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Effect of Base Pairing on the Electrochemical Oxidation of Guanine

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Abstract: The effect of base pairing by cytosine on the electrochemical oxidation of guanine is examined by means of cyclic voltammetry on carefully purified reactants in a solvent, $CHCl_3$, which strongly favors the formation of an H-bonded pair. The thermodynamics and kinetics of the oxidation reaction are not strongly influenced by the formation of the pair. They are actually similar to those of the reaction in which 2,6-lutidine, an encumbered base that cannot form a pair with guanine, replaces cytosine. The reaction does not entail a concerted proton−electron mechanism, as attested by the absence of H/D isotope effect. It rather involves the rate-determining formation of the cation radical, followed by its deprotonation and dimerization of the resulting neutral radical in competition with its further oxidation.

The mechanisms of electron and hole transfer through DNA are the object of active current attention in connection with damage and repair processes.[1] In these charge-transfer processes, the key intermediates are usually radical cations of DNA bases formed upon electron loss following photoionization or oxidative attack of a nucleobase. The oxidation potentials of the bases vary in the order guanine < adenine < thymine ≈ cytosine.[2] It follows that random oxidation of DNA, followed by electron transfer between the bases, is expected to eventually end with the formation of the most stable product, i.e., the guanine cation radical, which therefore plays an essential role in DNA damages. Further hole transfer between guanine moieties is controlled by two proton-transfer processes. One takes place within a base pair, as for example in the guanine cation radical/cytosine pair, and has been proposed to slow or stop the hole transfer.[3] The other concerns the transfer of a proton between the cation radical/intrabase pair and the surrounding water.[4] The rate of proton loss is a matter of discussion,[3a,4a,5] being affected by pairing with cytosine[3,4a] or by the neighboring bases on the same strand of DNA.[4b]

Direct electrochemical studies of guanine and derivatives are not very numerous;[6] until recently, only few studies of the effect of intrabase pairing on the oxidation of guanine were available.[7] The effect on the electrochemistry of guanine of the prior formation of a guanine-cytosine pair was reported in a solvent, $CHCl_3$, that favors the formation of an H-bonded pair as shown by NMR spectroscopy.[8] Two successive cyclic or differential pulse voltammetric waves of comparable height were observed, which were assigned to the oxidation of the paired and unpaired guanine successively.[8] This report and assignments are in fact subject to serious inconsistencies, which will be detailed in the following sections together with our own observations on the effect of guanine−cytosine pairing in $CHCl_3$, compared with the behavior of guanine in the presence of a base, 2,6-lutidine, that cannot form an H-bonded pair with guanine (as checked by NMR).

Results and Discussion

Equilibrium Association of Guanine and Cytosine in $CHCl_3$. As detailed in the following, a crucial factor in the discussion of the effect of the association between guanine and cytosine

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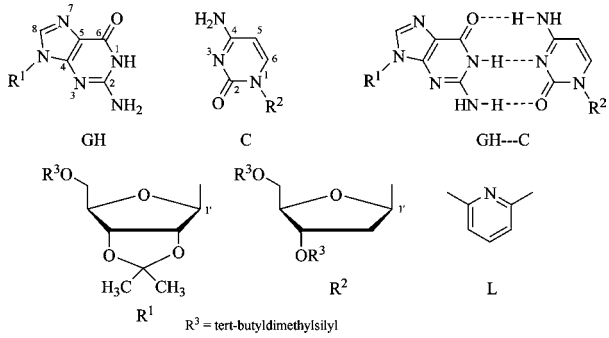
Chart 1



(Chart 1) on the oxidation characteristics of guanine in $CHCl_3$ is the purity of reactant as it can be checked by NMR spectroscopy. In fact, rather than guanine and cytosine, their $CHCl_3$-soluble derivatives shown in Chart 1 were used. They are designated in the following by GH and C, respectively. The NMR spectra of the pure species C and GH and the GH-C complex shown in Figure 1 served two purposes. They were recorded before each cyclic voltammetric experiment to make sure that pure reactants are dealt with. We indeed noted that aging and/or traces of acids produced spurious cyclic voltammetric responses involving additional waves. The second purpose was the determination of the GH/C association constant. The shift of characteristic bands from GH to the GH-C pair points to a rapid association, in a time shorter than 10^{-3} s,[9] as expected for the formation of H-bonds, in agreement with previous observations on analogous molecules.[10] Following the shift of the 10.5 ppm frequency of the N_1H proton of GH upon addition of C led to an association constant $K_A = 600\ M^{-1}$, in the presence of the supporting electrolyte used in the cyclic voltammetric experiments (see Figure 2 and Supporting Information for details). The proportion of associated and nonassociated guanine can be derived from this value of K_A at any reactant concentrations. For example for a mixture of 1 mM GH with 1 mM C, 30% of the GH is associated and 70% remains unpaired.

Appearance of Two Successive Waves in the Cyclic (or Differential Pulse) Voltammetry of GH-C Mixtures and Their Meaning. The two waves previously observed for a 1 mM GH −1 mM C mixture were interpreted as representing the oxidation of the associated and unpaired GH, respectively, the most positive wave corresponding to the unpaired GH and the least positive assigned to the oxidation of the GH-C complex.[8] In fact, as seen before, the two forms of GH are in rapid equilibrium: the scan rate of the cyclic voltammetric experiment, $\upsilon = 0.05$ V/s, corresponds to a time constant $RT/F\upsilon \simeq 0.5$ s, much larger than the upper limit of the NMR time constant. It follows that in the framework of the reaction square scheme in Scheme 1, oxidation is expected to go entirely through the oxidation of the most oxidizable species,[11] i.e., the GH-C complex, with instant displacement of the association equilibrium. According to the interpretation given in ref 8, a single wave is thus expected in contrast with the observed two waves.

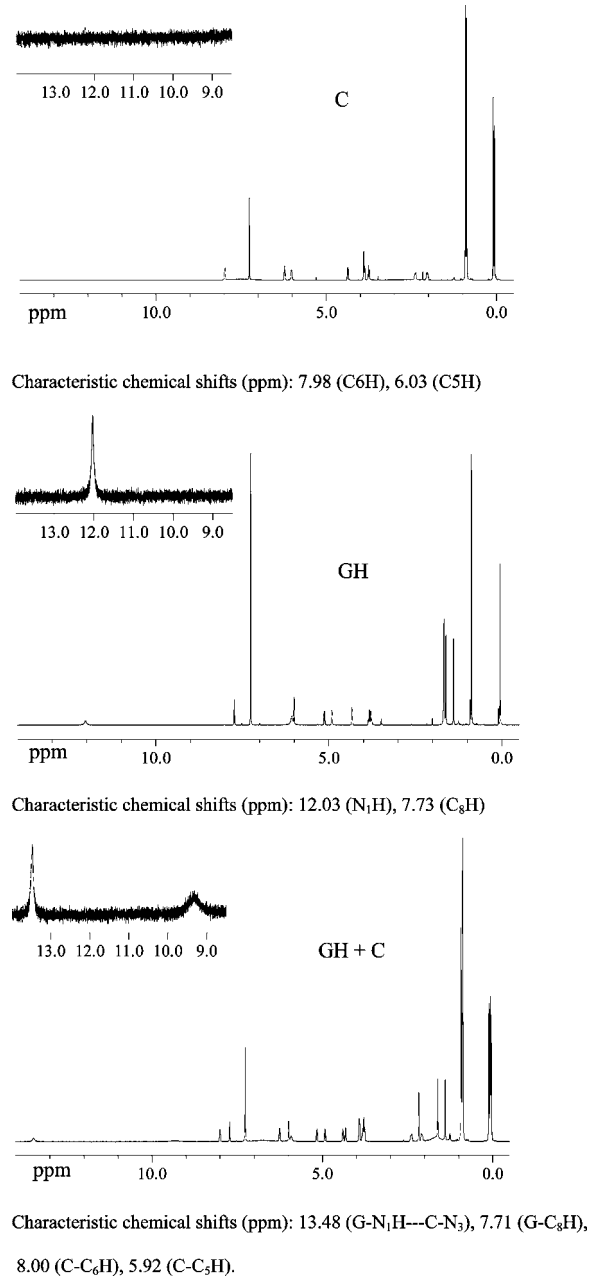


Figure 1. 400 MHz NMR spectra of the indicated species (see Chart 1 for definitions) in $CDCl_3$.

In other words, the alleged mechanism in ref 8 is not consistent with the observation of two waves reported in the same reference. Interpretation and experimental observation can actually be reconciled upon considering that, in fact, a single wave is observed when carefully purified reactants are used as shown in the next sections.

Cyclic Voltammetry of GH in the Presence of C or 2,6-Lutidine (Chart 1). The first test was to repeat the experiment reported in Figure 2 of ref 8 with carefully purified reactants (see Experimental Section). Addition of 1 mM C to a 1 mM solution of GH resulted in a single cyclic voltammetric wave, with a peak potential very close to the peak potential observed for GH alone (Figure 3) in the same conditions as those of the experiment reported in Figure 2 of ref 8. The height of the GH wave in the absence of C corresponds to a 0.5 electron stoichiometry, by reference to the ferrocene wave at the same

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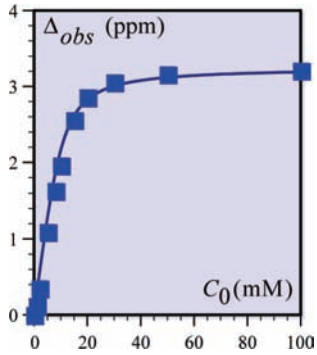
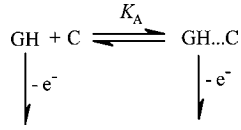


Figure 2. Variation of the observed chemical shift of the 10.5 ppm frequency of the N_1H proton of GH with the concentration of C. Squares: experimental data. Solid line: fitting (see Supporting Information).

Scheme 1



concentration, approximating the ratio of the diffusion coefficients by the inverse ratio of the equivalent radii according to the Einstein−Smoluchovski relationship and estimating the equivalent radii, *a*, by means of simple quantum chemical calculation (we used the PM3 method: $a_{ferrocene} = 4.7$ Å, $a_G = 5.9$ Å). This observation can be interpreted by the occurrence of a "father−son" reaction[11] in which GH itself deprotonates the initially formed cation radical, before dimerization of the deprotonated radical thus produced[12] (Scheme 2).

Upon addition of an equivalent of C, the peak potential does not vary significantly, but the peak height increases slightly over a one-electron stoichiometry.

Before analyzing further the effect of C on the electrochemistry of GH, it is interesting to observe and interpret the effect of an encumbered base, such as 2,6-lutidine (L), which cannot form an H-bonded complex with GH. Typical results are shown in Figure 4, noting that the oxidation current for 2,6-lutidine is small in the potential range where the GH wave takes place,

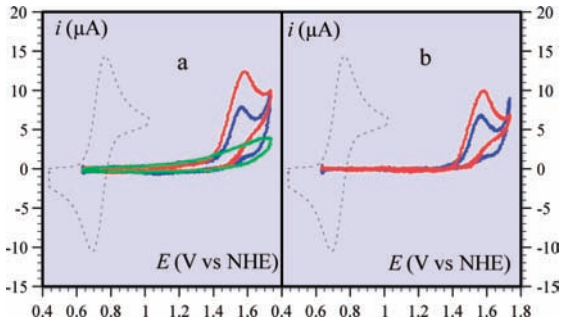
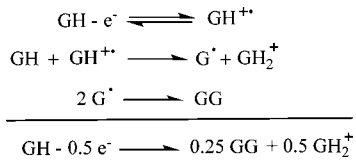


Figure 3. Cyclic voltammetry in $CHCl_3$ of (a) 1 mM GH (blue), GH + C (red), C (green) and (b) GH (blue) and GH + C (red) corrected from the oxidation of C. Scan rate: 0.05 V/s. Dotted reversible wave: 1 mM ferrocene.

Scheme 2



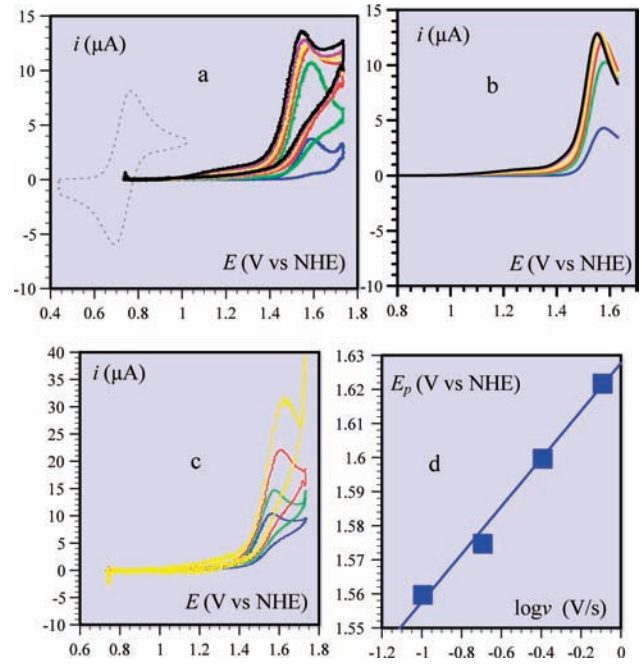
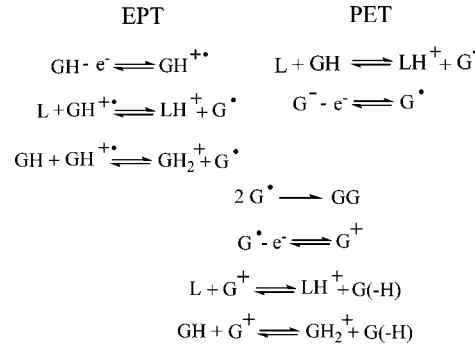


Figure 4. Cyclic voltammetry in $CHCl_3$. (a) 0.4 mM GH alone (blue) and with 1 (green), 2 (red), 4 (yellow) 8 (magenta), and 10 (black) equiv of L (see Chart 1). Scan rate: 0.1 V/s. Dotted reversible wave: 0.4 mM ferrocene. (b) Simulation (see text). (c) 0.4 mM GH + 0.4 mM L at 0.1 (blue), 0.2 (green), 0.4 (red), and 0.8 (yellow) V/s. (d) Peak potential as a function of the scan rate.

Scheme 3



except for the highest L concentrations, for which the rise of current past the GH peak corresponds to L oxidation as derived from cyclic voltammetric experiments with L alone.

Upon addition of L, the wave of GH becomes slightly less positive and increases in height, tending toward an electron stoichiometry of 2 (Figure 4a), which can be interpreted as the result of a competition, taking place after deprotonation of the initially formed cation radical, between the dimerization of the neutral G radical and its further one-electron oxidation according to an "ECE" mechanism,[11] followed by an additional deprotonation step. More precisely, the reactions that are involved are listed in Scheme 3. The thermodynamics of this set of reactions has been already worked out in the symmetrical case of reductions.[13] It can be straightforwardly adapted to the present oxidation scheme, showing that upon increasing the medium basicity, the system tends to pass from predominating elec-

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(13) Nadjo, L.; Savéant, J. M. *J. Electroanal. Chem.* **1971**, *33*, 419.

Table 1

reaction	thermodynamic and kinetic characteristics
electron transfer	standard potential, $E°$, in V vs NHE; standard rate constant, k_S, in cm/s
$GH - e^- \rightleftharpoons GH^{+\bullet}$	$E° = 1.70$, $\alpha = 0.5$, $k_S = 1$
$G^\bullet - e^- \rightleftharpoons G^+$	$E° = 1.53$, $\alpha = 0.5$, $k_S = 1$
$G^- - e^- \rightleftharpoons G^\bullet$	$E° = 0.93$, $\alpha = 0.5$, $k_S = 0.001$
proton transfer	rate constants in $M^{-1}\ s^{-1}$
$L + GH^{+\bullet} \rightleftharpoons LH^+ + G^\bullet$	$k_+ = 10^8$, $k_- = 10$
$GH + GH^{+\bullet} \rightleftharpoons GH_2^+ + G^\bullet$	$k_+ = 2 \times 10^8$, $k_- = 2$
$L + G^+ \rightleftharpoons LH^+ + G(-H)$	$k_+ = 10^9$, $k_- = 0.1$
$GH + G^+ \rightleftharpoons GH_2^+ + G(-H)$	$k_+ = 2 \times 10^8$, $k_- = 0.02$
$LH^+ + G^- \rightleftharpoons L + GH$	$k_+ = 10^8$, $k_- = 5 \times 10^3$
dimerization	rate constant in $M^{-1}s^{-1}$
$2G^\bullet \rightharpoonup GG$	$k_+ = 10^8$
diffusion	diffusion coefficients in cm^2/s
GH and all G's, L	$D = 8 \times 10^{-6}$, 2×10^{-5}

trodimerization to predominating ECE (see cases 5 and 6 in Figure 2 of ref 13). Within this thermodynamical framework, one can attempt to simulate the cyclic voltammograms shown in Figure 4a, noting that the proton-transfer reactions involves father−son steps in the absence of L or when the amount of L is small.

We also note in Figure 4a that a small but distinct pre-wave develops in front of the main wave as the concentration of base is increased. This is a manifestation of a proton−electron transfer (PET) pathway (Scheme 3) in which GH is first deprotonated by 2,6-lutidine, giving rise to the corresponding, more easily oxidized, anion. A complete simulation[14] involving the EPT and PET pathways, with the parameter values listed in Table 1, is shown in Figure 4b, leading to a satisfactory reproduction of the experimental data. It should however be emphasized that satisfactory simulations might be obtained with an infinite number of other combinations of parameter values as well. In other words, the parameters values in Table 1 are meaningless: obviously one cannot expect to determine an univoqual set of parameters when, as is the case here, the number of parameters is so much larger than the available experimental observables. Indeed, the number of parameters is 25 and the observables are 4 (location of the peak potential, variation of the peak potential with scan rate, and variation of the peak potential and the peak current with the base concentration). At best, the interest of simulation, in such cases, is to illustrate the possibility of passage from a 0.5 to a 2 electron stoichiometry upon addition of the base. No real additional knowledge is then acquired as compared to a simple qualitative description of the competition between ECE and dimerization pathways. Other dimerization pathways could have been added to the reaction scheme (dimerization of the cation radicals, cross-dimerization of a cation radical and a neutral radical) with no result other than increasing the number of parameters, and therefore the pointless character of simulations in such cases.

The variations of the cyclic voltammetric response with the scan rate shown in Figure 4c,d indicate that the oxidation reaction is kinetically controlled by the initial electron-transfer step with a transfer coefficient close to 0.5 (0.43 from the slope of the plot in Figure 4d, also consistent with the thickness of the waves).

The effect of C on the oxidation of GH is summarized in Figure 5. C gives rise to an oxidation current in the available

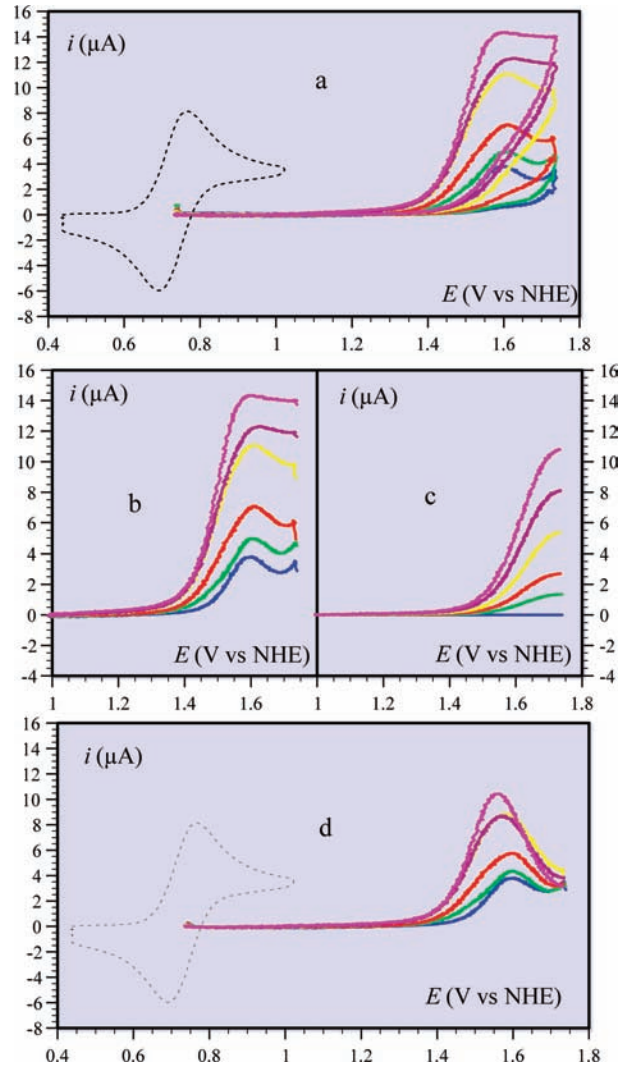

Figure 5. Cyclic voltammetry in $CHCl_3$ at 0.1 V/s of 0.4 mM GH alone (blue) and with 1 (green), 2 (red), 4 (yellow) 6 (black), and 8 (magenta) equiv of C (Scheme 1). (a,b) Raw data. (c) C alone. (d) GH response corrected by subtraction of the C response in panel c. Dotted reversible wave: 0.4 mM ferrocene.

potential range (Figure 5c). Although it appears at more positive potentials than the oxidation of GH, it cannot, unlike the case with L, be neglected when the excess of C increases. Figure 5 summarizes the procedure used to correct the oxidation response of GH.

It is remarkable that C, similarly to L, renders the wave only slightly less positive. The electron stoichiometry is raised upon addition of C, although less rapidly than with L. A small pre-wave also appears but to a lesser extent than with L. The reaction mechanism is therefore similar to the mechanism proposed in the case of L (Scheme 3), taking into account, however, that in the range of concentrations of interest, the GH-C complex predominates over free GH (taking $K_A = 600\ M^{-1}$, the degree of complexation is indeed 17, 29, 46, 57, and 64% for the successive additions of C in the experiments of Figure 5). The lesser importance of the PET pathway is a consequence of the lesser basicity of C as compared to L (the pK's are 4.2 and 6.75 in water, respectively[1e,15]). For the same reason, the

(14) (a) Using the Digielch software.[14b](b) Rudolph, M. *J. Electroanal. Chem.* **2003**, *543*, 23.

(15) *Handbook of Chemistry and Physics*, 82nd ed.; CRC: Boca Raton, 2001−2002; pp 8−51.

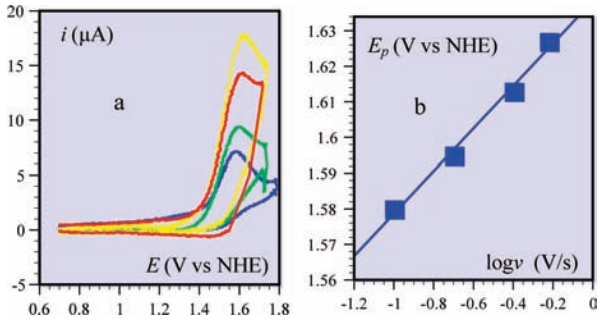

Figure 6. Cyclic voltammetry in $CHCl_3$ (corrected from the C oxidation current in Figure 5) of 0.2 mM GH + 0.8 mM C at 0.1 (blue), 0.2 (green), 0.4 (red), and 0.6 (yellow) V/s. (b) Peak potential as a function of the scan rate.

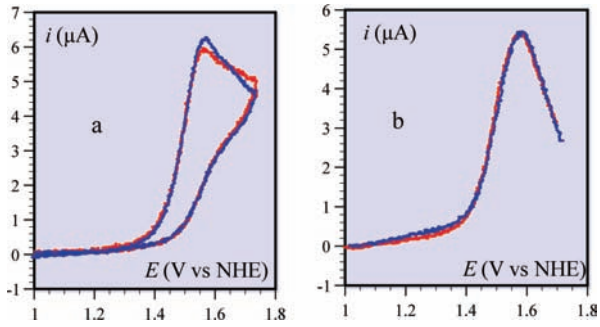

Figure 7. H/D kinetic isotope effect. Cyclic voltammetry in $CHCl_3$ at 0.1 V/s of 0.2 mM GH and, in a: 0.2 mM L + 50 eq MeOH (red) or MeOD (blue), in b: 0.8 mM C + 50 eq MeOH (red) or MeOD (blue), after subtraction of the C oxidation current.

competition between oxidation and dimerization of G$^{\bullet}$ is less in favor of oxidation than with L. The complexation of GH by C multiplies the number of possible pathways, rendering any simulation even more illusory than in the case of lutidine.

As with L, the electrochemical reaction is kinetically controlled by the initial formation of the cation radical, as results from the experiments depict in Figure 6. The transfer coefficient is 0.5 in this case, as results from the slope of the peak potential vs scan rate plot in Figure 6b, consistent with the peak widths.

That the rate-determining step of the oxidation of GH in the presence of C is the initial electron-transfer step is confirmed by the absence of H/D isotopic effect (Figure 7), which would be expected in the case of a concerted proton-electron transfer reaction.[16] In order to safely establish the absence of H/D isotopic effect, tests of reproducibility were carried out as detailed in the Supporting Information.

It is remarkable that the peak potential for GH oxidation into its cation radical is affected only to a small extent by the addition of C under conditions where an H-bonded pair is formed totally or predominantly. Once the H-bond pair is predominant and when the father−son reactions have been minimized upon addition of C, the potential shift reaches ca. 60 mV, in line with the 3.5-fold increase of the rate constant of quenching of the triplet state of *N*,*N*′-dibutylnaphthaldiimide by GH and GH + C under similar pairing conditions.[7a] This observation implies that the H-bond pairing is not strongly affected by the removal of one electron from the conjugated core of GH.

Conclusions

The effect of the addition of C on the electrochemical oxidation of GH was examined on carefully purified reactants in a reaction medium, $CHCl_3$, which favors pairing of the two molecules. The oxidation peak in cyclic voltammetry is very weakly shifted toward less positive potential. The reaction is kinetically controlled by the initial formation of the cation radical, as attested by a scan rate dependency investigation and by the absence of H/D isotopic effect. The main effect of the addition of C is the increase of the electron stoichiometry from 0.5 to values above 1, which indicates a competition between the dimerization of the neutral G radical formed upon deprotonation of the initial cation radical and its further oxidation at the electrode surface. This behavior is similar to that observed upon addition to GH of 2,6-lutidine, an encumbered base which cannot form an H-bonded pair with GH. In no case did we find that addition of C leads to two oxidation waves, the less positive of which would represent the GH-C complex and the second free GH, as previously alleged.

In fact, the H-bonded pairing between GH and C does not exert a strong influence on the thermodynamics and kinetics of guanine oxidation, the reaction mechanism being controlled by the initial formation of the cation radical.

Experimental Section

Chromatography and NMR. Flash chromatography was performed using Macherey-Nagel Kieselgel (SiO_2) 60 (70−230 mesh). NMR spectra were recorded at 298 K on a Bruker Avance III 400 MHz spectrometer and were referenced to the resonances of the solvent used. The data are reported as follows: chemical shift in ppm, integration, multiplicity (br = broad, s = singlet, d = doublet, m = multiplet) and coupling constants (Hz).

Chemicals. All chemicals were purchased from Sigma-Aldrich and used as received.

Synthesis of 2′,3′-*O*-Isopropylidene-5′-*O*-(*tert*-butyldimethylsilyl)guanosine (Molecule Previously Described in Ref 17). To a solution of 2′,3′-*O*-isopropylideneguanosine (0.5 g, 1.54 mmol) in pyridine (5 mL) was added *tert*-butyldimethylsilyl chloride (0.35 g, 2.32 mmol). The mixture was stirred at 50 °C for 48 h. The pyridine was then evaporated under reduced pressure, and the residue was dissolved in chloroform and washed with cold 1 M HCl (25 mL) and water (3 × 25 mL). It is important to note that the persistence of residual traces of HCl may cause a modification of our product a few weeks after synthesis (see Supporting Information); for this reason the pH of washing water was carefully checked after extraction. The organic layer was dried over Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by crystallization from chloroform to give a white solid (0.35 g, 0.79 mmol, 52%). ^{1}H NMR (400 MHz, $CDCl_3$, δ): 12.03 (1H, br s), 7.73 (1H, s), 6.07 (2H, br s), 6.00 (1H, s), 5.13 (1H, m), 4.91 (1H, m), 4.33 (1H, m), 3.81 (2H, m), 1.62 (3H, s), 1.39 (3H, s), 0.88 (9H, s), 0.05 (6H, s).

Synthesis of 3′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-2′-deoxycytidine (Molecule Previously Described in Ref 17). To a solution of 2′-deoxycytidine (1 g, 4.4 mmol) in pyridine (10 mL) was added *tert*-butyldimethylsilyl chloride (1.72 g, 11.4 mmol), and the mixture was stirred at 50 °C for 48 h. The pyridine was then evaporated, the residue was washed with cold 1 M HCl (50 mL) and water (3 × 50 mL), and the pH of the aqueous phase was checked. The residue was purified by chromatography (CH_2Cl_2/MeOH 9/1) to give a white solid (1.44 g, 3.2 mmol, 73%). ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.97 (1H, d, 6.9 Hz), 7.45 (2H, br s), 6.22 (1H, m), 6.02 (1H, d, 6.3 Hz), 4.36 (1H, s), 3.88 (2H, d, 11.2 Hz), 3.75

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(1H, d, 11.2 Hz), 2.38 (1H, m), 2.03 (1H, m), 0.91 (9H, s), 0.88 (9H, s), 0.09 (6H, s), 0.06 (6H, s).

Cyclic Voltammetry. Cyclic voltammetry experiments were carried out at 25 °C in a water-circulation thermostated double-wall jacketed cell under an argon atmosphere. A platinum wire was used as counter electrode and an aqueous saturated calomel electrode as reference electrode; the working electrode was a 3 mm glassy carbon electrode (Tokai), carefully polished and ultrasonically rinsed in absolute ethanol before use. The supporting electrolyte used was tetrabutylamonium perchlorate at 0.2 M concentration. A homemade instrument was used. It was equipped with positive feedback to compensate the effect of ohmic drop, which is particularly important in $CHCl_3$.[18] Purity of products was verified before each experiment via NMR spectrometry. For the H/D isotope effect, NMR was also used to check that addition of small amounts of MeOH and MeOD does not affect base pairing. With the aim of obtaining reproducible cyclic voltammetric responses, the working electrode was scanned three times in a solution containing only supporting electrolyte and chloroform before each scan. $CHCl_3$ was distilled under an argon atmosphere over calcium hydride prior to use.

Note Added after ASAP Publication. Table 1 contained typographical errors in the version published ASAP July 2, 2010; the corrected version reposted on July 7, 2010.

Supporting Information Available: Tests of reproducibility in the determination of the H/D isotope effect; effect of traces of acid on GH and C NMR spectrometry. This material is available free of charge via the Internet at http://pubs.acs.org.

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