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Long-Distance Charge Transport in DNA: The Hopping Mechanism

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ABSTRACT

Long-distance charge transport from a guanine radical cation ($G^{\bullet+}$) to a G-rich sequence is of biological importance. This reaction was studied by selective charge injection into a G, monitoring the charge transport to a GGG sequence by competing H_2O -trapping. The efficiency of the charge transport diminished dramatically with increasing number of A:T base pairs between $G^{\bullet+}$ and GGG. But in DNA strands where G's are located between the $G^{\bullet+}$ and GGG sequence, long-distance charge transport occurred by a multistep hopping mechanism.

I. Introduction

Deoxyribonucleic acid (DNA), which stores our genetic information, is a very stable polymeric biomolecule. Nevertheless, DNA damage can occur under the conditions of oxidative stress¹ and UV irradiation.^{2a} A major target for oxidants is guanine (G), the base with the lowest ionization potential of the four DNA bases.³ This leads, among other oxidation products, to 8-oxoguanine, which reveals a lower fidelity in the replication process and enhances the probability for adenine (A) incorporation into the complementary strand.¹ Thus, under conditions of oxidative stress, mutations from guanine-cytosine (G:C) base pairs into thymine-adenine (T:A) base pairs occur.

Under UV irradiation^{2a} and in the presence of certain oxidants,^{2b} the first step of the oxidation process is the formation of a guanine radical cation ($G^{\bullet+}$). Because GG and GGG sequences have lower ionization potentials than single G's,⁴ the positive charge should migrate from the single $G^{\bullet+}$ to G clusters if long-distance electron transport through DNA is possible.^{2,5,6} As a consequence, mutations will occur predominantly at G clusters. This is very dangerous, since several hot spot codons of p53 tumor suppressor genes as well as human ras proto-oncogenes contain GG sequences (Figure 1).² A mutation in these codons increases carcinogenesis.

The question of whether and how electrons migrate over long distances through DNA was raised over 30 years ago,⁷ and is still a matter of controversial debate.⁸ Different experiments in the 1990s have led to conclusions that DNA can function as a “ π -way” over which electron-transfer

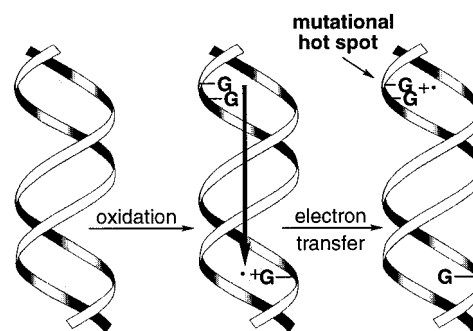


FIGURE 1. Oxidation of a single G and long-distance charge transport to a GG mutational hot spot.

reactions might be promoted efficiently,⁹ as an insulator,¹⁰ or both as wire and insulator.¹¹ The discussion is focused on the β -value of the Marcus–Levich–Jortner correlation (eq 1) that establishes an exponential rate decrease of the electron-transfer step with increasing distance. Depending

$$k \propto e^{-\beta \Delta r} \quad (1)$$

on the experiment, β -values between 1.4 and 0.1 \AA^{-1} have been reported for DNA double strands.^{11–16} These differences in β -values demonstrate dramatic divergent effects of the distance on the electron-transfer rate.

Because of the biological implications, our interest was in determining the possibility of charge transfer from a single $G^{\bullet+}$ to a GGG cluster. To answer this question, we developed an assay which enabled site-selective oxidation of single G bases.

II. Charge Injection into a Single G

Our method of charge injection is based on the spontaneous heterolytic cleavage of the phosphate ester C,O-bond in a 4'-DNA radical, **2**.^{16a} This reaction generates an enol ether radical cation in **3**, which triggers electron transfer through DNA from the nearest G. As a result, the radical cation in **3** is reduced to the enol ether unit in **4**, and the guanine radical cation ($G^{\bullet+}$) is formed (Figure 2).

In competition with this electron-transfer step (**5** \rightarrow **7** in Figure 3), the radical cation (**5** in Figure 3) is trapped by H_2O , which leads via radicals **8** and **10** to the stable products **9** and **11**. Careful HPLC analyses showed that the yield of 5'-phosphate **6** is equal to the combined yields of **7** + **9** + **11**, the products of electron transfer (**7**) and water addition (**9** + **11**) to the radical cation **5**.¹⁷ Thus, we observed a quantitative product balance. Because the reactions of radical cation **5** are irreversible and of first order (electron transfer) or pseudo-first order (trapping reaction by H_2O), the ratio of the products **7**/(**9** + **11**) is equal to the relative rate of the electron-transfer reaction step **5** \rightarrow **7**.¹⁸

We have measured these relative electron-transfer rates in several double-stranded 20mers that contained one 4'-acylated thymidine unit, as in **1**.^{17,18} Photolytic generation of the radical cation **3** occurred in 70–90% yield, which triggered the electron transfer from the

Bernd Giese was born in Hamburg, Germany, and educated in Heidelberg, Hamburg, and Munich, where he received his Ph.D. (1969) working under the guidance of Rolf Huisgen. After two years in the pharmaceutical industry (BASF), he became Privat-Dozent in Freiburg, Germany, in 1976. From 1977 to 1988 he was full professor in Darmstadt and joined the University of Basel, Switzerland, in 1989. His research interests are focused on radicals in chemical and biological systems.

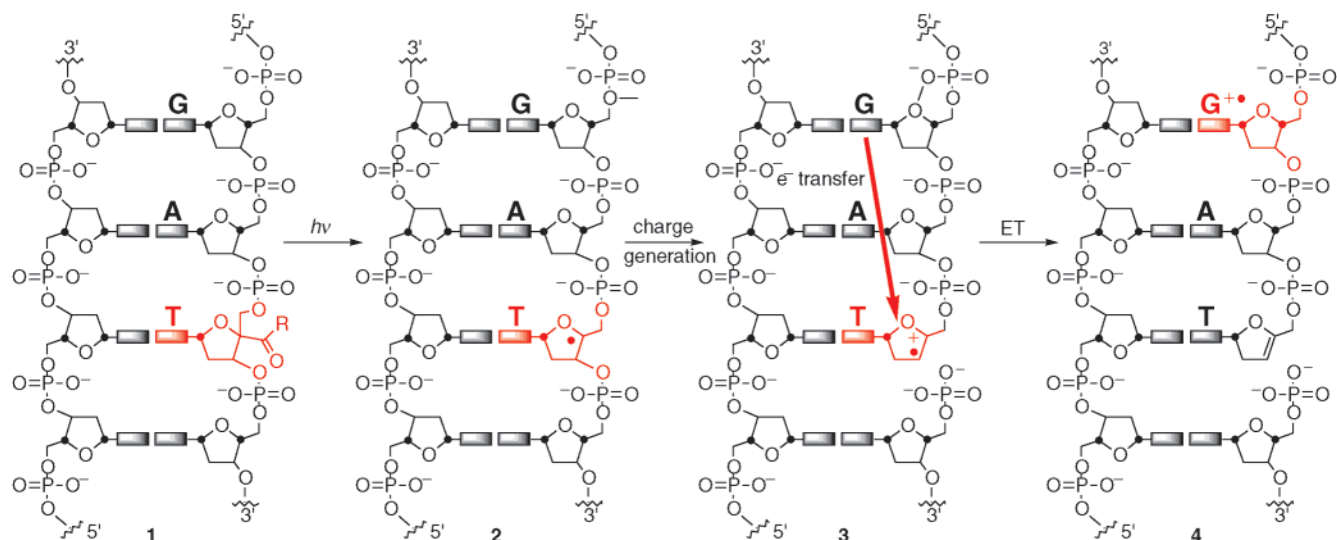


FIGURE 2. Photolytic generation of the 4'-DNA radical **2** from **1**: charge generation by heterolytic cleavage (**2** → **3**), and electron transfer (ET) through DNA (**3** → **4**). This assay is used for site-selective charge injection into a single G.

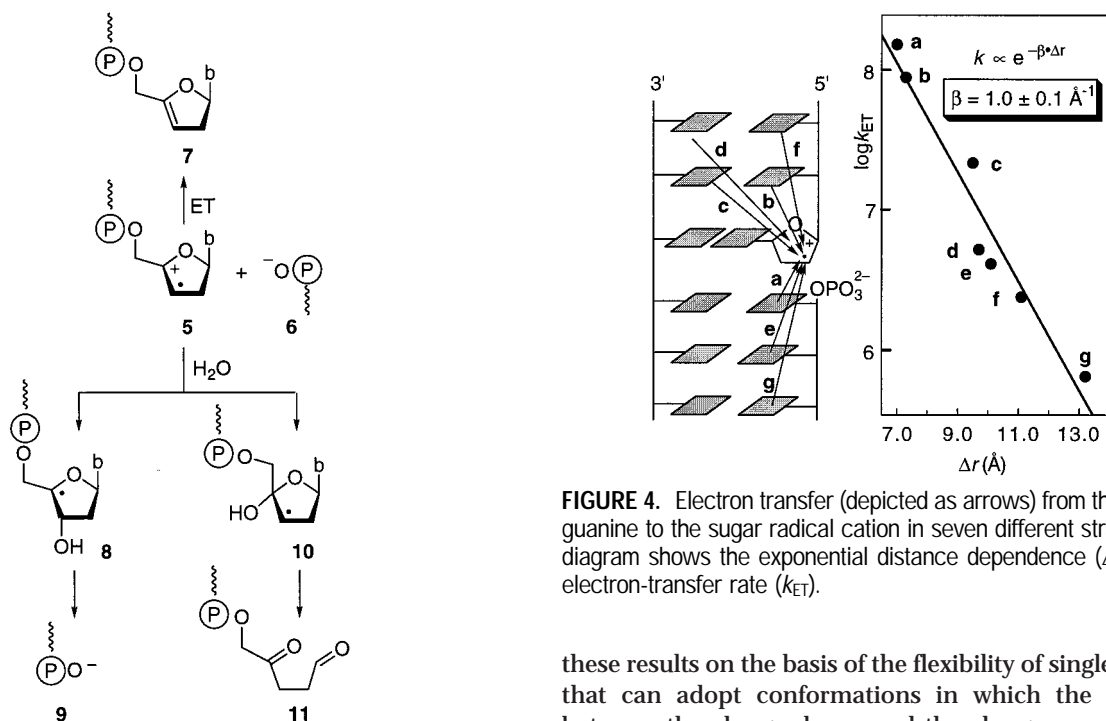


FIGURE 3. Competition between electron transfer (ET) and H₂O-trapping of the sugar radical cation **5** that is formed together with the 5'-phosphate **6** during the spontaneous cleavage of a 4'-DNA radical.

nearest G through DNA. The product ratio **7**/(**9** + **11**) depends on the distance of the nearest G in the oligonucleotide, and a plot according to eq 1 revealed a β -value of $1.0 \pm 0.1 \text{ \AA}^{-1}$ (Figure 4).

Analogous experiments with single strands showed a completely different influence of the base sequence on the electron-transfer rate: the number of T nucleotides between the radical cation and the G has only a weak influence on the rate.¹⁷ There are nearly no rate differences in strands where G is separated by two, three, or four T's from the radical cation site (Figure 5). We explained

FIGURE 4. Electron transfer (depicted as arrows) from the nearest guanine to the sugar radical cation in seven different strands. The diagram shows the exponential distance dependence (Δr) of the electron-transfer rate (k_{ET}).

these results on the basis of the flexibility of single strands that can adopt conformations in which the distance between the charge donor and the charge acceptor can be small, even if they are separated by several T units. Recent experiments by Kan and Schuster¹⁹ led to the same conclusion. The results demonstrate how important it is to exclude reactive single strands in double-strand experiments.

The ionization potential of the electron donor also influences the rate. Thus, if guanine was substituted by 8-oxoguanine, which has a 0.5 V lower ionization potential,²⁰ the rate of the electron transfer was increased by a factor of 4 in our assay.¹⁷ Nevertheless, the β -value remained unchanged within the experimental error.

III. Charge Transfer between G^{•+} and GGG

The method of charge injection described above offers the possibility for a site-selective formation of a single

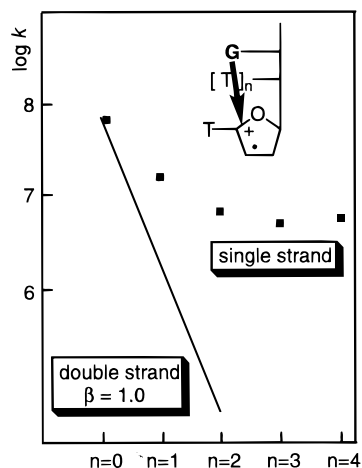


FIGURE 5. Influence of the number (n) of intervening thymines (T) between the sugar radical cation and the nearest guanine (G). The straight line is the distance dependence in double strands (see Figure 4). The squares are the results for single strands.

$G^{+\bullet}$. To study the biologically important question of whether this $G^{+\bullet}$ stimulates electron transfer from a G-rich sequence through DNA, we developed the assay shown in Figure 6.^{16b}

Photolysis of double strand **12** generated $G^{+\bullet}$ in **14** by electron transfer to the enol ether radical cation in **13**. For analytical reasons, the charge was transferred to the complementary strand (**14** \rightarrow **15**) that was radiolabeled at the 5'-end. For $G^{+\bullet}$ in **15**, a competition exists between two first- or pseudo-first-order reactions: electron transfer (**15** \rightarrow **16**) and water addition (trapping of $G^{+\bullet}$ with H_2O). The H_2O reaction of $G^{+\bullet}$ leads to an oxidatively modified guanine that can be cleaved off selectively by base treatment.²¹ The relative rate (k_{rel}) of the electron transfer from $G^{+\bullet}$ to the GGG sequence (**15** \rightarrow **16**) is given by the ratio of the cleavage products at the GGG unit and the single G base in the radiolabeled strand that can be analyzed by gel electrophoresis.^{16b} Figure 7 shows the

results for double strands **17**–**20**, where the distances between the $G^{+\bullet}$ and the GGG units increase from 7 to 17 Å.

The rate of the charge-transfer step decreased by about a factor of 10 per each intervening A:T base pair.^{16b,22} Thus, the amount of charge (ϵ) trapped by the H_2O reaction at the GGG unit decreased from 97% at 7 Å to 3% at 17 Å (Figure 7). At distances longer than 17 Å, a charge transfer from $G^{+\bullet}$ to a GGG sequence could not be detected by our assay. Recently, Saito *et al.*²³ observed similar effects in an assay where the charge was injected from a photoexcited benzophenone system. The distance influence on the charge transfer led to a β -value of $0.7 \pm 0.1 \text{ Å}^{-1}$ which is in very good accord with experiments of Lewis and Wasielewski *et al.*¹² where the electron transfer was triggered by a photoexcited stilbene (Figure 8).

IV. Hopping Mechanism

In our assay the efficiency of the charge transfer (ϵ)—that is, the amount of charge trapped by the H_2O reaction at the GGG unit—decreased from 97% at 7 Å to 3% at 17 Å (Figure 7).^{16b} In experiments with strands having five and more A:T base pairs between $G^{+\bullet}$ and the GGG unit, a charge transfer could no longer be detected.^{16b,23} Nevertheless, we observed a very efficient long-distance charge transport (70%) in double strand **21**, although the charge donor $G^{+\bullet}$ and the charge acceptor GGG are separated from each other by 15 base pairs (54 Å) (Figure 9).^{16b} This was a surprising result and showed that it is not the distance alone that determines the efficiency of the long-range charge transport; the sequence also has to play a decisive role.

DNA **21** contains 8 G's between the first $G^{+\bullet}$ and the GGG unit. We assume that these intervening G's can be oxidized by the $G^{+\bullet}$; thus, they act as relay stations for the charge on the way to the GGG unit.^{16b} As a result, the charge transport from the first $G^{+\bullet}$ to the GGG occurs not

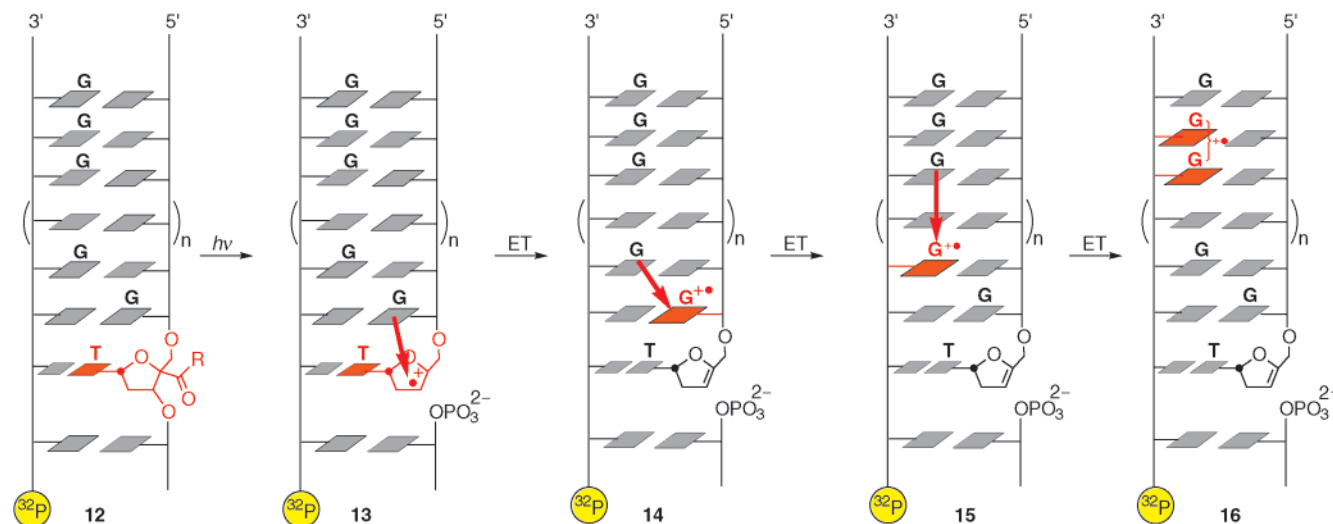


FIGURE 6. Charge injection into a single G (**12** \rightarrow **14**), charge transport to the complementary, radiolabeled strand (**14** \rightarrow **15**), and charge transport from a single $G^{+\bullet}$ to a GGG sequence (**15** \rightarrow **16**). This assay is used to determine the relative rates and efficiencies of the charge transport from a single $G^{+\bullet}$ to a GGG sequence.

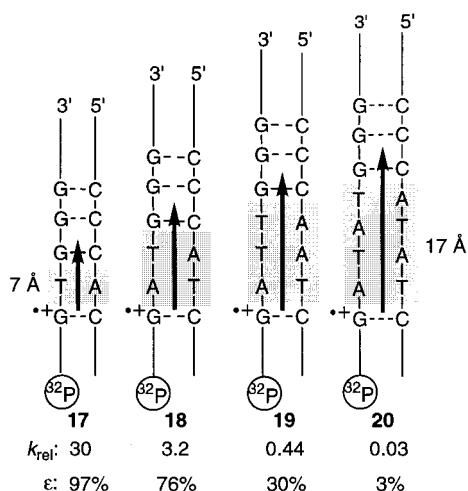


FIGURE 7. Relative rates (k_{rel}) and efficiencies (ϵ , amount of charge detected at the GGG unit) for the charge hopping from G^+ to the GGG sequence in double strands 17–20.

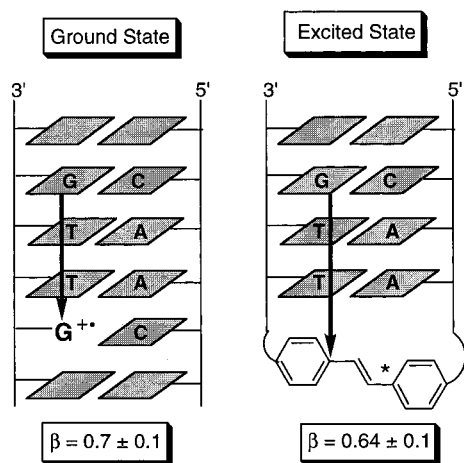


FIGURE 8. Assays for the determination of β -values in the ground state^{16b} and the photoexcited state.¹²

in one step but in a multistep reaction. Jortner et al.²⁴ have characterized such a situation by correlation (2), where a

$$\ln E \propto -\ln N \quad (2)$$

charge migrates by a random walk (linear diffusion) through DNA. In eq 2, E is the efficiency of the charge transport expressed as the ratio between the trapped GGG sequence and the single G's. The number of the equidistant hopping steps is N .

We proved correlation (2) in experiments with four different double strands, where the number (N) of the electron-transfer steps, each of them over a distance of 10 Å, increased from 1 to 4.²⁵

Equation 1 gives the distance dependence of each single step, and eq 2 describes the overall charge transport via several steps. Whereas the rate of each charge-transfer step depends exponentially on the distance (eq 1), the efficiency of the overall, long-distance charge transport of the multistep reaction has an algebraic dependence on the number of steps (eq 2). Therefore, the multistep hopping process reduces dramatically the influence of the

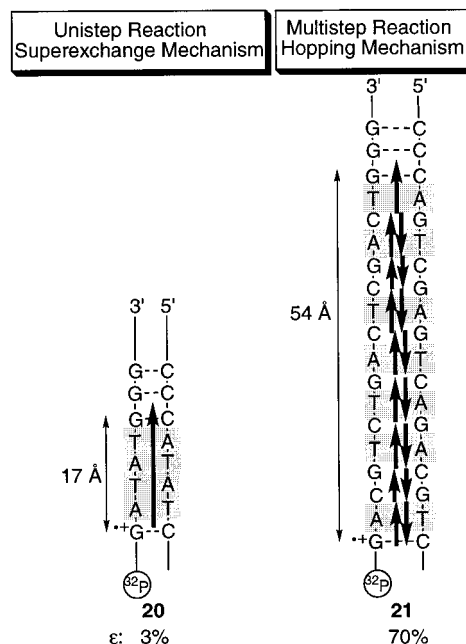


FIGURE 9. Efficiencies (ϵ , amount of charge detected at the GGG unit) of the charge transfer in a unistep and a multistep reaction over 17 and 54 Å, respectively.

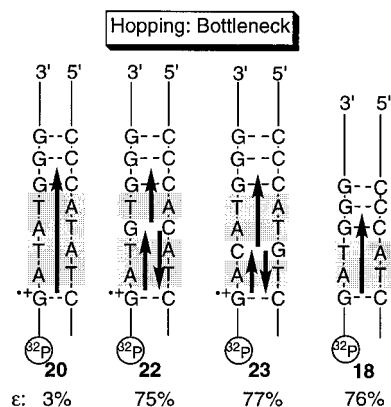


FIGURE 10. Sequence influence on the efficiency (ϵ , amount of charge detected at the GGG unit) of the charge transport over 17 Å in strands 20–23.

distance on the overall transport efficiency.²⁶ Actually, in mixed DNA double strands, the efficiency of the long-distance charge transport is determined by the longest hopping step. Such a “bottleneck” situation could be demonstrated by exchanging A:T pairs by G:C pairs in strand 20 or by exchanging G:C pairs by A:T pairs in 21.^{16b} This AT–GC exchange led to systems 22–24, which contained intervening A:T sequences of different lengths. The data of Figures 10 and 11 show that the efficiencies of the long-distance charge transport in 22–24 are nearly the same as those through the “bottleneck” sequences (the longest individual step between two G's).

This hopping model was supported by the kinetic analysis of Jortner and Bixon et al.,²⁷ who treated the hopping process as a sequential reaction which is characterized by the rates of the electron-transfer steps and the trapping steps. Another breakthrough is the absolute rate measurements of Lewis and Wasielewski et al.,²⁸ who

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- (22) In experiments with two A:T base pairs between G^{•+} and GGG (double strand **18**) the sequence was varied: G^{•+}NNGGG, NN = AA, AT, TA, TT. This variation changed the ratio of the trapping products by less than a factor of 2. Thus, the orientation of the two A:T base pairs has only a small effect on the charge-transfer step. Similar experiments with longer AT sequences are under way.
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