Ruthenium Mediated Guanine Oxidation in Reverse Micelles

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One electron guanine (G) oxidation, a reaction linked to mutagenic events, is usually investigated in dilute solution. To model the compact environment of DNA in a cell, G oxidation has been investigated in reverse micelles (RMs). A photochemical method to generate Ru^{3+} using bis(2,2'-bipyridine)dipyridophenazine ruthenium(II) chloride, $[Ru(bpy)_2dppz]Cl_2$, was used to oxidizes G's in DNA. The environment of $[Ru(bpy)_2dppz]^{2+}$ in RMs was characterized by emission spectroscopy in anionic and cationic RMs. In buffer solution, $[Ru(bpy)_2dppz]^{2+}$ exhibits an emission spectrum when intercalated into DNA; no emission spectrum is observed without DNA. In anionic RMs, $[Ru(bpy)_2dppz]^{2+}$ gave identical emission spectra in the absence or presence of DNA, showing that $[Ru(bpy)_2dppz]^{2+}$ is not bound to DNA in this system, but is instead bound to the anionic RM headgroups. In cationic RMs, $[Ru(bpy)_2dppz]^{2+}$ behaves as in buffer solution, but with a 70% decrease in emission intensity. Cationic RMs were used in subsequent experiments to ensure binding of the $[Ru(bpy)_2dppz]^{2+}$ to DNA. In RMs, only $[Fe(CN)_6]^{3-}$ efficiently quenches the $[Ru(bpy)_2dppz]^{2+}$ excited state to create the 3+ oxidation state. The Stern-Volmer plot of $[Ru(bpy)_2dppz]^{2+}$ and $[Fe(CN)_6]^{3-}$ in RMs is curved, supporting a Poissonian distribution of reactants in the RMs (Atik, S.S, Thomas J.K, *J. Am. Chem. Soc*., **1981,** *103*, 7403). The optimal quencher concentration to generate the maximum Ru^{3+} yield was determined from the Stern-Volmer plot. Circular dichroism spectra of DNA are the same in buffer and in cationic RMs. High-resolution denaturing polyacrylamide gel electrophoresis (PAGE) is used to detect G oxidation products in buffer solution and RMs using identical concentrations of reactants. In RMs, two to three-fold longer illumination times were required to detect G oxidation products; however, similar levels of oxidation products in RMs were observed with or without $[Fe(CN)_6]^{3-}$ present. This result indicates that the Ru^{3+} form of $[Ru(bpy)_2dppz]^{2+}$ does not oxidize G in RMs, but that singlet oxygen is most likely the G oxidant. By encapsulating DNA in cationic RMs, G oxidation by $[Ru(bpy)_2dppz]^{2+}$ follows a different mechanism than in buffer solution. Together, these results show that the levels and mechanism of G oxidation depend intimately on the conditions under which these reactions are probed.