DNA-PROTEIN CROSS-LINKS INDUCED BY *BIS*-ELECTROPHILES

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ABSTRACT

Diepoxybutane is a mutagenic and carcinogenic oxidation product of the important industrial chemical and environmental contaminant butadiene. The mutagenic potential of diepoxybutane is thought to be due in part to its bifunctional electrophilic character. One mechanism by which *bis*-electrophiles can exert their toxic effects is through the induction of genotoxic and mutagenic DNA-protein or –peptide cross-links. This mechanism has been shown in systems overexpressing the DNA repair protein O^6-alkylguanine DNA-alkyltransferase (AGT) or glutathione transferase and involves reactions with nucleophilic cysteine residues. The hypothesis that DNA-protein crosslink formation is a more general mechanism for genotoxicity by *bis*-electrophiles was investigated by screening nuclear proteins for reactivity with model monofunctional electrophiles. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was identified as a candidate due to the nucleophilicity of two cysteine residues (Cys^{152} and Cys^{246}) in reaction screens with model electrophiles (Dennehy, M. K. et al. (2006) *Chem. Res. Toxicol. 19*, 20-29). Incubation of GAPDH with *bis*-electrophiles resulted in inhibition of its catalytic activity but only at high concentrations of diepoxybutane. *In vitro* assays indicated DNA-GAPDH crosslink formation in the presence of diepoxybutane, and *bis*-electrophile reactivity at Cys^{246} was confirmed using mass spectral analysis. In contrast to AGT, overexpression of human GAPDH in *Escherichia coli* did not enhance mutagenesis by diepoxybutane. The candidate proteins histones H2b and H3 were identified in screens using human liver nuclei and the *bis*-electrophile 1,2-dibromoethane. Incubation of these proteins with diepoxybutane resulted in DNA-protein cross-links and

produced protein adducts, and DNA-histone H2b cross-links were identified (immunochemically) in *E. coli* cells expressing histone H2b. However, heterologous expression of histone H2b in *E. coli* failed to enhance *bis*-electrophile-induced mutagenesis, although histone H2b bound DNA with even higher affinity than AGT. The extent of DNA cross-linking of isolated histone H2b was similar to that of AGT, suggesting that differences in post-cross-linking events explain the difference in mutagenesis. In a related experiment, reactive diepoxybutane-glutathione conjugates believed to contribute to enhanced mutagenesis observed in bacterial cells overexpressing glutathione transferases were investigated. Mass spectral analysis of incubations containing purified glutathione transferase, glutathione, and diepoxybutane yielded a glutathione conjugate that retained the epoxide. Diepoxybutane also produced glutathione-DNA cross-links upon incubation.

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ABBREVIATIONS

AGT	O^6-Alkylguanine-DNA alkyltransferase
BER	Base excision repair
CID	Collision-induced dissociation
DEB	1,2,3,4-Diepoxybutane
DMSO	Dimethyl sulfoxide
DTA	Desktop Auditor
DTT	1,3-Dithiothreitol
G3P	Glyceraldehyde 3-phosphate
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GSH	Glutathione
GST	Glutathione transferase
HRR	Homologous recombination repair
IR	Ionizing radiation
IOA	Iodoacetamide
IPTG	Isopropyl β-D-thiogalactopyranoside
LB	Luria-Bertani
NER	Nucleotide excision repair
PAGE	Polyacrylamide gel electrophoresis
PCR	Polymerase chain reaction
SCE	Sister chromatid exchange
SDS	Sodium dodecyl sulfate

SRM	Selective reaction monitoring
UV	Ultraviolet