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Effects of base pairing on the one-electron reduction rate of cytosine

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Using nucleoside derivatives, which are soluble in dichloromethane, we have experimentally demonstrated that the reduction potential of cytosine is lowered by base pairing with guanine.

The one-electron reduction and oxidation of DNA has been extensively studied as it leads to mutagenic base modification and strand scission that cause carcinogenesis and aging.[1] The one-electron oxidation of DNA yields the radical cation of guanine, the most easily oxidized base, either directly or through hole transfer from the radical cation of adenine.[2–6] Therefore, guanine is the most subject to oxidative damage.[7] In the case of the one-electron reduction process, cytosine and thymine have similar electron affinities and there has been some discussion about which of the pyrimidine bases serves as the electron sink. A computational experiment suggested that the base pairing makes cytosine the base with the most electron affinity.[8] Indeed, a careful EPR experiment using a deuterated-thymine containing duplex oligodeoxynucleotide demonstrated that the radical anion mainly populate in cytosine.[9] However, there have been a few experimental studies specifically addressing the effects of base pairing on the one-electron reduction of nucleic acids. Recently, to evaluate the effect of base pairing on the one-electron oxidation rate of guanine, we designed an experiment in dichloromethane that mimics the hydrophobic environment of the base moiety in duplex DNA.[10–12] Here, the effect of base pairing on the one-electron reduction rate of cytosine and bromocytosine was investigated.

The silylated nucleoside derivatives (Nu) of cytosine (**C**), 5-bromocytosine (**brC**), guanine (**G**), and 8-bromoguanine (**brG**) were used for the experiments in dichloromethane (Fig. 1). As previously reported, more than 85% of the cytosine-derivatives form selective hydrogen bonding with guanine-derivatives under the present experimental conditions.[10] The one-electron reduction rate of cytosine was investigated by a fluorescence quenching experiment. For the one-electron reduction of cytosine derivatives proceeding slower than the diffusion-controlled rate in dichloromethane, pyrene (Py) in the singlet excited state (^{1}Py*) was selected as the reductant.[13,14] Since the fluorescence of pyrene can also be weakly quenched by the oxidative electron transfer from guanine,[15–17] bromocytosine, which has a lower reduction potential, was mainly used to verify the effect of base pairing on the one-electron reduction rate of cytosine.

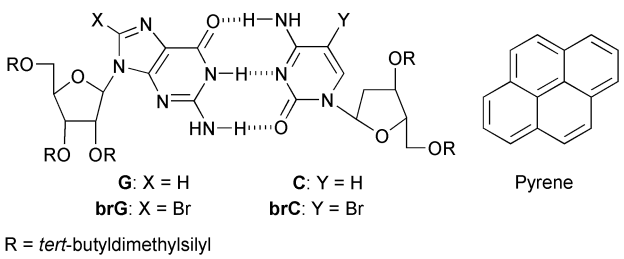

Fig. 1 Structure of cytosine derivatives, guanine derivatives, and pyrene.

The steady-state fluorescence spectra of Py were measured in the presence of **brC**, **G**, and the **brC**:**G** base pair (Fig. 2). **brC** slightly quenched the fluorescence intensity of Py, while negligible quenching was observed for **G**. Interestingly, the fluorescence intensity of Py significantly decreased in the presence of the **brC**:**G** base pair. This indicates an acceleration of the electron transfer from ^{1}Py* to **brC** upon base pairing. The fluorescence lifetimes of Py in the absence (τ_0) and in the presence (τ) of various concentrations of Nu were measured by the single photon counting method. The electron transfer quenching constants were determined using the Stern–Volmer equation (eqn. (1), Table 1),

$$\tau_0/\tau = 1 + k_q\tau_0[\mathrm{Nu}] \quad (1)$$

and the typical Stern–Volmer plots are shown in Fig. 3. Remarkably, the one-electron reduction rate of **brC** was accelerated about 10 times upon base pairing with **G**. This demonstrates a considerable decrease in the reduction potential of cytosine as a result of base pairing.[10–12] When a bromo group was introduced as an electron-accepting group at C8 of guanine, the electron transfer rate was further accelerated. Thus, this enhancement of the fluorescence quenching was attributed to the reductive charge transfer from ^{1}Py* to **brC**.

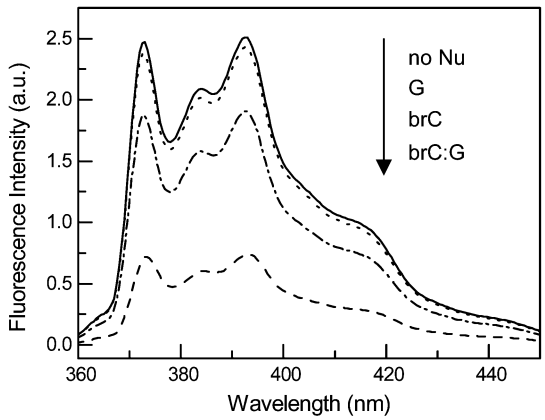

Fig. 2 Fluorescence quenching (λ_{ex} = 337 nm) of ^{1}Py* by **G**, **brC**, and **brC**:**G** base pair (12 mM) in dichloromethane.

Table 1 Bimolecular rate constants (k_q) for electron transfer quenching of ^{1}Py* by nucleoside derivatives

Nu	$k_q/10^8$ M^{-1} s^{-1}	$k_q/10^8$ M^{-1} s^{-1} (+ 5% methanol)
C	0.5	0.1
brC	1.6	3.5
G	< 0.1	0.3
brG	< 0.1	0.7
C:G	4.1	2.7
brC:G	15.2	12.8
C:brG	3.3	2.4
brC:brG	15.8	12.7

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In the case of **C**, acceleration of the quenching rate was also observed upon base pairing with **G**. However, the introduction of a bromo group at guanine resulted in a decrease in the quenching rate. Therefore, the fluorescence quenching of the **C**:**G** base pair is partly a consequence of the oxidative electron transfer from **G** to ^{1}Py*, since base pairing also lowers the oxidation potential of guanine.[10–12] An important observation is that the fluorescence quenching is consistently higher for the **brC**:**G** base pair than for the **C**:**G** base pair. In addition, the introduction of a bromo group at C5 of **C** in the **C**:**G** base pair should significantly decrease the rate of the oxidative electron transfer from **G** to ^{1}Py*.[10,18] Thus oxidative electron transfer from **G** to ^{1}Py* is not as high as the reductive electron transfer from ^{1}Py* to **brC**, and this dominates the observed quenching for the **brC**:**G** base pair. Hence, the fluorescence quenching of the **brC**:**G** base pair is largely a consequence of the reductive electron transfer from ^{1}Py* to **brC**, and this is consistent with the highest fluorescence quenching in the **brC**:**brG** base pair.

Experiments were also carried out in the presence of methanol which interacts with the hydrogen-bonding sites and partly disrupts the base pair formation.[19] The addition of 5% methanol decreased the electron transfer quenching of the base pairing Nu, while it tended to increase that of the non-base pairing Nu. Thus, the observed acceleration of the one-electron reduction of **brC** in the presence of **G** is clearly attributed to the base pairing between **brC** and **G**.

In the guanine:cytosine base pair, guanine serves to decrease the electron density of cytosine upon hydrogen bonding, as predicted by the computational calculations[8] and demonstrated by the results of our present experiments. At the same time, guanine stabilizes the reduced cytosine through protonation of the N3 of cytosine. Indeed, Wagenknecht *et al*. demonstrated that the one-electron reduction of cytosine in water is coupled to the proton transfer process.[20,21] Therefore, guanine may also aid in the proton-coupled electron-transfer reaction. Such a proton-coupled process was also demonstrated to contribute to the one-electron oxidation of DNA,[22–24] hole transfer,[25–28] and excess electron transfer in DNA.[29]

In summary, we have experimentally demonstrated that the reduction potential of cytosine is lowered by base pairing with guanine, thus base pairing makes cytosine the base with the most electron affinity in duplex DNA. Our results further support the importance of hydrogen bonding and/or proton transfer in the guanine:cytosine base pair for the one-electron redox properties of DNA.

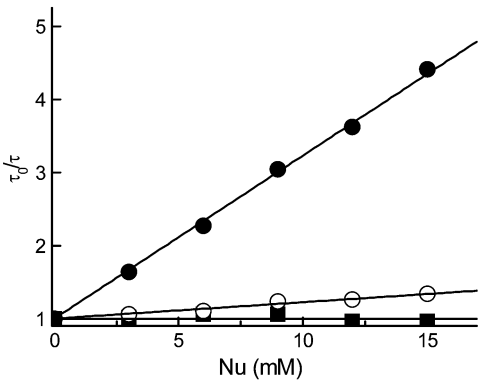

Fig. 3 Bimolecular quenching plots for the electron transfer reaction between ^{1}Py* and nucleoside derivatives (Nu) as electron acceptors: **brC** (○), **G** (■), and **brC**:**G** base pair (●).

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