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Lead(II)-Catalyzed Oxidation of Guanine in Solution Studied with Electrospray Ionization Mass Spectrometry

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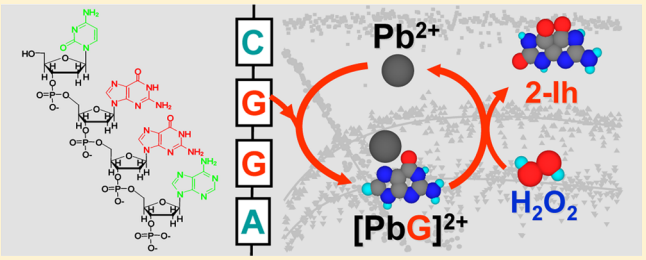
Supporting Information

ABSTRACT: The oxidation of guanine was investigated in water/methanol solution both in the absence and in the presence of Pb(II) with a variable temperature reactor coupled to a tandem mass spectrometer that allowed signature ions of solution reagents and products to be monitored by electrospray ionization (ESI). Two different oxidizing agents were employed, one strong (peroxymonosulfuric acid) and one weaker (hydrogen peroxide). Peroxymonosulfuric acid was observed to oxidize guanine rapidly at room temperature, $k_{app} > 10^{-2}$ s^{-1}, whether in the absence or in the presence of Pb(II), to produce spiroiminohydantoin. Guanine did not show measurable oxidation by hydrogen peroxide in the absence of Pb(II) at concentrations of H_2O_2 up to 1 M at temperatures up to 333 K ($k_{app} < 3 \times 10^{-8}$ s^{-1} at 298 K), but in the presence of Pb(II), it was observed to produce both 5-carboxamido-5-formamido-2-iminohydantoin (2-Ih) and imidazolone (Iz) in a ratio of 2.3 ± 0.1 with a total rate enhancement of more than 4×10^3. The activation energy was measured to be 82 ± 11 kJ mol^{-1} and is more than 120 kJ mol^{-1} lower than that for the uncatalyzed oxidation with hydrogen peroxide measured to be at least 208 ± 26 kJ mol^{-1}. An activation energy of 113 ± 9 kJ mol^{-1} has been reported by Bruskov et al. (*Nucleic Acids Res.* **2002**, *30*, 1354) for the heat-induced oxidation by hydrogen peroxide of guanine embedded as guanosine in DNA which leads to the production of 8-oxo-7,8-dihydro-guanine (8-oxo-Gua). The atomic lead dication lowers the activation energy by activating the hydrogen peroxide oxidant, possibly by O−O bond activation, and by directing the oxidation, possibly through coordination to the functional groups adjacent to the carbon C5: the C6 carbonyl group and the N7 nitrogen. The coupling of tandem mass spectrometry (MS^2) with a simple variable temperature reactor by ESI proved to be very effective for measuring reaction kinetics and activation energies in solution. Signature ions of both reagents and products, as well as the catalyst, could be identified, and the data were acquired in real time. The technique should be suitable for exploring other chemical and biochemical reactions that occur on similar time scales (minutes to hours).



INTRODUCTION

Oxidative damage of DNA could play a key role in mutagenesis, carcinogenesis, and cellular aging.[1] Such damage can occur at several positions in the DNA strand: at a nucleobase, at a sugar, or at a phosphate−sugar linkage. Nucleobase oxidation can be especially damaging, as it leads to formation of lesions, regions where the DNA structure is disrupted.[2,3] Guanine (Gua) is the nucleobase most susceptible to oxidation due to its low oxidation potential and high electron density.[4] The oxidation of guanine is commonly detected through 8-oxo-7,8-dihydro-guanine (8-oxo-Gua),[5−7] one of its oxidation products, that has been shown to be easily oxidized further due to a redox potential lower than that of guanine.[8,9]

While numerous guanine oxidation products are known[10] (see Scheme 1), only a few of these have been quantified in DNA oxidation experiments. Generally, guanine oxidation products can be divided into two distinct groups: (1) products arising from the oxidation at carbon C8 leading to the formation of 8-oxo-Gua, spiroiminohydantoin (Sp), guanidino-hydantoin (Gh), and dehydroguanidinohydantoin (dGh);[10] (2) products arising from the oxidation at carbon C5 leading to the formation of imidazolone (Iz), oxazolone (Z), and 5-carboxamido-5-formamido-2-iminohydantoin (2-Ih).[11,12] Quantification of guanine oxidation products in experiments with calf thymus DNA has shown that, while 8-oxo-Gua and imidazolone are the main products of guanine oxidation, they do not account for all DNA lesions formed in the experiments.[11,13]

Metal complexes have been shown to mediate guanine oxidation in the presence of radiation, photosensitizers, and oxidants.[14] Ghude et al.[15] have investigated the oxidation of modified guanosines and of single-strand oligodeoxynucleotides (ODN) and have illustrated the catalytic role that transition metals can play in the formation of DNA lesions. Experiments have demonstrated that metals can mediate the oxidation process in two ways: by interacting with the oxidant ($CoCl_2$/$KHSO_5^-$, Cr^{5+}/H_2O_2)[16,17] and creating a reactive oxidative species or by binding directly to guanine and catalyzing the

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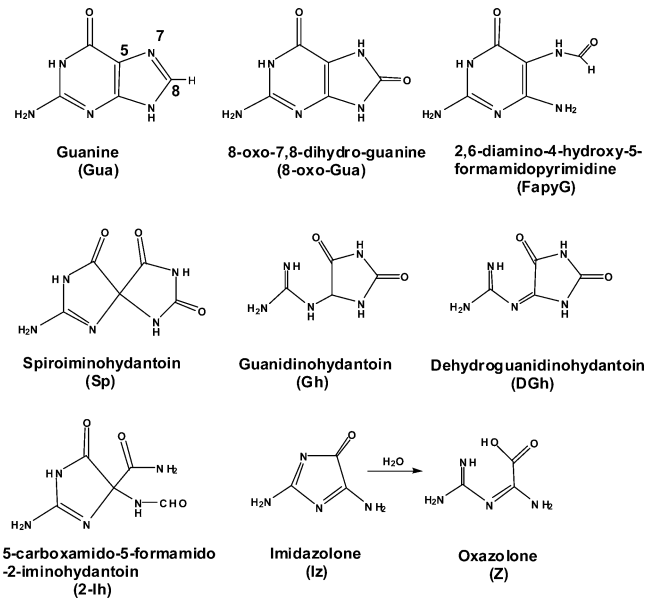
Scheme 1. Structures of Known Guanine Oxidation Products[a]



[a]The structures are adapted from ref 10, except that for 2-Ih which is derived from ref 12.

oxidation (NiCR/$KHSO_5$).[15,18,19] The former (indirect) pathway generally leads to the formation of 8-oxo-Gua or spiroiminohydantoin as a major product, whereas the latter leads to the formation of 2-Ih. Spiroiminohydantoin is known to be produced by various oxidative systems in either guanosine nucleosides, oligonucleotides, or DNA,[16,17,20−23] either from guanine directly or as a product of the further oxidation of 8-oxo-Gua. 2-Ih was recognized first as an intermediate[11] and later as a final product[1,15,24,25] of oxidation in oligodeoxynucleotides. The structure of this product was established by Ye et al.[12] based on mass spectrometry and NMR studies on 2-Ih species and on its isotopomers (^{13}C4) and (^{15}N7).

Lead(II) is also associated with the oxidative damage of DNA, as it leads to an increase in cellular oxidants and free radicals in the cell.[26] For instance, lead(II) can deplete glutathione and protein-bound sulfhydryl groups and produce reactive oxidative species (ROS) such as superoxide ion, hydrogen peroxide, and hydroxyl radicals.[27] In the presence of hydrogen peroxide, lead(II) induces cleavage of DNA strands in cultured cells.[28] The formation of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodGuo) has been observed in DNA exposed to lead(II) and hydrogen peroxide; ROS such as those generated by Fenton-like reactions and singlet oxygen ($^1O_2$) were proposed to be the principal cause of DNA damage in this case.[29] Such reactions may account for the genotoxicity and carcinogenocity associated with lead pollution.[29,30] While an excess of other metals, such as copper and iron, also can cause damage by creating ROS,[31] their levels in the body are regulated by homeostasis.[32] Since lead(II) plays no major function in the body, its levels are not regulated and can be dangerous at much lower concentrations.[33]

A major experimental challenge in studies of oxidative damage of DNA has been the detection and identification of reaction products; the large variety of possible products makes it difficult to identify and quantify all of them individually. For example, in studies using high performance liquid chromatography (HPLC), the detection of some products, including (2-Ih), was not possible,[34] as the electrochemical detection technique (HPLC-ECD) used was restricted to only a few electroactive DNA lesions.[35] On the other hand, the use of electrospray ionization/tandem mass spectrometry (ESI/MS^2) offers high sensitivity and specificity of product detection and identification aided by collisional dissociation measurements.[36] Furthermore, ionic signatures of the reagents and even the catalyst could be monitored concomitantly. In the pilot study reported here, we have successfully modified the electrospray sampling configuration and operation also to allow measurements of reaction kinetics and activation energies. In combination, these measurements offer insight into the mechanism of oxidation beyond what is possible by the observation or quantitation of products alone. Our pilot study is directed to the oxidation of guanine by two oxidative systems associated with lead(II): lead acetate−peroxymonosulfuric acid (a stronger oxidant) and lead acetate−hydrogen peroxide (a weaker oxidant).

EXPERIMENTAL METHODS

A schematic of the experimental setup is shown in Figure 1. Dilution of the reagent solution with solvent prior to

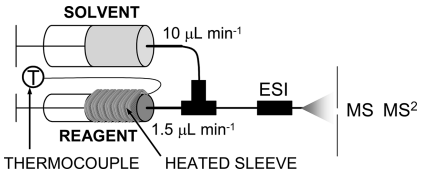


Figure 1. Schematic of the experimental setup. Luer-lock syringes were used to eliminate any metal surfaces in the system. The volumes of these syringes were 250 μL and 1 mL for the reagent and solvent, respectively.

electrospray provided kinetic and electrospray conditions suitable for experimentation. Direct injection of the reagent into the electrospray was found to be problematic from two points of view. Sufficient concentrations to achieve observable rates of reaction while also maintaining the resistivity of the solution for stable electrospray operation could not be achieved. The syringe containing the reagent solution was fitted with a heated sleeve to provide temperature control from room temperature upward (298 ± 0.5 and 333 ± 0.5 K). The temperature was monitored continuously with a low-temperature thermocouple. The two syringes were operated independently with separate syringe pumps. Typically flow rates were 1.5 μL min^{-1} for the flow of the reagent solution and 10 μL min^{-1} for the solvent (water:methanol). Reaction times were typically 30−60 min, much longer than the time necessary to heat the reaction mixture to the desired temperature (less than 30 s).

Mass spectra were obtained using an AB SCIEX QTrap 2000 hybrid triple quadrupole−linear ion trap mass spectrometer. Mass spectra were recorded in real time. Typical instrument parameters were the following: electrospray potential, 5 kV; interface temperature, approximately 120 °C; declustering potential, 30 V; curtain nitrogen gas flow rate, approximately 2 L min^{-1}. MS^2 measurements were conducted separately by diluting the reaction mixture after the reaction had gone to completion (60 min at 333 K). Structures of both reactant and product ions were analyzed by MS^2. The collision energy (CE)

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instrument parameter was varied between 0 and 50 V, with nitrogen as the collision gas.

Guanine base, lead(II) acetate, and lead(II) nitrate were purchased from Sigma-Aldrich (all ≥99% purity). The peroxymonosulfuric acid was made by mixing concentrated sulfuric acid (95−98%, Caledon) and hydrogen peroxide (30%, Caledon) in a 3:1 ratio. Due to degradation, this solution had to be used within 3 h from its preparation. Stock solutions of lead acetate (100 mM) and peroxymonosulfuric acid (0.12 M) were prepared in water (Millipore 18.2 MΩ). A saturated solution of guanine (approximately 100 μM) was prepared from a guanine suspension. This suspension was sonicated at 70 °C for 30 min, allowed to cool down, and centrifuged for 5 min to remove the remaining solids. The typical final concentration of lead acetate was 1 mM, of peroxymonosulfuric acid 0.012−0.060 M, and of hydrogen peroxide 0.1−1 M. The prepared acidic solutions (pH 1−2 for those containing H_2SO_5 and pH 4−5 for those containing H_2O_2) were diluted prior to injection for electrospray mass spectrometry (ESI-MS) with 80:20 water (Millipore 18.2 MΩ):methanol (HPLC grade, Sigma).

RESULTS AND DISCUSSION

Oxidation with Peroxymonosulfuric Acid. The electrosrayed ion profiles in Figure 2 show that the oxidation of guanine (Gua) with peroxymonosulfuric acid (H_2SO_5) leads to oxidation products with signature ions at m/z = 184 and 202. These were assigned to protonated spiroiminohydantoin, Sp (see Scheme 1), and its water adduct, respectively, on the basis of their mass and their collision-induced dissociation spectra (see Figure S1, Supporting Information). The observed low-energy loss of water from m/z = 202 indicates that the water adduct is most likely proton-bound and is a byproduct of electrospray ionization.

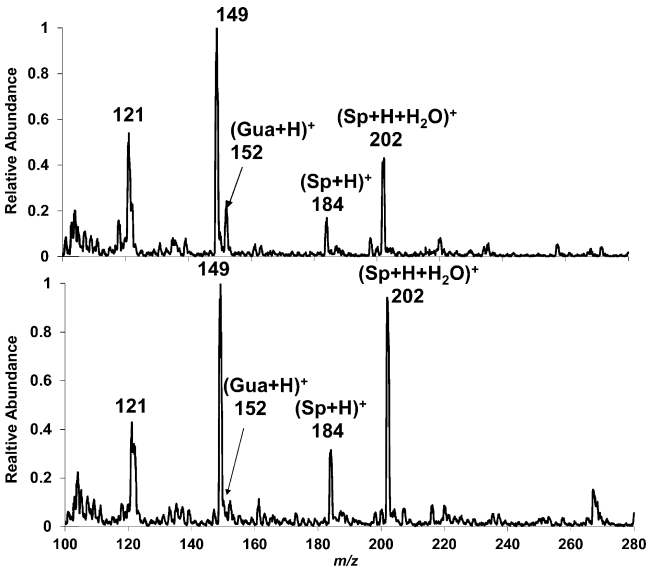

Figure 2. Mass spectra for reaction solutions containing guanine (Gua, 100 μM) and peroxymonosulfuric acid (0.024M) in the absence (top) or presence (bottom) of lead acetate (1 mM) collected approximately 10 min after sample preparation at room temperature (at which point the oxidation should be complete). The observed products are protonated spiroiminohydantoin, $(Sp+H)^+$, and its water adduct. The ion observed at m/z = 149 can be attributed to protonated phthalic anhydride due to the plastic implements used in the sample preparation.[37] The ion at m/z = 121 is assigned as the protonated acetic acid cluster from the lead acetate salt.

The electrosprayed ion profiles recorded for the guanine oxidation by peroxymonosulfuric acid in the presence or absence of Pb^{2+} show that the concentrations of both product signature ions, $(Sp+H_2O+H)^+$ and $(Sp+H)^+$, do not change with time (see Figure 3), and this indicates that the reaction is fast and complete before the solution is introduced into the electrospray. We estimate a lower limit to the apparent rate coefficient for the room-temperature oxidation of $k_{app} > 2 \times 10^{-2}$ s^{-1}. Experiments were attempted in which the two reagent solutions were mixed online to reduce the reaction time before electrospraying, but these experiments failed due to the corrosive nature of the peroxymonosulfuric acid.

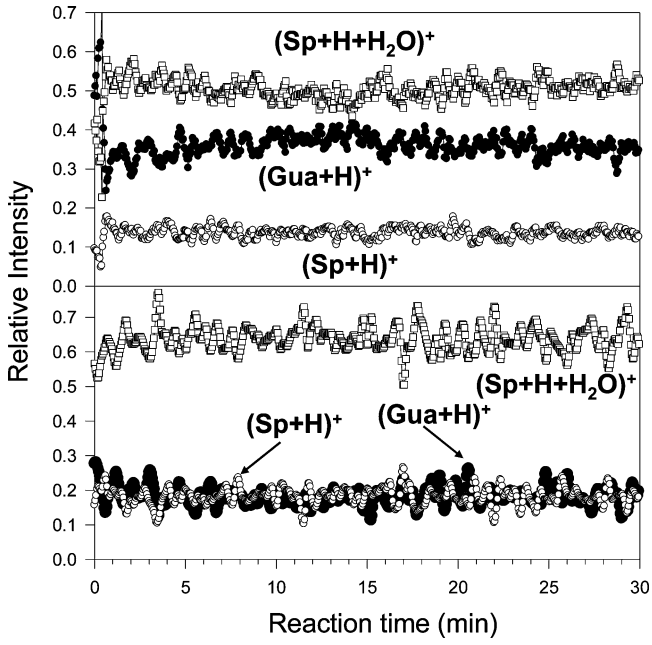

Figure 3. Reaction profiles recorded for the oxidation of guanine by peroxymonosulfonic acid in solutions of guanine (100 μM) and peroxysulfuric acid (0.024 M) in the absence (top) and presence (bottom) of lead acetate (1 mM). The spectra were recorded 5 min after preparation at room temperature. The solution was diluted on line 1:7. The declustering potential was 100 V. The reaction is fast and is complete before the solution is sampled by the mass spectrometer. The difference in relative intensities of protonated guanine and the two product ions in the two graphs are likely due to the presence of Pb^{2+}. Cationic species, such as lead(II), change the ionic strength of the electrosprayed solution and can lead to ion suppression of protonated ions.

Further experiments in which mass spectra were recorded in the presence of various concentrations of Pb^{2+} indicated that these spectra are not affected significantly by the concentration of lead(II) in the solution from 0 to 1 mM (see Figure 4) and that oxidation is essentially complete already in the absence of Pb^{2+}. Although the oxidation product ions appear to have greater intensities in the presence of lead(II), we cannot attribute these to the catalytic effect of lead(II). Such differences in intensities can easily be a consequence of the presence of lead(II) in the solution, which suppresses the intensity of protonated ions by approximately 95%. Due to such a high level of suppression, the relatively small differences in Figures 3 and 4 should not be considered important. So again,

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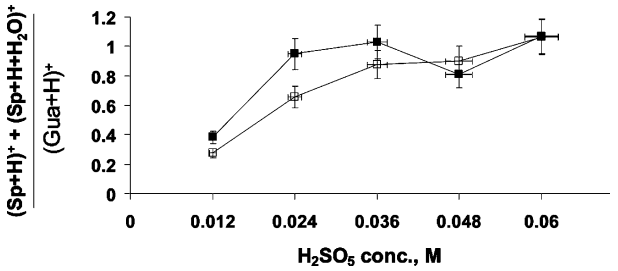

Figure 4. Comparison of intensities of the sum of product ions, $[(Sp+H)^+ + (Sp+H+H_2O)^+]$, relative to that of guanine, $(Gua+H)^+$, as a function of H_2SO_5 concentration in the presence (solid symbols) and absence (open symbols) of lead. The guanine concentration is 100 μM, and that of lead acetate is 1 mM. Each point represents an average of three measurements, and the error bars are the standard deviation for those measurements. The peroxymonosulfuric acid was diluted before mixing with guanine and lead(II) solutions in order to slow down the reaction. Dilution is likely to increase the rate of destruction of peroxymonosulfuric acid with dissolved oxygen and CO_2, reducing the amount of oxidation product.

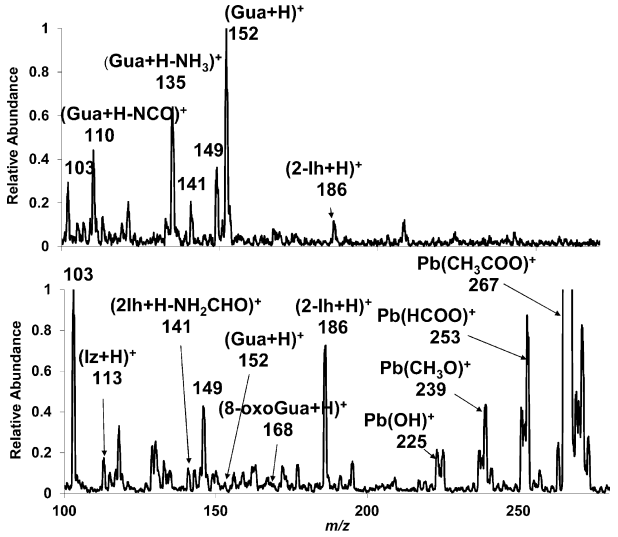

Figure 5. Mass spectra for reaction solutions containing guanine (Gua, 100 μM) and hydrogen peroxide (1.0 M) in the absence (top) and presence (bottom) of lead(II) (1.0 mM). The main oxidation products are the protonated 5-carboxamido-5-formamido-2-iminohydantoin $(2\text{-}Ih+H)^+$ and the protonated imidazolone $(Iz+H)^+$. The ion observed at m/z = 149 is attributed to phthalic anhydride formed from phthalate contaminants from the plastic implements used in the sample preparation. Ions corresponding to $Pb(Gua\text{-}H)^+$ and $Pb(Gua\text{-}H)^{2+}$ were not observed.

we can only report a lower limit to the apparent rate coefficient for the room-temperature oxidation, in this case in the presence of Pb^{2+}, of $k_{app} > 2 \times 10^{-2}$ s^{-1}.

Oxidation with Hydrogen Peroxide. Guanine did not show measurable oxidation by hydrogen peroxide in the absence of Pb(II) at concentrations of H_2O_2 up to 1 M at temperatures up to 333 K. The mass-spectrometric observation of very small amounts of signature ions of some of the reaction products could be attributed to the influence of metal impurities known to be present in the hydrogen peroxide: Fe: ≤0.5 ppm, heavy metals ≤1 ppm (certificate of analysis of H_2O_2 from supplier).

In the presence of Pb(II) with addition of 1 mM lead acetate, the oxidation of guanine was observed to proceed slowly at room temperature and became much faster as the temperature was increased. The mass spectra of various signature ions recorded at 333 K after 60 min are shown in Figure 5 in the presence and the absence of Pb^{2+}. The neutral reagents and products appear in these spectra in their protonated form. Pb(II) appears as various complexes of deprotonated acetic acid and methanol, viz., $Pb(CH_3COO)^+$, $Pb(CH_3COO)(CH_3OH)^+$, and $Pb(CH_3O)^+$. In-source dissociation products of protonated guanine, $(Gua+H-NH_3)^+$ and $(Gua+H-NCO)^+$, are present also in the mass spectrum of a mixture of guanine and lead (see Figure S2, Supporting Information), indicating that these are not signature ions of other products of the oxidation.

The apparent rate coefficients measured in our experiments for the oxidation of guanine by hydrogen peroxide in the absence and presence of Pb^{2+} at 298 K are included in the summary given in Table 1.

The major protonated oxidation product observed as a signature ion in the presence of Pb(II) was attributed to protonated 5-carboxamido-5-formamido-2-iminohydantoin, $(2\text{-}Ih+H)^+$, at m/z = 186. The collision-induced spectrum of this ion is given in Figure S4 in the Supporting Information and is in agreement with CID results reported previously.[25] Observed minor signature ions were attributed to protonated imidazolone $(Iz+H)^+$ at m/z = 113 and protonated 8-oxo-7,8-dihydroguanine $(8\text{-oxo-Gua}+H)^+$ at m/z = 168 (see Scheme 1). Observed H/D exchange products induced in D_2O exhibited the increase in m/z expected from the assignments of m/z = 113 and 186: five exchanges with $(Iz+H)^+$ and seven exchanges with $(2\text{-}Ih+H)^+$.

Table 1. Summary of Rate Coefficients Measured for the Oxidation of Guanine at 298 K

reaction	k_{app} (s^{-1})
Gua + H_2SO_5	>2 × 10^{-2}
Gua + Pb^{2+} + H_2SO_5	>2 × 10^{-2}
Gua + H_2O_2	<3 × 10^{-8}
Gua + Pb^{2+} + H_2O_2	1.2 × 10^{-4}

The reaction profile shown in Figure 6 for the guanine oxidation by hydrogen peroxide in the presence of Pb^{2+} indicates that the concentrations of both $(2\text{-}Ih+H)^+$ and $(Iz+H)^+$ increase with reaction time, while that of $(8\text{-oxo-Gua}+H)^+$ decreases almost parallel to the decrease in $(Gua+H)^+$. The latter suggests that $(8\text{-oxo-Gua}+H)^+$ is a byproduct of oxidation in the electrospray source, rather than in the heated syringe. Such oxidations, initiated by radical formation in a corona discharge in the vicinity of the needle tip in an electrospray source, are well-known,[38] even under normal instrument operating conditions with electrospray voltages as low as 3.5 kV.[39] Gas phase electrons liberated in the discharge are accelerated by the positive voltage applied to the electrospray needle and induce, in our case, the formation of OH radicals through inelastic collisions with solvent molecules and hydrogen peroxide (e.g., $H_2O_2 + e^- \rightarrow HO\bullet + \bullet OH + e^-$). Others have shown that the oxidation of guanosine by OH radicals results in the formation of 8-oxo-guanosine (8-oxo-Guo).[10] We can expect a similar oxidation reaction to occur with the closely related guanine molecule to form 8-oxo-Gua.

In our experiments, the production of 8-oxo-G would be limited by the amount of OH radicals produced by the corona discharge. We observe a constant amount of $(8\text{-oxo-Gua}+H)^+$ ion, 4−5% relative intensity, until the concentration of guanine

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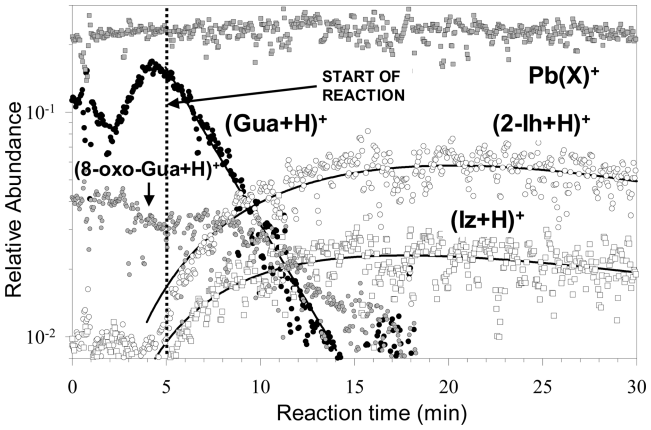

Figure 6. Reaction profile for the lead-catalyzed oxidation of guanine by hydrogen peroxide at 328 K. Guanine appears protonated $(Gua+H)^+$. The major observed product signature ion is attributed to protonated 5-carboxamido-5-formamido-2-iminohydantoin $(2\text{-Ih}+H)^+$, while protonated imidazolone $(Iz+H)^+$ is assigned as a minor product signature ion. The $(8\text{-oxo-Gua}+H)^+$ ion is thought to be a byproduct of oxidation in the electrospray source (see text). The profile for $Pb(X)^+$ represents the sum of Pb^{2+} containing ions, including $Pb(CH_3COO)^+$, $Pb(CH_3COO)(CH_3OH)^+$, and $Pb(CH_3O)^+$, originating from the lead(II) acetate and methanol used in the experiments. The solid lines represent kinetic fits of the data points. The start of reaction indicates the point where the temperature was rapidly increased from room temperature to the reaction temperature.

in solution is reduced by the reaction. As the guanine concentration falls below a critical value, an intensity of $(Gua+H)^+$ below approximately 4% relative intensity, the OH radical concentration ceases to be the limiting factor in the reaction and a reduction in the ion intensity of $(8\text{-oxo-Gua}+H)^+$, concurrent to the decay of the ion intensity of $(Gua+H)^+$, is observed. Thus, as both guanine and hydrogen peroxide are present in the solution being electrosprayed, the oxidation of guanine by hydroxide radicals is promoted near the tip of the electrospray needle. As guanine is consumed by the reaction in the heated syringe, the amount of (8-oxo-Gua) observed consequently also is reduced.

The order of the reaction was determined to be one with respect to all reagents (Pb^{2+}, guanine, and hydrogen peroxide) and this corresponds to the rate law $R = k_{app}[Pb^{2+}][Gua][H_2O_2]$. The order was determined by measuring apparent rate coefficients of the reaction while varying each of the reactant concentrations and keeping the other two constant (see Table 2).

The activation energy for the oxidation of guanine by hydrogen peroxide in the presence of Pb(II) was obtained by measuring apparent rate coefficients at different reaction temperatures. The apparent rate coefficients were found to vary from 0.02 s^{-1} at 308 K to 0.23 s^{-1} at 333 K. The standard deviation is 3% for measurements performed with the same stock solutions and 13% with different stock solutions. The Arrhenius plot in Figure 7 provides an activation energy of E_a = 82 ± 11 kJ mol^{-1} for the oxidation of guanine by hydrogen peroxide in the presence of Pb(II). Measured rate coefficients were much smaller in the absence of Pb(II) and provided an activation energy of E_a = 208 ± 26 kJ mol^{-1}. This latter value may be a lower limit because of the known presence of metal impurities, including lead, in the hydrogen peroxide. It is interesting to compare this value to the somewhat lower value of 113 ± 9 kJ mol^{-1} reported by Bruskov et al.[40] for the heat-induced oxidation by hydrogen peroxide of guanine embedded as guanosine in DNA which leads to the production of 8-oxo-G attributed to C8 oxidation.

Table 2. Apparent Rate Coefficients Measured for the Oxidation of Guanine by Pb^{2+}/H_2O_2[a]

$[Pb^{2+}]$ (mM)	[Gua] (μM)	$[H_2O_2]$ (M)	T (K)	k_{app} (s^{-1})
2.0	100	1.0	323	3.0×10^{-3}
1.0	100	1.0	323	1.6×10^{-3}
0.50	100	1.0	323	0.64×10^{-3}
0.25	100	1.0	323	0.34×10^{-3}
1.0	100	1.0	328	1.8×10^{-3}
1.0	50	1.0	328	0.89×10^{-3}
1.0	25	1.0	328	0.51×10^{-3}
1.0	100	2.0	323	2.2×10^{-3}
1.0	100	1.0	323	1.1×10^{-3}

[a]Experiments were performed at different concentrations of each reagent while keeping the others constant in order to determine the order of reaction.

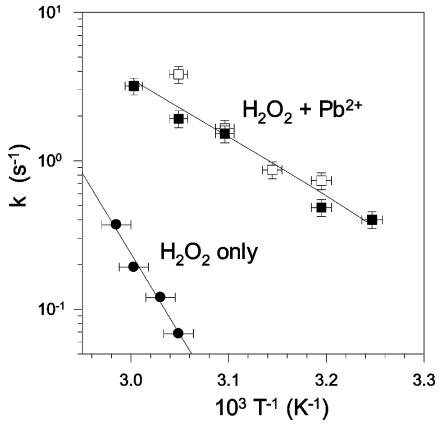

Figure 7. Arrhenius plot of k_{app} vs the inverse of temperature for the oxidation of guanine by hydrogen peroxide in the presence (squares) and the absence (circles) of Pb^{2+}. Data from measurements taken on two separate days (open and solid symbols) are combined. Each point represents an average of three measurements. Rate coefficients in the absence of lead are given as lower limits due to the presence of small amounts of metals (Cu, Fe, Pb, and others) in the hydrogen peroxide solution that are known to catalyze the oxidation. Calculated activation energies are E_a = 82 ± 11 kJ mol^{-1} in the presence of Pb^{2+} and E_a = 208 ± 26 kJ mol^{-1} in the absence of Pb^{2+}. The error bars for k_{app} represent the cumulative error associated with calculating the rate, such as the errors in the mass and volume measurement. The error bar for the inverse temperature represents the ±0.5 K uncertainty in the temperature.

Lead(II) Catalyzed Oxidation of Guanine with Hydrogen Peroxide. The rate enhancement apparent from Table 1 for the oxidation of guanine by hydrogen peroxide in the presence of Pb^{2+} is at least 4×10^3 at 298 K. The measured reduction in the activation energy also is substantial, more than 100 kJ mol^{-1}. This is indicative of the occurrence of catalysis. We suggest a cyclic reaction mechanism according to reactions 1 and 2 and Scheme 2 in which Pb(II) is the catalyst and $Pb(Gua)^{2+}$, not $Pb(Gua\text{-}H)^+$, is the reaction intermediate. We expect that guanine ($pK_a(G)$ = 9.92 at 313 K)[41] is neutral at the pH of 4–5 measured for the solutions containing hydrogen peroxide. Acidification of guanine by metal dications is known but typically is only of the order of 1–2 log units.[42]

$$Pb^{2+} + Gua \rightarrow Pb(Gua)^{2+} \quad (1)$$

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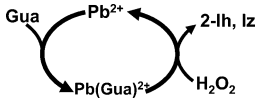
Scheme 2. Schematic Representation of the Lead(II) Catalyzed Oxidation of Guanine by Hydrogen Peroxide



$$Pb(Gua)^{2+} + H_2O_2 \rightarrow 2\text{-Ih} + Pb^{2+} \quad (2a)$$

$$\rightarrow Iz + (NH_2CHO + CO) + Pb^{2+} \quad (2b)$$

Figure 6 shows that the signature ions $Pb(X)^+$ of Pb^{2+} are intense and remarkably constant with time, as would be expected if Pb^{2+} is the catalyst. Ions corresponding to the intermediate $Pb(Gua)^{2+}$ (or $Pb(Gua\text{-}H)^+$) were not observed in the ESI spectrum in the presence of Pb(II) and oxidant. Figure 5 (and 6) also shows some IzH^+ (a signature ion for Iz, imidazolone), in the absence of $(2\text{-Ih+H}-NH_2CHO)^+$. It is much larger than the Iz+H in the CID spectrum of $(2\text{-Ih+H})^+$ in which it is produced from $(2\text{-Ih+H})^+$ at relatively high collision energies (see Figure S3, Supporting Information). Thus, Iz also is produced in reaction 2 and we can report a branching ratio (BR) for (2a)/(2b) = 2.3 ± 0.1 which was found to be independent of temperature. The parallel rise with time in the ion signals of $(2\text{-Ih+H})^+$ and Iz+H rules out the secondary production of Iz+H from $(2\text{-Ih+H})^+$.

Since the product of oxidation (2-Ih) is observed (as $(2\text{-Ih+H})^+$), even with low concentrations of Pb^{2+}, it is unlikely that Pb^{2+} is creating an oxidizing reagent through an independent reaction with H_2O_2, as that would quickly consume Pb^{2+} reagent.

Mechanism of Oxidation of Guanine by Hydrogen Peroxide in the Presence of Pb(II). The binding of Pb^{2+} in guanine (Gua) is expected to be similar to that in guanosine (Guo). Stability constants of the $[Pb(Guo)]^{2+}$ complex have been reported previously and are indicative of Pb^{2+} binding at N7 in guanine with the participation of the (C6)O group.[42] We envisage the conversion of guanine to 2-Ih in the presence of Pb(II) and H_2O_2 that is analogous to that proposed for the oxidation in the presence of NiCR and $KHSO_5$.[15] In this mechanism, the hydrogen peroxide oxidant is delivered to the exposed guanine by the Pb^{2+} chelated to N7 and (C6)O. The ensuing interaction of Pb^{2+} with H_2O_2 makes an oxygen free radical at C5 of guanine available. A control experiment, using the lead(II) nitrate salt instead of the acetate, was performed under similar experimental conditions of temperature, pH, and concentration. No differences were observed in reaction products, and the reaction rate was similar. Thus, it seems that acetate does not play a significant role in the oxidation.

For the guanine oxidation by peroxymonosulfuric acid in which spiroiminohydantoin (Sp) is produced by oxidation at the C8 carbon,[10] we were not able to determine whether lead(II) acts as a catalyst. The reaction was observed to be too fast, both in the absence and in the presence of lead(II). Peroxymonosulfuric acid is a stronger oxidant than hydrogen peroxide and guanine is expected to be mainly protonated, pK_a $[(Gua+H)^+ \rightarrow Gua + H^+] = 3.3$,[43] at the lower pH (pH ~ 2) of the solution.

CONCLUSIONS

ESI-MS has allowed us to monitor both the room-temperature kinetics and reaction products for the oxidation of guanine by peroxymonosulfuric acid (a strong oxidizing agent) and by hydrogen peroxide (a weaker oxidant) in water/methanol solution in the absence and presence of Pb(II). The former was found to be rapid ($k_{app} > 10^{-2}$ s^{-1}) both in the absence and presence of Pb(II) and results in the formation of spiroiminohydantoin (Sp) that has been reported to occur by oxidation at the C8 carbon.[10] The room-temperature oxidation of guanine by hydrogen peroxide was found to be immeasurably slow ($k_{app} < 3 \times 10^{-8}$ s^{-1}) but appeared to be induced under electrospray conditions, as some formation was observed of a signature ion of 8-oxo-Gua (also a C8 oxidation product). A dramatic enhancement of 4×10^3 was measured for the room-temperature oxidation of guanine by hydrogen peroxide in the presence of Pb(II) which was accompanied by the production of both 5-carboxamido-5-formamido-2-imino-hydantoin (2-Ih) and imidazolone (Iz) in a ratio of 2.3 ± 0.1. Temperature dependent measurements provided an activation energy of 82 ± 11 kJ mol^{-1} which is more than 120 kJ mol^{-1} lower than that measured for the uncatalyzed oxidation with hydrogen peroxide, of at least 208 ± 26 kJ mol^{-1}. Lead dication lowers the activation energy by activating the hydrogen peroxide oxidant, possibly by O−O bond activation, and directs the oxidation, possibly through coordination to the functional groups adjacent to the carbon C5: the C6 carbonyl group and the N7 nitrogen. An activation energy of 113 ± 9 kJ mol^{-1} has been reported by Bruskov et al.[40] for the heat-induced oxidation by hydrogen peroxide of guanine embedded as guanosine in DNA which leads to the production of 8-oxo-7,8-dihydro-guanine (8-oxo-Gua). This is 38% higher than that for the oxidation of "free" guanine, presumably as a consequence of the stereochemistry and bonding when embedded in DNA.

Coupling of tandem mass spectrometry (MS^2) with a simple variable temperature reactor by electrospray ionization has proven to be very successful for measuring reaction kinetics and activation energies. ESI tandem mass spectrometry allowed reagents and products in solution to be identified with considerable confidence. Although some instrumental effects, such as oxidation under electrospray conditions in the presence of trace amounts of metal ions, were observed, and these were easy to account for due to the real-time nature of the acquired data. This technique should be suitable for generally exploring kinetics of reactions in solution that occur on similar time scales (minutes to hours), especially the dynamics of catalytic systems. This might include the oxidation of DNA strands, even long ones. Reaction solutions could be diluted online with appropriate buffers, passed over an immobilized digestion enzyme (e.g., DNase) in a microfluidic device, and then analyzed by ESI/MS/MS. The high selectivity of the mass spectrometer would provide detailed information about the oxidation products, while accurate quantification can be achieved through addition of appropriate internal standards, either to the reaction solution or online before the ESI stage.

ASSOCIATED CONTENT

Supporting Information

Data for the collision-induced dissociation of the signature ions observed for the products in the oxidation of guanine with peroxymonosulfuric acid and with hydrogen peroxide, and the mass spectrum for a solution containing guanine and lead acetate. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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