Nucleoside Oxidation and Hydrolysis Induced by a Coordinated Metal Ion: Xanthine Oxidase Activity in a Simple Metallonucleoside Complex

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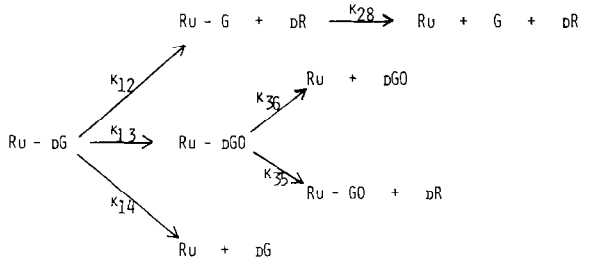
Both alkylating agents and transition metal antitumor drugs are thought to act by forming covalent links to the N_7 of guanine residues in cellular DNA and crosslinking to other G sites**. It has long been known that protonation or alkylation at the N_7 of deoxyguanosine catalyzes cleavage of the sugar–purine bond and depurination of DNA [1, 2]. We now report on the quantitation of the analogous reaction catalyzed by a metal ion coordinated to dG at N_7 as well as a competitive reaction which mimics the activity of xanthine oxidase in that the metal ion catalyzes the autooxidation of the nucleoside to yield 8-hydroxydeoxyguanosine (dGO). Both of these reactions are also in competition with the base-catalyzed dissociation of the metal–purine complexes as summarized in Scheme 1.



Scheme. 1. Reactions of 7-[(dG)$(NH_3)_5$Ru(III)] at pH 6.0 to 8.0.

The various reaction products were monitored by HPLC [3] from solutions containing 7-[(dG)$(NH_3)_5$-Ru(III)] at 56 °C buffered at pH's 6–8 at 0.5 pH intervals with $[O_2]$ held at approximately 1.8×10^{-4} *M* by air saturation [4]. The product concentrations were fitted to the appropriate integrated kinetic rate equations as a function of time using standard regression techniques to extract the observed rate constants†. Product analysis was effected by running large scale reactions in the pH ranges which favored particular products and isolating these by ion-exchange chromatography. Ru–G was identified by its UV-vis spectra, HPLC, ion-exchange and pK_a behavior [5], while free dG and G were identified by HPLC. Elemental analysis of Ru–dGO isolated as the chloride salt was consistent with oxidation and ammine retention rather than deammination, which had been originally speculated [3]. PMR of Ru–dGO yielded a spectrum similar to that of Ru–dG except that there was no peak corresponding to C_8–H and additional deoxyriboside peaks occurred, which are attributed to the presence of both *syn* and *anti* conformers with interconversion probably being hindered by the O_8. Large downfield shifts are observed for the 1′-proton (14.1 and 12.8 ppm), which are in the range expected for an alkyl proton adjacent to an aromatic ring coordinated to Ru(III). Since it has only exchangeable protons, Ru–GO yielded no PMR spectrum in D_2O solution. Comparison of GO, which was prepared by acid hydrolysis of Ru–dGO or dGO, against an authentic commercial sample revealed their IR and UV-vis spectra, pK_as and HPLC to be identical.

The observed rate constant for the hydrolysis of the sugar-purine bond (k_{12}) was 5.4×10^{-6} sec^{-1} over the pH range 6–7.0, but this decreased in the range of the pK_a (7.6) for proton loss from N_1. Comparison with the analogous rate constants (corrected to 56°) for sugar hydrolysis from $[H–dG]^+$ and $[Me–dG]^+$ indicates that $(NH_3)_5$Ru(III) is a factor of 1850 and 185 less efficient at catalyzing this reaction than the proton or methyl group, respectively [6]. This decrease is probably due to delocalization of the cationic charge over the ammine protons resulting in a relatively low charge to radius ratio for the metal ion. For this reason metal ions tend to stabilize the nucleoside in acid media by

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**Abbreviations: G, guanine; dG, deoxyguanosine; dGO, 2-amino-9-(β-D-2′-deoxyribofuranosyl)-purine-6,8(1H,7H)-dione; GO, 2-amino-purine-6,8(1H,7H,9H)-dione; Ru preceding a ligand indicates N_7-coordination of $(NH_3)_5$Ru-(III), Me indicates N_7 methylation.

†Rate constants were fit using the SAS NLIN procedure from the SAS Institute, Cary, NC, using the following integrated rate equations, where $[Ru–dG]_o$ is the initial concentration of the starting material:

$$[Ru–dG] = [Ru–dG]_o e^{-(k_{12}+k_{13}+k_{14})t}$$

$$[Ru–G] = [Ru–dG]_o[k_{12}/(k_{28} - k_{12} - k_{13} - k_{14})] [e^{-(k_{12}+k_{13}+k_{14})t} - e^{-k_{28}t}]$$

$$[dG] = [Ru–dG]_o[k_{14}/(k_{12} + k_{13} + k_{14})] [1 - e^{-(k_{12}+k_{13}+k_{14})t}]$$

$$[dGO] = [Ru–dG]_o k_{13} k_{36} [1/(k_{12} + k_{13} + k_{14})(k_{35} + k_{36}) - (1/(k_{35} + k_{36} - k_{12} - k_{13} - k_{14})(1/(k_{12} + k_{13} + k_{14})) e^{-(k_{12}+k_{13}+k_{14})t} - (1/(k_{35} + k_{36})e^{-(k_{35}+k_{36})t}]$$

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preventing proton coordination to the N_7 position [5, 7]. In contrast, the hydrolysis of Ru–dG at 56° and pH 7.4 is approximately 10^3 to 10^4 times more rapid than that of free dG [5] or dG residues in DNA [1, 2]. The overall rate law for the hydrolysis of Ru–dG can now be taken to be††:

$$-d[\text{Ru–dG}] = k_{12}[\text{Ru–dG}] =$$

$$= (k_1/K_1[H^+] + k_2 + k_3K_2/[H^+])[\text{Ru–dG}]$$

where K_1 and K_2 are the ionization constants for deprotonation from N_3 and N_1, respectively, k_1/K_1 can be estimated [5] to be 4.0×10^{-3} M^{-1} sec^{-1}, $k_2 = 5.4 \times 10^{-6}$ sec^{-1}, and k_3K_2[Ru–dG] is smaller than the observed rate constants for the competing dissociation and oxidation reactions.

The observed rate constants for oxidation of the coordinated nucleoside (k_{13}) increased with pH (2.1×10^{-5} sec^{-1} at pH 6 to 1.1×10^{-4} sec^{-1} at pH 7), indicating this reaction to be base catalyzed. This suggests that oxidation of the nucleoside is preceded by deprotonation of the C_8 followed by hydroxide or water attack at this site. The proximity of the metal ion to the C_8–H should enhance the acidity of this proton by over 10 orders of magnitude [8]. The reaction ceases in the anaerobic solutions, so that O_2 appears to be the oxidant. Since [Me–dG]$^+$ undergoes imidazole ring opening, rather than oxidation in basic media [6], it is likely that the presence of the transition metal ion is responsible for the catalysis of autooxidation, possibly by facilitating electron transfer to O_2 to yield superoxide [9]. Aside from the details of O_2 reduction, this mechanisms is similar to those that have been proposed for the action of xanthine oxidase [10], but the simplicity of the present model indicates that this activity only requires the presence of a fairly strongly polarizing metal ion at the N_7 position, OH^- and O_2. Studies are now underway to determine the precise oxygen dependence of this reaction and the oxygen reduction product.

††This rate expression holds over the experimental pH range where $[H^+] \gg [H^+]^2$, K_1K_2. The value of pK_1 can be estimated to be –0.85 (see ref. 5) and that of pK_2 has been measured as 7.6 ± 0.2.

Since monomethylation of dG sites on chromatin material is usually not mutagenic, possibly due to an efficient cellular mechanism for the repair of depurinated sites [1], and the rate of metal–ion induced depurination is both slow and comparable to the rate of metal dissociation ($k_{14} = 5.3 \times 10^{-6}$ sec^{-1} at pH 7), this may not be an important mutagenic mechanism. However, ammineruthenium(III) complexes also induce the SOS repair mechanism [11] and mutations resulting from this mode of repair may be important in the mutagenic behavior of transition metal ions. Autooxidation of coordinated dG represents a heretofore unsuspected mode of mutagenic or chemotherapeutic action, which could severely alter the base pairing properties of these residues.

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