO tested alone gives *ess* numbers of 0.15, 0.25 and 0.38 for pBR322 pretreated with 0, 10, and 50 mM pBQ, respec-

tively. The effect is not caused by mutagen itself, since the incubation of pBQ-treated plasmid does not give the increase of *ess* numbers. This rather unexpected finding can be explained on the ground of recent works from Dr. B. Singer's laboratory [8, 25]. The authors found that major human apurinic/apyrimidinic site endonuclease (HAP1), *E. coli* exonuclease III (EXO) and *E. coli* endonuclease IV catalyze incision of DNA at 5'-side of pBQ adducts without prior generation of an abasic site.

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REFERENCES

1. Lindahl, T. (1993) *Nature* **362**, 709–715.
2. Chaudhary, A.K., Nokubo, M., Reddy, G.R., Yeola, S.N., Morrow, J.D., Blair, I.A. & Marnett, L.J. (1994) *Science* **265**, 1580–1582.
3. Nath, R.G., Ocando, J.E. & Chung, F.-L. (1996) *Cancer Res.* **56**, 452–456.
4. Randerath, K., Reddy, M.V. & Disher, R.M. (1986) *Carcinogenesis* **7**, 1615–1617.
5. Wang, M.-Y. & Liehr, J.G. (1995) *Arch. Biochem. Biophys* **316**, 38–46.
6. Nair, J., Barbin, A., Guichard, Y. & Bartsch, H. (1995) *Carcinogenesis* **16**, 613–617.
7. Demple, B. & Harrison, L. (1994) *Annu. Rev. Biochem.* **63**, 915–948.
8. Singer, B. & Hang, B. (1997) *Chem. Res. Toxicol.* **10**, 713–732.
9. Singer, B., Antoccia, A., Basu, A.K., Dosanjh, M.K.,, Fraenkel-Conrat, H., Gallagher, P.E., Kuśmierek, J.T., Qiu, Z.-H. & Rydberg, B. (1992) *Proc. Natl. Acad. Sci. U.S.A.* **89**, 9386–9390.
10. Dosanjh, M.K., Chenna, A., Kim, E., Fraenkel-Conrat, H., Samson, L. & Singer, B. (1994) *Proc. Natl. Acad. Sci. U.S.A.* **91**, 1024–1028.
11. Hang, B., Chenna, A. & Singer, B. (1996) *Carcinogenesis* **17**, 155–157.
12. Mroczkowska, M.M., Kolasa, I.K. & Kuśmierek, J.T. (1993) *Mutagenesis* **8**, 341–348.
13. Borys, E., Mroczkowska-Słupska, M.M. & Kuśmierek, J.T. (1994) *Mutagenesis* **9**, 407–410.
14. Epe, B., Hegler, J. & Wild, D. (1998) *Carcinogenesis* **10**, 2019–2024.
15. Epe, B., Pflaum, M. & Boiteux, S. (1993) *Mutation Res.* **299**, 135–145.
16. Wójcik, A., Grzesiuk, E., Tudek, B. & Janion, C. (1996) *Biochimie* **78**, 85–89.
17. Grzesiuk, E. & Janion, C. (1996) *Mutat. Res.* **362**, 147–154.
18. Tudek, B., VanZeeland, A.A., Kuśmierek, J.T. & Laval, J. (1998) *Mutation Res.* **407**, 169–176..
19. O'Connor, T.R. (1993) *Nucleic Acids Res.* **21**, 5561–5569.
20. Skoneczny, M. (1995) in *"Genetic Engineering and Molecular Biology. Methods (A Laboratory Manual)"* (Palamarczyk, G., Rytka, J. & Skoneczny, M., eds.) pp. 1.8–1.11, Institute of Biochemistry and Biophysics PAS, Techgen, Warsaw (in Polish).
21. Matijasevic, Z., Sekiguchi, M. & Ludlum, D.B. (1992) *Proc. Natl. Acad. Sci. U.S.A.* **89**, 9331–9334.
22. Saparbaev, M., Kleibl, K. & Laval, J. (1995) *Nucleic Acids Res.* **23**, 3750–3755.
23. Marnett, L.J., Hurd, H.K., Hollstein, M.C., Levin, D.E., Esterbauer, H. & Ames, B.N. (1985) *Mutation Res.* **148**, 25–34.

24. Curren, R.D., Yang, L.L., Conklin, P.M., Grafstrom, R.C. & Harris, C.C. (1988) *Mutation Res.* **209**, 17–22.

25. Hang, B., Rothwell, D.G., Sagi, J., Hickson, I.D. & Singer, B. (1997) *Biochemistry* **36**, 15411–15418.

Note added in proof:

When this communication was being edited, a paper describing a low, but measurable level of excision of normal bases from intact DNA by *E. coli* AlkA and related glycosylases of higher organisms, was published – Berdal, K.G., Johansen, R.F. & Seeberg, E. (1998) *EMBO J.* **17**, 363–367. In the view of conclusions presented in that paper, at least part of the effects observed by us in experiments with intact plasmids and ascribed to the presence of unknown endogenous adducts, could be explained as the result of enzymatic release of normal, unmodified bases.