A Platinum(IV) Complex Oxidizes Guanine to 8-Oxo-Guanine in DNA and RNA

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The oxidative damage of DNA by metal complexes including Cr, Mn, Re, Fe, Ru, Os, Co, Rh, Ni, and Pd has been an active research area.[1] In this communication, we report that a platinum complex with a high reduction potential,[2] *d*,*l*-1,2-diaminocyclohexyltetrachloroplatinum(IV) (Pt^{IV}(dach)Cl_4), oxidizes guanine in guanosine-5′-monophosphate (GMP), 2′-deoxyguanosine-5′-monophosphate (dGMP), d(GG), and a double-stranded oligonucleotide to 8-oxo-guanine. To the best of our knowledge, this is the first report that provides unambiguous evidence of DNA oxidation by a Pt^{IV} complex. This oxidative damage may differentiate the anticancer activity of Pt^{IV} complexes from that of their Pt^{II} analogues.

When Pt^{IV}(dach)Cl_4 (10 mM) was reacted with dGMP or GMP (10 mM) at 37 °C, pH 8.3, after 4 days the pH dropped to 3.4, and yellow crystals were formed. These crystals were identified as Pt^{II}(dach)Cl_2 by IR analysis (3267, 3190, 3066, 2936, 2863, and 1564 cm^{-1}). This indicates that both dGMP and GMP reduced Pt^{IV} to Pt^{II}. High performance liquid chromatography (HPLC) was used to analyze a $t = 1$ day reaction mixture of Pt^{IV}(dach)Cl_4 and dGMP using both a diode array detector (DAD) and an electrochemical detector (ECD)[3] (Figure 1).

The DAD chromatogram displays a new weak peak at 5.8 min. The absorption spectrum of this new peak shows two maxima at 248 and 294 nm. The ECD chromatogram displays a strong peak at 5.8 min. The identical retention time, absorption spectrum, and electrochemical behavior were exhibited by an authentic sample of 8-oxo-dGMP. Therefore, we conclude that the peak at 5.8 min is due to 8-oxo-dGMP. The concentrations of 8-oxo-dGMP, dGMP, and Pt^{IV}(dach)Cl_4 in the reaction mixture were quantitated using calibration curves obtained from each standard solution.[4a] The concentration of dGMP decreased, while that of 8-oxoG increased up to a certain time and then decreased due to overoxidation or degradation.[4b] The maximum conversion of dGMP to 8-oxodGMP varies between ∼30 and 75% depending on the reaction conditions and the reaction time.

Cyclic voltammetry confirms these HPLC results. Figure 2 displays the cyclic voltammograms (CV) of Pt^{IV}(dach)Cl_4/GMP mixtures. The CV at $t = 0$ sample shows an anodic peak at 1.21 V and a cathodic peak at −0.25 V, which are assigned to GMP and Pt^{IV}(dach)Cl_4, respectively. The CV at $t = 2$ days sample shows a new anodic peak at 0.57 V assigned to 8-oxo-GMP. It is 0.64 V lower than that of GMP, which is consistent with the literature value of 0.64 V.[5] The new peak due to 8-oxo-GMP eventually disappears after a long reaction time.

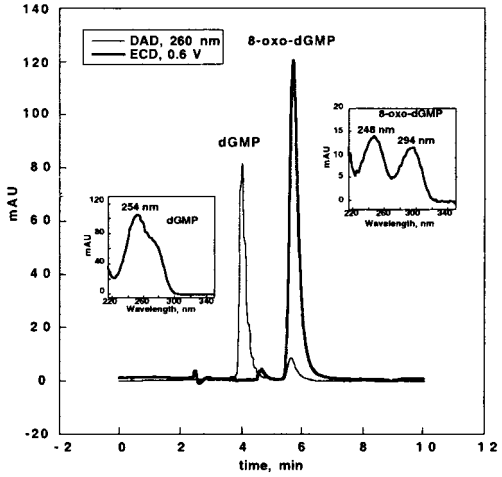

Figure 1. DAD (λ = 260 nm) and ECD (E = 0.6 V) HPLC chromatograms of Pt^{IV}(dach)Cl_4/dGMP (5 mM/20 mM) after 1 day. Zorbax C18 column (5μ, 4.6 × 200 mm); 99% 0.1 M ammonium acetate (pH 6.4) and 1% acetonitrile; and 1 mL/min.

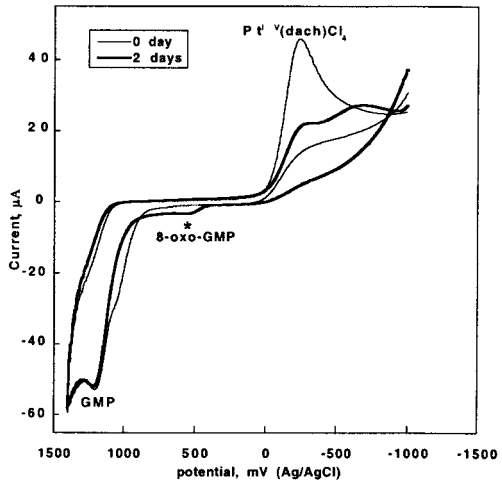

Figure 2. Cyclic voltammograms of Pt^{IV}(dach)Cl_4/GMP (10 mM/20 mM), pH 8.3, at $t = 0$ and 2 days. Glassy carbon electrode; BAS 100 electrochemical analyzer; supporting electrolyte: 0.1 M KCl, pH 8.3; and sweep rate of 100 mV/s.

Pt^{IV}(dach)Cl_4 also oxidizes guanine in d(GG) and a double-stranded oligonucleotide, where the double guanine containing strand is (5′−d[TGATCGGTGCGTCTGAGACT]-3′), to 8-oxo-G. When Pt^{IV}(dach)Cl_4 was reacted with d(GG) or the oligonucleotide, there was little reaction. However, addition of a small amount of *cis*-$Pt^{II}(NH_3)_2Cl_2$ (cisplatin) increased the reaction rate, suggesting a Pt^{II}-assisted Pt^{IV} substitution reaction occurred.[6,7] Figure 3 displays the HPLC chromatogram (DAD) of nucleosides obtained by enzymatic digestion of the oligonucleotide after 3

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(4) (a) See Supporting Information #1 for the calibration curve. (b) See Supporting Information #2 for the reaction profiles of Pt^{IV}(dach)Cl_4/dGMP (5 mM/10 mM, pH 8.6) and Pt^{IV}(dach)Cl_4/dGMP/cisplatin (20 mM/20 mM/2 mM, pH 8.6).
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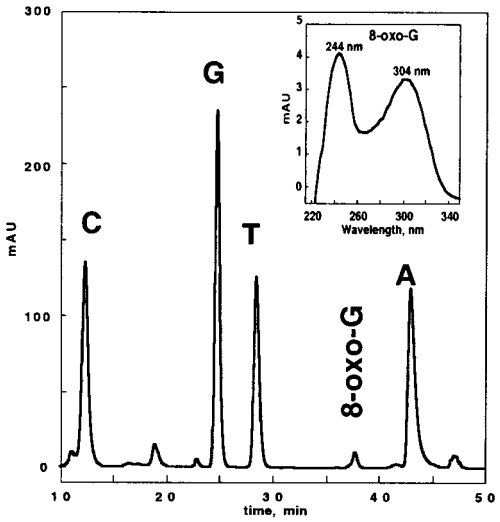


Figure 3. HPLC chromatograms ($\lambda = 260$ nm) of enzyme-digested 3 day old reaction mixture of $Pt^{IV}(dach)Cl_4$/oligonucleotide/cisplatin, (20 μM/10 μM/0.1 μM). Linear gradient from 100% 12.5 mM citric acid/25 mM ammonium acetate to 96% 12.5 mM citric acid/25 mM ammonium acetate and 4% methanol. Peaks were identified by comigration with authentic nucleoside standards. Control experiments on the reaction mixture of oligomer/cisplatin (10 μM/0.1 μM) showed no peak due to 8-oxo-G.

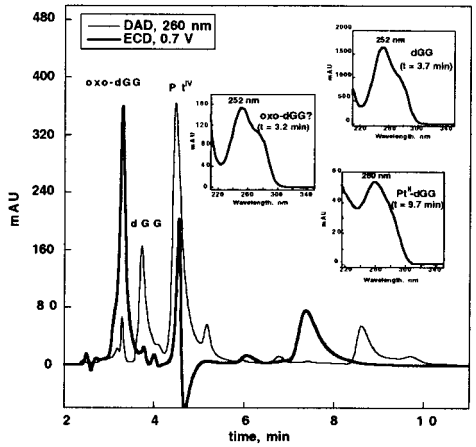
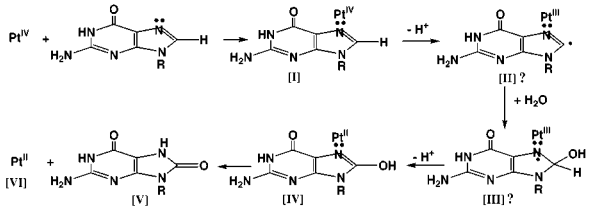


Figure 4. DAD and ECD HPLC chromatograms of $Pt^{IV}(dach)Cl_4$/d(GG/cisplatin, (4 mM/4 mM/0.4 mM) after 6 days. The control experiment for the d(GG)/cisplatin (4 mM/0.4 mM) reaction showed no other peak except a peak due to the unreacted d(GG). Experimental condition were the same as those given in Figure 1.

days of reaction for $Pt^{IV}(dach)Cl_4$/oligomer/cisplatin. It shows a peak at 38.0 min whose absorption spectrum matches with that of 8-oxo-G.

Figure 4 shows the HPLC results of the 6 day reaction mixture of $Pt^{IV}(dach)Cl_4$/d(GG)/cisplatin. It displays several peaks resulting from the redox reaction between $Pt^{IV}(dach)Cl_4$ and d(GG). The one product at 9.7 min has an absorption maximum of 260 nm, which is indicative of Pt^{II}-bound d(GG). The identical retention time and absorption spectrum were seen for the reaction mixture of $Pt^{II}(dach)Cl_2$/d(GG). There are two products detected by ECD. The peak at 3.2 min shows the same absorption spectrum as that of d(GG) at 3.7 min. However, its absorptivity is much lower than that of d(GG). One of the guanines may be oxidized by Pt^{IV}

Scheme 1. Proposed Mechanism



and the other is left intact, giving the same absorption spectrum as d(GG) with low absorptivity. The peak at 7.7 min is assigned to Pt^{II} bound to oxo-d(GG).

8-Oxoguanine is reported to be formed by at least four different pathways.[1] Among these, the one-electron abstraction pathway seems to fit the Pt^{IV}/G system (Scheme 1). The intermediate [I] was detected by ^{1}H NMR.[6] The second intermediate [II], G$^\bullet$, is possible on the basis of the similar kinetics of the reaction of Pt^{IV} with ascorbic acid[8] and with GMP to form the Pt^{II} species.[6] Both reactions have a long induction period, which is catalyzed by Pt^{II}, followed by fast reduction. Both ascorbic acid and GMP produced $Pt^{II}(dach)Cl_2$ when they were reacted with $Pt^{IV}(dach)Cl_4$. A long-lived ascorbate radical was detected by EPR spectroscopy in a Pt^{IV}/ascorbic acid reaction mixture.[8] Although ascorbic acid functions as a two-electron reductant, a sequential internal one-electron-transfer process including Pt^{III} intermediates is suggested to be responsible for the ascorbate radical.[8] By analogy, guanine may function as a two-electron reductant enabled by a sequential internal one-electron-transfer process including a Pt^{III}/G$^\bullet$ intermediate, [II]. The existence of the intermediate [IV] was shown by ^{1}H NMR and HPLC.[6] The final Pt^{II} product [VI] depends on the stoichiometric ratio of the initial concentration of $Pt^{IV}(dach)Cl_4$ and GMP. At equal concentrations, $Pt^{II}(dach)Cl_2$ is predominant, and with excess GMP, $Pt^{II}(dach)(GMP)_2$ is predominant.[6] The overall reaction generates two protons per one Pt^{IV}/G. Indeed, the pH of all of our reaction mixtures in nonbuffered solutions drops to a pH of ~4 from an initial pH of ~8. A very similar mechanism was reported for Ru^{III}-nucleoside reaction.[9] Ru^{III} binds to N_7G, goes through $1e^-$, $1H^+$ to generate a radical at C8 that can be attacked by water to yield the 8-hydroxylated free radical, which, in turn, undergoes a second $1e^-$, $1H^+$ oxidation to give the 8-keto nucleoside.

In conclusion, our study showed that $Pt^{IV}(dach)Cl_4$ oxidizes guanine mainly to 8-oxo-G in DNA and RNA. It will be interesting to see if $Pt^{IV}(dach)Cl_4$ can produce other oxidation products, such as imidazolone/oxazolone.[3,10] Further work to explore details of this reaction is underway.

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Supporting Information Available: Calibration curves and reaction profiles of $Pt^{IV}(dach)Cl_4$/dGMP and $Pt^{IV}(dach)Cl_4$/dGMP/cisplatin (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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