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Fate of the 2′-Deoxyadenosin-5′-yl Radical under Anaerobic Conditions

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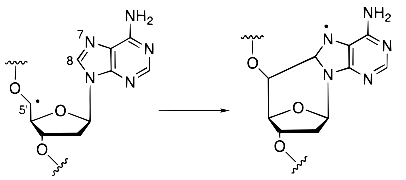
Free radicals and ionizing radiations induce damage in the DNA of living cells resulting in mutation, chromosomal aberration, carcinogenesis, and aging.[1] Understanding the chemical nature of radiation/free radical-induced DNA lesions and the elucidation of the mechanisms which are involved in DNA damage are essential for the assessment of the possible biological consequences and functions of the enzymatic repair processes. 5′,8-Cyclic adenine derivatives are observed among the decomposition products of DNA,[2] nucleotides,[3] and nucleosides[4] when irradiated in deaereated aqueous solutions. These adducts, when formed in DNA, could have significant biological impact on the function and conformation of the double helix. It has been proposed that HO• radicals from the radiolysis of water might generate a C5′ radical, which intramolecularly attacks the C8,N7 double bond of the adenine moiety, thus explaining the formation of 5′,8-cyclo-2′-dAdo (Scheme 1).[2−4] Depending on the substrate and the experimental conditions the ratio of the (5′*S*)- and (5′*R*)-isomers changes substantially.[5] The synthesis of (5′*S*)-5′,8-cyclo-2′-dAdo was recently obtained in seven steps starting from *N*^{6}-benzoyl-2′-dAdo in an overall yield <10%,[6] whereas the (5′*R*)-isomer was isolated in very low yields either from photolysis (at 254 nm)[6] of 8-bromo-2′-dAdo or the γ-radiolysis[4] of 2′-dAdo in a deaereated aqueous solution.[7]

We report herein a selective generation of C5′ radical, its fate under anaerobic conditions, and an efficient one-pot synthesis of (5′*R*)-5′,8-cyclo-2′-dAdo.

One hundred milliliters of a deaereated aqueous solution containing 1.5 mM of 8-bromo-2′-dAdo (**1**), 0.25 M *t*-BuOH, and 4 mM $K_4Fe(CN)_6$ at pH ~7 was γ-irradiated with a total dose up to 1.5 kGy at a dose rate of 25 Gy/min.[8,9] Workup of the reaction mixture afforded the cyclic nucleosides **2** and **3** in 79 and 13% yield, respectively (Scheme 2).[11]

Scheme 1



Additional experiments were performed: (i) The analogous reaction without $K_4Fe(CN)_6$ afforded **2** in 26% yield [$G(\mathbf{2}) = 0.82 \pm 0.03$], whereas the radiation chemical yield for the formation of bromide was equal to the consumption of **1** [$G(-\mathbf{1}) = G(Br^-) = 3.10 \pm 0.30$];[12] traces of 2′-dAdo were also detected. (ii) Regarding this latter experiment, when $K_4Fe(CN)_6$ was added to the reaction mixture after its irradiation and after a standing time equal to the irradiation time, no changes were detected with respect to the product yields. The absence of the post-irradiation effect means that the Fe^{2+} must be involved in the free radical transformation. (iii) γ-Radiolysis of 2′-dAdo in N_2O-saturated aqueous solution gave rise to **2** in 10% yield.[13] (iv) Formation of $Fe(CN)_6^{3-}$ in steady-state experiments was checked spectroscopically at 420 nm[14] and was found to be negligible.

Rationalization of the results suggests the reaction mechanism drawn in Scheme 2. The electron adduct undergoes fast bromide release, giving a vinyl- or aryl-type radical **4**. Such a reaction is well-established for bromo derivatives including bromouracil.[15,16] Radical **4** abstracts a hydrogen atom either from *t*-BuOH to give 2′-dAdo[17] (traces detected by HPLC) or intramolecularly from the 5′ position to generate the C5′ radical (**5**). Cyclization of radical **5** is expected to produce a mixture of radicals **6** and **7**, i.e., a mixture of the (5′*S*)- and (5′*R*)-isomers with defined stereochemistry at C8. Aromatic aminyl radicals such as **6** and **7** can be reduced by Fe^{2+} followed by fast protonation to give compounds **8** and **9**, respectively. Compound **9** can readily eliminate H_2O (the OH group in the 5′ position being in the anti arrangement with the H atom in the 8 position) and give the cyclonucleoside **3** after tautomerization. On the other hand,

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(1) For some relevant reviews, see: (a) Lindahl, T. *Nature* **1993**, *362*, 709. (b) Burrows, C. J.; Muller, J. G. *Chem. Rev.* **1998**, *98*, 1109.

(2) (a) In DNA: Dirksen, M.-L.; Brakely, W. F.; Holwitt, E.; Dizdaroglu, M. *Int. J. Radiat. Biol.* **1988**, *54*, 195. (b) In polyA: Fuciarelli, A. F.; Shum, F. Y.; Raleigh, J. A. *Biochem. Biophys. Res. Commun.* **1986**, *134*, 883.

(3) (a) Keck, K. *Z. Naturforsch.* **1968**, *23B*, 1034. (b) Raleigh, J. A.; Kremers, W.; Whitehouse, R. *Radiat. Res.* **1976**, *65*, 414. (c) Cadet, J.; Berger, M. *Int. J. Radiat. Biol.* **1985**, *47*, 127. (d) Raleigh, J. A.; Fuciarelli, A. F. *Radiat. Res.* **1985**, *102*, 165.

(4) Marriagi, N.; Cadet, J.; Teoule, R. *Tetrahedron* **1976**, *32*, 2385.

(5) For example, the (5′*R*)-isomer predominates in ss-DNA, whereas (5′*S*)-isomer slightly prevails in ds-DNA.[2a]

(6) Romieu, A.; Gasparutto, D.; Molko, D.; Cadet, J. *J. Org. Chem.* **1998**, *63*, 5245.

(7) 2′-dAdo is an abbreviation of 2′-deoxyadenosine.

(8) In all systems studied free radicals were generated by means of radiolysis of deaereated aqueous solutions containing **1** and *t*-BuOH. Radiolysis of neutral water leads to the species e_{aq}^- (2.6) HO• (2.7) and H• (0.6) where the values in parentheses represent the yields expressed in terms of *G*-values (molecules/100 eV of absorbed radiation).[10] In the presence of *t*-BuOH, HO• radicals are scavenged with a rate constant of $6.0 \times 10^8\ M^{-1}\ s^{-1}$ to give $Me_2C(OH)CH_2^\bullet$ and H_2O.[10]

(9) $K_4Fe(CN)_6$ was chosen as a suitable electron donor since it reacts slowly with e_{aq}^-.[10]

(10) Buxton, G. V.; Greenstock, C. L.; Helman, W. P.; Ross, A. B. *J. Phys. Chem. Ref. Data* **1988**, *17*, 513 and references therein. Ross, A. B.; Mallard, W. G.; Helman, W. P.; Buxton, G. V.; Huie, R. E.; Neta, P. *NDRL-NIST Solution Kinetic Database*, ver. 3; Notre Dame Radiation Laboratory, Notre Dame, IN and NIST Standard Reference Data: Gaithersburg, MD, 1998.

(11) Workup: The reaction mixture was lyophilized, and the residue was taken up in water and purified by reverse-phase column chromatography, using a peristaltic pump, a UV detector (RP18, eluted in water with a 0−30% methanol nonlinear gradient over 5 h at a flow rate of 4 mL/min, detector at 260 nm). The appropriate fractions were collected and lyophilized to give pure compounds **2** and **3**. The spectral data of **2** and **3** were comparable to those reported in the literature.[6] Yields were based on the recovered starting bromide.

(12) The quantitative analysis of the products was performed by HPLC injection using authentic samples. In the presence of $K_4Fe(CN)_6$ the $G(\mathbf{2})$ was found to be 2.44 ± 0.14.

(13) Similar results have been reported.[4]

(14) The expected $K_3Fe(CN)_6$ product can be conveniently monitored by UV−Vis spectroscopy at 420 nm ($\epsilon = 1020\ M^{-1}\ cm^{-1}$), see: Schuler, R. H.; Hartzell, A. L.; Behar, B. *J. Phys. Chem.* **1981**, *85*, 192.

(15) Nese, C.; Yuan, Z.; Schuchmann, M. N.; von Sonntag, C. *Int. J. Radiat. Biol.* **1992**, *62*, 527.

(16) Sugiyama, H.; Fujimoto, K.; Saito, I. *J. Am. Chem. Soc.* **1995**, *117*, 2945. Sugiyama, H.; Fujimoto, K.; Saito, I.; Kawashima, H.; Sekine, T.; Ishido, Y. *Tetrahedron Lett.* **1996**, *37*, 1805. Cook, G. P.; Greenberg, M. M. *J. Am. Chem. Soc.* **1996**, *118*, 10025. Sugiyama, H.; Fujimoto, K.; Saito, I. *Tetrahedron Lett.* **1997**, *38*, 8057. Fujimoto, K.; Sugiyama, H.; Saito, I. *Tetrahedron Lett.* **1998**, *39*, 2137.

(17) Similar reactions were observed in the case of bromouracil derivatives, see; Adams, G. E.; Willson, R. L. *Int. J. Radiat. Biol.* **1972**, *22*, 589. Patterson, L. K.; Bansal, K. M. *J. Phys. Chem.* **1972**, *76*, 2392. Bansal, K. M.; Patterson, L. K.; Schuler, R. H. *J. Phys. Chem.* **1972**, *76*, 2386. Bhatia, K.; Schuler, R. H. *J. Phys. Chem.* **1973**, *77*, 1888. Wagner, B. O.; Klever, H.; Schulte-Frohlinde, D. *Z. Naturforsch.* **1974**, *28b*, 86. Rivera, E.; Schuler, R. H. *J. Phys. Chem.* **1983**, *87*, 3966.

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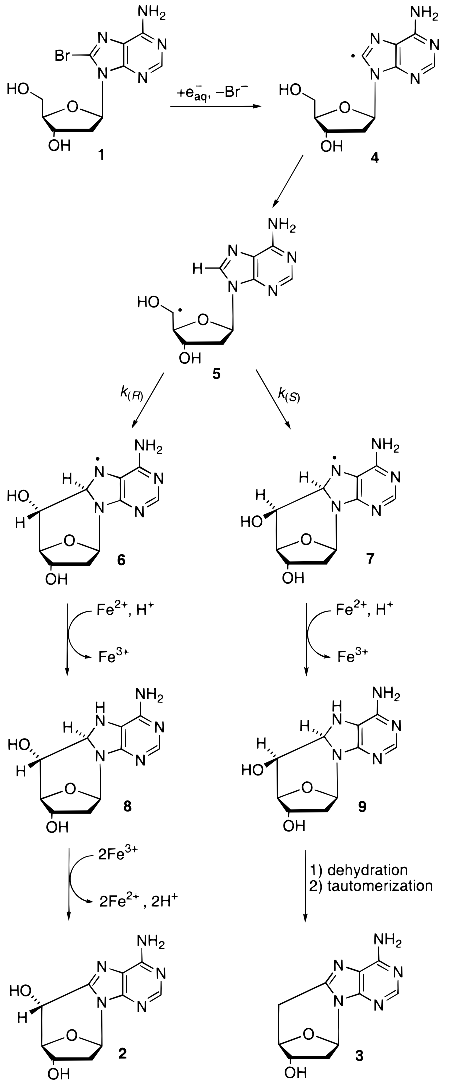
Scheme 2



compound **8**, for which the dehydration is not allowed, can be oxidized by Fe^{3+} to afford the (5′*R*)-5′,8-cyclo-2′-dAdo (**2**).[18]

To acquire more evidence for the proposed mechanism in Scheme 2 we also carried out pulse radiolysis studies. A rate constant of 1.5×10^{10} M^{-1} s^{-1} was determined for the reaction of **1** with e_{aq}^- at pH ∼7 by measuring the rate of the optical density decrease of e_{aq}^- at 720 nm ($\epsilon = 1.9 \times 10^4$ M^{-1} cm^{-1})[20] as a function of [**1**]. Compared with the analogous reaction with 2′-dAdo ($k = 8.2 \times 10^9$ M^{-1} s^{-1}),[21] the presence of bromine increased the rate constant of the reduction by a factor of 2. The reaction of a 1 mM solution of **1** with e_{aq}^- was complete in ∼0.3 μs. At this time, no significant absorption was detected in the 300−750 nm region. However, a spectrum containing two bands centered at 360 and 470 nm, respectively, developed in 17 μs (Figure 1). An extinction coefficient of $\sim 1 \times 10^4$ M^{-1} cm^{-1} at 360 nm was calculated. The time profile for the formation of the transient with $\lambda_{max} = 360$ nm (Figure 1, inset) follows the first-order kinetics with a rate constant of 2.5×10^5 s^{-1} which was independent of [**1**] and the dose/pulse. Furthermore, no kinetic isotopic effect was found by replacing H_2O with D_2O as the reaction medium.[22] The transient decays by second-order kinetics (first half-life ∼0.5 ms), giving species that do not absorb significantly.

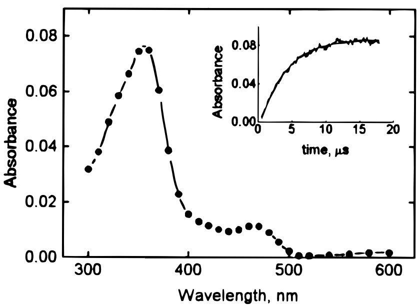
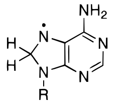


Figure 1. Absorption spectrum obtained from the pulse radiolysis of an Ar-purged solution containing 1 mM 8-bromo-2′-dAdo and 0.25 M *t*-BuOH at pH ∼7, taken 17 μs after the pulse; dose = 21 Gy; optical path = 2.0 cm. Inset: time profile of the absorption at 360 nm. The solid line represents the first-order kinetic fit to the data.

It is well-known that neither vinyl radicals nor α-hydroxylalkyl radicals possess any significant absorbance in the region 300−500 nm.[23] This was also confirmed for the vinyl radicals of type **4** (without the sugar moiety) by the reaction of e_{aq}^- with 8-bromoadenine. Therefore, we assigned the transient in Figure 1 to the mixture of radicals **6** and **7** (Scheme 2) and the rate constant of 2.5×10^5 s^{-1} to the cyclization step.[24] In this respect, it is gratifying to see that the absorption spectra in Figure 1 strongly resemble in shape and ϵ-values those assigned to the isostructural radical **10** obtained by reaction of adenine derivatives



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either with $H^\bullet$ or with e_{aq}^- followed by protonation at C8.[25] It is also worth mentioning that the stereochemical outcome of the cyclization should reflect the ratio of products **2** and **3**, i.e., $k_{(R)}/k_{(S)} = 6$.

The possible reactions of the 2′-deoxyadenin-5′-yl radical in DNA are cyclization, repair reaction by hydrogen abstraction from glutathione, and trapping by O_2 to give the corresponding peroxyl radical.[26] Assuming the rate constant for the cyclization of 2′-deoxyadenin-5′-yl radical in DNA is close to 2.5×10^5 s^{-1} as seen from the present study, it can be deduced that both the repair reaction by glutathione[27] and the trapping by O_2 will be competitive with cyclization.[28]

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[18] Fe^{3+} is expected to be produced not only from the reaction of radical **6** and **7** with Fe^{2+} but also from the reaction of the $HO^\bullet$ radical with $K_4Fe(CN)_6$ ($k = 1 \times 10^{10}$ M^{-1} s^{-1});[10] it can be calculated that 21% of the $HO^\bullet$ radicals are consumed via this path under our experimental conditions. However, attempts to spectroscopically detect Fe^{3+} failed.[14] The removal of Fe^{3+} from our system is probably due to the oxidation of **8** and to the reaction with the $Me_2C(OH)CH_2^\bullet$ radical whose rate constant is known to be 3×10^6 M^{-1} s^{-1}.[19]

[19] Candeias, L. P.; Wolf, P.; O'Neill, P.; Steenken, S. *J. Phys. Chem.* **1992**, *96*, 10302.

[20] Hug, G. L. *Natl. Stand. Ref. Data Ser. (U.S. Natl. Bur. Stand.)* **1981**, No. 69.

[21] Hissung, A.; von Sonntag, C.; Veltwisch, D.; Asmus, K.-D. *Int. J. Radiat. Biol.* **1981**, *39*, 63.

[22] Chemical radiation studies of 8-bromoguanosine in aqueous solutions have shown that it behaves in a completely different manner, see: Ioele, M.; Bazzanini, R.; Chatgilialoglu, C.; Mulazzani, Q. G. *J. Am. Chem. Soc.* **2000**, *122*, 1900.

[23] Chatgilialoglu, C. In *Handbook of Organic Photochemistry*; Scaiano, J. C., Ed.; CRC Press: Boca Raton, 1989; Vol. 2; pp 3−11.

[24] No significant changes were observed in the presence of 2 mM of $K_4Fe(CN)_6$ which suggests a rate constant for the reaction of radical **6** and **7** with $Fe(CN)_6^{4-}$ lower than 7×10^4 M^{-1} s^{-1}. It is worth pointing out that in the steady-state experiments the stationary concentrations of the radicals are a few orders of magnitude lower compared to those of the pulse radiolysis.

[25] (a) Candeias, L. P.; Steenken, S. *J. Phys. Chem.* **1992**, *96*, 937. (b) Steenken, S. *Chem. Rev.* **1989**, *89*, 503 and references therein.

[26] Goldberg, I. H. *Acc. Chem. Res.* **1991**, *24*, 191.

[27] (a) The rate constant for the reaction of $CH_3^\bullet CHOH$ with glutathione is 1.1×10^8 M^{-1} s^{-1}.[10] (b) The intracellular level of glutathione in mammalian cells is in the 0.5−10 mM range, see: Meister, A.; Anderson, M. E. *Annu. Rev. Biochem.* **1983**, *52*, 711.

[28] The oxygen concentration is low in the nucleus, see: Zander, R. *Z. Naturforsch.* **1976**, *31C*, 339. Zander, R. *Adv. Exp. Med. Biol.* **1976**, *75*, 463.