Mechanism-Based DNA−Protein Cross-Linking of MutY via Oxidation of 8-Oxoguanosine

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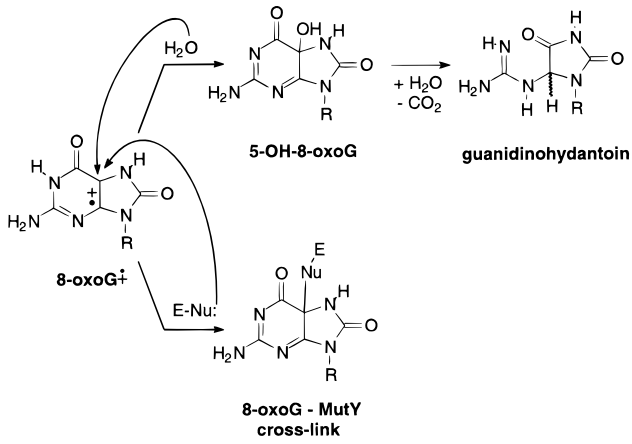
Introduction of a covalent bond cross-linking a protein to its nucleic acid target can be used to stabilize a protein−DNA complex or to investigate contacts between the two biopolymers.[1,2] Many cross-linking methods rely on photochemical triggers to generate reactive intermediates of suitably modified nucleotides or amino acid residues,[3] while other DNA−protein cross-links are a natural result of oxidative damage to chromatin arising from endogenous metal ions, oxidants, or ionizing radiation.[4,5] Of the four bases, guanine is the most susceptible to oxidative damage, but one of its common oxidation products, 8-oxoG,[6] is dramatically more reactive due to its low redox potential (∼0.6 V[7−9] compared to 1.29 V vs NHE for guanosine[10]). Thus, placement of an 8-oxoG:C pair into a DNA duplex yields little perturbation of DNA structure but significantly enhances DNA reactivity. Even though the one-electron oxidation of a DNA base may initially occur at a distant site, rapid electron transfer in the duplex will result in exclusive formation of $[8\text{-oxoG}]^{+\bullet}$.[11,12]

The ultimate fate of $[8\text{-oxoG}]^{+\bullet}$ appears to mimic the urate oxidation pathway leading, via 5-hydroxy-8-oxoG, to a guanidinohydantoin moeity (Scheme 1).[13] This mechanism likely involves trapping of the initially formed radical cation by a solvent water molecule; in the presence of bound protein, we reasoned that an active site nucleophile might participate instead, leading to a covalent DNA−protein cross-link analogous to 5-hydroxy-8-oxoguanosine.[14]

The *E. coli* DNA repair enzyme MutY[15] provides an ideal system in which to test this cross-linking hypothesis. MutY binds to 8-oxoG:A and G:A mispairs in duplex DNA and catalyzes deglycosylation of the 2′-deoxyadenosine.[16] Importantly, release

Scheme 1



of the product is slow, providing a relatively long-lived E·P complex.[17] The high mutagenicity of 8-oxoG stems from the misincorporation of A to form 8-oxoG:A mispairs, and the activity of MutY is therefore critical for prevention of deleterious DNA mutations.[18] Thus, specific 8-oxoG cross-linking will also provide insight into the amino acid residues involved in the recognition of damaged and mismatched DNA by MutY.

In the experiment, four duplex oligomers were tested for specific cross-linking triggered by the one-electron oxidant Na_2IrCl_6[19] (Figure 1). Oxidation of duplex **1·2** bound to MutY led to formation of a cross-link, observed as a higher band in an SDS-PAGE experiment.[20] That the cross-link was formed to 8-oxoG is supported by comparison of lanes 1 and 2 (Figure 1) in which cross-linking only occurred to the 8-oxoG-containing strand. Modest cross-linking was also observed with the 8-oxoG:C duplex **5·6**, which exhibits weaker binding to MutY.[21] The G:A mispaired substrate **3·4** can also undergo A deglycosylation but does not provide an oxidative cross-link to MutY since 8-oxoG is absent, and Ir^{IV} does not appreciably oxidize Gs.[19] Like guanidinohydantoin, the cross-linked lesion was substantially alkali labile giving ∼75% DNA strand scission after treatment with 0.2 M piperidine at 90 °C for 30 min (see Supporting Information).[22]

To investigate the protein residue involved in cross-linking, we prepared a series of MutY mutants. Earlier studies provided evidence that Lys-142 resides near the 8-oxoG:A binding site in MutY by virture of its ability to participate in formation of a stable covalent adduct with DNA in the presence of $NaBH_4$.[23] Furthermore, a recent crystal structure of the catalytic core of the protein crystalized with adenine[24] suggests a cleft region near Lys-142 that may accommodate the DNA substrate (Figure 2a). Accordingly, four Lys→Ala mutants and one Ser→Lys mutant, all of which were properly folded and active enzymes, were tested for cross-linking to substrate **1·2**. All except the K142A mutant were able to form cross-links in yields ranging from 4 to 15% (Figure 2b),[25] strongly suggesting that Lys-142 is positioned close to the 8-oxoG residue. The two bands observed on the gel for

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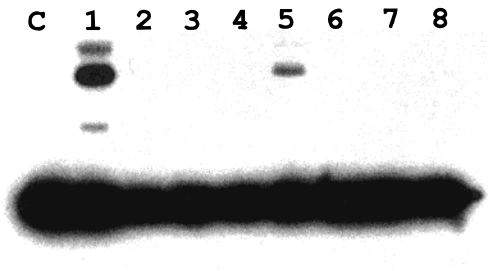

		Kd (nM)
1)	5′ TCATGGGTC**O**TCGGTATA	0.1
2)	3′ AGTACCCAG**A**AGCCATAT	
3)	5′ TCATGGGTC**G**TCGGTATA	35
4)	3′ AGTACCCAG**A**AGCCATAT	
5)	5′ TCATGGGTC**O**TCGGTATA	26
6)	3′ AGTACCCAG**C**AGCCATAT	
7)	5′ TCATGGGTC**G**TCGGTATA	150
8)	3′ AGTACCCAG**C**AGCCATAT	

Figure 1. Cross-linking of wild-type MutY (600 nM) with various duplex DNA substrates (20 nM) using 100 μM Na_2IrCl_6 (10 mM NaP_i buffer, pH 7, containing 100 mM NaCl, 15 min.). Lanes 1−8 represent experiments with duplex substrates in which strands **1**−**8**, respectively, were 5′ end-labeled with ^{32}P. The control lane was carried out with DNA plus MutY in the absence of oxidant. K_d values are approximate and were taken from similar oligodeoxynucleotides containing the same mispairs.[21]

each cross-link may represent different stable conformers of the cross-linked protein, perhaps due to protein oxidation with Ir^{IV}, but this remains to be confirmed experimentally.

Complete characterization of the cross-link is in progress; however, these initial studies demonstrate that a mild one-electron oxidant, Ir^{IV}, can be used to generate an 8-oxoG−lysine cross-link in yields comparable to other cross-linking agents. Given the minimal structural perturbation of replacing a G for an 8-oxoG, the preponderance of lysine residues in nucleic acid binding domains, and the commercial availability of both the 8-oxoG phosphoramidite and Na_2IrCl_6, this cross-linking method should be generally applicable to many questions concerning protein−nucleic acid interactions. Furthermore, it provides additional support for both the mechanism of 8-oxoG oxidation and the identity of putative 8-oxoG recognition elements in the active site of MutY.

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(25) Final enzyme concentrations used in the cross-linking reactions were based upon total protein in each preparation. As a result, the lower percent cross-linking seen in the case of wild-type relative to some mutated forms might be attributed to a lower percent active enzyme in the wild-type enzyme preparation.

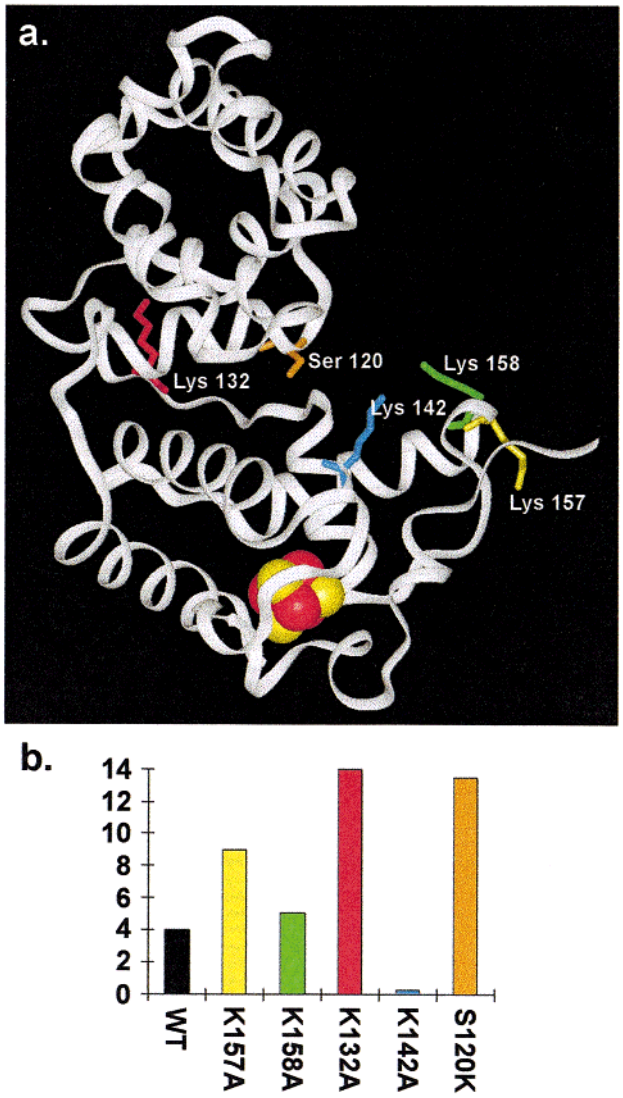

Figure 2. (a) Crystal structure[24] of truncated wildtype *E. coli* MutY with highlighted side chains of residues mutated for cross-linking experiments. The DNA binding site is believed to be on the right-hand side near K142. (b) Comparison of cross-linking ability of mutant forms of MutY to duplex **1·2** using reaction conditions specified in Figure 1.

Supporting Information Available: Experimental procedure and gels showing cross-linking to MutY mutants and piperidine treatment (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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