

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

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Dissertation

Submitted to the Faculty of the
Graduate School of Vanderbilt University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

in

Biochemistry

May, 2010

Nashville, Tennessee

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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 (Cys¹⁵² and Cys²⁴⁶) 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 Cys²⁴⁶ 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.

ACKNOWLEDGMENTS

First and foremost, I would like to thank my advisor, Professor F. P. Guengerich. He has provided endless support and encouragement throughout my graduate career, as well as constant patience and guidance. His dedication to research and to the members of his laboratory is inspiring, as well as concern for both the academic success and personal wellbeing of his students. I am also sincerely grateful for being given the opportunity to pursue my interest in biochemistry in such a remarkable environment.

I would like to thank the many past and present members of the Guengerich Laboratory who have been endlessly supportive and helpful, especially Martha Martin, Karen Angel, and Lindsay Folkman. I am also indebted to the staff of the Center in Molecular Toxicology, including Kathy Trisler, Kakie Mashburn, Ellen Rochelle, and Wil Comstock for taking care of all my administrative and computer needs.

I would like to acknowledge my committee members, Professors Richard Armstrong, David Hachey, Daniel Liebler, and Carmelo Rizzo for their constant direction and encouragement. The biannual meetings elevated my enthusiasm for my project and gave me new focus. I am also grateful for our collaborators at Vanderbilt, especially Drs. Donald Stec and Wade Calcutt.

Finally, I would like to thank my family. I cannot express the gratitude I feel toward my parents, Mary and James Loecken, who have sacrificed so much for me. The endless love and guidance they provide made any obstacle seem manageable. I would also like to thank my loving sister, who has served as an inspiration, as well as being a

consistent and loving supporter of mine. I am truly grateful for her insight and reassurance, as well as the ceaseless encouragement from my entire family.

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ABBREVIATIONS

AGT	<i>O</i> 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