DOE/ER/45723-1

FINAL REPORT

DE-FG02-98ER45723

Structure/Function Analysis of DNA-glycosylases that Repair Oxidized Purines and Pyrimidines and the Influence of Surrounding DNA Sequence on their Interactions

University of Vermont

Principal Investigator: Susan S. Wallace, Ph.D.
Department of Microbiology and Molecular Genetics
The University of Vermont
Stafford Hall
Burlington, Vermont 05405

Phone: (802) 656-2164
Fax: (802) 656-8749
e-mail: swallace@zoo.uvm.edu

National Lab Collaborator: Michael Kennedy
Battelle Memorial Institute
Pacific Northwest National Laboratory
P.O. Box 999
Richland, WA 99352

Phone: (509) 372-2168
Fax: (509) 376-2303
e-mail: ma_kennedy@pnl.gov

DISCLAIMER

INTRODUCTION

The overall goal of this project was to elucidate the structure/function relationships between oxidized DNA bases and the DNA repair enzymes that recognize and remove them. The NMR solution structure of formamidopyrimidine DNA glycosylase (Fpg) that recognizes oxidized DNA purines was to be determined. Furthermore, the solution structures of DNA molecules containing specific lesions recognized by Fpg was to be determined in sequence contexts that either facilitate or hinder this recognition. These objectives were in keeping with the long-term goals of the Principal Investigator's laboratory, that is, to understand the basic mechanisms that underpin base excision repair processing of oxidative DNA lesions and to elucidate the interactions of unrepaired lesions with DNA polymerases. The results of these two DNA transactions can ultimately determine the fate of the cell. These objectives were also in keeping with the goals of our collaborator, Dr. Michael Kennedy, who is studying the repair and recognition of damaged DNA. Overall the goals of this project were congruent with those of the Department of Energy's Health Effects and Life Sciences Research Program, especially to the Structural Biology, the Human Genome and the Health Effects Programs. The mission of the latter Program includes understanding the biological effects and consequences of DNA damages produced by toxic agents in the many DOE waste sites so that cleanup can be accomplished in a safe, effective and timely manner.

SPECIFIC AIM 1: To solve the three dimensional structure of Fpg protein using NMR spectroscopy

Escherichia coli formamidopyrimidine-DNA glycosylase (Fpg), a 269-residue, 30.2 kDa metalloprotein, is a trifunctional DNA base excision repair (BER) enzyme that binds double-stranded DNA and performs 3 catalytic activities: (i) DNA glycosylase, (ii) AP lyase, and (iii) deoxyribophosphodiesterase. In the process, Fpg recognizes and removes a wide variety of oxidative DNA lesions with its primary biological substrate the promutagenic 7,8-dihydro-8-oxoguanine (8-oxoG). Removal of 8-oxoG and other oxidative DNA lesions (2,6-diamino-4-hydroxy-5-formamido pyrimidine, the adenine equivalents, triaminooxazolone, 5-hydroxyuracil, 5-hydroxycytosine, 5,6-dihydrothymine, and α-R-hydroxy-β-ureidoisobutyric acid) is important because cellular DNA is continuously being exposed to endogenous and exogenous agents that damage DNA. Unrepaired, oxidative DNA lesions may have deleterious cellular consequences, including cell death and mutagenesis. In eukaryotes, such damage also plays a role in a number of diseases, including cancer and aging. Furthermore, it has been observed that the DNA sequence context plays a role in the efficiency of 8-oxoG repair by Fpg (Hatahet *et al.*, 1998). In order to understand how Fpg performs its many biological activities on DNA in solution, the structure and function of Fpg was studied by NMR spectroscopy.

We unambiguously confirmed the existence of a C4-type zinc-binding domain in Fpg using EXAFS spectroscopy . The metal-binding domain of Fpg allows for substitution of diamagnetic Zn^{2+} with paramagnetic ions such as Co^{2+}, which can be used to produce pseudocontact shifts which are a useful source of structural information in proteins (Gochin and Roder, 1995) and DNA-protein complexes (Buchko *et al.*, 1999). We successfully expressed Fpg containing Co^{2+} and shown the enzyme to be equally active as the native Zn^{2+} form (Buchko *et al.*, 2000). This work was published in *Biochemistry* (39:12411-12449, 2000).

The solution-state NMR studies of *E. coli* Fpg indicate a dynamic process that may be key to understanding the function of Fpg that is invisible to the X-ray crystallographic studies (presumably due to crystal packing effects). We discovered this process after assigning the majority (86%) of the backbone ^{15}N, $^{1}H^{N}$, $^{13}C^{\alpha}$, $^{13}C^{\beta}$, and ^{13}CO resonances of Fpg for which cross peaks were observed in the $^{1}H/^{15}N$ HSQC spectrum (only 83% of the possible cross peaks were visible). A manuscript describing the backbone assignments of Fpg was published in the *Journal of Biomolecular NMR* (22:301-302, 2002).

SPECIFIC AIM 2 To determine the NMR structures of dodecamers that contain the benchmark oxidative lesion, 8-oxoguanine (8-oxoG), surrounded by sequence contexts that either hinder or facilitate repair.

Table 1. DNA Sequences that were synthesized and purified for NMR studies.

Sequence #	DNA Sequence	Base Across from 8-oxoG or AP
1	CTAGTG(8-oxoG)ATCCC	C
2	CTAGTGGATCCC	C
3	CTAGTG(8-oxoG)ATCCC	A
4	CTAGTGGATCCC	A
5	CTAGTT(8-oxoG)TTCCC	C
6	CTAGTTGTTCCC	C
7	CTAGTT(8-oxoG)TTCCC	A
8	CTAGTTGTTCCC	A
9	CTCTTT(AP)TTTCTC	C
10	CTCTTTGTTTCTC	C
11	GTCACCGTG(AP)TACGACTC	C

NMR studies were initiated for eight DNA oligonucleotides (see Table 1) containing 8-oxoG lesions in sequence contexts that have been shown to be either efficiently or inefficiently excised by Fpg (Hatahet *et al.*, 1998). These sequences are being studied to determine if sequence context modulates the intrinsic structural features of the DNA surrounding 8-oxoG lesions in a way that that might correlate with, and potentially help understand, the sequence context dependence of Fpg processing of 8-oxoG lesions. The proton resonances for the sequence #1 in the list was completely assigned. In addition, the phosphorous spectrum of sequence #1 was examined. A single phosphorous resonance is deshielded by greater than 0.5 ppm related to the other 21 phosphorous resonances. This ^{31}P spectrum bears a striking resemblance to that of the DNA Dickerson sequence engineered to contain an artificial extrahelical residue, CGCAGAATTCGCG, in which the A4 residue is forced to adopt an extrahelical conformation forming a single nucleotide bulge (Nikonowicz, *et al.*, 1989). In the later sequence, the deshielded ^{31}P signal was assigned to the phosphorous linking the extrahelical A4 nucleoside to the adjacent 3′ nucleoside, G5. In sequence #1, the deshielded ^{31}P signal was assigned to the 8-oxoG-p-A8 phosphorous linkage from a two-dimensional proton-detected ^{31}P-^{1}H heteronuclear

correlation experiment shown. Another example is a three-carbon propanediol linker that acts as an abasic-site analog and is bound with high specificity by Fpg but is not cleaved (Castaing *et al.*, 1993). The ^{31}P spectrum of this sequence shows two deshielded resonances which likely correspond to the 5' and 3' phosphodiester linkages of the abasic site. It will not be surprising if the two deshielded resonances correlate with complete freedom of backbone motion at the abasic site. These data, along with the data from Nikonowicz, *et al.* (1989) indicate that the ^{31}P chemical shift could be a sensitive reporter of extrahelical nucleoside character in DNA sequences containing 8-oxoG lesions. Here, by extrahelical, we mean a nucleoside that because of backbone distortion and/or dynamics results in the increased the propensity of the entire nucleoside flipping out in the presence of a suitable perturbation of the DNA helix. These data are in preparation for publication.

Literature Cited

Buchko, G.W., Daughdrill, G.W., Rao, S., Isern, N.G., Lorimier, R.de, Limbeck, J., Taylor, J-S., Wold, M.S., Gochin, M. Spicer, L. D., Lowry, D.F., and Kennedy, M.A. (1999) The minimal DNA-binding domain (M98-F219) of human nucleotide excision repair protein XPA: Chemical shift mapping of the DNA and RPA binding surfaces and backbone dynamics of free and DNA-complexed protein studied by ^{15}N NMR relaxation. *Biochemistry*, **38**, 15116-15128.

Buchko, G.W., Bandaru, V., Wallace, S.S., Hess, N.J., and Kennedy, M.A. (2000) Extended X-ray absorption fine structure evidence for a metal-binding domain in *E. coli* formamidopyrimidine-DNA glycosylase. Submitted to *Protein Sci.*

Castaing, B., Geiger, A., Seliger, H., Nehls, P., Laval, J., Zelwar, C., and Boiteux, S. (1993) Cleavage and binding of a DNA fragment containing a single 8-oxogunanine by wild type and mutant Fpg proteins. *Nucl. Acids Res.*, **21**, 2899-2905.

Gochin, M. and Roder, H. (1995) Protein structure refinement based on paramagnetic NMR shifts: applications to wild-type and mutant forms of cytochrome c. *Protein Sci.*, **4,** 296-305.

Hatahet, Z., M. Zhou, L.J. Reha-Krantz, S.W. Morrical, S.S. Wallace (1998) In search of mutational hotspots. *Proc. Natl. Acad. Sci. USA*, **95**, 8556-8561.

Nikonowicz, E., Roongta, V., Jones, C.R., Gorenstein, D.G. (1989) Two-dimensional 1H and 31P NMR spectra and restrained molecular dynamics structure of an extrahelical adenosine tridecamer oligodeoxyribonucleotide duplex. *Biochemistry*, **28,** 8714-8725.

PUBLICATIONS

Buchko, G.W., Hess,N.J., Bandaru,V., Wallace,S.S. and Kennedy,M.A. (2000) Spectroscopic studies of zinc(II)- and cobalt(II)-associated *Escherichia coli* formamidopyrimidine-DNA glycosylase: Extended X-ray absorption fine structure evidence for a metal-binding domain. *Biochemistry*, **39**, 12441-12449.

Buchko,G.W., Wallace,S.S. and Kennedy,M.A. (2002) Base excision repair: NMR backbone assignments of *Escherichia coli* formamidopyrimidine-DNA glycosylase. *J. Biomol. NMR,* **22**, 301-302.

Conference presentations:

Buchko,G.W., McAteer, K, Wallace,S.S. and Kennedy,M.A. (2002) Interaction of formamidopyrimidine-DNA glycosylase (Fpg) with damaged DNA: Backbone sssignments, chemical shift mapping, and relaxation studies. 43rd Experimental Nuclear Magnetic Resonance Conference, March 24-29, Asilomar, CA.

Buchko,G.W., McAteer, K, Wallace,S.S. and Kennedy,M.A. (2002) Interaction of formamidopyrimidine-DNA glycosylase (Fpg) with DNA containing an abasic site analogue: Backbone assignments, chemical shift mapping, and relaxation studies. 20th International Conference on Magnetic Resonance in Biological Systems, Aug. 25-30, Toronto, ON, Canada.