Nucleoside Complexes

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The Charge-Transfer Band of an Oxidized Watson–Crick Guanosine–Cytidine Complex

*Amedeo Capobianco, Maurizio Carotenuto, Tonino Caruso, and Andrea Peluso**

One-electron oxidation of DNA gives rise to an electron hole that easily migrates over distances of up to 200 Å,[1–3] and ends up at a guanine site, the nucleobase with the lowest oxidation potential.[4,5] Long-range hole transfer depends on the energy distribution of the low-lying electronic states, which in turn is strongly affected by intermolecular interactions—π stacking and H-bonding—between nucleobases.[6–9] Herein, we report for the first time the detection of a low-energy spectroscopic signal of the oxidized Watson–Crick complex of guanosine and cytidine derivatives, attributed to the charge-transfer (CT) localizing the hole on the cytidine moiety.

The UV/Vis absorption spectra of electrooxidized 2′,3′-*O*-isopropylidene-5′-*O*-(*tert*-butyldimethylsilyl)guanosine (referred to here as Guo) in 10 mM solution (with 0.1M tetrabutylammonium perchlorate (TBAP) as the supporting electrolyte) in DMSO, CH_2Cl_2, and $CHCl_3$ are shown in Figure 1.[10] The spectra were recorded in an electrochemical cell equipped with an optically transparent thin-layer electrode (OTTLE), at a controlled potential of +0.91 V versus the ferrocenium/ferrocene (Fc^+/Fc) couple.[8,11,12]

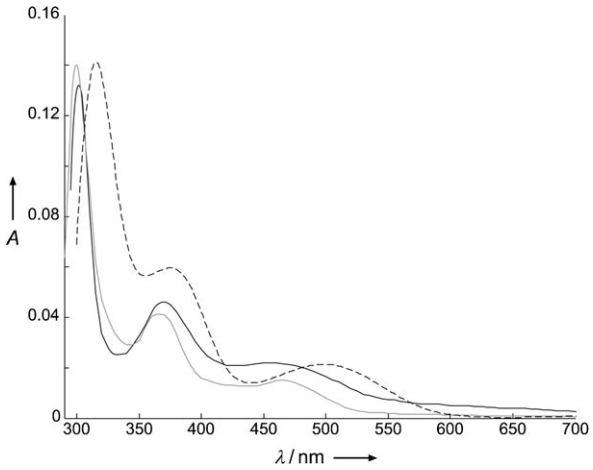

Figure 1. UV/Vis absorption spectra of the electrogenerated $Guo^{\bullet+}$ radical cation in various solvents. Dashed line: DMSO, black line: $CHCl_3$, gray line: CH_2Cl_2.

Although cyclic voltammetry indicates that Guo oxidation is an irreversible process, so that the guanosine radical cation should be rapidly consumed,[8] the spectra in all the solvents show the three characteristic absorption peaks of oxidized Guo in water (320, 390, and 500 nm), which were unequivocally attributed to the radical cation of guanosine.[13–15] In DMSO the three peaks exhibit the same intensity ratios as those obtained by pulse radiolysis of an aqueous solution of guanosine at acidic and neutral pH. In $CHCl_3$ and CH_2Cl_2, the peak frequencies are slightly blue-shifted because of the different solvent polarization. The relative intensity of the peak at 310 nm is slightly higher and increases as the electrooxidation proceeds, which suggests that an oxidation product is also absorbing at that wavelength region (see below).

The FTIR absorption spectrum of neutral Guo (10 mM) and TBAP (0.1M) in $CHCl_3$, in the solvent-free region 1600–1800 cm^{-1}, is reported in Figure 2 (curve a). The band at 1690 cm^{-1} is assigned to the carbonyl stretching mode[16] and

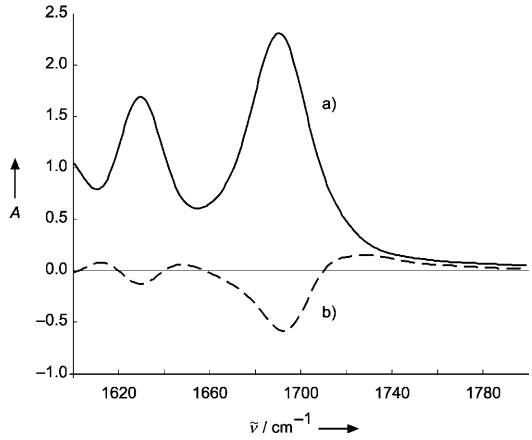

Figure 2. a) IR spectrum of Guo in $CHCl_3$, and b) the difference spectrum obtained by subtracting the spectrum in (a) from that recorded at 0.91 V vs. Fc^+/Fc.

the other, at 1630 cm^{-1}, to the bending vibration of the amine group, since it disappears upon deuteration.[17] The difference spectrum obtained by subtracting the spectrum of neutral Guo from that recorded during oxidation at controlled potential is also shown in Figure 2 (curve b; see also the Supporting Information). The parent absorption at 1690 cm^{-1} is bleached and a peak around 1710 cm^{-1} appears. The latter is considered to be the IR spectroscopic signature of $Guo^{\bullet+}$.[18]

Both UV/Vis and FTIR measurements clearly show that the spectrum in Figure 1 is that of oxidized Guo and a

[*] Dr. A. Capobianco, Dr. M. Carotenuto, Dr. T. Caruso, Prof. A. Peluso
Dipartimento di Chimica, Università di Salerno
84084 Fisciano (SA) (Italy)
E-mail: apeluso@unisa.it

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reaction product. No other signals are observed in the 300–700 nm region, both in $CHCl_3$ and CH_2Cl_2 (see the Supporting Information). When the potential is switched off, only the signal at 316 nm persists. The relative intensity of the peak at shorter wavelength increases as the electrooxidation proceeds, and its frequency slightly shifts from that of $Guo^{\bullet+}$ (around 300 nm) to that of the oxidation product (316 nm). The product has been identified by using HPLC–mass spectrometry (MS) and NMR spectroscopy (see the Supporting Information) as the 8-(8-guanosyl)guanosine derivative (8-8 Guo), which indeed also exhibits a strong absorption at 320 nm in water.[19] HPLC separation of electrolyzed solutions of Guo/TBAP showed a well-defined peak in addition to that of Guo. Electrospray ionization (ESI) MS revealed the presence of ions at m/z 874.3, the value expected for $[M+H]^+$ of 8-8 Guo.

Furthermore, the signal of the aromatic proton at $\delta =$ 7.72 ppm is not observed in the ^1H NMR spectra of oxidized solutions of Guo in $CDCl_3$, which suggests that oxidized Guo has been substituted at C8. ^{13}C NMR spectra also confirm the formation of 8-8 bipurinyl compounds on the basis of existing data.[20,21]

The UV spectrum of an equimolar (10 mM) solution of Guo and 3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-2-deoxycytidine (here referred to as dCyd) in CH_2Cl_2 with TBAP (0.1M), recorded at a potential of +0.57 V versus Fc^+/Fc, is reported in Figure 3. Cyclic voltammetry measurements showed that at

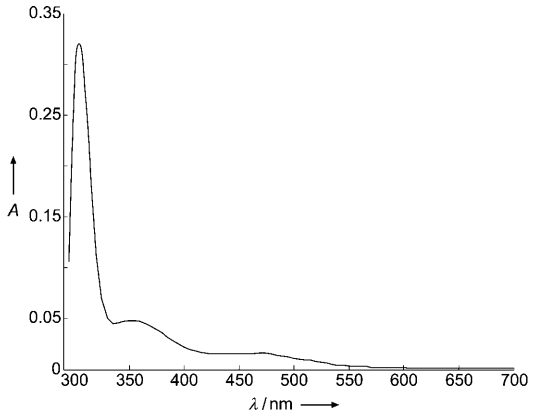

Figure 3. UV/Vis spectrum of Guo and dCyd (20 mM) in CH_2Cl_2 recorded at controlled potential (+0.57 V vs. Fc^+/Fc).

this potential solutions containing only Guo or only dCyd are not oxidized, whereas solutions containing both species show a well-resolved peak.[8] Voltammograms obtained in the OTTLE cell are reported in the Supporting Information. The UV/Vis spectrum of the Watson–Crick Guo–dCyd complex, the predominant species in solution,[8,22] is very similar to that of $Guo^{\bullet+}$, but the peak at 470 nm is slightly less intense for the complex. No other signals are observed in the region 300–700 nm.

The ^1H NMR and ^{13}C NMR spectra of exhaustively electrolyzed solutions of the Watson–Crick Guo–dCyd complex in $CDCl_3$ show that dCyd is not altered by electrooxidation, whereas all the other signals are the same as those observed in electrolyzed solutions containing only Guo.

The gas-phase ionization potential of cytosine is about 0.9 eV higher than that of guanine;[23] therefore, the CT in the oxidized Watson–Crick complex is expected to fall in the near-infrared (NIR) region. We have thus investigated the region 6000–11 500 cm^{-1} using the same conditions as for the UV spectra. A positive broad band, which is not observed during the oxidation of solutions containing only Guo or only dCyd, is indeed clearly observed in the difference spectrum at approximately 10 600 cm^{-1} in CH_2Cl_2 and at 10 200 cm^{-1} in $CHCl_3$ (see Figure 4, Table 1, and the Supporting Information).

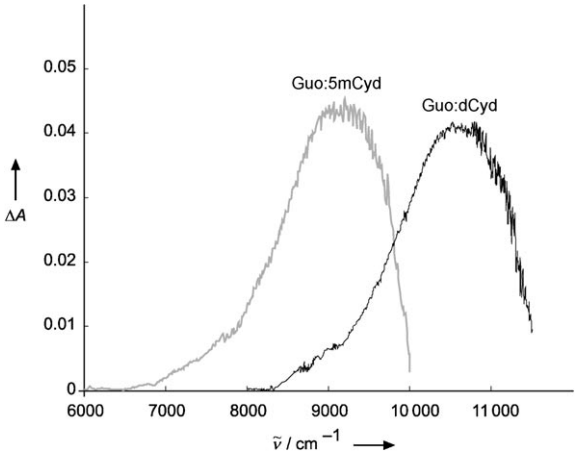

Figure 4. NIR absorption spectra of the Watson–Crick Guo–dCyd (black line) and Guo–5mCyd (gray line) complexes in CH_2Cl_2, recorded at +0.57 V vs. Fc^+/Fc. Gua, C: 10 mM; TBAP: 0.1 M.

Table 1: Predicted and observed wavenumbers [cm^{-1}] for the CT band of guanine–cytosine (Gua–Cyt) and guanine–5-methylcytosine (Gua–5mCyt) complexes.

	Gas phase	$CHCl_3$		CH_2Cl_2	
	DFT	DFT	obs.[a]	DFT	obs.[a]
Gua–Cyt	9917	11 218	10 200	11 385	10 600
Gua–5mCyt	8382	9645	8700	9890	9100

[a] Guo–dCyd and Guo–5mCyd.

As a first reasonable hypothesis, this band has to be assigned to the CT of the oxidized Guo–dCyd Watson–Crick complex. To better support this assignment, we also recorded the spectrum of the Watson–Crick complex of Guo with 2′,3′,5′-tri-*O*-(*tert*-butyldimethylsilyl)-5-methylcytidine (5mCyd). The CT is expected to shift at lower energy when dCyd is replaced by 5mCyd, because the experimental ionization potentials of methylated pyrimidine bases are lower than those of unsubstituted ones.[23] Computed and available experimental ionization potentials of cytosine and methylcytosines are reported in Table 2. From these data, it is expected that the replacement of dCyd by 5mCyd should shift the CT band at lower wavenumbers by about 1400 cm^{-1}.

Table 2: Theoretical and experimental vertical (adiabatic) ionization potentials [eV] of cytosine (Cyt), and 5- and 6-methylcytosine (mCyt).

	Gas		$CHCl_3$	CH_2Cl_2
	DFT[a]	exp.[b]	DFT	DFT
Cyt	8.75 (8.60)	8.94 (8.68)	6.99 (6.83)	6.79 (6.62)
6mCyt	8.53 (8.34)	– (8.38)	6.93 (6.74)	6.75 (6.54)
5mCyt	8.42 (8.25)		6.81 (6.61)	6.62 (6.42)

[a] Computed adiabatic ionization potentials include zero-point vibrational energies. [b] Ref. [23].

Indeed, the absorption band is now found at 9100 cm^{-1} in CH_2Cl_2, as shown in Figure 4, and at 8700 cm^{-1} in $CHCl_3$ (see the Supporting Information), in very good agreement with the expected frequency shifts.

Time-dependent density functional theory (TDDFT) computations, carried out using the simpler nucleobases as a model of the nucleosides, further support the above assignment. A weak band (oscillator strength 0.06) at 11385 cm^{-1} is predicted for the Watson–Crick complex between guanine (Gua) and cytosine (Cyt) in CH_2Cl_2, which corresponds to the excitation of an electron from the singly occupied HOMO, a π orbital localized on dCyd, to the LUMO, a nonbonding π orbital localized on Guo (see the Supporting Information). That transition shifts at 9890 cm^{-1} by replacing Cyt with 5mCyt, in optimum agreement with the observed values.

The low-energy absorption of the Watson–Crick Guo–dCyd complex can therefore be reasonably assigned to the CT of the oxidized complex. It provides the first experimental estimate of the energy of one of the low-lying electronic states of oxidized duplex DNA, important information toward a better understanding of the mechanism of hole transfer in DNA.[24] The formation of 8-8 Guo as an oxidation product of the Watson–Crick Guo–dCyd complex could also be an important hint for a deeper understanding of oxidative DNA damage; in that field spectroelectrochemistry could play an important role in the future.

Experimental Section

To increase their solubility in $CHCl_3$ and CH_2Cl_2, 2′,3′-*O*-isopropylideneguanosine, 2-deoxycytidine, and 5-methylcytidine (Sigma–Aldrich) were derivatized with *tert*-butyldimethylsilyl groups on the ribose unit to yield Guo, dCyd, and 5mCyd, respectively, by using the procedure described in ref. [8]. Guo, dCyd, and 5mCyd were purified by recrystallization from 2-propanol and characterized by one- and two-dimensional NMR spectroscopy. Ferrocene (Sigma–Aldrich) was used as internal standard only in voltammetric measurements, not in spectroscopic ones (see the Supporting Information). UV/Vis spectra were recorded by a Varian-Cary 50 spectrophotometer, NIR and IR spectra by a Bruker Vertex 70 spectrometer, and NMR spectra by a Bruker DRX 400 MHz instrument. The scheme of the OTTLE cell and details concerning HPLC–MS measurements are given in the Supporting Information.

All computations were carried out with the Gaussian 03 package.[25] The B3LYP/6-311++G(d,p) level of theory was used in all computations. Effects arising from solvent polarization were estimated by the polarizable continuum model.[26] Additional computational details are given in the Supporting Information.

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