Conformation, Lifetime, and Repair of 4′-DNA Radicals

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Received August 8, 1997

The 4′-DNA radical **2** is exceptional among the DNA radicals since it is involved in the cleavage of the strand even in the absence of O_2 by a heterolytic C,O-bond scission yielding radical cation **3** and the 5′-phosphate **4**.[1] We have measured the rate of this spontaneous scission reaction and studied the question of whether H-donors can regenerate stereoselectively the DNA **5** from radical **2**. Trapping reactions that yield the isomeric strand **6** are not repair processes because the incorporation of 2-deoxy-α-L-*threo*-pentofuranosyl units into a template DNA strand leads to an arrest in the enzymatic DNA synthesis.[2] Thus, H-donors that are able to repair DNA radicals have to combine high reactivity with high stereoselectivity. This communication demonstrates that 4′-DNA radicals of double strands have a much better chance of being repaired by H-donors than single strands.The single- and double-stranded 4′-DNA radicals **2** were generated by photolysis of 4′-substituted radical precursors **1a,b**[3] and trapped with the diethyl ester of glutathione (GSH). From the ratio of the trapped products **5** and **6** to the oligonucleotide **4** the competition constants k_H/k_E could be measured by pseudo-first-order kinetic experiments.[4] Using a k_H value of 1.0×10^7 M^{-1} s^{-1} for the H-trapping with GSH[5] the cleavage rate constants k_E of Table 1 were determined. The rate data show that the double-stranded 4′-DNA radicals ($k_E \approx 10^2$ s^{-1}) undergo the scission reaction about 10 times slower than the single-stranded radicals ($k_E \approx 10^3$ s^{-1}).[6] Presumably, the H-bridging between the two strands slows the C,O-bond cleavage reaction (**2** → **3** + **4**). The same template effect also influences the stereoselectivity of the H-trapping reaction.[7] The selectivity data of Table 2 demonstrate that single-stranded radicals **2** react nearly unselectively with H-donors. In contrast to this unselective behavior the double-stranded 4′-DNA radicals lead predominantly to the natural 2′-deoxyribonucleotides **5**. Presumably, the GC and AT hydrogen bonds between the two strands induce a preferred conformation of the 4′-(2′-deoxy)-ribonucleotide radical. Only if the radical center is sp^3-hybridized (as in the DNA itself) is the H-bridging optimal. In conformation **7**, the larger loop of the singly occupied radical orbital points into the minor groove of a B-DNA (Figure 1) and the attack of the radical trap occurs predominantly from this side.[8]

Scheme 1

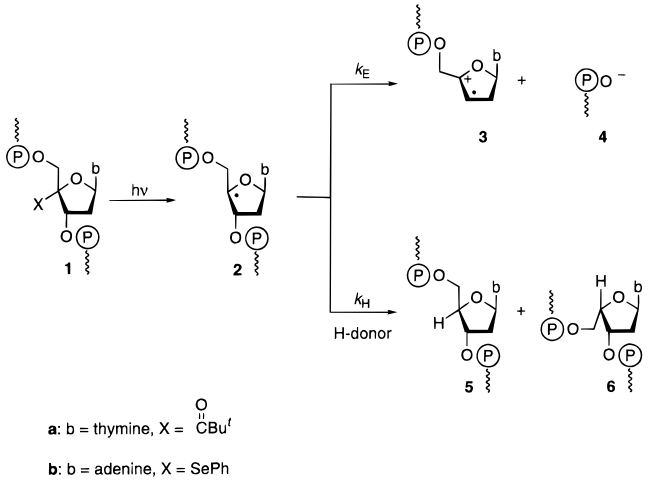

Table 1. Rate Constants k_E (s^{-1}) of the Spontaneous Cleavage Reaction of Single-Stranded and Double-Stranded 4′-DNA Radicals **2** in Water (20 mM phosphate buffer, pH = 7.0; 0.1 M NaCl; 20 °C)

	k_E (s^{-1})	
radical precursor[a]	single strand	double strand[b]
5′-T_2A_3T*T_9-3′	0.8×10^3	0.2×10^2
5′-T_2C_3T*T_9-3′	1.3×10^3	0.8×10^2
5′-T_2CGA*CTAACTG-3′	1.9×10^3	2.1×10^2

[a] The modified nucleotides T* and A* correspond to the modified thymidine and 2′-deoxyadenosine as in **1a,b**. [b] The double strands were formed by annealing the modified strands with the unmodified complementary strands.

(1) (a) For reviews, see: von Sonntag, C.; Hagen, U.; Schön-Bopp, A.; Schulte-Frohlinde, D. *Adv. Radiat. Biol.* **1981**, *9*, 109. (b) Giese, B.; Beyrich-Graf, X.; Erdmann, P.; Petretta, M.; Schwitter, U. *Chem. Biol.* **1995**, *2*, 367.

(2) Hess, M. T.; Schwitter, U.; Petretta, M.; Giese, B.; Naegeli, H. P. *Biochemistry* **1997**, *36*, 2332.

(3) The single strands were synthesized according to: (a) Giese, B.; Erdmann, P.; Schäfer, T.; Schwitter, U. *Synthesis* **1994**, 1310. (b) Marx, A.; Erdmann, P.; Senn, M.; Körner, S.; Jungo, T.; Petretta, M.; Imwinkelried, P.; Dussy, A.; Kulicke, K. J.; Macko, L.; Zehnder, M.; Giese, B. *Helv. Chim. Acta* **1996**, *79*, 1980. For double-strand formation, the modified oligonucleotides were annealed to their complementary strands.

(4) A buffered aqueous solution (200 μL, 20 mM phosphate, pH = 7.0, 0.1 M NaCl) of 0.3−2 nmol of single- or double-stranded oligonucleotides and 3−150 nmol of glutathione diethyl ester (GSH) were deoxygenated by treatment with argon. The thermostated solution (20 °C) was irradiated for 3−20 min (Osram 500 W, 320 nm cutoff filter) in a 4 × 5 mm quartz cuvette and directly injected on an anion exchange HPLC column (Macherey-Nagel Nucleogen 60-7 DEAE, 125 × 4 mm; eluent A: 20 mM KH_2PO_4, 20% acetonitrile, pH 7.0; eluent B: 20 mM KH_2PO_4, 20% acetonitrile, pH 7.0, 1 M KCl; gradient: 0 min 90% A, 20 min 52% A, 25 min 30% A, 28 min 10%A; flow rate: 1 mL/min) or on a reversed phase HPLC column (Waters Symmetry C18, 5 μm, 150 × 3.9 mm; eluent A: 0.1 M triethylammonium acetate (TEAA); eluent B: acetonitrile; gradient: 6% B to 14% B in 30 min, flow rate: 1 mL/min; column temperature: 30 or 50 °C). The amounts of **4**−**6** were determined from the peak areas divided by the calculated extinction coefficient of the corresponding oligonucleotides. Their identities were confirmed by MALDI-TOF MS and by comparison with independently synthesized oligonucleotides. The competition constants k_H/k_E were determined from the dependence of the product ratio ((**5** + **6**)/**4**) on the GSH concentration according to a pseudo-first-order kinetic treatment.

(5) Tronche, C.; Martinez, F. N.; Horner, J. H.; Newcomb, M.; Senn, M.; Giese, B. *Tetrahedron Lett.* **1996**, *37*, 5845.

(6) These absolute rate measurements with selectively generated 4′-DNA radicals are in the same order as those from radiolysis experiments: Behrens, G.; Koltzenburg, G.; Ritter, A.; Schulte-Frohlinde, D. *Int. J. Radiat. Biol.* **1978**, *33*, 163.

(7) A buffered aqueous solution (200 μL, 20 mM phosphate, pH = 7.0, 0.1 M NaCl) of about 4 nmol of the single- or double-stranded oligonucleotides and a 1000-fold excess of glutathione diethyl ester (GSH) were deoxygenated by treatment with argon. The thermostated solutions (20 °C) were irradiated 10−20 min (Osram 500 W, 320 nm cutoff filter) in a 4 × 5 mm quartz cuvette. After irradiation the oligonucleotides were precipitated with isopropyl alcohol. The dried residues were redissolved (50 μL, 35 mM Tris-HCl, pH = 7.5, 16 mM $MgCl_2$) and digested with 1 μg of *Snake Venom Phosphodiesterase* and 1 unit of *Calf Intestinal Alkaline Phosphatase* yielding the nucleosides. The digest was analyzed by RP-HPLC (LiChrospher RP 18, 100-5, 250 × 4 mm; eluent A: 0.1 M triethylammonium acetate (TEAA); eluent B: acetonitrile; gradient: 2% B to 15% B in 30 min, flow rate: 1 mL/min). The HPLC peaks were identified by comparison with authentic nucleosides. Experiments with independently synthesized oligomers containing 2′-deoxy-α-L-*threo*-pentafuranosyl nucleotides have demonstrated that enzymatic digestion yields the natural and unnatural nucleosides according to their content in the oligomers. Alternatively, a direct determination of the ratio of the isomers was possible by applying reversed phase HPLC with the irradiation solution as described in ref 4.

(8) Another reason for the cleavage rate difference between the single- and double-stranded 4′-DNA radical could be the biased conformation of the double-stranded 4′-DNA radical **7** that forces the cleavage to follow a *cis*-elimination step. A biased conformation of the double-stranded 4′-DNA radicals has been proposed by Akhlaq, M. S.; Schuchmann, H.-P.; von Sonntag, C. *Int. J. Radiat. Biol.* **1987**, *51*, 91.

S0002-7863(97)02769-8 CCC: $14.00

Table 2. Stereoselectivity (**5**:**6**) of the Trapping Reaction of Single- and Double-Stranded 4′-DNA Radicals **2** in Water (20 mM phosphate buffer, pH = 7.0; 0.1 M NaCl; 20 °C) with Glutathione Diethyl Ester and a Water Soluble Trialkyltin Hydride (in Brackets)

	stereoselectivity **5**:**6**	
radical precursor[a]	single strand	double strand[b]
5′-CGCCA*GCGCGGC-3′	1.2 (1.4)	10.9 (10.5)
5′-CGCCT*GCGCGGC-3′	1.4	8.1
5′-CGCCA**GCGCGGC-3′	1.6 (1.0)	8.4 (10.1)
5′-CGCCT**GCGCGGC-3′	2.0	8.5

[a] The modified nucleotides T* and A* correspond to the modified thymidine and 2′-deoxyadenosine as in **1a,b**; the modified nucleotide T** and A** correspond to modified nucleotides which are inverted at the 4′-position (2′-deoxy-α-L-*threo*-pentofuranosyl nucleoside derivatives). [b] Double strands were synthesized by annealing the modified strands with the unmodifed complementary strands.

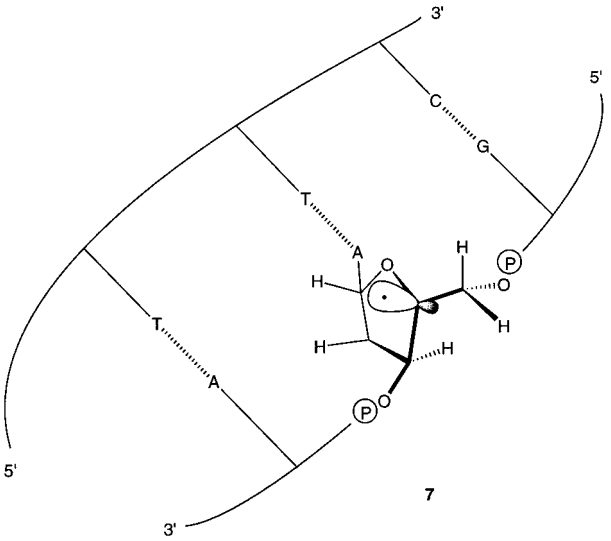

Figure 1. Conformation of the 4′-DNA radical **7** in double strands.

The stereochemistry does not change if the H-donor is varied. Thus, a water-soluble trialkyltin hydride (R_3SnH)[9] is as selective as GSH (Table 2). In addition, radicals generated from 4′-substituted 2′-deoxy-α-L-*threo*-pentofuranosyl nucleotides, where the configuration at the 4′-position is inverted,[10] react with nearly the same stereoselectivity as those generated from 4′-substituted D-deoxyribonucleotides **1** (Table 2). The CD spectra of the unmodified and the modified double strands are very similar and prove a B-DNA conformation for these oligonucleotides (see the Supporting Information). Thus, the kind and the position of the radical-forming functional group in the 4′-DNA radical precursor do not influence the stereochemistry of the trapping reaction. This proves that the 4′-DNA radical rapidly adopts its optimal conformation in which the singly occupied orbital points into the minor groove of the DNA (Figure 1). The stereoselectivities of the trapping reactions decrease only moderately with increasing temperature over a wide temperature range. However, when the melting temperature (T_m)[11] is reached, the high selectivity of the double strand drops abruptly to the low selectivity of the single strand (Figure 2).

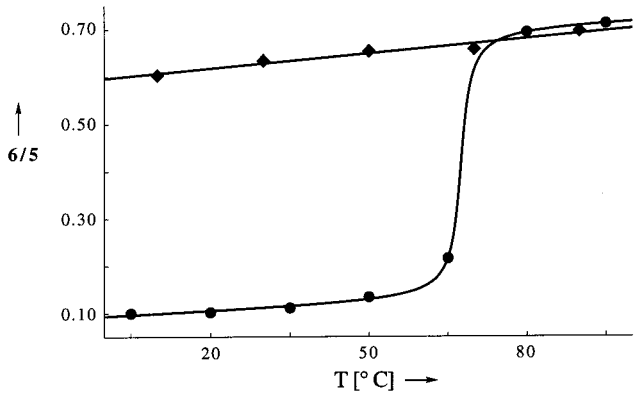

Figure 2. Stereoselectivity (**6**/**5**) of the H-trapping of single- (◆) and double-stranded (●) 4′-DNA radicals generated from the modified strand 5′-CGCCA*GCGCGGC-3′ and the corresponding double strand which is annealed with the complementary strand.

Conclusion: Double-stranded 4′-DNA radicals are pyramidalized and have half-lives of milliseconds in the absence of radical traps. With H-donors these radicals are repaired to the natural DNA double strands with high stereoselectivity.

Acknowledgment. This work was supported by the Swiss National Science Foundation. The authors thank Dr. A. A. Stämpfli and R. Dahinden, Novartis Crop Protection AG, Basel, for performing the exact mass measurements.

Supporting Information Available: CD spectra, HPLC, and kinetic plots (5 pages). See any current masthead for ordering information and Internet access instruction.

JA972769Q

(9) Ligh, J.; Breslow, R. *Org. Synth.* **1995**, *72*, 199.

(10) For the synthesis of the substituted 2′-deoxy-α-L-*threo*-pentofuranosyl nucleosides see ref 3.

(11) The UV melting temperature for the modified double strand of Figure 2 is 63.6 °C. The unmodified double strand has a melting temperature of 67.5 °C.