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Formation of *S*-[1-(*N*^{2}-Deoxyguanosinyl)methyl]glutathione between Glutathione and DNA Induced by Formaldehyde

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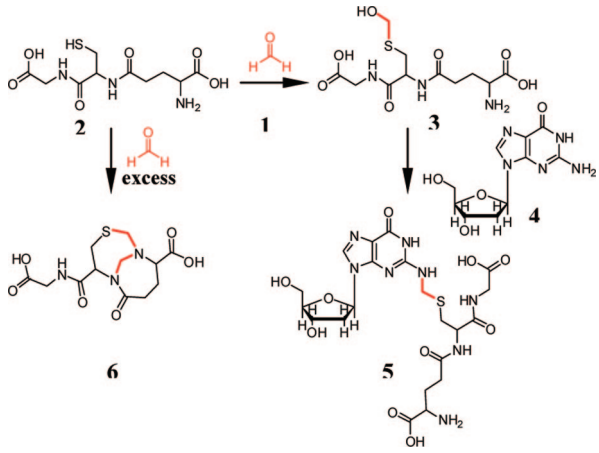
Formaldehyde, an essential metabolic intermediate generated endogenously from serine, glycine, methionine, and choline and also produced from some metabolites and proteins by demethylation,[1] is present in human blood at ~0.1 mM.[2] Formaldehyde can also enter the body through environmental exposures. Formaldehyde forms DNA and protein adducts and DNA−protein cross-links, and its toxicity has been the object of intensive investigation.[3−9] Previous studies have shown that formaldehyde is genotoxic.[1] Although *N*^{6}-dA, *N*^{2}-dG, and *N*^{4}-dC adducts of formaldehyde are found *in vitro*,[10−13] no exogenous formaldehyde-induced DNA adducts have ever been detected in animals exposed by inhalation. The inability to detect DNA adducts may result from rapid binding of formaldehyde by the tripeptide glutathione (GSH), which significantly decreases the chance that exogenous formaldehyde will attack DNA directly.

GSH is a major reducing thiol present in all human cells at a concentration of ~5 mM. Formaldehyde (**1**; Scheme 1) reacts spontaneously with GSH (**2**) to form *S*-hydroxymethylglutathione (**3**).[14] Formaldehyde dehydrogenase (ADH3) oxidizes **3** to *S*-formylglutathione, which is then hydrolyzed to formate by *S*-formylglutathione hydrolase, regenerating free glutathione.[1] The *S*-hydroxymethyl group of **3** is a reactive target for nucleophilic substitution. Recent work in our laboratory on formaldehyde-induced DNA−protein cross-links shows that the thiol groups of cysteine residues can readily cross-link with DNA bases in the presence of formaldehyde, raising the possibility that **3** can conjugate with DNA as shown in Scheme 1.

Scheme 1. Formation of *S*-[1-(*N*^{2}-Deoxyguanosinyl)-methyl]glutathione Induced by Formaldehyde



To characterize potential cross-linked products, excess GSH (5 mM) was allowed to react with formaldehyde in 10 mM potassium phosphate buffer (pH = 7.2) for 4 h at 37 °C, followed by incubation with deoxyguanosine (dG) (**4**) for another 8 h. A single coupling product eluted at 11.9 min on a C18 reversed phase column, giving a UV spectrum similar to that of dG, with an absorbance maximum at 260 nm (Figure S1). The exact mass of the protonated molecule was 587.1896 Da, consistent with elemental composition $C_{21}H_{30}N_8O_{10}S$ expected for *S*-[1-(*N*^{2}-deoxyguanosinyl)methyl]glutathione (**5**).

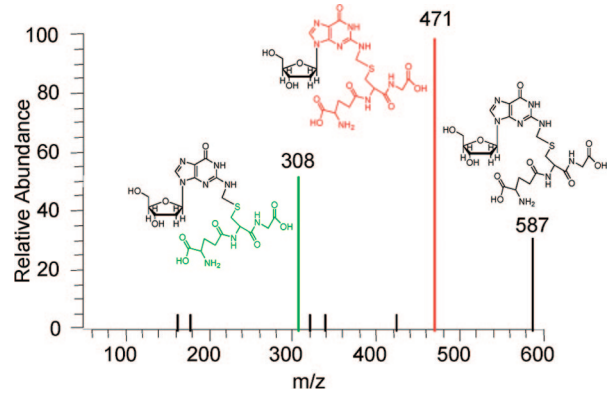


Figure 1. ESI-MS/MS of the protonated molecular ion of **5**.

The ESI-MS/MS of the protonated molecule (Figure 1) shows major product ions corresponding to the loss of deoxyribosyl and deoxyguanosine *N*^{2} Schiff base fragments, in accord with structural

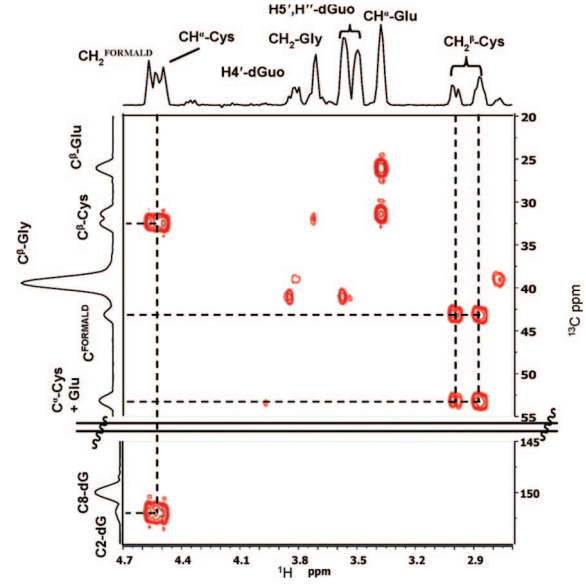


Figure 2. Expansion of the HMBC spectrum of **5** to show the Cys-*β*-methylene-formaldehyde linker-*N*^{2}-dG connectivity.

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assignment **5**. Definitive structural characterization was provided by NMR analysis of product isolated from a larger-scale reaction, which also served as standard for subsequent quantitation. The formaldehyde-derived methylene linkage between the β methylene carbon of the Cys residue and the exocyclic N^2 of dG is established by C−H connectivities in the HMBC spectrum (Figure 2), which shows the expected cross peaks between the diastereotopic Cys β-methylene protons and the formaldehyde-derived carbon of the methylene linker and between the protons of the methylene linker and C2 of dG. Adduct **5** was stable in aqueous solution at room temperature over 16 h at pH 4; an ~40% loss was observed at pH 7.2 (see Figure S6), supporting the hypothesis that if formed, this adduct would be detectable in DNA.

To test this hypothesis, DNA was incubated with GSH in the presence of formaldehyde. GSH (5 mM) in 10 mM potassium phosphate buffer (pH = 7.2) was treated with different concentrations of formaldehyde (0.1, 0.5, 1, 5, 50 mM) for 4 h at 37 °C, followed by incubation with 100 μg of calf thymus DNA for 12 h. After extensive washing and digestion, the resultant adduct **5** was collected by HPLC and quantified by triple quadrupole mass spectrometry using the selected reaction monitoring (SRM) mode (*m/z* 587 → *m/z* 308) and the previously generated **5** from the larger-scale reaction as a standard. Figure 3 shows that production of **5** rises with increasing formaldehyde concentration from 0.1 to 5 mM and then declines when the formaldehyde concentration is further increased to 50 mM. Mass spectrometry analysis of the 50 mM reaction mixture showed the presence of a species consistent with the bicyclo[4.4.1]undecane structure (**6**), previously reported to be formed from reaction of GSH with two formaldehyde molecules when formaldehyde is present in excess.[15,16]

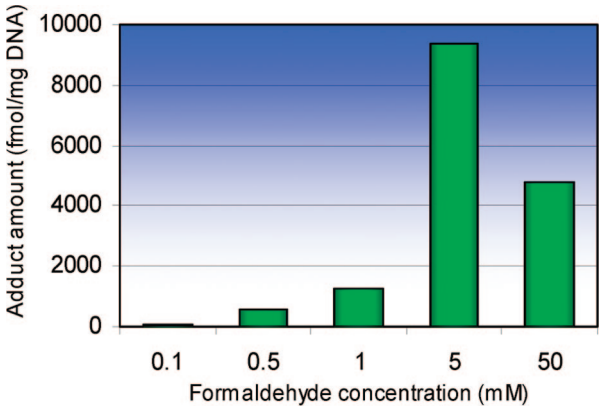

Figure 3. Influence of formaldehyde concentration on the formation of **5** in DNA.

This study establishes that **5** is formed as a consequence of formaldehyde scavenging by the cellular antioxidant GSH *in vitro*. Both formaldehyde and GSH are ubiquitous cellular components; thus, this DNA adduct is expected to form endogenously in cells. The involvement of the cysteine residue of GSH in coupling suggests that other thiols may participate in the formation of this type of DNA damage from formaldehyde.

The formation of **5** from exogenous formaldehyde may serve as a biomarker to evaluate formaldehyde exposure. Concentrations of formaldehyde in the blood of humans and of rats after formaldehyde exposure (1.9 ppm for 40 min and 14.4 ppm for 2 h) were not different from the pre-exposure concentration,[17] consistent with extensive formation of **3**. It has also been shown that formaldehyde exposure depletes GSH levels in cells and tissues,[1] suggesting that GSH was not completely regenerated. Therefore, the scavenging of formaldehyde-induced **3** by ADH3 may be limited, which would allow opportunity for reaction between **3** and DNA to form **5**. Also, adduct **5** is of potential importance for investigating effects of formaldehyde at distal sites. This issue remains one of the biggest challenges for understanding formaldehyde toxicity, carcinogenicity and epidemiology, which have been controversial for many years.[1,18,19] Formaldehyde exposure by inhalation results in decreases in cellular GSH concentration in the liver,[20] a remote site that inhaled formaldehyde is unlikely to reach by simple diffusion. Detection of **5** in distant tissues will shed light on the intriguing question of whether formaldehyde exhibits systemic toxicity.

In summary, we have demonstrated that formaldehyde can cross-link GSH with DNA by forming *S*-[1-(N^2-deoxyguanosinyl)methyl]glutathione. This adduct may form endogenously since formaldehyde and GSH are ubiquitous in human cells. This adduct is unique because of the involvement of the reactive *S*-hydroxymethylglutathione intermediate that normally serves for formaldehyde detoxication. Since *S*-hydroxymethylglutathione is expected to be relatively abundant and highly reactive, and the adduct *S*-[1-(N^2-deoxyguanosinyl)methyl]glutathione is reasonably stable, the adduct may serve as a biomarker to understand formaldehyde toxicity and to evaluate formaldehyde exposure if coupled with the application of isotope-labeled formaldehyde to differentiate between endogenous and exogenous formaldehyde-derived adducts.

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Note Added after ASAP Publication. After this paper was published ASAP February 18, 2009, the structure of formaldehyde was corrected in Scheme 1 and the table of contents graphic. The corrected version was published February 24, 2009.

Supporting Information Available: Experimental procedures, Figures S1−S6: HPLC analysis, exact mass, ^{1}H NMR and 2D NMR spectra of **5**, calibration curve and evaluation of the stability of **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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