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

Shuping Bi,[†] Biao Liu, Fu-Ren F. Fan, and Allen J. Bard*

Department of Chemistry and Biochemistry, University of Texas at Austin, Austin, Texas 78712

Received December 16, 2004; E-mail: ajbard@mail.utexas.edu

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$^{\bullet+}$. Gs is an important nucleoside[1,2] because oxidation of the base guanine to the radical cation has been implicated in the transfer of charge and in radiative damage of DNA.[3] 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 photo-irradiation in the presence of photosensitizers.[4−7] However, direct electrochemical measurement of the potential has been thwarted by the instability of Gs$^{\bullet+}$ and distortions of the voltammetric waves in water because of adsorption of Gs or its products.[8,9] 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,[10,11] 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$^{\bullet+}$ 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,[12−14] 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$_3$PO$_4$ to adjust the solution pH from that of dry DMF (11.85) to pH 7.10 (0.1% 1.2 M H$_3$PO$_4$) and pH 4.23 (3.5% 1.2 M H$_3$PO$_4$). 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$_6$ yielded a well-defined "S-shaped" steady-state CV with a half-wave potential, $E_{1/2}$, of 1.3 V versus a Ag quasireference electrode (AgQRE). The potential of the AgQRE was −0.43 V versus the $E^{\circ\prime}$ of ferrocene/ferrocenium (Fc/Fc$^+$) couple in DMF. At a given sweep rate, v, the steady-state current, i_{ss}, depended linearly on the concentration of Gs (Figure S2). Also, at Gs concentrations ≤10 mM, the current response of Gs increased with increasing $v^{1/2}$ 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.
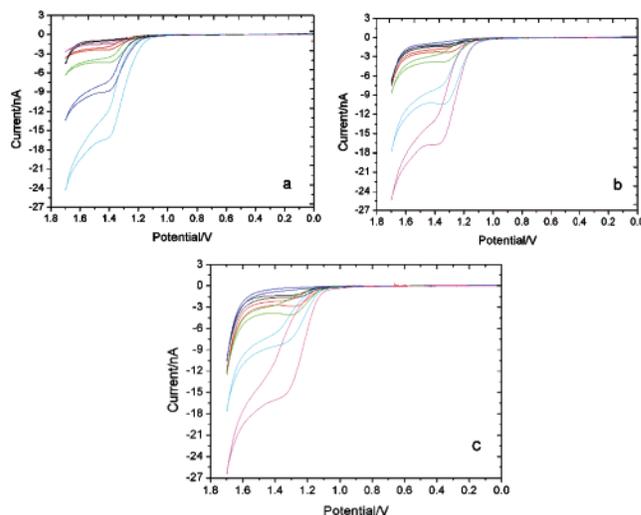
† Department of Chemistry, Nanjing University, Nanjing, 210093, China.

Figure 1. Plot of i_{ss} vs C_{Gs}* at three different water contents in DMF with different pH values at the CF-UME (8-μm diameter) in DMF/0.1 M TBAPF$_6$ 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$_3$PO$_4$ (pH 7.10). From bottom to top: 10, 5, 2, 1, 0.5, 0 mM. (c) 3.5% 1.2 M H$_3$PO$_4$ (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_{Gs}, of Gs in DMF with a Pt-UME of radius, a, of 12.5 μm based on comparing the transient and steady-state currents.[15] A normalized plot of $i(t)$ with respect to i_{ss} plotted versus $t^{-1/2}$ (Figure S5) yielded a straight line with a slope of 0.210, an intercept of 0.834, and a D_{Gs} of 4.5 × 10^{-6} cm^2 s^{-1}. This value is close to that reported previously by Faraggi et al.[16] in aqueous solution. From the steady-state current i_{ss} for a disk UME,

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

(where C_{Gs}* 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.[13]

SECM was used to study the oxidation of Gs in DMF. In particular, we wanted to detect the radical cation Gs$^{\bullet+}$ generated in the primary electrooxidation step of Gs by SECM. On the basis of the present and previous studies,[8,14] the overall electrooxidation of Gs in DMF is probably an ECE process, where the radical cation, Gs$^{\bullet+}$, 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$^{\bullet+}$, 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
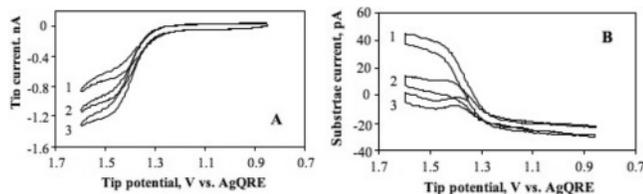
Figure 2. CVs for the oxidation of 2 mM guanosine in DMF/0.1M TBAPF$_6$ obtained at the tip (A) and substrate (B) electrodes. E_S = 1.1 V vs AgQRE. The scan rate of the tip potential was 20 mV/s. The distance between the tip and the substrate was (1) 0.15, (2) 0.3, and (3) 1 μm.

rapid deprotonation of Gs$^{•+}$, d must be small, so that 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 μm-diameter electrodes, as shown in Figure S6. A CF-UME tip (10-μm 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 into 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 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, Fc$^+$, as the redox mediator (2 mM ferrocene in DMF/0.1M TBAPF$_6$). The substrate potential was controlled such that Fc was oxidized to Fc$^+$ at a diffusion-controlled rate. A potential negative enough to reduce Fc$^+$ was applied to the tip, which was scanned in the vicinity of substrate surface (initially positioned about 5 μm away) to find the center of the 10-μm-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$_6$ 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 μm, 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 ($d \leq 1$ μm), 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, μ, of the intermediate Gs$^{•+}$ as ≤ 0.2 μm.[17] The lifetime, τ, of Gs$^{•+}$ can be estimated by the equation of $\tau \approx \mu^2/(2D_{Gs})$.[10] Thus, the Gs$^{•+}$ observed in this experiment has a $\tau \leq 40$ μs 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 Gs$^{•+}$ + e = Gs can be obtained from the SECM

data at d = 150 nm, where the observed half-wave potential (0.89 V versus Fc$^+$/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 $D_R/\pi d$][18] to yield $E°$ = 1.55 V versus NHE. This can be compared to the $E°$ estimated previously of 1.58 V versus NHE, from spectroscopic measurements in Ar-saturated aqueous solution of 2 mM Gs, 25 mM K$_2$S$_2$O$_8$, 0.1 mM 1,2,4-trimethoxybenzene, and 1 M 2-methyl-2-propanol.[4]

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, Gs$^{•+}$, has been electrochemically detected for the first time, with an estimated lifetime of ≤ 40 μs. 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.

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

References

(1) Masaya, S.; Yoshiko, N.; Nobuyoshi, N. *Ann. Neurol.* **2003**, *54*, S32−S45.
(2) Gian Piero, S.; Giovanni, G. *Synlett* **2004**, *4*, 596−602.
(3) Giese, B. *Acc. Chem. Res.* **2000**, *33*, 631−636.
(4) Steenken, S.; Jovanovic, S. V. *J. Am. Chem. Soc.* **1997**, *119*, 617−618.
(5) Steenken, S. *Chem. Rev.* **1989**, *89*, 503−520.
(6) Burrows, C. J.; Muller, J. G. *Chem. Rev.* **1998**, *98*, 1109−1151.
(7) Kino, K.; Saito, I. *J. Am. Chem. Soc.* **1998**, *120*, 7373−7374.
(8) Subramanian, P.; Dryhurst, G. *J. Electroanal. Chem.* **1987**, *224*, 137−162.
(9) Goyal, R. N.; Jain, N.; Grag, D. K. *Bioelectrochem. Bioenerg.* **1997**, *43*, 105−114.
(10) *Scanning Electrochemical Microscopy*; Bard, A. J., Mirkin, M. V., Eds.; Marcel Dekker: New York, 2001; Chapters 1 and 7.
(11) (a) Unwin, P. R.; Bard, A. J. *J. Phys. Chem.* **1991**, *95*, 7814−7824. (b) Zhou, F. M.; Unwin, P. R.; Bard, A. J. *J. Phys. Chem.* **1992**, *96*, 4917−4924. (c) Zhou, F. M.; Bard, A. J. *J. Am. Chem. Soc.* **1994**, *116*, 393−394. (d) Engstrom, R. C.; Pharr, C. M. *Anal. Chem.* **1989**, *61*, 1099A−1104A. (e) Treichel, D. A.; Mirkin, M. V.; Bard, A. J. *J. Phys. Chem.* **1994**, *98*, 5751−5757.
(12) Oliveira-Brett, A. M.; da Silva, A. A.; Brett, C. M. A. *Langmuir* **2002**, *18*, 2326−2330.
(13) Oliveira-Brett, A. M.; Matysik, F. M. *Bioelectrochem. Bioenerg.* **1997**, *42*, 111−116.
(14) Dryhurst, G. *Anal. Chim. Acta* **1971**, *57*, 137−149.
(15) Denuault, G.; Mirkin, M. V.; Bard, A. J. *J. Electroanal. Chem.* **1991**, *308*, 27−38.
(16) Faraggi, M.; Broitman, F.; Trent, J. B.; Klapper, M. H. *J. Phys. Chem.* **1996**, *100*, 14751−14761.
(17) The reaction layer thickness was estimated from the d value at which significant amount of 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 μm).
(18) Bard, A. J.; Faulkner, L. R. *Electrochemical Methods*; Wiley: New York, 2001; p 37 where the Fc$^+$/Fc potential is taken as +0.424 V versus SCE.

JA042433Y