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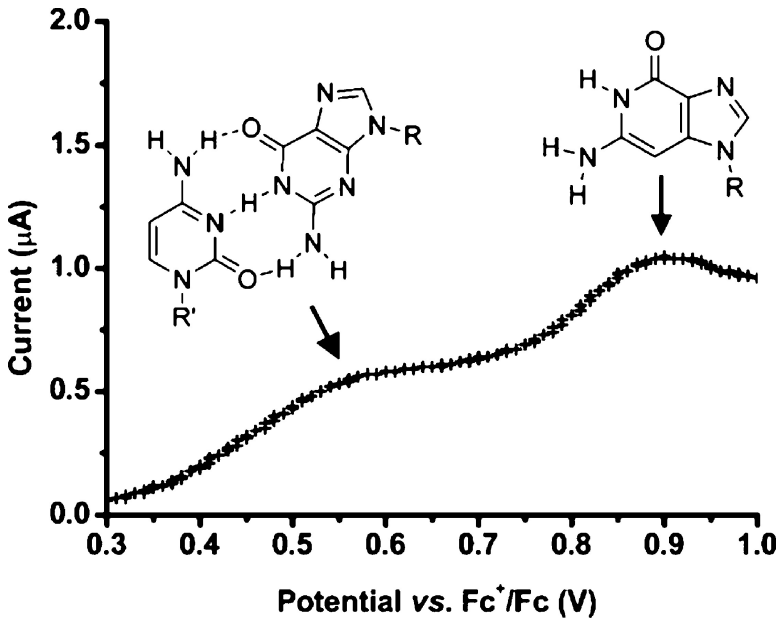

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Direct Experimental Observation of the Effect of the Base Pairing on the Oxidation Potential of Guanine

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Oxidation of DNA yields the radical cation of guanine (**G**), either directly or through hole transfer along the DNA π stack.[1] Oxidation of **G** has been, therefore, extensively studied for understanding the mechanism of DNA damage caused by ionizing radiation and oxidizing agents.[2] According to experimental evidence, **G** is the DNA base with the lowest oxidation potential.[3] Recently, photoelectron spectroscopy has shown the existence of a distinct low electron-binding energy band for oligonucleotides which contain one or more guanine bases, arising from the ionization of a π orbital of **G**.[3a] In aqueous solution, the oxidation potential of **G** is 1.29 V versus NHE at pH 7,[3b] but there are both experimental and theoretical evidences that its value in DNA could differ somewhat from that of the isolated nucleotide.

Two effects have been recognized to lower the oxidation potential of **G** in DNA: (i) the π stacking[4] with other nucleotides, and (ii) the formation of H-bonds at the N1 acidic site of **G**, the K_a of $\mathbf{G}^{+\bullet}$ being ca. 5 orders of magnitude higher than that of neutral **G**.[5] Both effects are expected to be significant. Concerning formation of H-bonds, the effect of pairing with the complementary cytosine (**C**) base on the oxidation potential of **G** has been investigated by determining the oxidation rate of **G** via quenching of triplet absorption of *N*,*N*′-dibutylnaphthaldiimide and fullerene, used as **G** oxidants in their triplet excited states.[6] It was estimated that the formation of the **G:C** complex lowers the oxidation potential of **G** by ca. 0.1 V, much less than what was estimated by computations, ca. 0.75 eV in the gas phase.[7]

So far, there are no direct measurements of the oxidation potential of **G** in the presence of **C**, except for 7,8-dihydro-8-oxoguanosine,[7b] whose π electron system is substantially different from that of **G**, as testified by its lower oxidation potential.[8] We have, therefore, performed electrochemical investigations of guanosine (**Gs**), cytidine (**Cd**), and **Gs** in the presence of **Cd**, both in dimethyl sulfoxide (DMSO) and $CHCl_3$, the latter solvent favoring the formation of the Watson−Crick H-bonded **Gs:Cd** complex, the most stable among the several H-bonded **G:C** complexes which can be formally written,[9] integrated by a theoretical study of the oxidation potential of **Gs** and **Gs:Cd** in solution.

Voltammetric measurements have been carried out by Metrohm 757 VA, by using both platinum and glassy carbon working electrodes. Suitable supporting electrolytes were chosen according to their purity and to their solubility and electrochemical stability in the chosen solvent. The working solutions were accurately purged from dissolved oxygen by bubbling N_2 for 5 min. Ag/AgCl with a 3 M aqueous KCl salt bridge was used as an external reference electrode and the ferrocenium/ferrocene half-couple (Fc^+/Fc) as internal reference in all the measurements.[10] To increase their solubility in $CHCl_3$, **G** and **C** were derivatized with *tert*-butyldimethylsilyl groups on the ribose unit to yield 2′,3′-*O*-isopropylidene-5′-*O*-(*tert*-butyldimethylsilyl)guanosine (**Gs**′) and 3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-2-deoxycytidine (**Cd**′), the former was synthesized from commercial 2′,3′-*O*-isopropylideneguanosine, the latter from 2-deoxycytidine.[11]

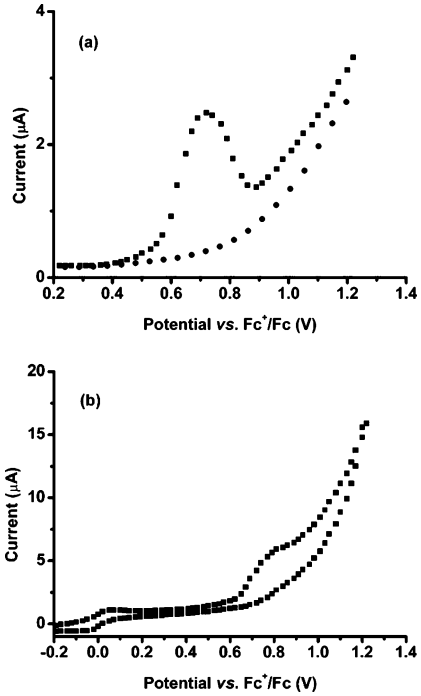

Figure 1. Voltammetric curves of nucleoside bases in DMSO at 25 °C. (a) Differential pulse voltammogram of: (■) 1 mM **Gs**; (●) 1 mM **Cd**. (b) Cyclic voltammogram of 1 mM **Gs** and 1 mM **Cd**. Working electrode, Pt; supporting electrolyte, 0.1 M Bu_4NClO_4; scan rate, 50 mV/s; internal reference half-couple, Fc^+/Fc, in both the experiments.

The formation of the **Gs′:Cd′** complex was studied in $CHCl_3$ containing 0.1 M tetrabutylammonium perchlorate, the supporting electrolyte used in voltammetric measurements, to evaluate the effect of the ionic strength of the solution on the **Gs′:Cd′** formation constant (K_{assoc}). The latter has been evaluated via ^{1}H NMR, by using the procedure outlined in ref 12. In 0.1 M tetrabutylammonium perchlorate, $K_{assoc} = 2 \times 10^4$ M^{-1}, very similar to that measured for 2′,3′,5′-tripentanoylguanosine with 4-ethylcytosine in $CDCl_3$ (1×10^4 M^{-1}).[13]

The voltammograms of **Gs** and **Gs:Cd** in DMSO and of **Gs′**, **Cd′**, and **Gs′:Cd′** in $CHCl_3$ are reported in Figures 1 and 2, respectively.

In DMSO, oxidation of **Gs** occurs at ca. 0.73 V versus ferrocenium/ferrocene (roughly 1.44 V vs NHE).[10] All of the oxidation potentials are referred to the peak of current/potential curve in the differential pulse voltammogram.[14] Oxidation of **Cd** is not observed in the allowed potential window. The addition of an equimolar amount of **Cd** to **Gs** does not show any relevant change in both shape and position of the current/potential curve of **Gs**, as

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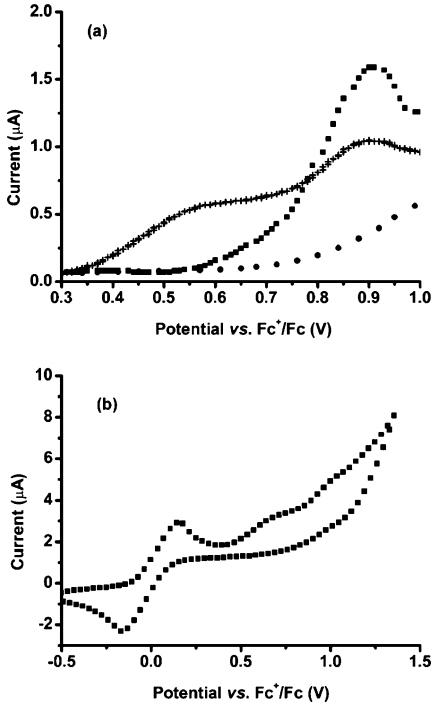

Figure 2. Voltammetric curves of nucleoside base derivatives in $CHCl_3$ at 25 °C. (a) Differential pulse voltammogram of: (■) 1 mM **Gs′**; (●) 1 mM **Cd′**; (+) 1 mM **Gs′**; 1 mM **Cd′**. (b) Cyclic voltammogram of 1 mM **Gs′** and 1 mM **Cd′**. Working electrode, Pt; supporting electrolyte, 0.1 M Bu_4NClO_4; scan rate, 50 mV/s; internal reference half-couple, Fc^+/Fc, in both the experiments.

Table 1. Computed (DFT/B3LYP/6-311++g**) Adiabatic and Vertical Ionization Potentials (eV) of **G**, **C**, and of the Watson and Crick **G:C** Complex in the Gas Phase ($\epsilon = 1$), $CHCl_3$ ($\epsilon = 4$), and DMSO ($\epsilon = 48$)

	Adiabatic			Vertical		
ϵ	1	4	48	1	4	48
G	7.66	6.02	5.63	7.91	6.32	5.86
C	8.62	6.87	6.44	8.74	6.99	6.61
G:C	6.92	5.77		7.28	6.05	

expected because of the low K_{assoc} in DMSO,[15] less than 1.5% of total **Gs** is in the form of **Gs:Cd** complex in the analyzed solution.

In $CHCl_3$ (Figure 2), the oxidation potential of **Gs′** is shifted toward more positive potentials, owing to the 10-fold lower dielectric constant of $CHCl_3$ with respect to that of DMSO and/or to specific solvent−solute interactions; the oxidation peak shifts at 0.91 V versus Fc^+/Fc couple.

Even in $CHCl_3$, oxidation of **Cd′** is not observed, but addition of **Cd′** to **Gs′** causes the appearance of a double signal, one occurring at the same potential of isolated **Gs′** (0.91 V), which can be therefore assigned to the fraction of isolated **Gs′** in solution, the other, at 0.57 V, which, on the basis of theoretical computations (vide infra), can be assigned to the formation of **Gs′:Cd′** complex.

To better assign the experimental signals, we have carried out a computational study of the ionization potentials of **G** and **C** and of the **G:C** Watson−Crick complex, in the gas phase and in the two solvents used in voltammetric measurements. All computations have been carried out by the Gaussian 03 package,[16] using hybrid density function theory (DFT) (B3LYP/6-31++g**) and the polarizable continuum medium method for evaluating solvent effects.[17] The results are reported in Table 1. Both the adiabatic and the vertical ionization potential of **G** in the gas phase are well reproduced by the adopted computational method, the experimental values being 7.77 and 8.24 eV, respectively.[18] The solvent polarity affects significantly the oxidation potential of both **G** and **G:C** complex (cf. Table 1).

Concerning the effect of the formation of the H-bond complex **G:C**, in $CHCl_3$, the computed IP of the pair **G:C** is 5.77 eV, 0.25 eV lower than that of isolated guanine, again in very good agreement with the voltammetric measurements ($\Delta E_{ox} = 0.34$ V).

In conclusion, we have shown that the oxidation potential of a guanine derivative is significantly affected by base pairing with a cytosine derivative; the lowering of the oxidation potential amounts to 0.34 V.

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Supporting Information Available: ^{1}H NMR spectra of **Gs′** and of the **Gs′:Cd′** Watson and Crick complex in 0.1 M Bu_4NClO_4; optimized geometries of **G** and of the Watson−Crick **G:C** complex; differential pulse and cyclic voltammograms of the **Gs** and the Watson−Crick **Gs:Cd** in DMSO and **Gs′** and the Watson−Crick **Gs′:Cd′** in $CHCl_3$ and in DMSO on glassy carbon electrode; complete ref 16. This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) (a) A quantity of 0.70 g (4.6 mmol) of *tert*-butyldimethylsilyl chloride (TBDMSCl) was added to a solution of 2′,3′-*O*-isopropylideneguanosine (Sigma-Aldrich) (1.0 g, 3.1 mmol) in pyridine (10 mL). The reaction mixture was stirred at 50 °C for 48 h and then evaporated to dryness. The residue dissolved in chloroform was washed with cold (4 °C) 1 N HCl (50 mL) and water (2 × 50 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated to dryness. The residue was purified by crystallization from hexane−acetate and characterized via ^{1}H NMR. The crystallization afforded **Gs′** (85%). (b) A quantity of 1.72 g (11.4 mmol) of TBDMSCl was added to a solution of 2-deoxycytidine (Sigma-Aldrich) (1.0 g, 3.8 mmol) in pyridine (10 mL); after 48 h at room temperature, the reaction yielded **Cd′** (95%). **Cd′** was purified by recrystallization from 2-propanol and characterized via ^{1}H NMR.
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