Selective Enhancement of the One-Electron Oxidation of Guanine by Base Pairing with Cytosine**

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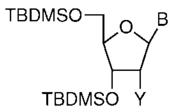
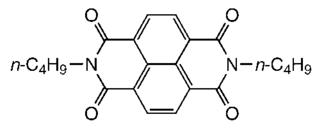
The one-electron oxidation of nucleobases has been extensively studied since it leads to strand scission and oxidative lesions.[1] Guanine, with the lowest oxidation potential,[2] is the base subjected to most oxidation and which leads to the formation of mutagenic 8-oxoguanine and imidazolone.[1a] Various studies suggest there is a lower oxidation potential for guanine in duplex DNA.[3] The π-stacking interactions, especially by the adjacent 5′ guanine residue, have been demonstrated to decrease the ionization potential of guanine.[4] A computational experiment has suggested that the ionization potential of the hydrogen-bonded guanine–cytosine pair is lower than that of guanine alone.[5] Modulation of electron transfer by hydrogen-bonding interactions has been suggested to play an important role in electron-transfer systems such as photosystem II in green plants.[6] However, there have been few experimental studies specifically addressing the effects of hydrogen bonding on the rate of electron transfer in nucleic acids. Since solvation also affects the redox properties of nucleobases,[7] it is difficult to evaluate the effect of hydrogen bonding by comparing the oxidation properties between single-stranded DNA and duplex DNA where the solvation and base stacking are significantly different. Therefore, in order to separate hydrogen bonding from stacking effects we have designed an experiment in dichloromethane which mimics the hydrophobic environment of the base moiety in duplex DNA. We report here the first experimental results of the effect of hydrogen bonding on the one-electron oxidation of nucleobases.

To facilitate the solubility of the nucleosides in dichloromethane substituted nucleoside derivatives with a *tert*-butyldimethylsilyl (TBDMS) group on the ribose unit, that is, tri-*tert*-butyldimethylsilylguanine (**G**), di-*tert*-butyldimethylsilylcytosine (**C**), di-*tert*-butyldimethylsilyladenine (**A**), and di-*tert*-butyldimethylsilylthymine (**T**), were prepared. *N,N′*-dibutylnaphthaldiimide (NDI) in the triplet excited state (^{3}NDI*) was selected as an oxidant for the selective and guanine-specific single electron oxidation that proceeds slower than the diffusion-controlled rate in dichloromethane (the diffusion-controlled rate constant $k_{\mathrm{diff}} = 1.6 \times 10^{10}\,\mathrm{M}^{-1}\,\mathrm{s}^{-1}$) under the present experimental conditions.[8] The third-harmonic oscillation (355 nm, 4 ns, 15 mJ) from a Q-switched Nd:YAG laser was used for laser flash excitation of NDI, and the rate constants of the electron transfer from nucleosides to ^{3}NDI* were measured.



G: B = guanine, Y= TBDMSO
C: B = cytosine, Y= H
T: B = thymine, Y= H
A: B = adenine, Y= H



NDI

A transient absorption spectrum with two bands with maxima at 455 and 485 nm was observed 20 ns after excitation of a solution of NDI in dichloromethane with a laser flash pulse with a wavelength of 355 nm. A depletion of the two absorption bands at 360 and 380 nm corresponding to NDI in the ground state were also evident in the spectrum. The spectrum formed is characteristic of the T_1-T_n absorption of *N,N′*-alkylatednaphtaldiimide derivatives in organic solvents and was assigned to ^{3}NDI* (Figure 1a).[8]Concomitant with the decay of the transient absorption peaks of ^{3}NDI* at 455 and 485 nm (Figure 1b), the formation of a transient absorption in the range of 470–600 nm was observed at 200 ns. The transient absorption spectrum was identical to that observed during the quenching of ^{3}NDI* by the well-known electron

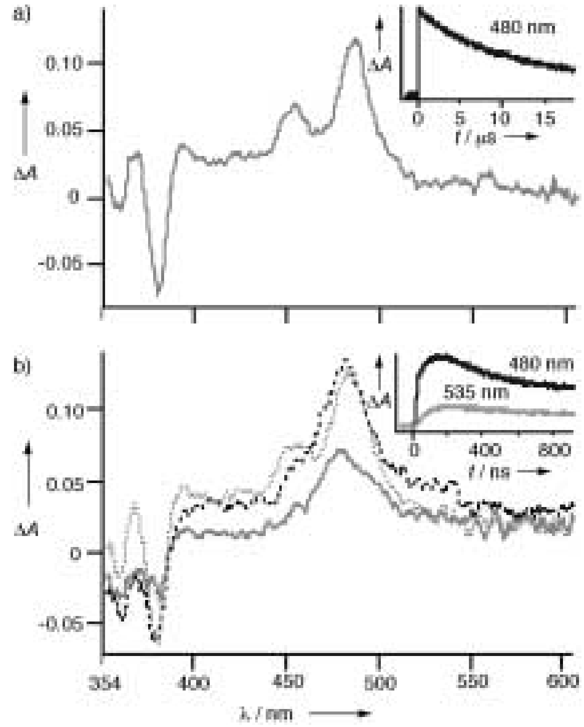


Figure 1. a) Transient absorption spectrum observed 20 ns after the pulsed excitation of 20 μM NDI in argon-saturated dichloromethane. The inset shows the triplet decay observed at 480 nm. b) Time-dependent transient absorption spectra recorded for 20 μM NDI in argon-saturated dichloromethane containing 9 mM **G** and **C** at 20 ns (••••), 200 ns (- - - -), and 1 μs (——) after the pulsed excitation ($\lambda_{\mathrm{exc}} = 355$ nm). The inset shows time profiles of the transient absorption peaks at 480 and 535 nm which correspond to ^{3}NDI* and NDI$^{\bullet-}$, respectively.

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donor 1,4-diazobicyclo[2,2,2]octane (DABCO). Thus the product from the quenching by the nucleosides is identified as the one-electron reduced forms of NDI ($NDI^{\bullet -}$).[9]

The bimolecular quenching plots for the electron-transfer reaction of $^3NDI^*$ with electron donors (D) were obtained from the rate constants for the decay (k_{obs}) at various concentrations of donors such as the nucleosides **G**, **C**, **A**, **T**, DABCO, 1,2,4-trimethoxybenzene (1,2,4-TMB), and 1,4-dimethoxybezene (1,4-DMB; Figure 2a). The rate constants (k_q) for the electron transfer in the forward reaction (from D to $^3NDI^*$) were calculated from the slope of the linear plots (Table 1).

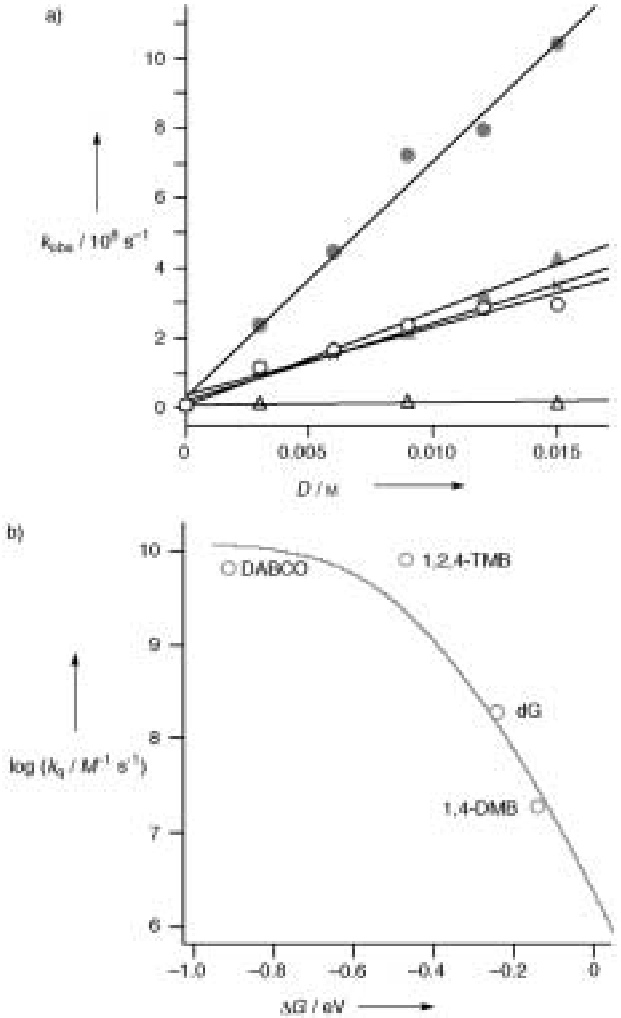

Figure 2. a) Bimolecular quenching plots for the electron-transfer reaction between $^3NDI^*$ and electron donors (D): **G** (○), **C** (△), **G** + **T** (▲), **G** + **A** (×), and **G** + **C** (●). b) Correlation between the intermolecular quenching rate constants (k_q) and free-energy change of the electron transfer ($-\Delta G$). The solid line was calculated according to the Marcus Equation assuming $\lambda = 1.0$ eV and $\tilde{\nu}K_D = 5.0 \times 10^{10}$ cm^{-1}.[15]

Quenching of $^3NDI^*$ by **G** was performed in the presence of **C**, **T**, and **A** to verify the effect of hydrogen bonding between **G** and other nucleosides on the rate constant. Of special interest was that an acceleration of the electron transfer was observed selectively for the **G:C** pair, whereas addition of **T** and **A** gave only negligible changes. The association constant of **G** with **C** was measured to be 6.1×10^3 M^{-1} in $CDCl_3$ by the NMR titration method.[10] Therefore, 80 to 95% of **G** is estimated to form the selective hydrogen bond with **C** under the present experimental conditions. Changing the concentration of **C** from 6 to 15 mM while the concentration of **G** was kept constant at 6 mM had only a negligible effect on k_q.[11] This observation corresponds to a 1:1 complexation between **G** and **C**. Thus, the observed acceleration of the one-electron oxidation of **G** in the presence of **C** is clearly attributed to the presence of the hydrogen bond between **C** and **G**.

Table 1. Bimolecular rate constant (k_q) for the electron-transfer reaction between $^3NDI^*$ and nucleoside analogues together with the electron donors (D) in dichloromethane, oxidation potential ($E_{1/2}$), and free-energy change for the electron transfer (ΔG).

D	k_q [10^8 M^{-1} s^{-1}]	$E_{1/2}$ [V][a]	ΔG [eV]
DABCO	65	0.81[12]	−0.92
1,2,4-TMB	80	1.26[13]	−0.47
G + **C**	6.8	1.37[b]	−0.36
G + **T**	2.7	1.44[b]	−0.29
G + **A**	2.2	1.46[b]	−0.27
G	1.9	1.47[7]	−0.26
1,4-DMB	0.19	1.59[13]	−0.14
A	< 0.05	1.94[7]	0.22
C	< 0.05	2.1[7]	0.38
T	< 0.05	2.1[7]	0.38

[a] Potential versus NHE in CH_3CN. [b] Estimated from k_q according to the relation between k_q and $-\Delta G$ (Figure 2b).

Thermodynamic driving forces of the electron transfer (ΔG) were estimated from the Rehm–Weller Equation [Eq. (1)] by using the reported reduction potential (1.81 V versus the normal hydrogen electrode (NHE))[8] of $^3NDI^*$, and oxidation potenitals of solutions of the donors D in acetonitrile (Table 1).[7, 12, 13] The electrostatic interaction term was estimated to be 0.085 eV.[14]

$$\Delta G = [E(D^{\bullet +}/D) - E(^3NDI^*/NDI^{\bullet -})] + 0.085 \quad (1)$$

The k_q values increased as the value of $-\Delta G$ for the electron transfer increased, which is consistent with the theoretical line obtained from the Marcus equation when it is assumed that $\lambda = 1.0$ eV and $\tilde{\nu}K_D = 5.0 \times 10^{10}$ M^{-1} s^{-1}.[15] From this relationship between k_q and $-\Delta G$ for the donors studied here, $E_{1/2}$ values were estimated for **G** in the presence of **C**, **A**, and **T**. Thus, **G**:**C** base pairing is found to lower the oxidation potential of **G** by 100 mV. The effects of hydrogen-bond interactions between the electron donor, a bacteriochlorophyll dimer, and histidine residues in the bacterial reaction center has been investigated by site-directed mutational experiments.[16] It has been demonstrated that the midpoint potential was increased by 60–125 mV for the addition of each hydrogen bond to histidine. In this case histidine serves as a hydrogen-bond donor which leads to the destabilization of the oxidized state of the electron donor. Since cytosine possess a donor-acceptor-acceptor hydrogen-bonding pattern, it behaves overall as a hydrogen-bond acceptor and results in a decrease in the oxidation potential of guanine upon hydrogen bonding.

In the present study, we have clarified that the oxidation potential of guanine is lowered by selective hydrogen bonding with cytosine. Our results suggest that the rate and direction of electron transfer could be regulated by hydrogen-bonding recognition. Hence, hydrogen bonding may play an important role in biological systems involving electron-transfer processes.

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[10] The ^{1}H NMR spectrum of a 2 mM solution of **G** was recorded to obtain the unbound chemical shift of the N(1)H of **G** (δ_G). The concentration of **G** was kept at 2 mM, and the chemical shift of the N(1)H of **G** (δ_{obs}) was measured in the presence of 0.2–5 mM **C**. The data was fitted to the following equation to determine K_a, where δ_{CG} is the chemical shift of the **G**:**C** complex: $\delta_{obs} = \delta_G + (\delta_{CG} - \delta_G)((0.002 + [\mathbf{C}] + 1/K_a)\text{-}((0.002 + [\mathbf{C}] + 1/K_a)^2 - 0.008[\mathbf{C}])^{1/2})/0.004$. S. C. Zimmerman, W. Wu, Z. Zheng, *J. Am. Chem. Soc.* **1991**, *113*, 196–201. Similarly, the association constant of 2′,3′,5′-tripentanoylguanosine with 4-ethylcytosine has been reported to be approximately $1 \times 10^4\,\mathrm{M}^{-1}$ from NMR titration experiments in $\mathrm{CDCl_3}$: T. J. Murray, S. C. Zimmerman, *J. Am. Chem. Soc.* **1992**, *114*, 4010–4011.

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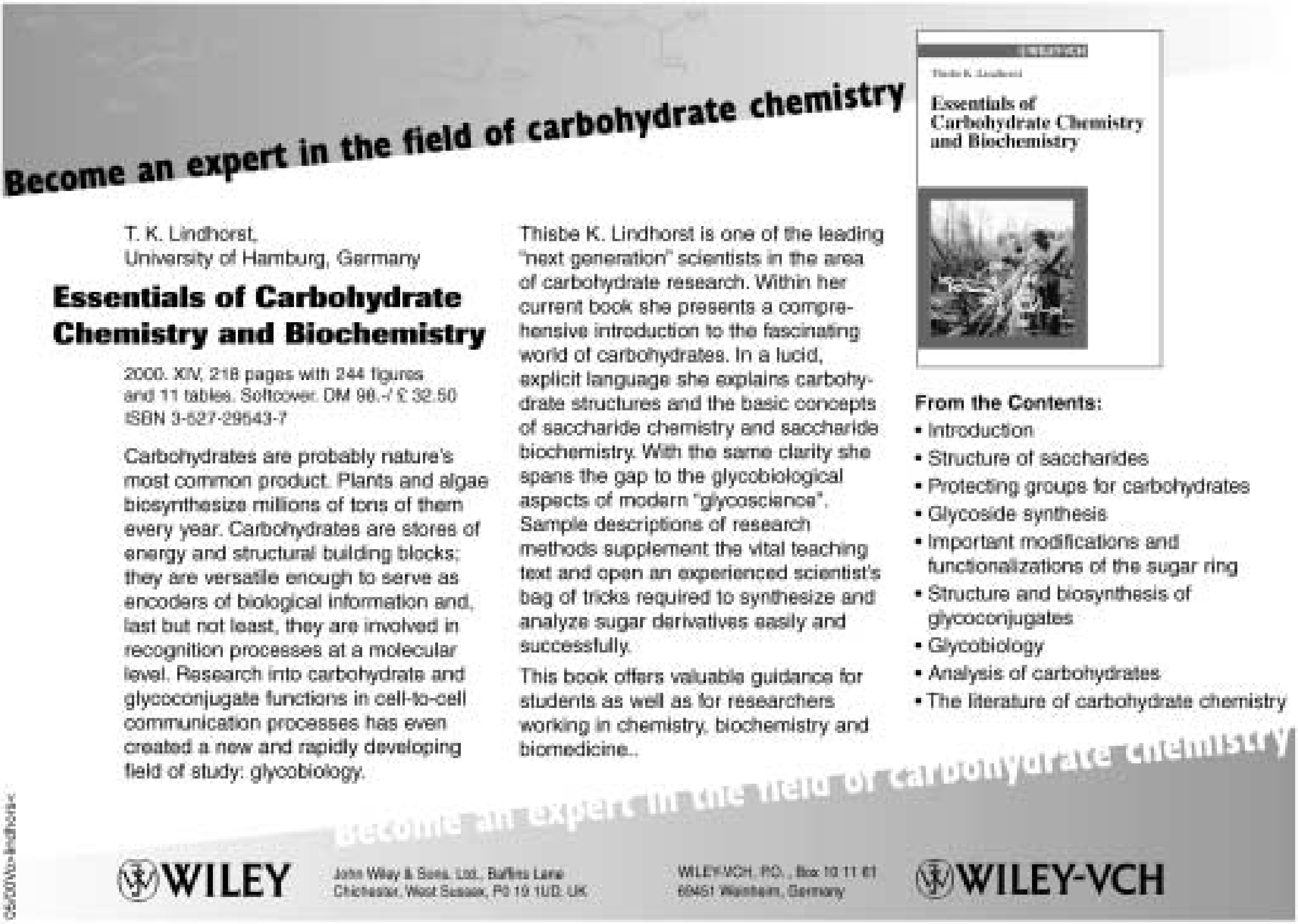

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