Sequence Dependent Long Range Hole Transport in DNA

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Abstract: A guanine radical cation ($G^{+\bullet}$) was site-selectively generated in double stranded DNA and the charge transfer in different oligonucleotide sequences was investigated. The method is based on the competition between a charge transfer from $G^{+\bullet}$ through the DNA and its trapping reaction with H_2O. We analyzed the hole transfer from this $G^{+\bullet}$ to a GGG unit through one, two, three, and four AT base pairs and found that the rate decreases by about 1 order of magnitude with each intervening AT base pair. This strong distance dependence led to a β-value of 0.7 ± 0.1 Å^{-1}. Within the time scale of this assay the charge transfer nearly vanished when the $G^{+\bullet}$ was separated by four AT base pairs from the GGG unit. However, if the second or the third of the four intervening AT base pairs was exchanged by a GC base pair, the rate of the hole transfer from the $G^{+\bullet}$ to the GGG unit increased by 2 orders of magnitude. In addition, a long-range charge transfer over 15 base pairs could be observed in a mixed strand that contained AT as well as GC base pairs. Because $G^{+\bullet}$ can oxidize G but not A bases, the long-range charge transport can be explained by a hopping of the positive charge between the intervening G bases. Thus, the overall charge transport in a mixed strand is a multistep hopping process between G bases where the individual steps contribute to the overall rate. The distance dependence is no longer described by the β value of the superexchange mechanism.

Introduction

Guanine (G) bases are a target for oxidative damage in DNA.[1] This damage is often the consequence of an oxidation of G to a guanine radical cation ($G^{+\bullet}$)[2,3] that reacts further with H_2O or O_2.[4] Barton *et al.*[5] as well as Schuster *et al.*[6] have observed that oxidation damage can occur at G bases that are far away from the oxidant. Such a far reaching translocation of charge[7−9] cannot be brought about by superexchange,[10] the mechanism which is considered to be responsible for the strong distance

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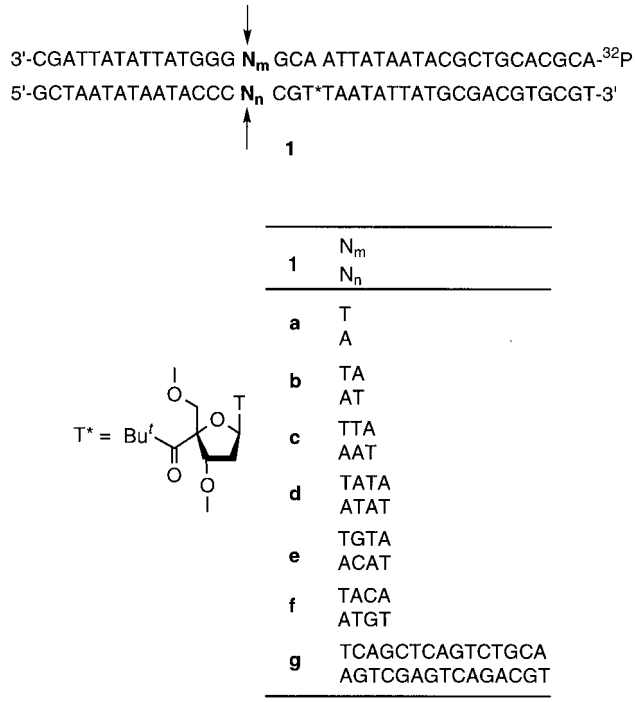
Chart 1



dependence of the hole transfer rates to G bases in the experiments of Lewis and Wasielewski *et al.*,[11] Tanaka *et al.*,[12] and Giese *et al.*[13] In the following, we report on a sequence dependent hole transport in DNA whereby the longest distance between G bases determines the overall rate. In the DNA duplexes presented, this phenomenon is attributed to hopping of holes between G sites. The strong distance dependence of the individual charge-transfer steps reflects superexchange interactions between G bases caused by intervening AT base pairs.

Experimental Section

General. Buffer solutions were prepared with Nanopure water from a Barnstead NANOpure water system. Unmodified oligonucleotides were obtained from Pharmacia Biotech (RP or PAGE grade). Modified oligonucleotides were synthesized with an Expedite 8909 synthesizer from Perseptive Biosystems applying standard phosphoramidite chemistry. Chemicals for DNA synthesis were purchased from MWG Biotech and Glen Research. HPLC purification of oligonucleotides was performed with a Waters Alliance HPLC and a Merck reverse phase column (LiChrospher 100 RP-18e, 5μm, 250 × 4 mm). Masses of oligonucleotides were determined with a Vestec Benchtop II MALDI-ToF MS (laser wavelength 337 nm, acceleration voltage 25 kV, negative ion mode) with 2,4-dihydroxyacetophenone as matrix. Radiolabeling at the 5′-ends of DNA was performed with [γ-^{32}P]ATP and T4 polynucleotide kinase (New England Biolabs) followed by purification with gel filtration with use of Quick Spin columns from Boehringer Mannheim. Polyacrylamide gel electrophoresis (PAGE): 12% under denaturating conditions with Accu Gel 40 from National Diagnostics. Photolyses were performed with an Osram high-pressure mercury arc lamp (500 W, 320 nm cutoff filter) in poly(methyl methacrylate) cuvettes from Semadeni. Oligonucleotide solution concentrations were determined by the absorbance at 260 nm.

Synthesis of the 4′-Modified Oligonucleotides. The syntheses of oligonucleotides were carried out on a DNA synthesizer in 0.2 μmol scales (500 Å controlled pore glass support). The standard method for 2-cyanoethylphosphoramidites was used, except that the coupling of the modified nucleotide T* (see Chart 1) was extended to 15 min.[14] With this modification there is no notable difference between the efficiency of coupling for this amidite and commercially available ones. Workup was done with standard procedures. The purity of all oligonucleotides was controlled by reverse phase chromatography and MALDI-ToF MS. The corresponding unmodified oligonucleotides where T* is substituted against T (RP grade) and complementary strands (PAGE grade) were purchased from Pharmacia Biotech. In Chart 1 the prepared double stranded oligonucleotides **1a**−**g** are shown.

Determination of Relative Rates of the Charge Transfer. Double stranded oligonucleotides **1a**−**g** were prepared by hybridizing 3 ± 0.5 pmol of 4′-pivaloyl modified strands with 1 ± 0.2 pmol of the corresponding freshly 5′-radiolabeled complementary strands in 100 μL of phosphate buffer (20 mM, 100 mM NaCl, pH 5.0). Annealing was achieved by heating the samples at 85 °C for 5 min and slowly cooling to room temperature. The solutions were purged with argon for 30 min and the experiments were carried out in the absence of O_2 under an argon atmosphere at 15 °C. After 10−12 min of irradiation with an Osram high-pressure mercury arc lamp (500 W, 320 nm cutoff filter), 40 μL of the solutions were incubated with 400 μL of 1 M piperidine at 90 °C for 30−35 min.[15] The samples were lyophilyzed and electrophorezed (3 h at 1500 V) on a 12% denaturating 19:1 acrylamide:bisacrylamide gel containing 7 M urea. In control experiments, which were carried out with the same procedure, the modified double strands **1a**−**g** were replaced by the corresponding unmodified double strands. The dried gels were analyzed by autoradiography with a Phosphorimager 425 from Molecular Dynamics (exposure times 1−5 h) in combination with the software ImageQuant. The intensities of the spots resulting from piperidine treatment were determined by volume integration. Quantitative data, shown in Tables 1 and 2, were obtained by subtracting intensities of the control irradiations (unmodified double strands) from those obtained from the photolysis experiments with modified double strands. To determine the error, six separate experiments with double strand **1b** were carried out under different conditions (Table 1).

Because the intensities depend on the exposure time of the gels, the ratios of the intensities of the cleaved strands to the total intensity were used for the analysis of the data. Table 1 shows that the ratios of the total intensities of the cleaved strands to the total intensities of all strands vary by ±35%.[16] Nevertheless, the error for the ratio of the intensities GGG/G_{23} is only ±20%.[17] About 5% of the total cleavage occurs at the A_{24} site. The concentration variation (Table 1, entries 5 and 6)

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(15) For piperidine treatment a standard procedure was applied. The radiolabeled cleaved strands appear as spots in the autoradiograms of Figures 1 and 5. See: Chung, M.-H.; Kiyosawa, H.; Ohtsuka, E.; Nishimura, S.; Kasai, H. *Biochem. Biophys. Res. Commun.* **1992**, *188*, 1. See also ref 21.

(16) The variation of the total intensity of the cleaved strands to the total intensity of all strands (Table 1) is caused by the different yields of the DNA strand cleavage (**2** → **3** + **4**, Scheme 1) in the different experiments. This is a consequence of the different irradiation conditions, because focusing the light in an identical way in the various experiments was not ensured.

(17) To determine the total yield of the oxidative cleavage caused by the charge injection, the yield of the enol ether (see **5** in Scheme 1) was measured. For the experimental conditions of entry 2 in Table 1 the yield of the enol ether was 25%. This yield of the charge injection on G (**2** → **4** + **5**, Scheme 1) and an intensity ratio $(GGG+G_{23})$/total of 0.02 (Table 1, entry 2) lead to a yield of 8% for the oxidative cleavage of the ^{32}P labeled strand. The yield of oxidative cleavage of both strands is higher because the cleavage of the unlabeled strand cannot be measured with this assay. If one assumes that the charge is equally distributed over G_{23} at the labeled strand and the adjacent G at the unlabeled strand (see Scheme 2), then the total yield of the oxidative cleavage would be about 10%. The relatively small yield of the oxidative cleavage shows that $G^{+\bullet}$ not only reacts with H_2O but also undergoes competing reactions, for example deprotonation (Steenken, S. *Biol. Chem.* **1997**, *378*, 1293) and subsequent H-abstraction. This is in accordance with our previous measurements[13] where we have observed that the major part of the oxidized $G^{+\bullet}$ is repaired under anaerobic conditions (deprotonation with subsequent H-abstraction). However, the ratio of these competing reactions (H_2O addition versus repair) should not change in slightly different strands. Therefore, with our assay relative yields of the charge transfer can be determined.

Table 1. Data of Experiments with Double Strand **1b**. Oxidative Damage at G_{23}, A_{24}, and GGG Sites Was Quantified by Phosphorimaging of Denaturing Polyacrylamide Gels after Photolysis and Subsequent Piperidine Treatment

entry	irradiation time (min)	piperidine treatment (min)	exposure time of the gel (h)	total intensity $\times 10^5$	intensity at GGG site $\times 10^3$	intensity at G_{23} site $\times 10^3$	intensity at A_{24} site $\times 10^3$	GGG/total $\times 10^{-2}$	G_{23}/total $\times 10^{-2}$	A_{24}/total $\times 10^{-2}$	GGG/G_{23}	$GGG/G_{23}A_{24}$
1	10	30	1	62	94	20.5	*c*	1.5	0.33		4.6	
2	10	30	5	334	540	132	*c*	1.6	0.40		4.1	
3	12	35	1.5	92	72	17	3.7	0.78	0.18	0.040	4.2	3.5
4	12	35	4	230	230	61	19	1.0	0.26	0.083	3.8	2.9
5[a]	10	30	1	64	74	19	*c*	1.2	0.30		3.9	
6[b]	10	30	1	67	60	13	*c*	0.90	0.19		4.6	

[a] Increased concentration of the modified strand by a factor of 10. [b] The concentration of the modified strand is increased by a factor of 33 and the concentration of the unmodified strand by a factor of 100. Thus, the unmodified/4′-pivaloyl modified strand ratio is 1:1. Only 1% of the unmodified strand is radiolabeled. [c] Intensity of A_{24} was not determined in these experiments.

Table 2. Quantification of Oxidative Cleavage at G, GA, and GGG Sites by Phosphorimaging of Denaturing Polyacrylamide Gels after Photolysis of Double Strands **1a**−**g** and Subsequent Piperidine Treatment

entry	double strand[a]	GGG/total[b] $\times 10^{-2}$	G_{23}/total[b] $\times 10^{-2}$	A_{24}/total[b] $\times 10^{-2}$	other G and GA sites/total[b] $\times 10^{-2}$	$GGG/G_{23}A_{24}$	distances Δr (Å)[c]
1	**1a**	0.74	0.025			30 ± 6	6.8
2	**1b**	0.89	0.22[d]	0.06[d]		$3.2^d \pm 0.6$	10.2
3	**1c**	0.22	0.45	0.05		0.44 ± 0.2	13.6
4	**1d**	0.019	0.59	0.10		0.03 ± 0.015	17
5	**1e**	1.08	0.27	0.047	0.038	$3.4\ (3.0) \pm 0.7^e$	17
6	**1f**	0.62	0.14	0.022		$3.8\ (3.4) \pm 0.8^{e,f}$	17
7	**1g**	0.66	0.18	0.013	0.1[g]	$3.4\ (2.3) \pm 0.7^e$	54

[a] For the base sequences see Chart 1. [b] Volume integration of the corresponding spots was carried out with the software ImageQuant. The data are differences between experiments with oligonucleotides containing 4′-pivaloylthymidine and the experiments with the corresponding unmodified strands. [c] Distance between the G_{23} and GGG unit. [d] Data taken from Table 1, entries 3 and 4. [e] Ratios $GGG/(G_{23}A_{24}$ + other G and GA sites) in parentheses. [f] The value in parentheses was estimated under the assumption that the single G, which is on the complementary strand, is damaged to nearly the same extent as the single G of double strand **1e** (entry 5). [g] The value was estimated under the assumption that the G and GA sites on the complementary strand are damaged to nearly the same extent as the G and GA sites on the radiolabeled strand.

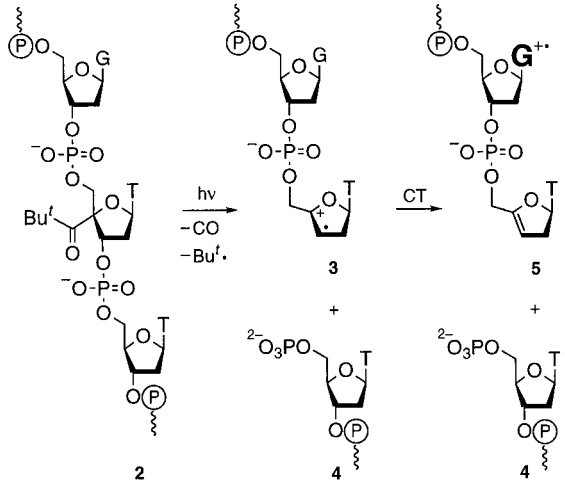
demonstrates that the charge transfer occurs intramolecularly. The experimental data for strands **1a**−**g** are compiled in Table 2. For most of the strands at least two independent experiments were performed. In some cases the error of these experiments is smaller than that of the six experiments with **1b**. To be on the safe side, we used for these cases the error determined by the six experiments with **1b**.[18]

Results and Discussion

Recently, we have developed a method to generate site selectively a guanine radical cation ($G^{+\bullet}$) in DNA double strands.[13] This assay utilizes the formation of a deoxyribose radical cation **3** by Norrish I cleavage of ketone **2** (**2** → **3** + **4**). Carbohydrate radical cation **3** then oxidizes the adjacent guanine to the guanine radical cation **5** (Scheme 1).

We have now studied how efficiently this $G^{+\bullet}$ can oxidize further G bases in DNA double strands. For analytical reasons an assay was used where the charge transfer is detected in the complementary strand. Irradiation of double stranded oligonucleotides **1a**−**g** cleaved the modified strands (analogous to **2** → **3** + **4** in Scheme 1) and generated the guanine radical cations **6** (analogous to **5** in Scheme 1). In a subsequent step, the positive charge of the first formed $G^{+\bullet}$ in **6** is transferred to the adjacent G_{23} of the complementary strand (**6** → **7**), and the hole

Scheme 1

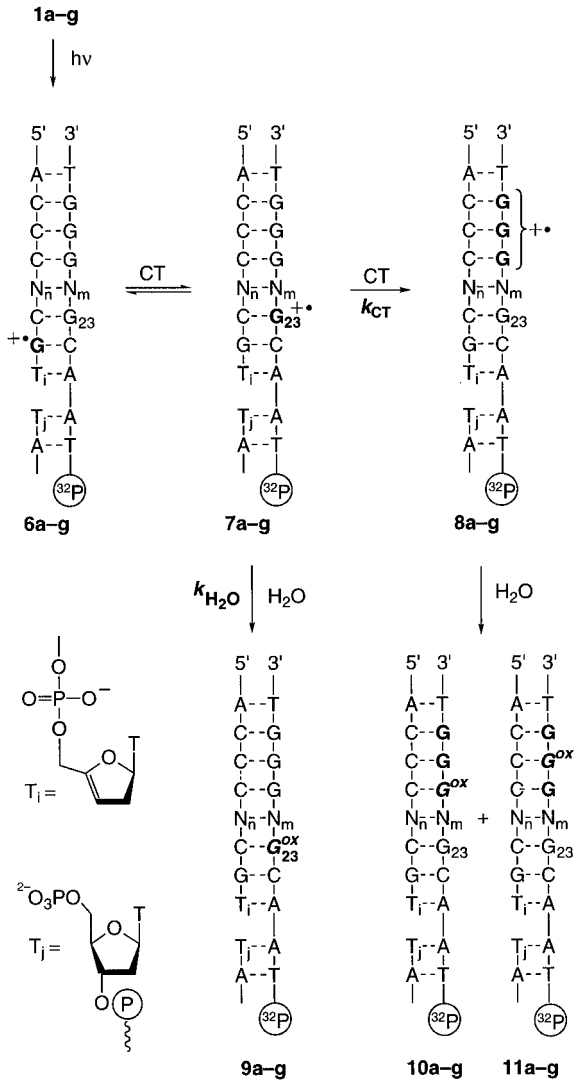


transfer between $G_{23}^{+\bullet}$ and the GGG site (**7** → **8**) was determined in this strand (Scheme 2). The GGG unit in **8** made this reaction step irreversible.[19] Different double strands were

(18) The data in Table 2 (entries 5 and 7) show that the intensity of the cleaved strands at intervening G bases is weaker than that of the starting base G_{23}. This can be explained by a higher rate of H_2O addition at $G_{23}^{+\bullet}$ compared to that of a $G^{+\bullet}$ that is further away from the charge injection site. For reactions of aromatic radical cations with H_2O it is known that the rate determining step is the deprotonation of the radical cation/H_2O complex (see: Oyama, M.; Nozaki, K.; Nagaoka, T.; Okazaki, S. *Bull Chem. Soc. Jpn.* **1990**, *63*, 33). For $G_{23}^{+\bullet}$ such a deprotonation can be performed by the monophosphate dianion (**4** in Scheme 1) that is set free in the radical induced DNA strand cleavage step **2** → **3** + **4**. This phosphate dianion is in the vicinity of $G_{23}^{+\bullet}$ and can act as a base for the deprotonation during the H_2O addition. The $G^{+\bullet}$ that is further away cannot be reached by this phosphate dianion and should therefore react slower with H_2O. Experiments are in progress to prove this effect.

(19) This G stacking decreases the ionization potential. From ab initio studies the oxidation potential of a GGG unit is calculated to be 0.7 eV below the oxidation potential of a single G unit (see: (a) Sugiyama, H.; Saito, I. *J. Am. Chem. Soc.* **1996**, *118*, 7063. (b) Prat, F.; Houk, K. N.; Foote, C. S. *J. Am. Chem. Soc.* **1998**, *120*, 845). This reduces the back hole transfer rate from $GGG^{+\bullet}$ to G by a factor of about 10^{-12} which makes the back hole transfer very unlikely. If one nevertheless assumes that the back hole transfer from $GGG^{+\bullet}$ to G is faster than the reaction of $GGG^{+\bullet}$ with H_2O, a "Curtin-Hammett" situation would exist where the transition state of the H_2O reactions with $G_{23}^{+\bullet}$ and $GGG^{+\bullet}$ is decisive for the cleavage ratio. Since the rate of the H_2O addition hardly depends on the distance between the GGG and the G_{23} site, nearly no distance effect should result. But the data in Table 2 (entries 1−4) demonstrate that the cleavage ratios ($GGG/G_{23}A_{24}$) decrease with increasing distance from 6.8 to 17 Å by 3 orders of magnitude.

Scheme 2



used in which the number of AT base pairs, separating $G^{+\bullet}$ from GGG in **7**, was increased. This allowed the measurement of the distance dependence of the charge transfer (Scheme 2).

The hole transfer **7** → **8** competes with the H_2O reaction **7** → **9** that generates an oxidized guanine (G^{ox}).[4] Since the steps **7** → **8** and **7** → **9** are of first or pseudo-first order, the ratio of damage products (**10** + **11**)/**9** is proportional to the competition constant k_{CT}/k_{H_2O} of $G^{+\bullet}$ in the double strand **7**. If the rates of the H_2O reactions of $G^{+\bullet}$ in **7** as well as of $GGG^{+\bullet}$ in **8** are not much dependent upon the base sequence, the product ratio (**10** + **11**)/**9** is proportional to the relative rate coefficient $k_{CT,rel}$ of the charge-transfer step **7** → **8**.[20] The amount of DNA strands **8**, **9**, and **10** that contained G^{ox} bases was determined by the piperidine method.[15,21] In Figure 1A autoradiograms of the

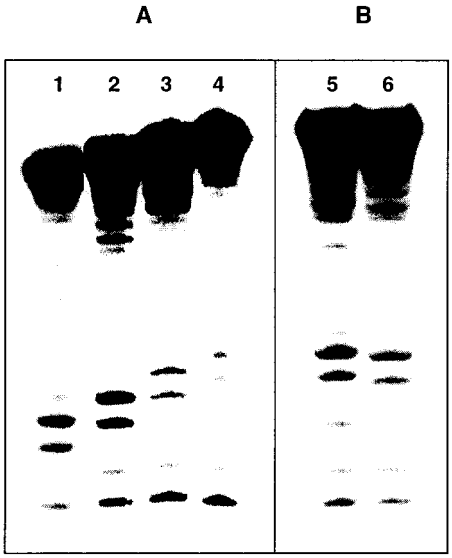


Figure 1. Denaturing polyacrylamide gel visualized by phosphorimagery. The ^{32}P-labeled strands after photolysis and subsequent piperidine treatment are shown. The spots on the top of the gel correspond to intact oligonucleotides. The additional spots result from piperidine cleavage at oxidized G, GA, and GGG sites. (A) Lanes 1−4: Sequences **1a**−**d** with one to four AT base pairs between the G and GGG site, respectively. (B) Lanes 5 and 6: Sequences **1e** and **1f** which result from **1d** by exchanging one AT for one GC base pair. See Chart 1 and Figure 4 for the sequences.

polyacrylamide gel electrophoresis after irradiation of **1a**−**d** and piperidine treatment are shown. The spots in lanes 1−4 result from piperidine cleavage in the ^{32}P-labeled single strands. The major bands belong to oxidative damage and piperidine cleavage at the single guanine sites G_{23} as well as the GGG units. A minor spot, having 10−20% of the G_{23} intensity, appears at the A_{24} site of **1b**−**d**. An occurrence of a minor band at the A_{24} site is reasonable because in GA sequences the positive charge at $G^{+\bullet}$ is slightly stabilized by the adjacent A so that H_2O should react also with A_{24}.[2c,d,19a,22] For the determination of the relative rate coefficients of the charge transfer $k_{CT,rel}$ we therefore used the intensity ratios $GGG/G_{23}A_{24}$.[23] The intensities of all spots, corrected by control experiments, are given in Table 2 and in the histograms of Figure 2.

The intensity ratio of $GGG/G_{23}A_{24}$ decreases from 30 via 3.2 and 0.44 to 0.03 if the number of AT pairs between $G_{23}^{+\bullet}$ and the GGG unit increases from one to four (Table 1, entries 1−4, and Figure 2). According to eq 1[10] these intensity ratios that are proportional to the relative rate coefficients $k_{CT,rel}$ are plotted against the distances Δr of the charge-transfer steps between $G_{23}^{+\bullet}$ and the GGG unit (Figure 3).

$$k_{CT,rel} = A \bullet e^{-\beta \Delta r} \qquad (1)$$

From the slope of Figure 3 a β-value of 0.7 ± 0.1 Å^{-1} was determined. Thus, for the first time, the distance dependence of the biologically relevant charge transfer between G bases has been measured. The value of $\beta = 0.7 \pm 0.1$ Å^{-1} is typical for hole transfer reactions through DNA via the superexchange mechanism[10−13] and means that the rate of the hole transfer

(20) The absolute rate constants k_{H_2O} of the H_2O reactions with $G^{+\bullet}$ and $GGG^{+\bullet}$ are not known. The H_2O addition rate of a single $G^{+\bullet}$ is presumably faster than that of $GGG^{+\bullet}$ because of the better stabilization of the positive charge in $GGG^{+\bullet}$.[19] Therefore, only relative rate constants $k_{CT,\,rel}$ of the charge transfer can be measured. In Table 2 the relative rate coefficients of the charge transfer $k_{CT,\,rel}$ were determined by the intensity ratios $GGG/G_{23}A_{24}$.

(21) The only oxidation product of G that is not cleaved efficiently with piperidine is 7,8-dihydro-8-oxoguanine (8-oxoG). We can rule out the formation of 8-oxoG as the major oxidative damage in our anaerobic experiments because treatment with the one electron oxidant K_2IrCl_6 after irradiation and subsequent piperidine treatment did not lead to enhanced cleavage. For the method, see: Muller, J. G.; Duarte, V.; Hickerson, R. P.; Burrows, C. J. *Nucleic Acids Res.* **1998**, *26*, 2247.

(22) This reasoning is supported by the observation that the base A_{26} in double strand **1d**, which has only neighboring T bases, shows no cleavage after piperidine treatment (Figure 1, lane 4).

(23) Double strand **1a** does not contain an A_{24} site so that the rate coefficient $k_{CT,\,rel}$ for **1a** was determined by the ratio of intensities GGG/G_{23}.

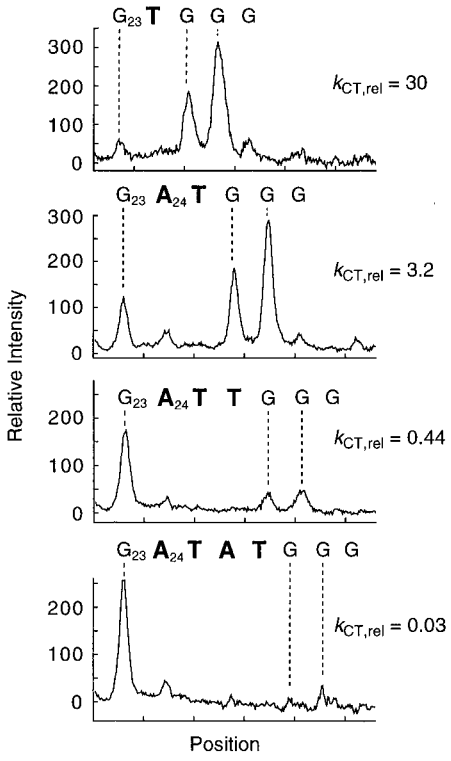


Figure 2. Histograms from lanes 1−4 of Figure 1A obtained by integration along a lane followed by subtraction from the control irradiation with unmodified double strands. The histograms demonstrate that with increasing numbers of intervening AT base pairs the ratio of damage GGG/G_{23} or GGG/$G_{23}A_{24}$ decreases. See Chart 1 and Figure 4 for the sequences.

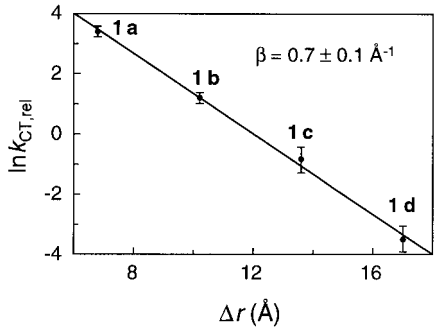


Figure 3. Distance dependence of the hole transfer from G_{23} to GGG. See Chart 1 and Figure 4 for the sequences.

between G bases decreases 1 order of magnitude with each intervening AT base pair.

In these experiments the hole transfer between $G_{23}^{+\bullet}$ and the GGG unit occurs through AT base pairs only. This is different in double strands **1e**,**f** where one intervening AT base pair is exchanged by one GC base pair, but the number of intervening base pairs between $G_{23}^{+\bullet}$ and GGG remains unchanged. As the data in Table 1 (entries 5,6) and the spots in Figure 1B demonstrate, the intensity ratios (GGG/$G_{23}A_{24}$) increase from 0.03 for **1d** to 3.4 and 3.8 for **1e** and **1f**, respectively.[24] Thus, although the distance of the charge transfer from $G_{23}^{+\bullet}$ to GGG remains the same (about 17 Å), nevertheless the rate of the charge transfer changes by 2 orders of magnitude. Because the charge donor ($G_{23}^{+\bullet}$) and the charge acceptor (GGG) have not changed in these experiments with DNA double strands **1d**−**f**,

(24) If one takes the cleavage intensities of the additional G sites in **1e** and **1f** into account, the $k_{CT,\,rel}$ data are about 10% smaller (Table 2, entries 5 and 6).

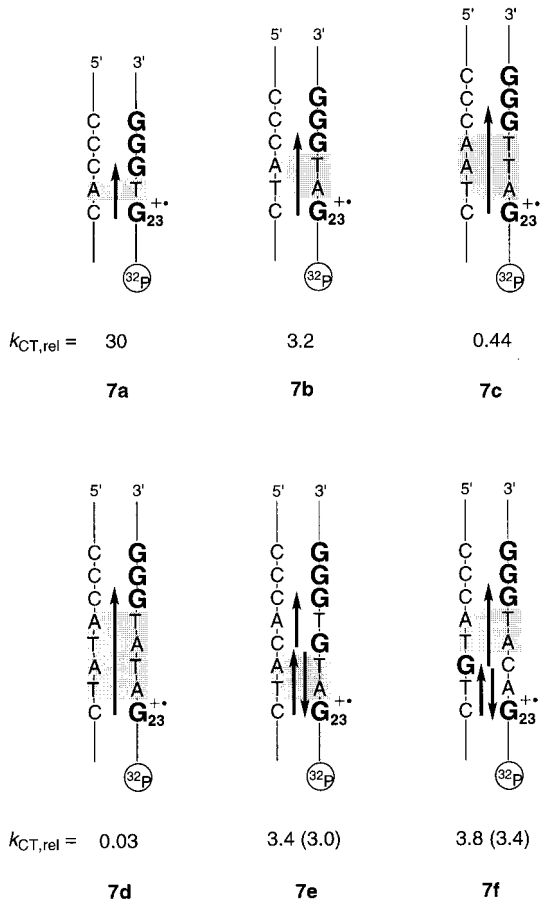


Figure 4. Hole transfer (indicated by arrows) from G_{23} to GGG in double strands **7a**−**f**. In **7a**−**d** the hole transfer takes place through AT base pairs by a single step superexchange mechanism. In double strands **7e**,**f** a two-step mechanism is proposed in which the intervening G base is oxidized in the first step followed by an irreversible hole transfer to the GGG unit. This hopping process increases the charge-transfer efficiency compared to the single superexchange step over the same distance.

this rate discrepancy must be caused by the differences in the base sequences. It is obvious that the exchange of one intervening AT by one GC base pair dramatically increases the efficiency of the overall hole transfer. The redox potential of A is at least 0.5 V higher than that of G,[3,25] so that A can be oxidized by $G^{+\bullet}$ only in a very slow reaction. Because $G^{+\bullet}$ easily oxidizes a G base, we assume that the charge transport in **1e,f** takes place via oxidation of the intervening G bases. According to this mechanism the hole transport from the starting $G_{23}^{+\bullet}$ to the GGG unit occurs by a hopping process[26,27] in which the intervening G base is oxidized to $G^{+\bullet}$. According to this mechanism, the one-step charge transfer via a superexchange mechanism between $G_{23}^{+\bullet}$ and GGG in double strands **1a**−**d** is turned into a two-step hopping process in double strands **1e,f**. The charge is transported between the G bases until the reaction stops at the GGG unit.[19] Each single hopping process occurs via a superexchange mechanism that is described by the large

(25) Calculations predict that the difference between the oxidation potentials of G and A (ca. 0.5 V for the deoxynucleosides, see ref 3) is even larger in GC and AT pairs, see: Hutter, M.; Clark, T. *J. Am. Chem. Soc.* **1996**, *118*, 7574.

(26) Hopping of positive charge in DNA/PNA hybrids between neighboring A bases has been proposed recently, see: Armitage, B.; Ly, D.; Koch, T.; Frydenlund, H.; Ørum, H.; Batz, H. G.; Schuster, G. B. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 12320.

(27) Jortner, J.; Bixon, M.; Langenbacher, T.; Michel-Beyerle, M. E. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 12759.

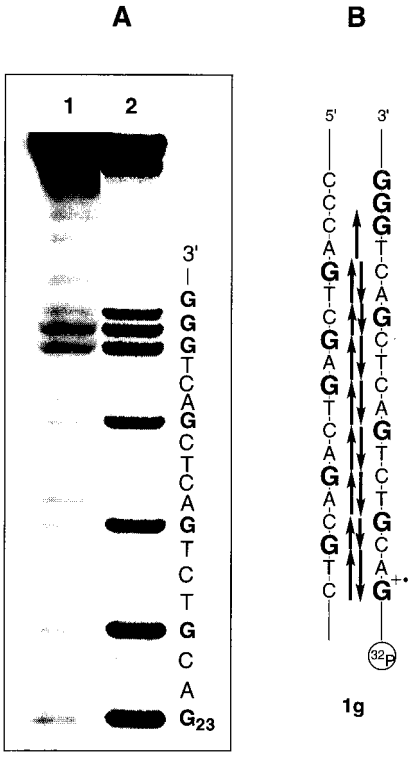


Figure 5. Denaturing polyacrylamide gel visualized by phosphorimagery. Lane 1 shows the ^{32}P-labeled strand after photolysis of double strand **1g** and subsequent piperidine treatment. The spot on the top of the gel corresponds to the intact oligonucleotide. The additional spots result from piperidine cleavage at the G, GA and GGG sites. Lane 2 gives the corresponding Maxam−Gilbert G sequencing reaction of the unmodified, ^{32}P-labeled single strand of **1g**.

distance dependence of eq 1.[10] Thus, the one-step charge transfer over 4 AT base pairs in double strand **1d** is divided into two subsequent steps in experiments with **1e**,**f**, one reversible charge transfer between two single G units[28] and one irreversible charge transfer from a single G to a GGG unit (Figure 4). Because each intervening AT base pair reduces the rate of the superexchange charge transfer by about 1 order of magnitude, the hole transfer over the longest sequence of AT base pairs is the bottleneck of the reaction (two AT base pairs in double strands **1e**,**f**). This explains why the charge transfer over 17 Å in **1e** and **1f** occurs with a similar efficiency as that over 10 Å in **1b**, where the total charge transfer takes place through two AT base pairs (Table 1, entries 2, 5, and 6; Figure 4).

A hopping mechanism could also explain hole transfer reactions over very long distances if in mixed DNA strands the GC pairs are separated from each other by only a few AT base pairs. We have demonstrated this in experiments with double strand **1g** where the charge transport between $G_{23}^{+\bullet}$ and the GGG unit takes place over 54 Å. In this strand the intervening GC pairs are separated from each other by not more than one AT base pair (Table 2, Figure 5). The data in Table 2 (entries 2 and 7) show that the charge transport over 54 Å in **1g** is nearly as efficient as that over 10 Å in **1b**. We suppose that the charge transport in **1g** contains several reversible charge-transfer reaction steps between single G sites and one irreversible step from a single G to the GGG unit (Figure 5B).

For a situation in which the total charge transport over the distance Δr occurs in several hopping steps of the same distance Δr_{hop}, the theory of random walk in its most simple form leads to eq 2,[27] where k and k_{hop} are the rate coefficients for the overall

$$\ln(k/k_{hop}) = -2\ln(\Delta r/\Delta r_{hop}) \qquad (2)$$

charge transport and the hopping steps, respectively. Equation 2 is indicative for a weak, algebraic distance dependence of the overall charge-transfer rate in a multistep hopping process. In contrast, a single step superexchange rate between the starting $G^{+\bullet}$ and the final trap (GGG) is expected to follow the well-known exponential distance dependence represented by eq 3.[10]

$$\ln(k/k_o) = -\beta\Delta r \qquad (3)$$

It is obvious that the influence of distance on the total charge transfer in a single step superexchange mechanism is incomparably larger than in a hopping process. Whereas a measurable unistep charge transfer over more than 50 Å cannot occur (the rate decreases by 1 order of magnitude per intervening AT base pair), the multistep hopping process described by eq 2 can still be efficient over long distances if the individual charge-transfer steps are faster than competitive chemical reactions such as those between $G^{+\bullet}$ and H_2O.

Conclusion

In biological relevant systems, where $G^{+\bullet}$ is the oxidant, long-range oxidation can occur in DNA double strands via a hopping process between the G bases. The number of AT pairs that separate the individual G bases governs the rate of individual charge-transfer steps according to the superexchange mechanism. The overall charge transport is a multistep hopping process between the G bases where the individual steps contribute to the overall rate. The efficiency of this overall charge transfer does not exhibit the strong exponential dependence on the distance which is characteristic for the individual charge-transfer steps.

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(28) In **7e** (Figure 4) the step between the single G units is presumably irreversible because of the fast subsequent hole tranfer to the GGG unit.