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Reactivity of Nucleosides with a Hydroxyl Radical in Non-aqueous Medium

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The hydroxyl radical (HO•) is an important reactive oxygen species (ROS), which is usually present at very low levels in biological systems, mainly arising from oxygen metabolism. Its concentration is markedly enhanced upon exposure of cells to exogenous chemical and physical agents, such as ionizing radiation. It is well established that HO• mediated damage to biomolecules is involved in deleterious phenomena such as aging, chronic inflammation, ischemia, autoimmune disease, cancer.[1] Indeed, DNA oxidation mediated by HO• may lead to sugar and base modifications that threaten genomic integrity due to their mutagenic potential.[2,3]

From a chemical point of view, HO• is an electrophilic radical that reacts with most targets at high rates. It may undergo addition to nucleobases and, in the case of thymine or guanine, H-abstraction from the C-5 methyl or the C-2 amino group, respectively.[3,4] In general, the reactivity of HO• with bases, nucleosides or nucleotides has been investigated in aqueous medium by means of ionizing radiation (γ-radiolysis) or by the Fenton reaction with iron(II) and hydrogen peroxide. Interestingly, radiation experiments lead mainly to nucleobase oxidation, whereas Fenton chemistry favors hydrogen abstraction from the sugar. Thus, the reaction conditions appear to play an important role in oxidatively generated DNA damage mediated by HO•. Indeed, hydrogen abstraction by HO• from ethanol or isopropanol is 25 times slower in acetonitrile than in water,[5] whereas addition of HO• to an aromatic system such as naphthalene is only 5 times slower in the organic solvent.[6] Therefore, it seems meaningful to investigate whether the reactivity of HO• with nucleosides in non-aqueous systems is actually dominated by addition to the base.

In this context, considerable effort has been devoted in recent years to include nucleic acids within lipophilic environments, such as liposomes (lipoplexes) and polymers (polyplexes) as vectors for gene delivery, in the non-viral approaches to gene therapy.[7] To enhance the transfection efficiency, photochemical internalization has been developed.[8] It is based on improved endolysosomal release by photoactivation of a sensitizer. However, a limitation of this technique relies on the potential loss of gene integrity by oxidatively generated damage.[9]

A quantitative estimation of the reactivity of HO• with nucleic acids or their building blocks can be obtained by determining the reaction rate constants ($k_{HO^\cdot}$). In aqueous medium, the $k_{HO^\cdot}$ values have been determined by means of pulse radiolysis; for nucleosides they range from 4.0 to $6.0\times10^9\,M^{-1}s^{-1}$, close to the diffusion control limit.[4b,10,11]

Related kinetic information in non-aqueous medium is essentially lacking. Hence, it seems relevant to check for the stability of nucleic acid components against oxidative degradation within lipophilic gene delivery vectors. Reactivity of DNA bases through another type of mechanism, that is, one electron oxidation, has been recently reported in organic solvents.[12] In the present work, acetonitrile has been selected as a simple non-aqueous system. An important advantage of this solvent is that it exhibits a low reactivity towards HO• ($k_{HO^\cdot}$ ca. $10^6\,M^{-1}s^{-1}$).[6] In addition, the diffusion rate constant in acetonitrile is higher than in water; this would provide a broader dynamic range for a possible differentiation between the intrinsic reactivity of the nucleobases.

Here, five nucleosides and three purine-derived lesions have been taken as targets for HO• attack. As their solubility in acetonitrile is insufficient to reach the concentrations required for kinetic experiments, they have been employed as their silylated 2′-deoxyribonucleosides (Figure 1, see preparation in the Supporting Information).

The reactivity with HO• radical was determined by laser flash photolysis (LFP) of *N*-hydroxypyridine-2-thione (NPT) at 355 nm (Scheme 1).[13] Homolytic cleavage of this thione generated HO• together with the unreactive pyrithiyl radical ($\lambda_{max}=490$ nm), which provided a quantitation of the process. Since HO• is undetectable, *trans*-stilbene (TS) was used as a trap; the adduct [TS-OH]• exhibited a maximum at 390 nm.[5,6,13,14] The kinetic traces at this wavelength were monitored in the absence and in the presence of increasing amounts of the modified nucleosides (0–34 mM). Following an established kinetic model, the rate constants for the reaction between HO• and the modified nucleobases were determined by using naphthalene as a standard.

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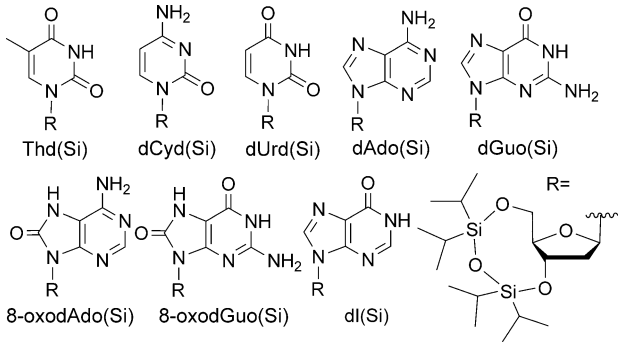
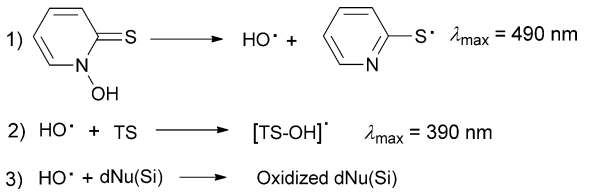


Figure 1. Structure of the modified 2′-deoxyribonucleosides dNu(Si) synthesized for the kinetic studies in acetonitrile.



Scheme 1. Experimental approach to determine the rate constants for the reaction of HO• with the 2′-deoxyribonucleosides dNu(Si).

The traces due to [TS-OH]• in the presence of increasing concentrations of Thd(Si) are shown in Figure 2 A. The competitive Stern–Volmer analysis for the three modified nucleosides (together with naphthalene) is given in Figure 2 B. It was performed by plotting the ratio between the transient absorbance at 390 nm in the absence (ΔA_o) and in the presence (ΔA) of each substrate versus concentration.

Determination of the absolute rate constants for the reaction between the modified nucleosides and HO• (Table 1) was based on the comparison between the obtained slopes and the known absolute rate constant for naphthalene ($k_{HO^\bullet} = 1.8 \times 10^9\ M^{-1}s^{-1}$).[14a]

The values obtained for the silylated 2′-deoxyribonucleosides in acetonitrile are of the same order of magnitude and remarkably lower than diffusion control in this solvent, pointing to a similar reactivity pattern. Furthermore, they are in general slightly lower than those reported in the literature for the natural nucleosides in water. It has been reported that HO• addition to aromatics is much faster in water than in acetonitrile, due to a differential stabilization of the relatively polar transition state.[6] In our case, this effect should be less pronounced, since the substrates are already markedly polar.

To determine whether the base or the sugar moiety are involved in the reaction with the HO• radical, steady-state irradiations of the 2′-deoxyribonucleosides dNu(Si) were carried out in the presence of equimolar amounts of NPT as a source of HO• in air-equilibrated acetonitrile. The resulting reaction mixtures were analyzed by UPLC-MS; typical chromatographic patterns are shown in Figure 3 for the case of Thd(Si). It became clear that the reaction takes place at

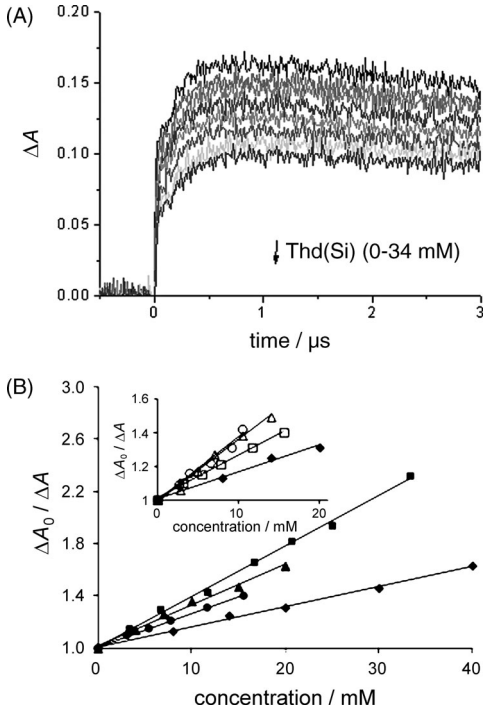


Figure 2. A) Kinetic traces recorded at 390 nm after laser flash photolysis ($\lambda_{exc} = 355$ nm) of NPT (0.29 mM), in the presence of TS (7.5 mM) and increasing concentrations of Thd(Si) in deaerated acetonitrile solutions. B) Stern–Volmer plots obtained as the ratio $\Delta A_o/\Delta A$ of the traces measured at 390 nm versus concentration of each modified nucleoside, Thd(Si) (■), dCyd(Si) (▲), dUrd(Si) (●) and naphthalene (◆), used as a reference. Inset: Stern–Volmer plots of dAdo(Si) (△), 8-oxodAdo(Si) (○), dI(Si) (□) and naphthalene (◆).

Table 1. Rate constants for the reaction of HO• with 2′-deoxyribonucleosides.

Nu(Si)	$k_{HO^\bullet}$ [$M^{-1}s^{-1}$] CH_3CN[a]	$k_{HO^\bullet}$ [$M^{-1}s^{-1}$] H_2O[a]
Thd(Si)	4.0	4.7[b]
dCyd(Si)	3.3	6.0[b]
dUrd(Si)	3.0	5.2[b]
dAdo(Si)	3.6	4.6[b]
dGuo(Si)	n.d.[c]	4.1[b]/5.7[d]
8-oxodAdo(Si)	4.7	4.3[e]
8-oxodGuo(Si)	n.d.[c]	4.8[e]
dI(Si)	2.9	–

[a] Rate constants are stated values $\times 10^9$. [b] These values correspond to the natural 2′-deoxyribonucleosides from ref. [10]. [c] Not determined: compounds not soluble in CH_3CN. [d] This value corresponds to the natural 2′-deoxyguanosine from ref. [4b]. [e] These values correspond to the 8-oxo-7,8-dihydro-2′-deoxyguanosine and 8-oxo-7,8-dihydro-2′-deoxyadenosine from ref. [11].

the base, since selected ion monitoring (SIM) at m/z 127.05 ± 0.05 (corresponding to unaltered thymine) gave a trace identical to unreacted starting material (Figure 3 A). Conversely, no significant reaction at the sugar moiety was detected, as demonstrated by the facts that 1) no free thymine was observed (see the Supporting Information), and 2) SIM at m/z 359.21 ± 0.05 (corresponding to unaltered silylated 2-deoxyribose) gave a complex chromatogram with two main peaks (1 and 2), in addition to starting material

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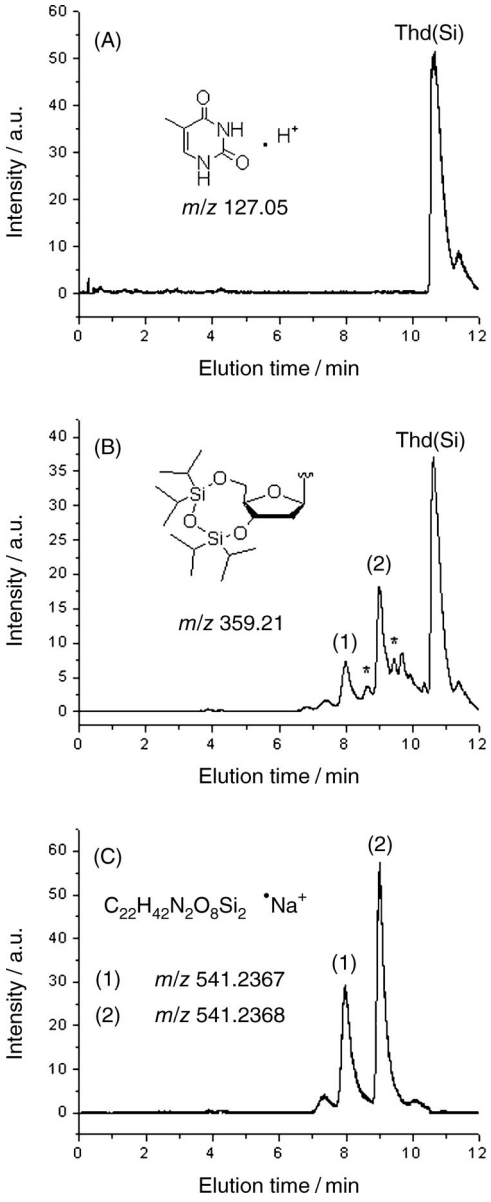

Figure 3. UPLC-MS analysis of the reaction mixture obtained after steady-state UVA-irradiation (60 min) of NPT (50 mM) in the presence of equivalent amounts of Thd(Si) in air-equilibrated acetonitrile. A) Selected ion monitoring (SIM) at m/z 127.05 ± 0.05, corresponding to the unaltered base; B) SIM at m/z 359.21 ± 0.05, corresponding to the unaltered sugar; C) SIM at m/z 541.23 ± 0.05, corresponding to the base peak of 1 and 2. Exact mass, together with the molecular formulae, are given.

(Figure 3B). Exact mass analysis of peaks 1 and 2 revealed that they are isobaric, with a molecular formula $C_{22}H_{42}N_2O_8Si_2$ (Figure 3C). This is compatible with the formation of thymidine glycols, which are well-known products of HO$^{\bullet}$ addition to natural thymidine in aerated solution. Moreover, 5-formyl-2′-deoxyuridine and 5-(hydroxymethyl)-2′-deoxyuridine (marked with an asterisk in Figure 3B) resulting from H-abstraction from the C5 methyl group were also observed as minor products (see the Supporting Information), whereas formamide or 5-hydroxy-5-methylhydantoin derived from the cleavage of the 5,6-bond were not detected (see the Supporting Information).

In summary, the reaction of a hydroxyl radical with 2′-deoxyribonucleosides is in general somewhat slower in acetonitrile than in water. Therefore, loss of gene integrity by oxidatively generated damage in lipoplexes or polyplexes is expected to be similar or even slightly lower than in aqueous medium. In addition, the main reaction pathway involves addition to the nucleobase, rather than hydrogen abstraction from the sugar. This is interesting in connection with the reactivity of nucleic acids within lipophilic gene delivery vectors in non-viral approaches to gene therapy.

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