Determination of the Quenching Rate Constants of Singlet Oxygen by Derivatized Nucleosides in Nonaqueous Solution

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Accurate determination of the rate constants for chemical reaction, k_R, and physical quenching, k_Q, of singlet oxygen, $O_2(^1\Delta_g)$, by the different nucleosides of DNA and RNA is necessary to understand photodynamic DNA damage.[1−3] Both chemical and physical interactions with singlet oxygen are important, and the ratio of these rate constants is connected to the balance between biological protection and damage.[4] Although photodynamic damage is usually considered as acting mainly *via* membrane damage, DNA damage can also occur. The main process by which DNA damage is induced is the singlet oxygen mechanism (type II photooxidation). A clear relation between induction of single-strand breaks and cellular uptake of photodynamic dyes has been established.[5]

While the photooxidation of guanosine has been extensively studied,[6,7] and its total quenching constant accurately determined ($k_R + k_Q = 5.3 \times 10^6\ M^{-1}\ s^{-1}$ for dG in an aqueous system),[8,9] the rate constants for the rest of the nucleosides are far lower, but either have not been reported or have a large uncertainty because of their small magnitude ($k_R + k_Q < 5 \times 10^5\ M^{-1}\ s^{-1}$) and the rapid competing decay of singlet oxygen in water and other protic solvents.[8,10] To measure these rate constants, we chose a solvent in which singlet oxygen has a long lifetime and used lipophilic derivatives of the nucleosides. We demonstrate later that, at least for guanosine, the rate constants are relatively independent of solvent and functionalization.

Scheme 1 shows the sensitized generation of singlet oxygen and the different pathways of its deactivation in solution. Since the type I mechanism is not likely to occur at low substrate concentration,[11] the reaction is expected to proceed via singlet oxygen. The value for the total rate constant ($k_R + k_Q$) for singlet oxygen quenching is given by the expression for the observed rate of decay of singlet oxygen luminescence, $k_{obsd} = k_d + (k_R + k_Q)[Q]$, where $(k_R + k_Q)$ is the slope of the plot of k_{obs} vs [Q] and k_d is its intercept.

The solvent was Freon 113 (1,1,2-trichlorotrifluoroethane), in which singlet oxygen has a very long lifetime ($\tau_\Delta \approx 18$ ms)[12,13] and which therefore allows measurements of small quenching rate constants. Because of the insolubility of the nucleosides in this solvent, derivatives were used. The derivatives chosen were the 2′,3′,5′-tris((*tert*-butyldimethylsilyl)oxy) derivatives of guanosine (**1**), adenosine (**2**), cytidine (**3**), and uridine (**4**) and 3′,5′-bis((*tert*-butyldimethylsilyl)oxy)thymidine (**5**), synthesized by procedures similar to that described by Sheu *et al.* for guanosine.[7] The guanosine derivative **1** has been previously reported[9] to give rate constants almost identical to those for guanosine (Scheme 2).

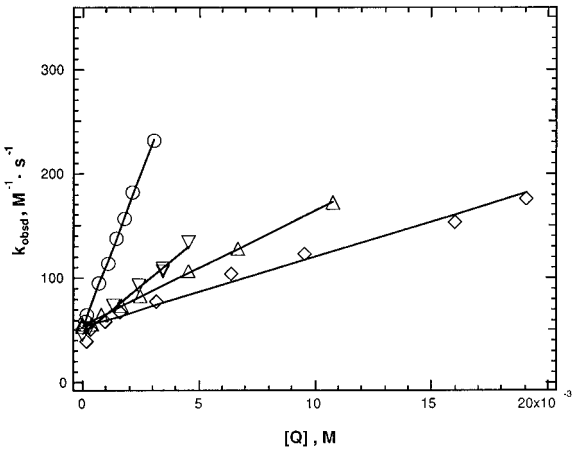
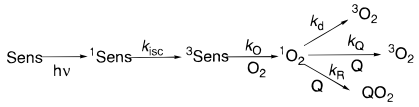
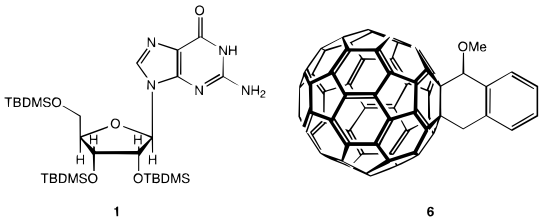


Figure 1. Observed rate constant (k_{obsd}) for luminescence decay of singlet oxygen for derivatives **2** (▽), **3** (○), **4** (△), and **5** (◇) in 1,1,2-trichlorotrifluoroethane. The plot for guanosine derivative **1** is off scale and was omitted from the plot (intercept 63.1 $M^{-1}\ s^{-1}$, $r^2 = 0.985$).

Scheme 1



Scheme 2



A sensitizer suitable for measuring such low rate constants must have a very low rate constant for singlet oxygen quenching and a high quantum yield of singlet oxygen formation (ϕ_Δ). Both conditions are met with C_{60} ($k_Q < 5 \times 10^5\ M^{-1}\ s^{-1}$, $\phi_\Delta \approx 1$).[14,15] However, C_{60} is only slightly soluble in 1,1,2-trichlorotrifluoroethane, and a derivative (**6**), also an excellent sensitizer, which also meets these criteria, was used instead.[16,17]

Stock solutions of **1** (1.7×10^{-2} M), **2** (2.4×10^{-2} M), **3** (5.3×10^{-2} M), **4** (6.6×10^{-2} M), and **5** (6.4×10^{-2} M) were prepared, and aliquots were added to various solutions containing **6** ($A_{355} < 0.3$) (Figure 1). The derivatives do not form aggregates at the concentrations used except for **2**, where the linearity limit of Beer's Law plots is 2×10^{-3} M. The samples were excited at 355 nm with low energy pulses (<100 μJ), and

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(13) Lifetimes up to 22 ms have been observed in this experiment and set a new value for the singlet oxygen lifetime (τ_Δ) in this solvent because of the extremely low $^1O_2$ quenching rate constant of the fullerene derivative.
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Table 1. Rates of Total Quenching of $O_2(^1\Delta_g)$ by the Nucleoside Derivatives in 1,1,2-Trichlorotrifluoroethane

substrate	related nucleoside	$(k_R + k_Q)/M^{-1}\ s^{-1}$
1	guanosine	$(3.0 \pm 0.2) \times 10^6$
2	adenosine	$(1.8 \pm 0.1) \times 10^4$
3	cytidine	$(5.8 \pm 0.1) \times 10^4$
4	uridine	$(1.1 \pm 0.1) \times 10^4$
5	thymidine	$(6.9 \pm 0.3) \times 10^3$

the 1270 nm emission of singlet oxygen was detected as previously described (Table 1).[7]

The value of $(3.0 \pm 0.2) \times 10^6\ M^{-1}\ s^{-1}$ for $(k_R + k_Q)$ in 1,1,2-trichlorotrifluoroethane observed for **1** is in agreement with that previously reported for the same compound by Sheu *et al.*,[9] since it falls between the reported values $1.75 \times 10^6\ M^{-1}\ s^{-1}$ in benzene-d_6 and $6.33 \times 10^6\ M^{-1}\ s^{-1}$ in acetone-d_6. It is also close to both the values of $5.3 \times 10^6\ M^{-1}\ s^{-1}$ for dG in an aqueous system[8] and $\approx 5 \times 10^6\ M^{-1}\ s^{-1}$ for dG, GMP, and dGMP in the same system.[10] Therefore, the values for the rest of the nucleoside derivatives found in this low-polarity solvent are also likely to be close to those with the free sugar in more polar ones. The values of total quenching for the rest of the nucleosides also agree with the previously reported 2.8×10^4 to $1.4 \times 10^5\ M^{-1}\ s^{-1}$ range.[10]

The reaction rates k_R for all but the guanosine derivative are too low to be accurately determined. Compounds **2**−**4** do not show any measurable reactivity toward singlet oxygen even on long irradiation, even though singlet oxygen has a lifetime on the order of milliseconds and a corresponding reactivity greater by $\sim 10^3$ than that in an aqueous system.[2,18] Therefore $k_R + k_Q \approx k_Q$ for these compounds. Exhaustive photooxygenation of **5** yields a very small amount of unidentified products. However, the reaction rate constant is too low to be measured accurately and is probably also negligible. The value of k_R for the guanosine derivative has been determined to be $(4.8 \pm 0.5) \times 10^4\ M^{-1}\ s^{-1}$ in 1,1,2-trichlorotrifluoroethane, about 1.6% of $(k_R + k_Q)$. This value is comparable to the percentage previously found for acetone-d_6 (2%).[9]

What is important about the results is not the absolute value of the total quenching, but the relative reactivity of the bases with singlet oxygen, which is G ≫ C > A > U > T, in the ratio 100:2.0:0.6:0.4:0.2. The magnitude of these ratios gives a qualitative idea of the selectivity for singlet oxygen mediated cleavage (without selective binding). Specific binding may alter this ratio significantly.[19] Furthermore, the number of reactions involved in this process makes it difficult to establish the real ratios of cleavage. Only a small fraction (10^{-2} to 10^{-3}) of the reactions between guanosine and $^1O_2$ seems to result in single-strand breaks or alkali labile sites.[5] However, this result rules out the possibility of cutting DNA with singlet oxygen at any base sequence that does not contain guanosine.

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(18) Photooxygenations of **2**−**5** were performed in $CDCl_3$ with TPP (5,10,15,20-tetraphenyl-21*H*,23*H*-porphine, 0.1 mM) or **6** as sensitizers and 2-methyl-1-pentene as a competitor, and their relative rate of disappearance was monitored by NMR. For the determination of the reaction rates of **1** in 1,1,2-trichlorotrifluoroethane, the value of k_R of the competitor 2-methyl-2-pentene (which is known to react only chemically) in this solvent was previously determined by monitoring the $^1O_2$ luminescence as described above ($k_R = 1.8 \times 10^6\ M^{-1}\ s^{-1}$). For the competition experiment between **1** and 2-methyl-2-pentene, a few drops of $CDCl_3$ were added for internal lock and C_{70} was used as the sensitizer.

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