Electronic Supplementary Information

Iron Fenton oxidation of 2′-deoxyguanosine in physiological bicarbonate buffer yields products consistent with the reactive oxygen species carbonate radical anion not hydroxyl radical

Aaron M. Fleming and Cynthia J. Burrows*

315 S. 1400 East Rm 2020, Department of Chemistry,

University of Utah, Salt Lake City, UT 84112-0850 USA

*To whom correspondence should be addressed E-mail: burrows@chem.utah.edu

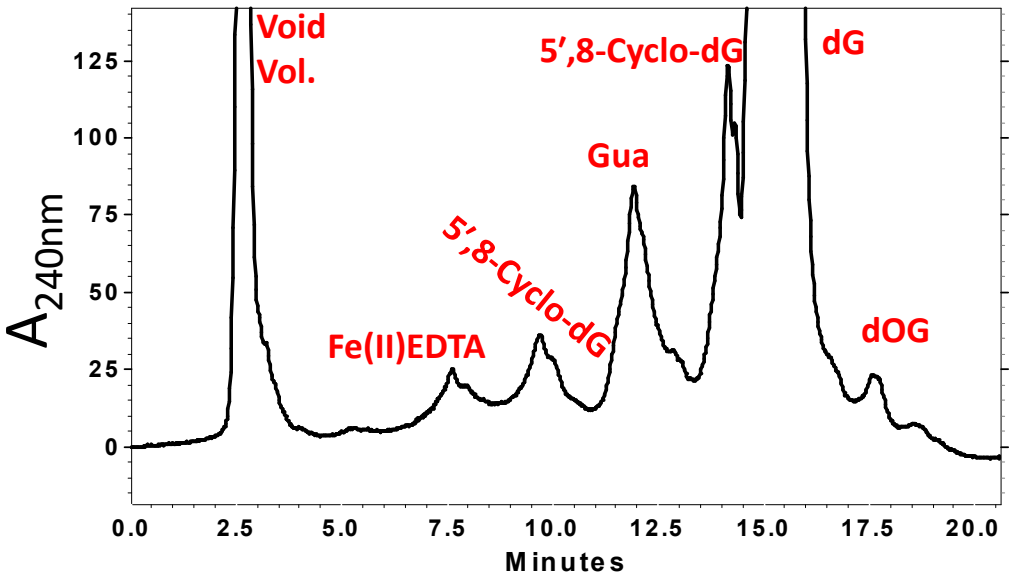
Methods

All reagents were obtained from commercially available sources and used without further purification unless otherwise stated. A 200-µL solution of dG (1.0 mM) in NaP_i or $NaHCO_3$ buffer (25.0 mM, pH 7.4) was allowed to react with a freshly prepared solution of Fe(II) catalyst (50 µM), ASC (3.0 mM), and H_2O_2 (1.0-3.0 mM). Preparation of the Fe(II) catalyst was achieved by mixing in NaP_i or $NaHCO_3$ buffer at pH 7.4 $FeSO_4$•7 H_2O with 1.1 equivalents of the ligand (EDTA, Cit, or αKG) under Ar atmosphere for 30 min prior to initiation of the reaction. The reactions were conducted by first mixing dG, ASC, and the Fe(II) catalyst in buffer followed by addition of the H_2O_2 as a bolus to initiate the reaction at 22 °C for 30 min. The reactions were quenched by the addition of 10 µL of EtOH followed by the addition of the post reaction internal standard etheno-2'-deoxyadenosine (εdA; 0.1 mM). The reaction mixture was analyzed via a dual HPLC column method previously reported and validated in our laboratory.[1-7] Each reaction was conducted in three independent trials to obtain average values that are reported in the graphs. The error for each measurement represents the standard deviation of the average values reported, which are ~10-15% of the average values reported in the bar graphs.

The RP-HPLC analysis retains dG, Gua, dOG, Fapy-dG, and the diastereomers of 5',8-cyclo-dG; however, in the present studies Fapy-dG was not found. The void volume from the RP-HPLC run on a Hypercarb HPLC column to analyze the diastereomers of d2Ih, dGh, and dSp, as well as dZ. The method employed provided time for dIz to hydrate and be converted dZ, and therefore, only dZ was inspected;[3] however, dZ was not found in the present study. Analysis of the reactions was first conducted by passing the sample down a C18 RP-HPLC column (250 X 4.6 mm, 5 µm) running the following solvents: A = 20 mM NH_4OAc (pH 7.0) in ddH_2O, B = CH_3CN, running at 1 mL/min, while monitoring the absorbance at 240 nm. The run was initiated at 1% B then after 3 min B increased to 10% over 10 min following a linear gradient, after which 10% B was held isocratic for 4 min. Next, B was increased to 65% over 10 min along a linear gradient then held at 65% for 10 min followed by termination of the run. The void volume from this RP-HPLC run was collected and lyophilized to dryness and resuspended in mobile phase A of the next HPLC run. Analysis of the void volume occurred on a Hypercarb HPLC column (150 X 4.6 mm, 5 µm, Thermo Scientific) that was running the following solvent systems: A = 0.1% acetic acid in ddH_2O, and B = MeOH, while running at a 1 mL/min flow rate, and monitoring absorbance at 240 nm. The run started at 0% B and after 10 min increased to 90% B following a linear gradient over 30 min. Characterization of the products was previously reported by our laboratory and the data are presented here for completeness.[1,3] HPLC-ESI^+-MS results for each identified compound are as follows: *m/z* $(M+H)^+$ 152.1 (Gua), 268.1 (dG), 284.1 (dOG), 266.3 (5',8-cyclo-dG diastereomers), 302.1 (d2Ih diastereomers), 274.1 (dGh diastereomers), 300.1 (dSp diastereomers), 247.1 (dZ), and 170.3 (Fapy-G free base); all values found matched their calculated values. Collected samples provided the following ESI^+-MS/MS data for the free bases of the following compounds: 2Ih enantiomers (186, 158, and 141; lit.,[8] 186, 158, 141) and Sp enantiomers (184, 156, 141, 113, 99, and 86; lit.,[9] 184, 156, 141, 113, 99, and 86). HRMS-ESI^+ (*m/z*) for nucleosides of dSp, $C_{10}H_{13}N_5O_6Na$ $(M)^+$ calcd 322.0764, found 322.0761; dGh, $C_9H_{15}N_5O_5Na$ $(M)^+$ calcd 296.0971, found 296.0980; dZ, $C_8H_{14}N_4O_5Na$ $(M)^+$ calcd 269.0862, found 269.0870. UV-vis profiles for each compound are shown in the ESI. Integrated peak areas obtained from absorbance at 240 nm on each HPLC run, were used to quantify the reaction yields through normalization of each area by its unique $\varepsilon_{240\,nm}$ (ddH_2O): dG (lit.,[10] 14,080 $dm^3\ mol^{-1}\ cm^{-1}$), dOG (lit.,[10] 14,300 $dm^3\ mol^{-1}\ cm^{-1}$), 5',8-cyclo-dG (lit.,[10] 14,080 $dm^3\ mol^{-1}\ cm^{-1}$), d2Ih (lit.,[11] 3,275 $dm^3\ mol^{-1}\ cm^{-1}$), dGh (lit.,[12] 2,412 $dm^3\ mol^{-1}\ cm^{-1}$), dSp (lit.,[9] 3,275 $dm^3\ mol^{-1}\ cm^{-1}$), dZ (lit.,[13] 1,778 $dm^3\ mol^{-1}\ cm^{-1}$), Gua (lit.,[10] 14,080 $dm^3\ mol^{-1}\ cm^{-1}$), εdA (lit.,[14] 7,300. $dm^3\ mol^{-1}\ cm^{-1}$).

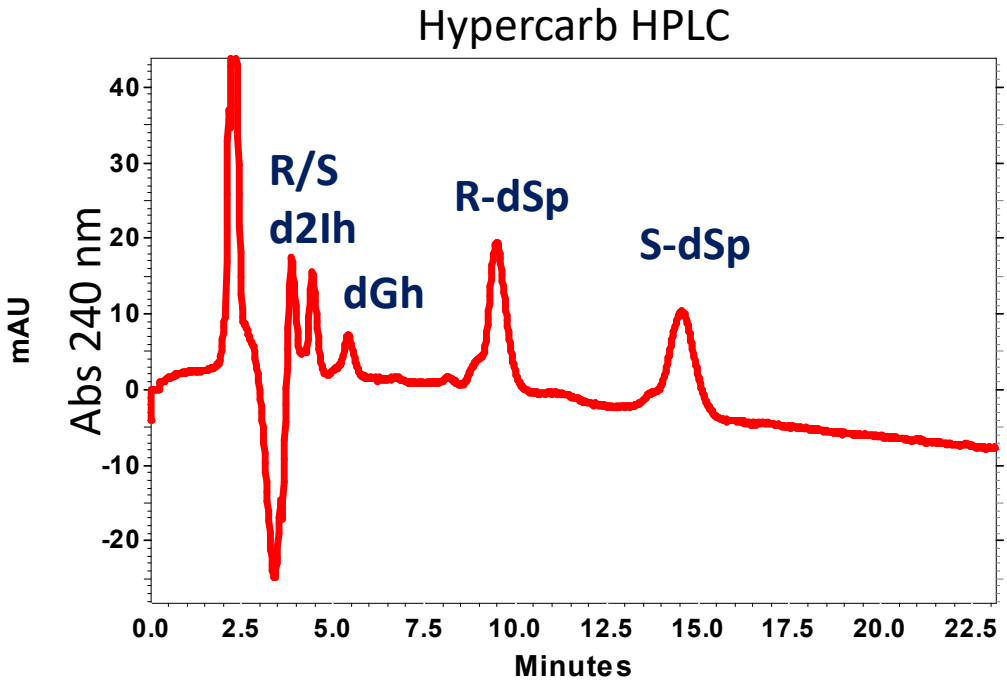
Fig. S1. Example HPLC chromatograms

A. RP-HPLC of the Fe(II)-EDTA in Phosphate Buffer



*5′,8-cyclo-dG is diastereotopic and elutes as two peaks from this RP-HPLC column.

B. Example Hypercarb HPLC chromatogram from the Fe(II)-EDTA reaction in phosphate buffer



*The products d2Ih and dSp are diastereotopic and elute as two peaks, while dGh is also diastereotopic, the two peaks are not resolvable on this column.

C. RP-HPLC of Fe(II)-EDTA in bicarbonate buffer

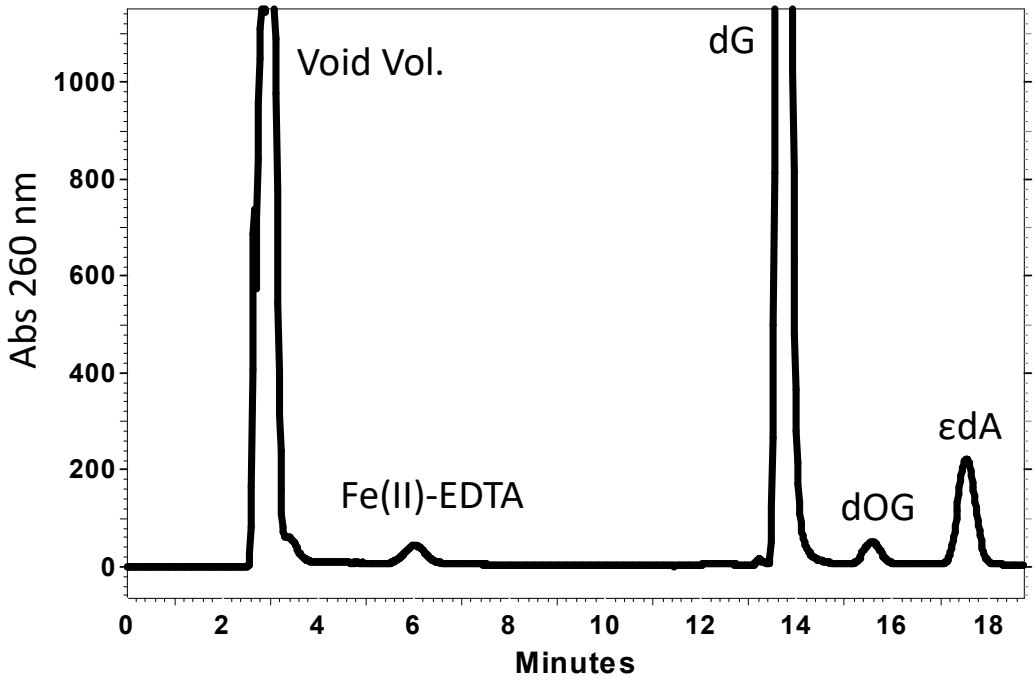
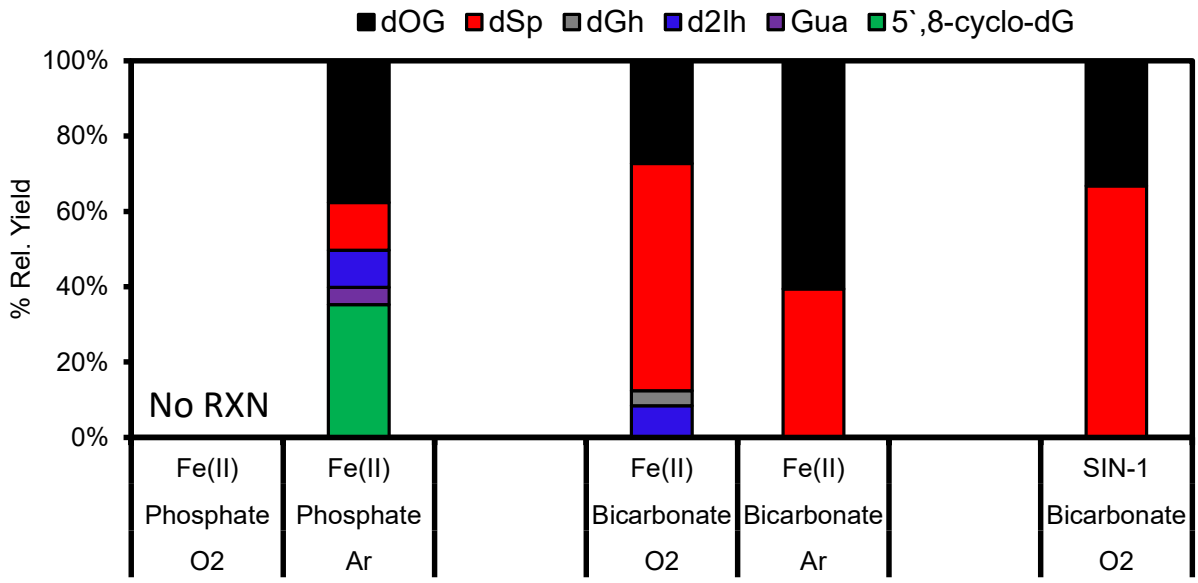
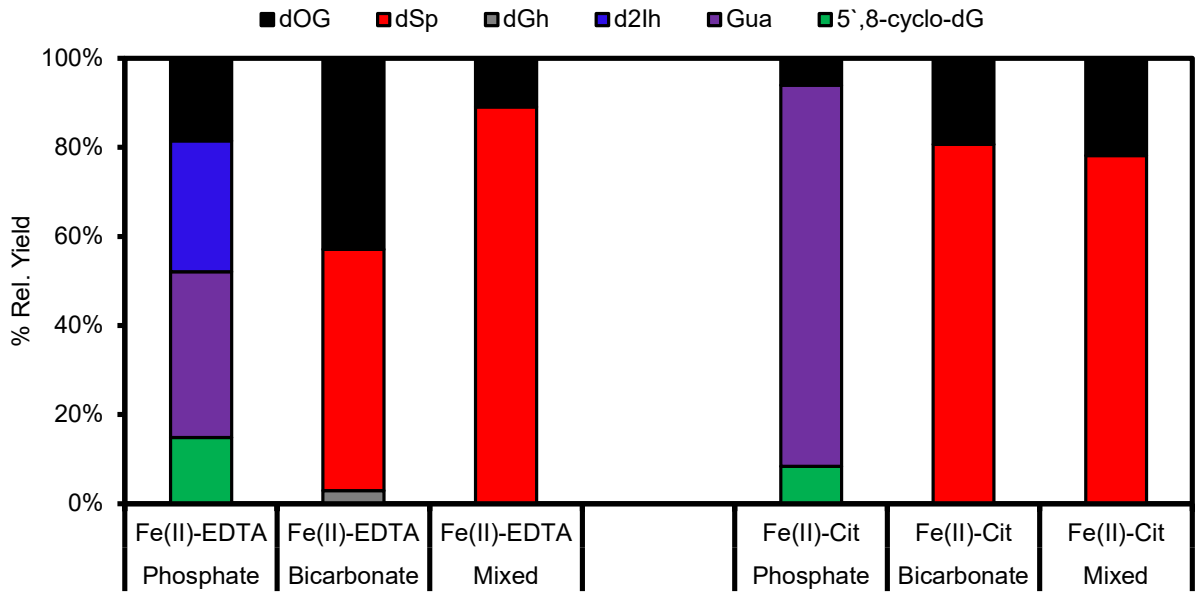


Fig. S2. Product analysis of Fe(II) Fenton oxidation of dG in phosphate buffer.



Relative product distribution from dG oxidation by the Fe(II) Fenton reaction in phosphate or bicarbonate buffer in the presence of ambient O_2 or bubbling Ar gas through the reaction for 10 min prior to addition of H_2O_2. The reaction was comprised of dG (1 mM), buffer (25 mM pH 7.4), ASC (3 mM), $FeSO_4 \bullet 7H_2O$ (50 µM), and H_2O_2 (3 mM) at 22 °C for 30 min prior to quenching with 10 µL of EtOH followed by HPLC analysis. The values reported represent the average of triplicate and independent trials. The error (i.e., standard deviation) on each value reported in the bar graph is ~10-15% of the values reported.

Fig. S3. Product analysis of reaction with mixed phosphate and bicarbonate buffers.



Relative product distribution from dG oxidation by the Fe(II) Fenton reaction in a mixture of phosphate bicarbonate buffer in the presence of ambient O_2. The reaction was comprised of dG (1 mM), buffer (12.5 mM each of sodium phosphate and sodium bicarbonate pH 7.4), ASC (3 mM), Fe(II) catalyst (50 µM), and H_2O_2 (3 mM) at 22 °C for 30 min prior to quenching with 10 µL of EtOH followed by HPLC analysis. The values reported represent the average of triplicate and independent trials. The error (i.e., standard deviation) on each value reported in the bar graph is ~10-15% of the values reported.

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