Voltammetric Studies of Guanine and Its Derivatives by Ru(bpy)$^{2+/3+}$ Mediator on Indium Tin Oxide Electrode

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Oxidizing metal complex mediates the electrochemical oxidation of guanine nucleotides. This catalysis results in an enhancement in cyclic voltammograms that yield the rate constant for the oxidation of guanine by the metal complex *via* digital simulation. The rate constant of oxidation of guanine by $Ru(bpy)_3^{3+}$ is 6.4×10^5 $M^{-1}s^{-1}$. The rate constant and the enhanced current depend on the number of phosphate groups on the sugar of nucleotide. Also the modified guanine bases show different oxidation rate constants following the trend guanine-5'-monophosphate (GMP) > 8-bromo-guanine-5'-monophosphate (8-Br-GMP) > xanthosine -5'- monophosphate (XMP) > inosine-5'-monophosphate (IMP). The guanine bases derivatized differently are all distinguishable from one another, providing a basis for studying electrochemistry of DNA and RNA and developing electrochemical biosensors.

Introduction

The detection of specific base sequences in human and bacterial nucleic acids is becoming increasingly important in the diagnosis of diseases. Interactions of DNA with chemicals and radiation can result in various types of DNA damage. The oxidation and alkylation of nucleic acids have been an interesting research field in toxicology in relation to cancer and aging.[1] Guanine shows higher reactivities than the other four bases in the reactions with alkylating agents, hydroxyl radicals, transition-metal complexes, and other oxidants.[2] The high reactivity of guanine over the other nucleotides results from the one-electron potentials which is 1.34 V (vs NHE) for guanosine, 1.79 V for adenosine, and higher for the pyrimidines.[3]

Bases of the nucleic acid components are known to undergo redox processes at mercury and carbon electrodes. Ribose and deoxyribose bound to nucleosides and nucleotides as well as phosphate groups are electroinactive, while guanine and adenine can be oxidized at carbon electrodes.[4] Reduction of guanine, cytosine, and adenine occurs at highly negative potentials that can be attained only with mercury electrodes. Nucleic acid bases and nucleosides are strongly adsorbed at the mercury electrode, and adenine and cytosine produce reduction signals.[5] In addition to the usual nucleic acid components a large number of purine and pyrimidine derivatives form sparingly soluble compounds with the mercury electrode, and can be determined by cathodic stripping voltammetry at nanomolar concentrations.[6,7]

Recently, developed was the electrochemical system, in which DNA can be detected electrochemically at bare and modified indium tin oxide (ITO) electrodes.[8-11] In this study, I wish to focus on the electrochemical behavior of various mononucleotides with an electron mediator on the ITO electrode. In this approach, the rate constant for one-electron oxidation of modified guanine bases by the transition-metal mediator is measured using catalytic cyclic voltammetry.

Experimental Section

Reagents. All reagents and solvents were purchased from commercial sources and used as received. Metal mediator of Ru(II) was recrystallized in ethanol before use. ITO electrodes were obtained from Delta Technologies (Stillwater, MN). Water was obtained from a Milli-Q purification systems.

Electrochemical Analysis. Cyclic voltammograms were obtained using a PAR 263A and 273A potentiostat/galvanostat with a single compartment voltammetric cell. The cell was equipped with an indium tin oxide working electrode (area 0.32 cm^2), a Pt-wire counter electrode, and a Ag/AgCl reference electrode. In a typical experiment, a sample containing 50 μM metal complex and 0.3 mM nucleotide phosphate dissolved in buffered aqueous solutions containing 700 mM NaCl and 50 mM Na-phosphate buffer (pH = 6.8, [Na] = 780 mM) was scanned at 25 mV/s from 0.0 V to about 200 mV beyond the redox couple of the metal complex. Scans of mononucleotides in the absence of metal complex showed no appreciable oxidative current to 1.3 V vs Ag/AgCl. A freshly-cleaned ITO electrode was used for each experiment, and a background scan of buffer alone was collected for each electrode and subtracted from subsequent scan. Second-order rate constants about base oxidations were determined by fitting of cyclic voltammetric data to a two-step mechanism using the DigiSim® (BAS) software package.

Results and Discussion

Electrochemical activation of nucleic acid oxidation has recently been studied to obtain kinetic information from the experimental current response.[8-11] The simplest mechanism for electrocatalytic DNA oxidation by an electron-transfer mediator (M) under a high salt condition is shown in Eq. (1) and Eq. (2). The mechanism consists of an electrochemical

oxidation step, and a homogeneous chemical oxidation step which regenerates the initial reduced species. A mechanism is suitable to take the second-order oxidation reaction into account.

$$M^{2+} \rightarrow M^{3+} \quad (1)$$

$$M^{3+} + DNA \rightarrow M^{2+} + DNA(ox) \quad (2)$$

As the electrocatalytic oxidation of DNA is mainly due to the presence of guanine bases in the sequence, mononucleotides can also be detected in solution *via* the catalytic oxidation of bases using $Ru(bpy)_3^{2+}$ as the mediator. The mediator exhibits a reversible redox couple at 1.05 V, which is very similar to the oxidation potential of guanine.[12] Addition of mononucleotide to a solution of $Ru(bpy)_3^{2+}$ leads to a catalytic enhancement in the oxidation current according to a two-step mechanism where G_{ox} is a molecule where guanine has been oxidized by one electron.

$$Ru(bpy)_3^{2+} \rightarrow Ru(bpy)_3^{3+} \quad (3)$$

$$Ru(bpy)_3^{3+} + G \rightarrow Ru(bpy)_3^{2+} + G_{ox} \quad (4)$$

The quasi-reversible cyclic voltammogrm of $Ru(bpy)_3^{2+}$ is shown in Figure 1A. Addition of GMP produces catalytic enhancement in the oxidation current (Figure 1C). No current above background was observed for the ITO electrodes in the absence of $Ru(bpy)_3^{2+}$ (Figure 1B). However, addition of adenosine-5'-monophsphate, cytidine-5'-monophosphate or thymidine-5'-monophosphate generates no current enhancement. These results suggest the large current enhancement would be produced only in the presence of GMP. This conclusion is consistent with the reported results that guanine base has the lowest redox potential (1.06 V *vs* Ag/AgCl).[12,13]

Even though phosphate was not oxidized by the mediator, the number of phosphate groups at the 5'-position of the sugar may affect cyclic voltammograms by the greater negative charge provided with the phosphate functional groups. In fact, addition of guanosine-5'-triphosphate (GTP) generates the cyclic voltammogram shaped differently in the catalytic current (Figure 2C). Since phosphate groups are not expected to reduce the oxidation potential of the base moiety, this effect should be due to electrostatic attraction between the positively charged mediator and nucleotide. Such observations were also reported in the rate constants for the reduction reaction of purines with the primary radical species from the radiolysis,[14] and in the electrochemical studies about the interactions of cationic and anionic metal mediators with oligonucleotides.[15,16]

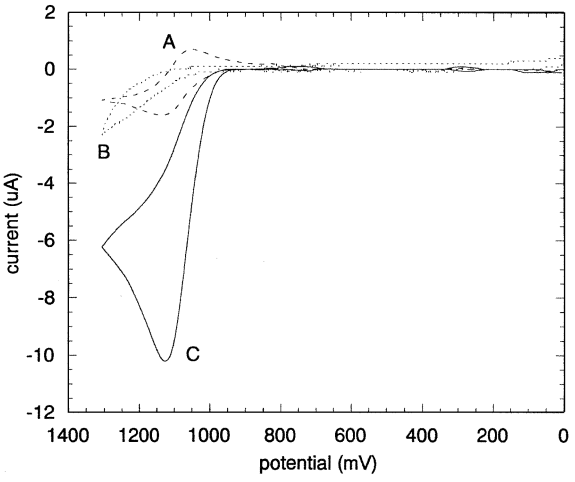

Figure 1. Cyclic voltammograms of 25 μM $Ru(bpy)_3^{2+}$ (A, dashed) and (B, dotted) after exposure of the electrode to GMP (0.3 mM). Curve C (solid) shows the voltammogram obtained at the electrode from (B) in the presence of $Ru(bpy)_3^{2+}$. Scan rate: 25 mV/s. Reference electrode: Ag/AgCl. Working electrode: unmodified ITO.

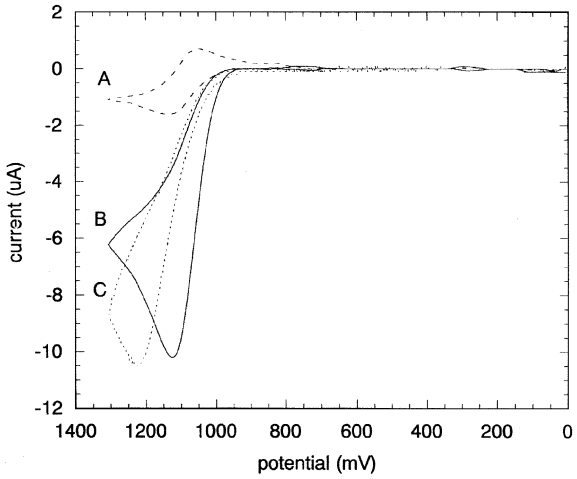

Figure 2. Cyclic voltammograms of (A) $Ru(bpy)_3^{2+}$ (25 μM) with (B) GMP (0.3 mM) and (C) GTP (0.3 mM). Scan rate: 25 mV/s. Reference electrode: Ag/AgCl. Working electrode: unmodified ITO.

Cyclic voltammograms were analyzed by fitting the background-subtracted current-potential curves using the fitting software. The input parameters were the $E_{1/2}$ for the metal complex (1.09 V) and the diffusion coefficients for the metal complex (5.85×10^{-6} cm^2/s) and the mononucleotides (1.19×10^{-6} cm^2/s). The simulated cyclic voltammogram of GMP using DigiSim is shown in Figure 3. The simulation gives

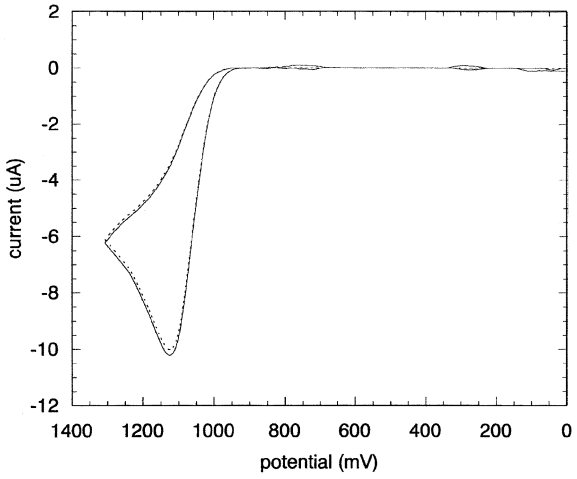

Figure 3. Voltammogram $Ru(bpy)_3^{2+}$ with GMP (solid line) and simulated cyclic voltammogram using DigiSim (dotted line).

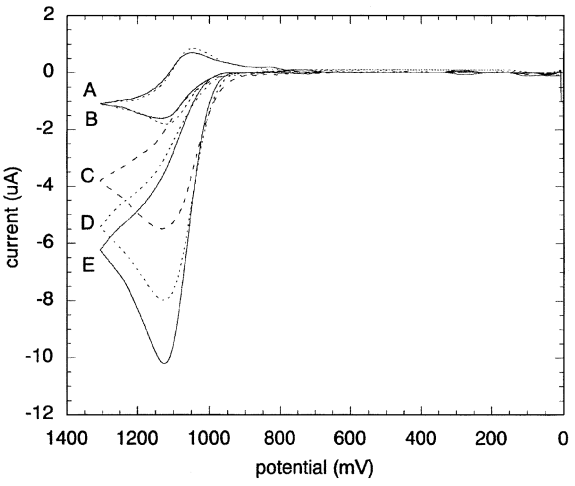

Figure 4. Cyclic voltammograms of (A) Ru(bpy)$_3^{2+}$ (25 μM) with (B) IMP, (C) XMP, (D) 8-Br-GMP, and (E) GMP (0.3 mM). Scan rate: 25 mV/s. Reference electrode: Ag/AgCl. Working electrode: unmodified ITO.

the second-order rate constant obtained from the fit for electron transfer to Ru(bpy)$_3^{3+}$ of $k_{GMP} = 6.4 \times 10^5$ M^{-1}s^{-1}. However, the curve generated with GTP was not well fitted using the analysis program.

Shown in Figure 4 are the cyclic voltammograms of Ru(bpy)$_3^{2+}$ in the presence of the mononucleotides, such as IMP, XMP, and 8-Br-GMP, in which the guanine bases have been modified. The structures of the mononucleotides are shown in Figure 5. Such a large current enhancement was not observed for IMP, which implies the exocyclic amine must play an important electronic role in the guanine oxidation. The current enhancements show the trend GMP > 8-Br-GMP > XMP > IMP. The presence of an extra carbonyl functional group to the pyrimidine ring (in XMP) makes the purine ring more electron deficient relative to the addition of Br to the ring (in 8-Br-GMP). Digital simulation of the scan rate dependences of the voltammograms shown in Figure 4 gives the second-order rate constants for electron transfer to Ru(bpy)$_3^{3+}$ of $k_{8\text{-Br-GMP}} = 3.5 \times 10^5$ M^{-1}s^{-1}, $k_{XMP} = 1.2 \times 10^5$ M^{-1}s^{-1}, and $k_{IMP} = 97$ M^{-1}s^{-1}.

The modified bases show the different electronic roles in the base oxidation and are all distinguishable from one another, providing a basis for studying electrochemistry of DNA and developing electrochemical biosensors. Such hypoxanthine (the base of IMP) and 8-bromo-guanine are still able to make the hydrogen bonds with cytosine, so guanine can be replaced with these bases when needed to detect RNA or DNA electrochemically. Detailed studies of the electrostatic interactions between mononucleotides (or DNA) and inorganic mediators and the ITO electrode are currently underway.

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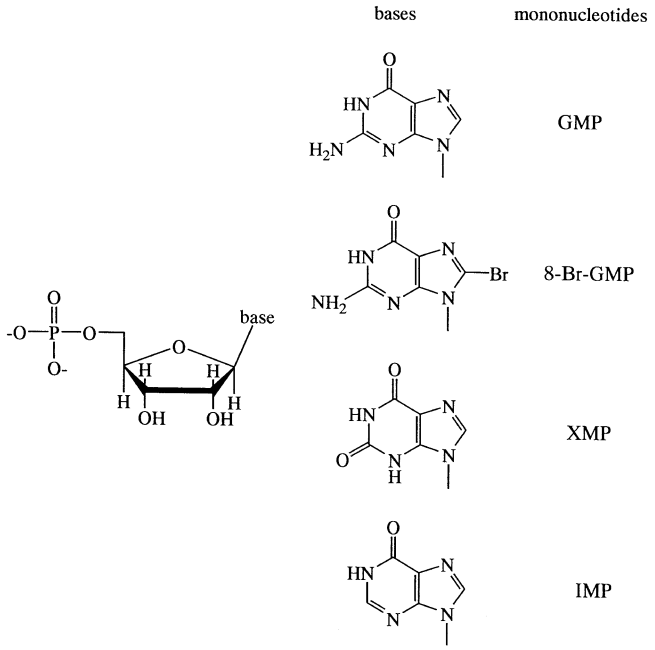

Figure 5. Structures of mononucleotides.

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