CHAPTER 7

An Electrochemical Probe of DNA Stacking in an Antisense Oligonucleotide Containing a C3′-Endo Locked Sugar

Adapted from Boon, E.M., Barton, J.K., Pradeepkumar, P.I., Isaksson, J., Petit, C., Chattopadhyaya, J. (2002) *Angewandte Chemie International Edition*, in press.

T phosphoramidite was synthesized and its biochemical and physical properties were characterized by P.I.P., J.I., and C.P.

INTRODUCTION

Charge transport (CT) through double helical DNA is now well established, but this reaction is extremely sensitive to DNA base pair stacking (1-11). Sequence dependent DNA structure, conformational dynamics, and local flexibility all contribute to coupling within the base pair π-stacked array, and thus to the efficiency of DNA CT chemistry. This sensitivity in base stacking also provides the foundation for applications of DNA CT that probe nucleic acid structure, particularly those utilizing electrochemistry experiments on DNA films as discussed in Chapters 2 and 3 (12-19). In these experiments, DNA oligonucleotide duplexes modified with a thiol linker are self-assembled on a gold electrode surface. A redox active intercalator, such as methylene blue (MB^+), bound to the close packed DNA films is electrochemically reduced and the reduced intercalator is used as a catalyst for the reduction of a species diffusing in solution outside of the DNA film (usually ferricyanide). Once reoxidized by ferricyanide, methylene blue is available for subsequent electrochemical reduction and the catalytic cycle continues (15-16). The electrochemical reduction of MB^+ takes place via CT through the DNA base stack, and thus perturbations in base pair stacking are repeatedly interrogated in this assay, rendering our electrocatalytic assay exquisitely sensitive to even the smallest disruptions in π-stacking. Using this technique, all single base mismatches have been detected as well as several common DNA base damage products (Chapter 3, ref. 16). Base stacking perturbations are also detected within DNA/RNA hybrid duplexes (Chapter

3, ref. 18). Furthermore, the electrochemical reduction of DNA intercalators bound to DNA-modified electrodes has also been used to monitor DNA-protein interactions in Chapter 8 (19).

Therefore, since DNA-mediated CT reactions are extremely sensitive to very small changes in DNA base pair stacking, we may exploit this assay generally in probing local perturbations in nucleic acid structure and stacking. Here we describe the application of electrocatalysis at DNA-modified electrode surfaces to probe stacking in an antisense oligonucleotide (AON) containing a 3′-locked endo conformation (Figure 7.1).

Hybrid duplexes formed by AON containing one North-form (3′-endo) locked 1-(1′,3′-*O*-anhydro-β-D-psicofuranosyl) thymine block **T** (Figure 7.2) paired with RNA (AON/RNA heteroduplex) are completely inactive to cleavage by RNase H in four nucleotides to the 5′ side of the conformationally constrained nucleotide (20). In fact, a single cleavage site can be engineered in a AON/RNA hybrid at the site of **T** incorporation (21). This protection from cleavage is proposed to result from the formation of a local RNA/RNA type duplex conformation in the vicinity of **T** as opposed to the normal B-DNA/A-RNA type hybrid conformation. This result underscores the importance of knowledge of local conformation for antisense design and recruitment of RNase H. Furthermore, these studies lead to the suggestion that incorporation of a 3′-endo type locked nucleotide (typical for A-form duplexes) into a nucleic acid duplex leads to formation of a *local* A-form duplex which, if true, could lead to a *junction* in base pair stacking.

Structural studies have also been performed on oligonucleotide duplexes containing **T** moieties (22). CD spectra show that there is more

Figure 7.1. Schematic illustration of our electrocatalytic method to directly measure stacking in oligonucleotide duplexes. Here we probe the stacking of T (*) within a DNA scaffold and within a DNA/RNA hybrid scaffold. Oligonucleotide duplexes are tethered to alkanethiol linkers and self-assembled onto Au electrode surfaces for use in electrochemical assays. Stacking is probed in a DNA-mediated charge transport reaction to reduce MB^+ that serves as a catalyst to reduce ferricyanide in solution. If stacking is disrupted, MB^+ is not efficiently reduced and less charge accumulates over the course of the catalytic assay.

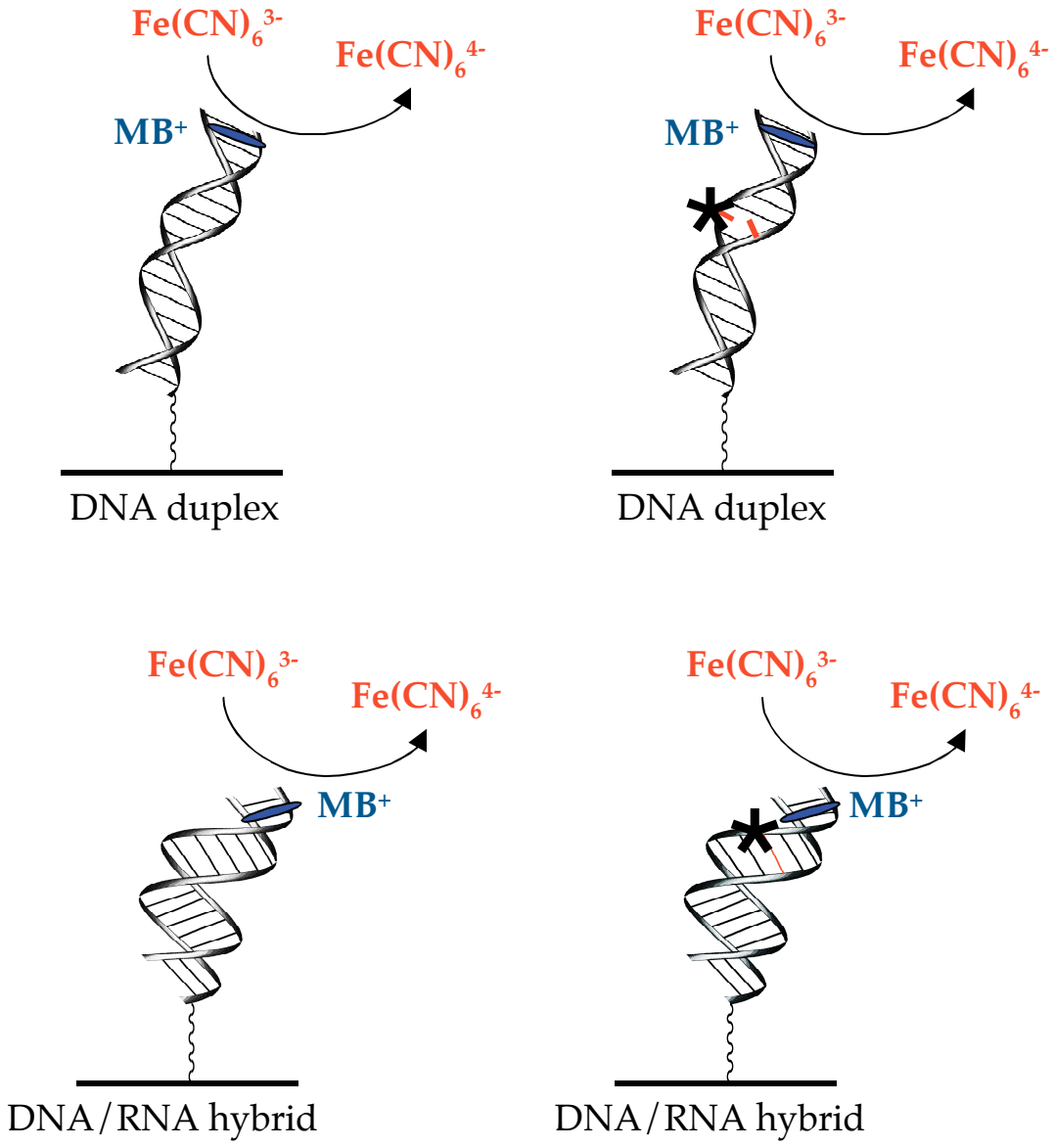
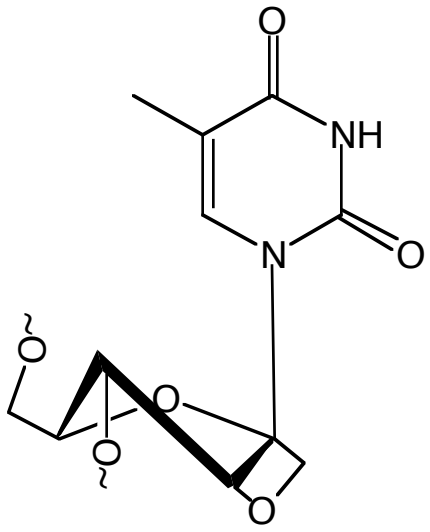


Figure 7.2. Chemical structure of **T**. The linker forces the sugar to adopt a 3′endo conformation.



distortion in a DNA/DNA duplex containing **T** than the corresponding DNA/RNA hybrid duplex. Furthermore, although a T_m drop of 6°C/**T** incorporation is observed, the Watson-Crick hydrogen bonding remains intact as is evident from ^{1}H NMR studies. This suggests that **T** is hydrogen bonded in the duplex base stack and the T_m drop is likely the result of a change in the stacking geometry of the duplex, not loss of hydrogen bonding as with mismatches in DNA. Given the differing behavior in a DNA/DNA versus DNA/RNA duplex, it seems likely that this change in stacking geometry of the duplex is orchestrated by the sugar conformation.

These structural data coupled with the RNase H cleavage protection studies suggest that **T** may introduce a junction in the base pair stacking of DNA. However none of these methods probe the base stacking of DNA directly. CT through DNA, however, does provide a direct assay of base pair stacking. So here we apply charge transport through DNA-modified surfaces to probe the base pair stacking of **T** within DNA/DNA and DNA/RNA hybrid duplexes.

MATERIALS AND METHODS

Materials

All DNA synthesis reagents were obtained from Glen Research. Methylene blue and potassium ferricyanide were purchased from Aldrich

and used as received. **T** was synthesized and incorporated into DNA by using a previously described procedure (23).

Preparation of DNA-modified surfaces

Thiol-modified oligonucleotides were prepared using phosphoramidite synthesis as described in the Appendix. Thiol-terminated linkers were attached to single stranded oligonucleotides, HPLC purified, and hybridized to unmodified complements. These duplexes (0.1 mM) were then deposited on polycrystalline gold electrodes for 12-24 hours, thoroughly rinsed with buffer and used for electrochemical experiments. These surfaces have been characterized by cyclic voltammetry, ellipsommetry, radiolabeling of the duplexes, and AFM. We have found densely packed monolayers with a 45° orientation of the helical axis with respect to the gold surface are formed when no potential is applied to the electrode surface.

Electrochemical measurements

Chronocoulometry was carried out anaerobically (bubbling argon) on 0.02 cm^2 gold electrodes using a Bioanalytical Systems (BAS) Model CV-50W electrochemical analyzer. Buffer and electrolyte conditions were 5 mM sodium phosphate buffer containing 50 mM NaCl, pH 7, ambient temperature. A normal three electrode configuration consisting of a modified gold disk working electrode, a saturated calomel reference electrode (SCE, Fisher Scientific), and a platinum wire auxiliary electrode was used. The working compartment of the electrochemical cell was separated from the

reference compartment by a modified Luggin capillary. Potentials are reported versus SCE.

RESULTS AND DISCUSSION

To determine whether **T** disrupts base pair stacking within a DNA scaffold and within a DNA/RNA hybrid scaffold, we incorporated **T** into DNA-modified electrode surfaces and monitored the electrocatalytic efficiency in these films (Figure 7.1). Electrode surfaces modified with duplexes **1**-**12** (Table 5.1) were interrogated in electrocatalytic chronocoulometric analyses at -350 mV (vs. SCE) with 0.5 μM methylene blue (MB^+) as the intercalated catalyst and 2.0 mM $Fe(CN)_6^{3-}$ as the solution-borne substrate (15,16).

Figure 7.3 shows the results of **T** incorporation into DNA/DNA duplexes (sequences **1**-**5**). In these DNA/DNA B-form duplexes, the current is attenuated, similarly to that in mismatch controls (Figure 7.4). Thus these data suggest that incorporation of a 3'-endo constrained pentoribofuranose unit into a duplex where all other deoxyribose units are 2'-endo creates a *base* stacking perturbation.

We further explored this phenomenon by incorporating **T** into DNA/DNA homoduplexes and DNA/RNA hybrid duplexes, as shown in Figure 7.5. In the DNA/DNA homoduplexes (**10**) **T**, again, yields a diminution in integrated charge. However, upon incorporation of **T** into a

DNA/RNA A-form heteroduplex (**12**), little diminution in integrated charge, consistent with little perturbation in stacking, is evident.

These electrochemical data are in excellent correlation with the RNase H protection studies (20,21) and NMR results (22). The electrochemical data suggest that ribose conformation of a nucleotide moiety dictates its stacking in a duplex. In B-form DNA/DNA duplexes, the sugar conformation is normally 2'-endo. Thus when a nucleotide constricted to 3'-endo type conformation is incorporated into the duplex, the associated base assumes typical A-form stacking (and possibly transfers this conformation to four other neighboring nucleotides (20)), which results in a stacking junction between the A- and B-form parts of the duplex. When **T** is incorporated into a hybrid duplex, where stacking is a mix between A- and B-form, this locked nucleotide is better accommodated by the neighboring nucleotides and does not lead to a stacking perturbation.

Thus, these observations suggest that the origin of the drive for helical order vis-a-vis disorder in determining the *local structure* of a nucleic acid duplex lies in the preferred nature of the sugar conformation, which can be designed, engineered and enforced to orchestrate the helix forming process. Importantly, these results illustrate the utility of DNA-mediated CT through DNA-modified surfaces as a tool for directly probing DNA base pair stacking.

Table 7.1

1	SH[i]-5'GAAGAAAAAATGAAG CTTCTTTTTTACTTC
2	SH-5'GAAGAAAAAATGAAG CTTCT**T**TTTTACTTC
3	SH-5'GAAGAAAAAATGAAG CTTCTTT**T**TTACTTC
4	SH-5'GAAGAAAAAATGAAG CTTCTTTTT**T**ACTTC
5	SH-5'GAAGAAAAAATGAAG CT**T**CTTTTTTACTTC
6	SH-5'GAAGAAAAAATGAAG CTTCT**C**TTTTACTTC
7	SH-5'GAAGAAAAAATGAAG CTTCT**A**TTTTACTTC
8	SH-5'GAAGAAAAAATGAAG CTTCT**G**TTTTACTTC
9	SH-5'AGTACAGTCATCGCG TCATGTCAGTAGCGC
10	SH-5'AGTACAG**T**CATCGCG TCATGTCAGTAGCGC
11	SH-5'AGTACAGTCATCGCG tcatgtcagtagcgc[ii]
12	SH-5'AGTACAG**T**CATCGCG tcatgtcagtagcgc

[i]SH-5' stands for $SH(CH_2)_2CONH(CH_2)_6NHCO$ attached to the 5' OH of the DNA single strand. [ii]Lowercase lettering indicates RNA.

Figure 7.3. Electrochemical results for T incorporated into DNA-modified surfaces. Sequences **1**-**5** were self-assembled on a clean gold electrode surfaces and used in electrocatalysis experiments. Chronocoulometric analyses took place at -350 mV with 0.5 μM methylene blue (MB^+) as the intercalated catalyst and 2.0 mM $Fe(CN)_6^{3-}$ as the solution-borne substrate. This chronocoulometry plot shows the results for T incorporated at a variety of positions within the duplex. Within a DNA/DNA duplex, T creates a stacking perturbation.

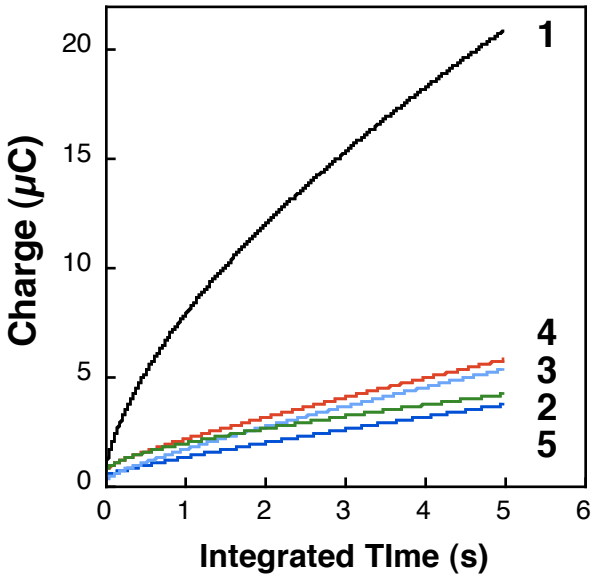


Figure 7.4. Electrochemical results for mismatches incorporated into DNA-modified surfaces. Sequences **1** and **6-8** were self-assembled on a clean gold electrode surfaces and used in electrocatalysis experiments. Chronocoulometric analyses took place at -350 mV with 0.5 □M methylene blue (MB^+) as the intercalated catalyst and 2.0 mM $Fe(CN)_6^{3-}$ as the solution-borne substrate. These data serve as a control for base stacking. Here the results for mismatches resulting in sequences **6**-**8** are compared to the perfectly complementary native sequence **1**. The mismatches presented here are similar to the results for placing T within a DNA duplex.

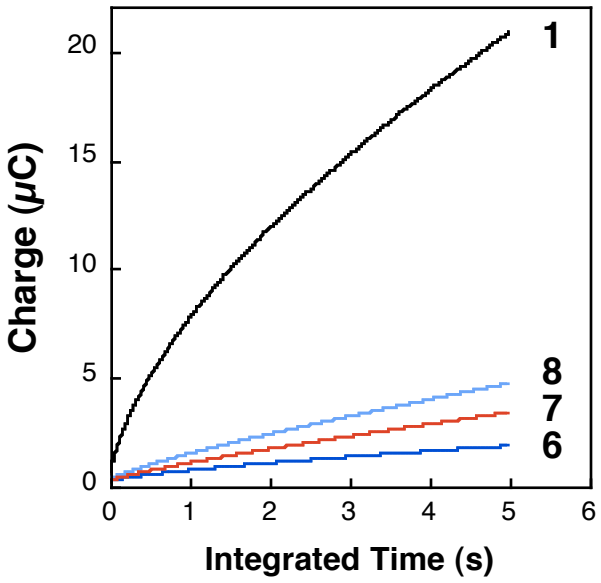
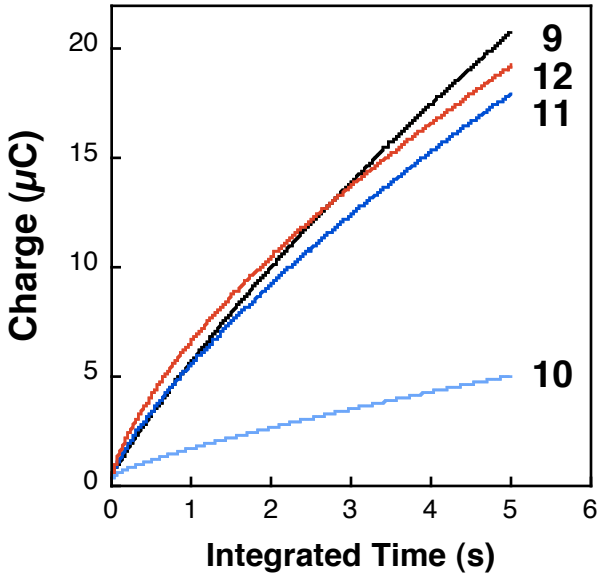


Figure 7.5. Electrochemical results for **T** incorporated into NA-modified surfaces. Sequences **9-12** were self-assembled on a clean gold electrode surfaces and used in electrocatalysis experiments. Chronocoulometric analyses took place at -350 mV with 0.5 □M methylene blue (MB^+) as the intercalated catalyst and 2.0 mM $Fe(CN)_6^{3-}$ as the solution-borne substrate. This plot contains the chronocoulometry results for **T** incorporated into DNA/DNA verses DNA/RNA modified surface. Within a DNA/DNA duplex, **T** creates a stacking perturbation (**10**), however, within a DNA/RNA duplex, stacking is normal (**12**).



SUMMARY

Charge transport through DNA-modified surfaces has been shown to sensitively detect base stacking perturbations such as single base mismatches (Chapter 2 and 3). In this chapter, this methodology is extended to probe the preferred base stacking orientation of a conformationally constrained nucleotide (3'-endo locked) within DNA/DNA and DNA/RNA duplexes. The conformation of the sugar is seen to sensitively determine the local stacking of the duplex. These results illustrate the utility of DNA-mediated charge transport through DNA-modified surfaces in characterizing small perturbations in DNA stacking and structure.

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