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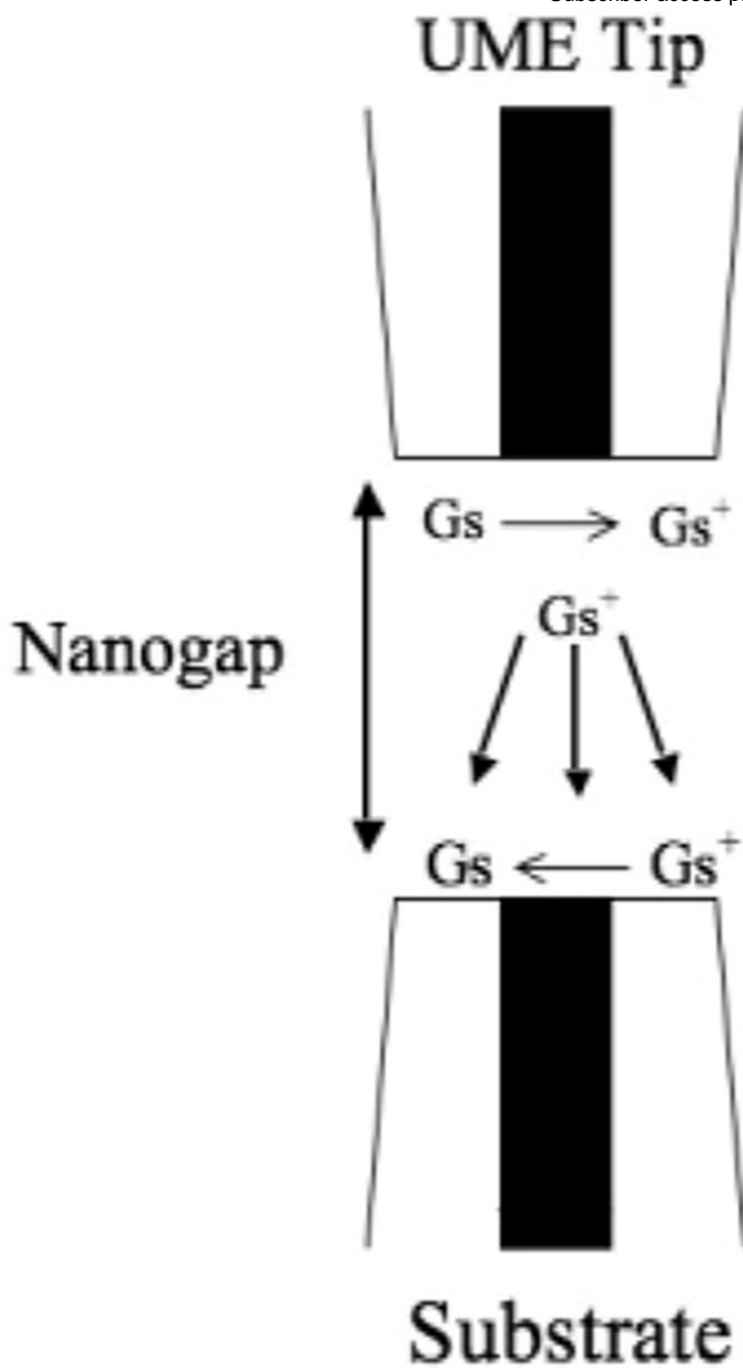
**Electrochemical Studies of Guanosine in DMF and Detection of Its Radical Cation in a Scanning Electrochemical Microscopy Nanogap Experiment**

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## Electrochemical Studies of Guanosine in DMF and Detection of Its Radical Cation in a Scanning Electrochemical Microscopy Nanogap Experiment

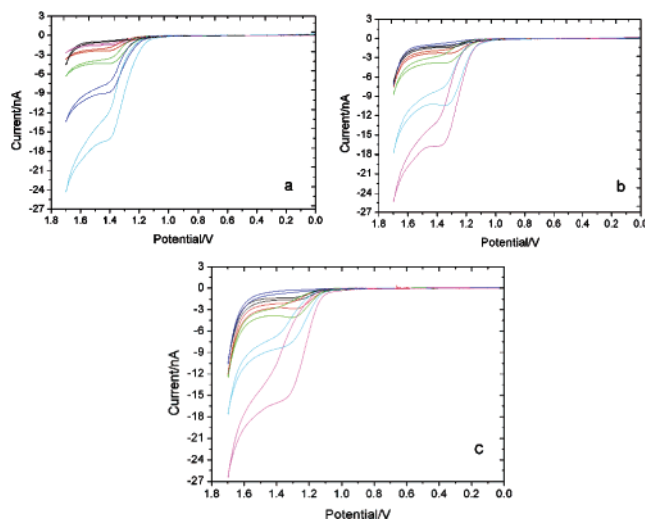
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We report the electrochemical oxidation of guanosine (Gs) and the application of scanning electrochemical microscopy (SECM) in a nanogap configuration to detect and measure the lifetime of the Gs radical cation, Gs<sup>•+</sup>. Gs is an important nucleoside<sup>1,2</sup> because oxidation of the base guanine to the radical cation has been implicated in the transfer of charge and in radiative damage of DNA.<sup>3</sup> The potential for Gs oxidation has been estimated by techniques, such as ionizing radiation, chemical oxidation, two-photon photoionization by a high-intensity laser pulse, and photoirradiation in the presence of photosensitizers.<sup>4–7</sup> However, direct electrochemical measurement of the potential has been thwarted by the instability of Gs<sup>•+</sup> and distortions of the voltammetric waves in water because of adsorption of Gs or its products.<sup>8,9</sup> We show here that the oxidation of Gs in *N,N*-dimethylformamide (DMF) yields a nearly steady-state cyclic voltammogram (CV) of Gs at a carbon-fiber ultramicroelectrode (CF-UME), which is a requirement for kinetic studies by SECM. SECM, a powerful tool for studying fast heterogeneous and homogeneous electron-transfer reactions,<sup>10,11</sup> with CF-UMEs was used in the feedback mode and in the generation/collection (G/C) mode in a nanogap configuration to detect the Gs<sup>•+</sup> directly.

The EC oxidation of Gs at a CF-UME in various aqueous solutions at different pH values did not yield normal “S-shaped” steady-state CVs (see Supporting Information Figure S1). These results, similar to those observed at carbon macroelectrodes,<sup>12–14</sup> suggest that Gs is adsorbed strongly at the CF-UME in aqueous solutions. Because of the adsorption problems encountered in aqueous solutions, DMF was used to study the EC oxidation of Gs. As a comparison with aqueous solution, we also added a small amount of an aqueous solution of 1.2 M H<sub>3</sub>PO<sub>4</sub> to adjust the solution pH from that of dry DMF (11.85) to pH 7.10 (0.1% 1.2 M H<sub>3</sub>PO<sub>4</sub>) and pH 4.23 (3.5% 1.2 M H<sub>3</sub>PO<sub>4</sub>). The pH value was measured with an Orion Research model 70/A-digital ionalyzer (Cambridge, MA). Commercially available highest grade dry DMF contains ~0.002% of water. Thus, the DMF solutions of the three pH values (pH 11.85, 7.10, and 4.23) contained about 1.1 mM, 56 mM, and 1.94 M water, respectively. As shown in Figure 1, the EC oxidation of Gs at a CF-UME in DMF/0.1 M TBAPF<sub>6</sub> yielded a well-defined “S-shaped” steady-state CV with a half-wave potential, *E*<sub>1/2</sub>, of 1.3 V versus a Ag quasireference electrode (AgQRE). The potential of the AgQRE was –0.43 V versus the *E*<sup>o</sup> of ferrocene/ferrocenium (Fc/Fc<sup>+</sup>) couple in DMF. At a given sweep rate, *v*, the steady-state current, *i*<sub>ss</sub>, depended linearly on the concentration of Gs (Figure S2). Also, at Gs concentrations ≤ 10 mM, the current response of Gs increased with increasing *v*<sup>1/2</sup> at high *v* values, indicating a diffusion-controlled process (Figures S3 and S4). However, adsorption of Gs on the CF-UME in DMF occurred when the Gs concentration was > 10 mM, especially when the water content was high.



**Figure 1.** Plot of *i*<sub>ss</sub> vs *C*<sub>Gs</sub><sup>\*</sup> at three different water contents in DMF with different pH values at the CF-UME (8- $\mu$ m diameter) in DMF/0.1 M TBAPF<sub>6</sub> solutions. (a) Dry DMF (pH 11.85). From bottom to top: 10, 5, 2, 1, 0.5, 0 mM. (b) 0.1% 1.2 M H<sub>3</sub>PO<sub>4</sub> (pH 7.10). From bottom to top: 10, 5, 2, 1, 0.5, 0 mM. (c) 3.5% 1.2 M H<sub>3</sub>PO<sub>4</sub> (pH 4.23). From bottom to top: 10, 5, 2, 1, 0.5, 0 mM. Scan rate, 20 mV/s.

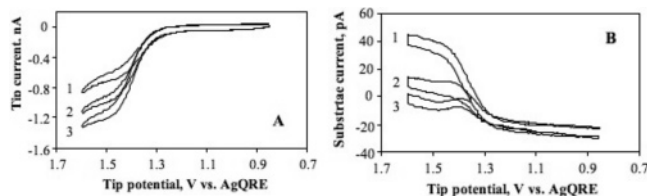
Chronoamperometry was used to determine the number, *n*, of electrons involved in the first oxidation wave and the diffusion coefficient, *D*<sub>Gs</sub>, of Gs in DMF with a Pt-UME of radius, *a*, of 12.5  $\mu$ m based on comparing the transient and steady-state currents.<sup>15</sup> A normalized plot of *i*(*t*) with respect to *i*<sub>ss</sub> plotted versus *t*<sup>-1/2</sup> (Figure S5) yielded a straight line with a slope of 0.210, an intercept of 0.834, and a *D*<sub>Gs</sub> of 4.5 × 10<sup>-6</sup> cm<sup>2</sup> s<sup>-1</sup>. This value is close to that reported previously by Faraggi et al.<sup>16</sup> in aqueous solution. From the steady-state current *i*<sub>ss</sub> for a disk UME,

$$i_{ss} = 4nFD_{Gs}aC_{Gs}^*$$

(where *C*<sub>Gs</sub><sup>\*</sup> is the Gs concentration and *F* is the Faraday constant), an *n* value of 2 can be obtained from the steady-state voltammetric response shown in Figure 1. This indicates that the overall first EC oxidation wave of Gs in DMF is a two-electron process, as reported for Gs oxidation in aqueous solution.<sup>13</sup>

SECM was used to study the oxidation of Gs in DMF. In particular, we wanted to detect the radical cation Gs<sup>•+</sup> generated in the primary electrooxidation step of Gs by SECM. On the basis of the present and previous studies,<sup>8,14</sup> the overall electrooxidation of Gs in DMF is probably an ECE process, where the radical cation, Gs<sup>•+</sup>, generated first, is unstable and quickly undergoes rapid deprotonation followed by fast loss of a second electron and subsequent hydration. This results in the formation of 8-oxo-Gs. To detect the unstable electrogenerated intermediate Gs<sup>•+</sup>, Gs was oxidized at one electrode (tip) and detected at a second one (substrate) held in close proximity at a distance, *d*. Because of the

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**Figure 2.** CVs for the oxidation of 2 mM guanosine in DMF/0.1M TBAPF<sub>6</sub> obtained at the tip (A) and substrate (B) electrodes.  $E_S = 1.1$  V vs AgQRE. The scan rate of the tip potential was 20 mV/s. The distance between the tip and the substrate was (1) 0.15, (2) 0.3, and (3) 1  $\mu\text{m}$ .

rapid deprotonation of  $\text{Gs}^{*+}$ ,  $d$  must be small, so that  $\text{Gs}^{*+}$  can diffuse across the gap before reacting. Moreover, to decrease the background current in the substrate (since small, pA-level, currents must be detected), the substrate must also be small. This situation is different from most SECM experiments of this type and basically requires forming a nanogap between two  $\mu\text{m}$ -diameter electrodes, as shown in Figure S6. A CF-UME tip (10- $\mu\text{m}$  diameter) was held in close proximity to a second tip of the same size immersed in the solution of interest. The tip potential was scanned in the region of Gs oxidation ( $E_T = 0.85$  to 1.6 V versus an AgQRE). The substrate electrode was held at a potential ( $E_S = 1.1$  V) where the tip-generated intermediate  $\text{Gs}^{*+}$  upon reaching the substrate surface is reduced back to Gs.

To form the nanogap by locating the small substrate electrode, SECM measurements were first performed in the substrate generation/tip collection (SG/TC) mode with Fc, which generates on oxidation a stable product,  $\text{Fc}^+$ , as the redox mediator (2 mM ferrocene in DMF/0.1M TBAPF<sub>6</sub>). The substrate potential was controlled such that Fc was oxidized to  $\text{Fc}^+$  at a diffusion-controlled rate. A potential negative enough to reduce  $\text{Fc}^+$  was applied to the tip, which was scanned in the vicinity of substrate surface (initially positioned about 5  $\mu\text{m}$  away) to find the center of the 10- $\mu\text{m}$ -diameter carbon substrate electrode, where the collection current is maximized. After the substrate electrode was found and the nanogap formed, the SECM Teflon cell was rinsed with DMF and 2 mM Gs in DMF/0.1M TBAPF<sub>6</sub> was added into the cell. SECM tip generation/substrate collection (TG/SC) experiments were then carried out. Figure 2 shows the CVs for the oxidation of 2 mM Gs obtained in this mode. When the distance between the tip and the substrate ( $d$ ) was larger than 2  $\mu\text{m}$ , no substrate collection current was observed, after correction for the nearly constant background of  $\sim -20$  pA probably contributed from a small offset of the SECM instrument and a slight amount of oxidation of Gs at the potential of 1.1 V. When the tip was closer to the substrate ( $d \leq 1$   $\mu\text{m}$ ), a substrate collection current was observed as shown in Figure 2, where the collection efficiency increased as the tip approached the substrate. We estimate the reaction layer thickness,  $\mu$ , of the intermediate  $\text{Gs}^{*+}$  as  $\leq 0.2$   $\mu\text{m}$ .<sup>17</sup> The lifetime,  $\tau$ , of  $\text{Gs}^{*+}$  can be estimated by the equation of  $\tau \approx \mu^2/(2D_{\text{Gs}})$ .<sup>10</sup> Thus, the  $\text{Gs}^{*+}$  observed in this experiment has a  $\tau \leq 40$   $\mu\text{s}$  or equivalently it undergoes a first-order reaction with a rate constant,  $k \geq 2.5 \times 10^4 \text{s}^{-1}$  in the solution studied. The reversible standard potential for the half reaction  $\text{Gs}^{*+} + e = \text{Gs}$  can be obtained from the SECM

data at  $d = 150$  nm, where the observed half-wave potential (0.89 V versus  $\text{Fc}^+/\text{Fc}$ ) can be corrected for the effect of following reaction [i.e., a shift of  $0.059 \log(1 + \mu k/m_R)$  or 0.02 V where  $m_R = 4 D_R/\pi d$ ]<sup>18</sup> to yield  $E^\circ = 1.55$  V versus NHE. This can be compared to the  $E^\circ$  estimated previously of 1.58 V versus NHE, from spectroscopic measurements in Ar-saturated aqueous solution of 2 mM Gs, 25 mM  $\text{K}_2\text{S}_2\text{O}_8$ , 0.1 mM 1,2,4-trimethoxybenzene, and 1 M 2-methyl-2-propanol.<sup>4</sup>

In conclusion, the SECM in a G/C nanogap configuration has been shown to be a useful technique for observing short-lived electrogenerated intermediates of Gs. The radical cation,  $\text{Gs}^{*+}$ , has been electrochemically detected for the first time, with an estimated lifetime of  $\leq 40$   $\mu\text{s}$ . Because SECM measurements can be made under steady-state conditions, short time measurements are not needed. This technique should also allow electrode reactions of other nucleosides to be investigated that were previously not addressable by other EC techniques.

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**Supporting Information Available:** Additional experimental procedures and figures. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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- The reaction layer thickness was estimated from the  $d$  value at which significant amount of  $\text{Gs}^{*+}$  was collected at the substrate after correction for its total background current (offset current of the SECM instrument plus a small amount of Gs oxidation) at 1.1 V bias. This  $d$  value was close to that for the case of curve 2 of Figure 2 ( $d = 0.3$   $\mu\text{m}$ ).
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