COMMUNICATION

ChemComm
www.rsc.org/chemcomm

Charge transfer through DNA triggered by site selective charge injection into adenine

Thomas Kendrick and Bernd Giese*

Department of Chemistry, University of Basel, St. Johanns Ring 19, CH-4056 Basel, Switzerland. E-mail: bernd.giese@unibas.ch; Fax: +41-61-267-1105; Tel: +41-61-267-1106

Received (in Cambridge, UK) 21st June 2002, Accepted 1st August 2002
First published as an Advance Article on the web 12th August 2002

A positive charge, which was injected site selectively into adenine (A) of a DNA double strand, migrates along $(A:T)_n$ sequences in a distance independent way.

Recently, we have found that hole transfer between guanines (G) in DNA double strands follow two different mechanisms.[1] In cases where the distance between guanines is short, the rates depend strongly upon the number of adenine:thymine (A:T) base pairs. This is in accord with a single step charge shift between the guanines where the A:T base pairs act as bridges that do not carry the charge.[2] But, if the guanines are separated by long A:T sequences as in DNA **1**, the distance effect vanishes.[1] We have explained this surprising result with a mechanism in which a guanine radical cation ($G^{\bullet+}$) oxidises the adjacent adenine in an endothermic reaction step (Fig. 1).[1,3] After this rate determining injection step the charge is transferred rapidly along the adjacent adenines until it is trapped by the GGG unit. The charge was detected at the guanines by their reactions with water, which led after base treatment to cleavage products P_G and P_{GGG}, respectively (Fig. 1).[4]

We have now checked this mechanism using a DNA double strand like **2** where the positive charge was directly injected into an adenine base, which is situated inside the A:T sequence. As injection system we synthesized the modified adenine **7** that was incorporated into DNA oligomers. As shown in Scheme 1, the synthesis of **7** starts from the deoxyribose derivative **3**, which was recently described by D. Crich.[5] After formation of the *tert*-butyl ketone and change of the protecting groups at C-5′ (**3**→**4**), the benzoylated adenine was introduced (**4**→**5**). In further steps the appropriate protecting groups for the DNA synthesis were attached at C-3′ and C-5′ (**5**→**7**). For experiments with the monomer the 3′-OH group was phosphorylated (**9**→**10**).[6]

We had observed earlier that photolysis of the 4′-pivaloylated nucleotide **10a**, which carries a guanine as base yielded the enol ether **13a** nearly quantitatively.[7] This is because the intermediate enolether radical cation **11a**, which is generated by photocleavage of the ketone and subsequent heterolysis, oxidises guanine at C-1′ much faster than it reacts with water. On the other hand, with thymine as base (**10b**) no enol ether (**13b**) was observed[8] because the ionisation potential of T is much higher than that of G.[9] The redox potential of adenine lies between that of thymine and guanine, and photolysis of **10c** gave enol ether **13b** in 45% yield (Scheme 2).[10] Thus, the oxidation of the adenine base (b = A) by the enol ether radical cation of **11c** competes successfully with its trapping reaction by water. This offers, for the first time, the possibility to inject site selectively a charge into adenine of DNA double strands.

We therefore introduced the pivaloylated building block **7** into DNA double strands **14a**,**b**, and generated the intermediate adenine radical cation in **15a**,**b** by photolysis.[11] The positive charge in **15a**,**b** migrated towards the 3′- and the 5′-end until it was trapped by the GGG sequences. The amount of the charge reaching the guanines was detected by reaction of the guanine

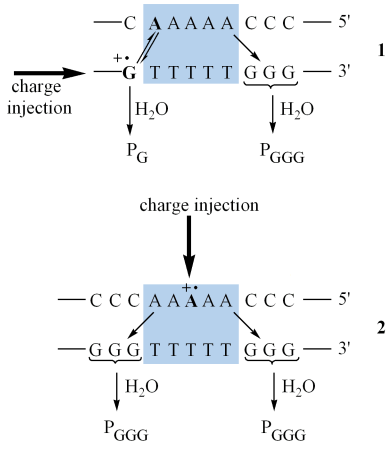
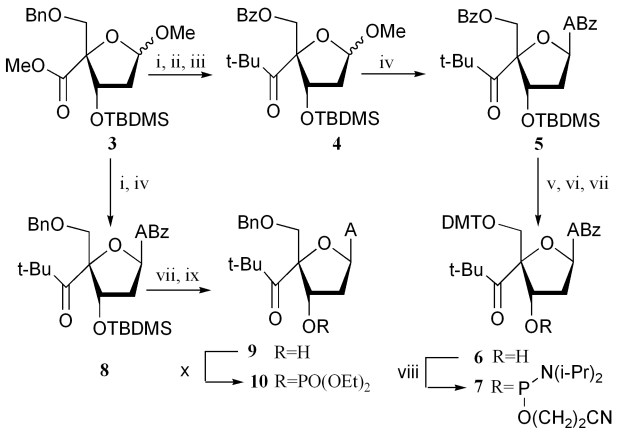
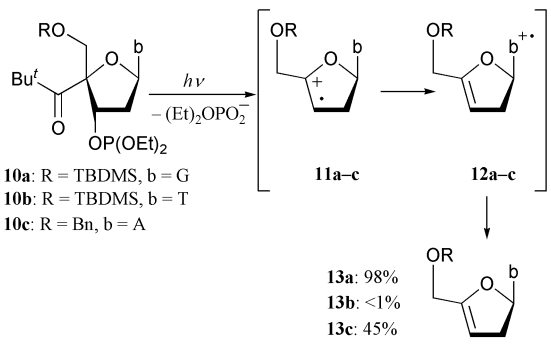


Fig. 1 Charge injection into adenine (A) at the end (strand **1**) or in the middle (strand **2**) of an $(A:T)_5$ sequence.



Scheme 1 Reagents and conditions: i, t-BuLi, THF, −78 °C, 20 min, 77%; ii, *hν*, NBS, $CaCO_3$, CCl_4, rt, 80%; iii, BzCl, DMAP (5%), 55 °C, 1 h, 82%; iv, N^6-benzoyladenine-TMS, $SnCl_4$, MeCN, rt to 40 °C, 48 h, 60% α:β(1:4); v, NaOH, Py/EtOH/H_2O, 0 °C, 20 min, 70%; vi DMTCl, Py/Col (1:1), MeCN, 60 °C, 83%; vii, TBAF, rt, 30 min, 80%; viii 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite, EtN(i-Pr)$_2$, CH_2Cl_2, rt, 30 min 84%; ix K_2CO_3, MeOH/H_2O, 0 °C to rt, 24 h, 89%; x t-BuMgCl, ClPO(OEt)$_2$, THF, rt, 85%.



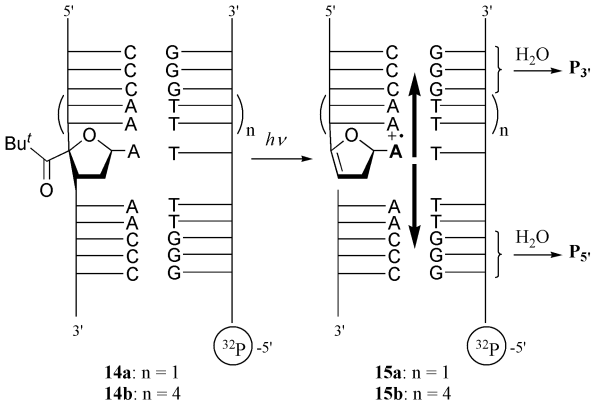
Scheme 2

DOI: 10.1039/b205928b

radical cations with water, which afforded products $P_{3'}$ and $P_{5'}$ after subsequent strand cleavage (Scheme 3).[12]

First, we checked whether the efficiency of the charge transfer towards the 5′-end is different from that towards the 3′-end. It turned out that photolysis of **14a**, where both sequences between $A^{\cdot+}$ and the GGG units contain two A:T base pairs, gave about the same amount of products $P_{3'}$ (55%) and $P_{5'}$ (45%) (Fig. 2). In strand **14b** one of the A:T sequences was extended from 2 to 8 A:T base pairs. Nevertheless, we observed nearly the same ratio of products $P_{3'}/P_{5'}$ (Fig. 2). Thus, the efficiency of the charge migration through the A:T sequences alters very little depending on the number of the A:T base pairs in these experiments.

If the positive charge hops reversibly between all adenines, the charge transport *via* the stretch of 8 A:T base pairs of **14b** should be less efficient than *via* the stretch of 2 A:T base pairs.[2,4]



Scheme 3

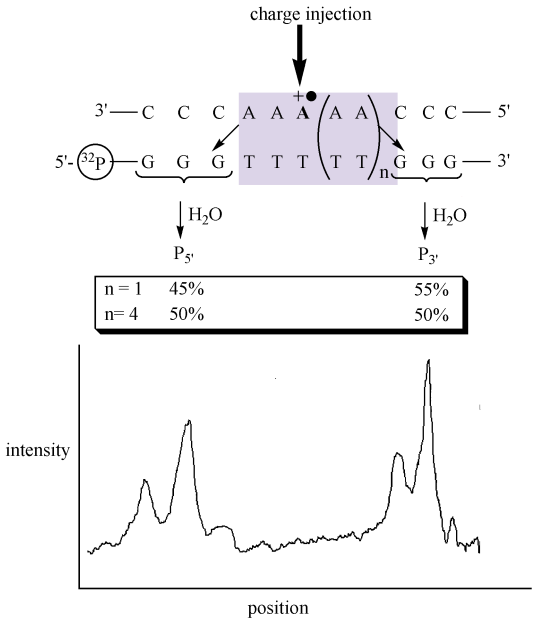


Fig. 2 Histogram of denaturing polyacrylamide gels, obtained by subtraction of control experiments (irradiation of unmodified strands) from irradiation experiments with the modified strand **14a** (n = 1), and relative yields of the strand cleavage products at the 5′- and 3′-GGG units for strands **14a** (n = 1) and **14b** (n = 4).

But this is not the case as the data in Fig. 2 show. A possible explanation is that the positive charge, injected into the adenine is delocalized over adjacent A:T base pairs. According to this suggestion, in well-organised sequences where the adjacent base pairs of the DNA have nearly the same redox potentials, a positive charge is not localised at one base pair but is distributed over several base pairs. This is reminiscent of the 'polaron' picture for long distance charge transfer through DNA that was discussed by G. B. Schuster[13] and E. M. Conwell,[14] or even of the conductive band in a semiconductor.[15] Further experiments with this new assay will show how robust this charge delocalisation is against changes in the A:T sequence.

Generous support by the Swiss National Science Foundation and the Volkswagen Foundation are gratefully acknowledged.

Notes and references

1 B. Giese, J. Amaudrut, A.-K. Köhler, M. Spormann and S. Wessely, *Nature*, 2001, **412**, 318.

2 J. Jortner, M. Bixon, T. Langenbacher and M. E. Michel-Beyerle, *Proc. Natl. Acad. Sci. USA,*, 1998, **95**, 12759.

3 B. Giese and M. Spichty, *ChemPhysChem*, 2000, **1**, 195; B. Giese and A. Biland, *Chem. Commun.*, 2002, 667.

4 E. Meggers, M. E. Michel-Beyerle and B. Giese, *J. Am. Chem. Soc.*, 1998, **120**, 12950.

5 D. Crich and X. Hao, *J. Org. Chem.*, 1999, **64**, 4016.

6 The debenzylation of **3** required NBS because standard deprotection methods like hydrogenation (Pd/C, H_2) were unsuccessful. The experimental details for the synthesis of modified nucleotides **7** and **10** will be described in a full paper. The configurations were confirmed by comparing the ^{1}H-NMR spectra of compound **8** with the analogous ester nuclosides described in ref. 5. The α- and β-anomers are easily distinguished by the chemical shifts of the OTBDMS group at C-3′.

7 M. Spormann and B. Giese, *Synthesis*, 2001, 2156.

8 E. Meggers, A. Dussy, T. Schäfer and B. Giese, *Chem. Eur. J.*, 2000, **6**, 485.

9 C. A. M. Seidel, A. Schulz and M. H. M. Sauer, *J. Phys. Chem.*, 1996, **100**, 5541; S. Steenken and S. V. Jovanovic, *J. Am. Chem. Soc.*, 1997, **119**, 617.

10 Photolysis of nucleotide **10c** was carried out following the procedure described in ref. 7.

11 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 **7** was done manually by removing the solid phase column from the machine and by passing the phosphoramidite into the column *via* two syringes, one containing **7** in MeCN and the second syringe containing coupling solution. With this modification there is no notable difference between the efficiency of coupling for this modified amidite and non-modified ones. Workup was done by standard procedures. The purity of all oligonucleotides was controlled by reverse phase chromatography and MALDI-ToF MS. Photolyses of the modified oligonucleotides were performed as described in ref. 4. The sequences upstream (5′-direction) and downstream (3′-direction) of the GGG units are ATATAATTTCG and ATATTATGCGA, respectively.

12 For the strand cleavage at the sites of oxidised guanines 30 μl of the probes were treated with 300 μl of 1 M piperidine at 90 °C for 30 min. With this assay we could not detect cleavage at the adenines. By this method the efficiencies of the charge transfer reactions are measured, which not only depend upon the charge transfer rate but also upon the rate of the irreversible trapping of the guanine radical cation ($GGG^{\cdot+}$) by H_2O. Only if this water trapping reaction is as fast or faster than the endothermic electron transfer from A to $GGG^{\cdot+}$, these yields can be correlated with charge transfer rate ratios. From our earlier experiments (ref. 1) it can be deduced that this might actually be the case.

13 G. B. Schuster, *Acc. Chem. Res.*, 2000, **33**, 253.

14 S. V. Rakhmanova and E. M. Conwell, *J. Phys. Chem. B*, 2001, **105**, 2056.

15 D. Porath, A. Bezryadin, S. de Vries and C. Dekker, *Nature*, 2000, **403**, 635.