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Artificial Sandwich Base for Monitoring Single-Nucleobase Changes and Charge-Transfer Rates in DNA

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Abstract

Developing a convenient method to discriminate among different types of DNA nucleotides within a target sequence of the human genome is extremely challenging. We herein report an artificial ferrocene-base (Fe-base) that was synthesized and incorporated into different loci of a DNA strand. The Fe-base replacement on a nucleobase can interact with DNA bases and efficiently discriminate among A, T, G, and C DNA bases of the complementary locus on the basis of interacting electrochemical properties. Furthermore, cyclic-voltammetry (CV) studies demonstrated the electrochemical stability of DNA strands incorporated with Fe-bases and the reversibility of the incorporation. Square-wave voltammetry (SWV) was performed to measure current changes between Fe-bases and bases of interest in the DNA duplex. The changes in the charge-transfer rates appeared to be correlated with the position of the Fe-base in the DNA strand, allowing rapid and efficient sensing of single-nucleobase changes in DNA and showing promise for the design of Fe-oligomer chip technology as a tool for DNA sequencing.

Graphical Abstract

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R.A. and S.X. are co-first authors.

Supporting Information

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Additional experimental details, synthesis of Feposphoramidite, DNA complementary sequences, redox properties of the ferrocene base incorporated into a DNA strand, squarewave voltammograms, cyclic voltammograms, scan-rate dependence of the anodic and cathodic peaks of the cyclic voltammograms for Fe-DNAs, and MALDI-TOF analyses (PDF)

The authors declare no competing financial interest.

In the past few decades, many modifications have been made on natural bases and nucleobase replacements, including mimics, simple hydrocarbons, heterocycles, and fluorescent species.¹ These new constructs have a range of novel properties that can be exploited for biological sensing and biotechnology.^{2–4} Ferrocene (Fe), a metallocene, has attracted the attention of the scientific and technical communities because of its interesting chemistry. In particular, its remarkable redox properties have been widely utilized in chemical sensors as well as in biosensors. Therefore, it is not surprising that incorporation of ferrocene moieties into DNA has become a highly attractive field of study given the wide range of potential applications,⁵ including the development of electrochemical sensors⁶ and DNA nanomaterials.⁷

Over the last two decades, scientists have succeeded in conjugating ferrocene with DNA through its backbone sugar and nucleobases in order to monitor signal changes with the aim of detecting targets.^{8–13} Previously, deoxyuridine was conjugated with ferrocene by a C–C linkage to study the positional charge-transfer effect of dsDNA. Even though this study showed the charge-transfer effect of dsDNA, it seemed to be a kind of extended uridine base, and the interaction or charge-transfer effect could be altered by uridine-complementary-base-pair interactions.¹⁴ However, to date, no studies have reported the replacement of ferrocene on nucleobases for the purpose of studying interactions with DNA bases. Consequently, the present study may overcome the drawbacks of most studies that have reported on the linkage of Fe groups to DNA by flexible alkyl chains or C–C connections, making it difficult to precisely detect charge-transfer interactions between DNA bases.

In particular, if our artificial ferrocene base (Fe-base) shows interactions with both single- and double-stranded DNA bases, it could be used as an effective method to detect DNA base-specific signatures at the atomic scale, a primary goal of DNA sequencing. The incorporation of Fe-bases in DNA could offer further advantages, such as electronic detection, tunable sizing, stability at high temperatures and moderate pHs, and high throughput, thereby optimizing conventional sequencing methods. For instance, nanopore sequencing is a successful third-generation sequencing method,¹⁵ but in some cases it exhibits poor temporospatial resolution for single-base sequencing.^{16–19} However, these

resolution issues could be resolved by interactions between Fe-bases and their adjacent bases, as well as their complementary (opposite Fe-base) bases, in DNA.

Recently, Tucker et al. reported that ferrocene could be used as a DNA-backbone component owing to the similarity in the Cp-ring-gap size between adjacent bases in B-DNA.²⁰ Following the logic of this strategy, we aimed to use ferrocene as a functional artificial base to determine the nature of its interactions with natural DNA bases. We reasoned that direct interactions between Fe-bases and DNA bases could lead to the development of new applications, quite apart from bioanalysis and sensor chemistry, as previously demonstrated. Studies on ferrocene have focused on its conjugation with DNA to read out signal changes.^{21–24} However, aside from the known uses of ferrocene in electrochemistry based on its simplicity and sensitivity, thus far, no studies have reported the effects of direct interactions between artificial Fe-bases and natural DNA bases. Therefore, in the present paper, we have designed and synthesized an artificial and functional sandwich base, termed Fe-base, and inserted it at various positions in a DNA strand to determine the nature of its direct interactions with DNA A, T, C, and G bases in the context of quantifiable charge-transfer rates, subsequently exploring the rapid and accurate detection of changes at the single-nucleobase level. Results showed that the charge-transfer rate in DNA upon hybridization mainly relies on the position of the functional Fe-base in a DNA strand.

EXPERIMENTAL SECTION

Synthesis of the Fe-Base.

Fe-base synthesis is described in the Supporting Information. Briefly, commercially available (*S*)-3-amino-1,2-propanediol was coupled with compound 1, and the corresponding phosphoramidite (4, Scheme 1) was prepared for DNA solid-phase synthesis. All compounds were characterized by standard methods (Figures S1–S7).

Following the DNA-synthesis protocols (SI), the incorporation of an Fe-base into a DNA strand is simple and similar to that of other natural bases. After HPLC purification and UV analysis, each strand was characterized by cyclic voltammetry (CV) and matrix-assisted-laser-desorption–ionization time-of-flight (MALDI-TOF) analysis (Figures S17–S20).

RESULTS AND DISCUSSION

To address the question of Fe-base interactions with DNA bases, we designed four DNA sequences (Table 1) with Fe- bases in different positions and complementary DNA (cDNA) sequences (Table S1) with different bases (A, T, G, and C) opposite each Fe-base. Current changes were then measured for Fe-DNA sequences alone and after the addition of cDNA to the solution. Schematic representation of these studies showed the interactions between Fe-base and both adjacent and complementary bases (Figure 1). Furthermore, it is possible to predict the efficiency of charge transfer when, as noted above, duplex formation primarily relies on the position of the Fe-base in the DNA strand. Indeed, the four DNA bases display different charge-transfer rates with the Fe-base to and from the working electrode, owing to its redox processes.

Cyclic-Voltammetry Studies of the Fe-Base.

CV studies showed that the electron-donating groups of ferrocene derivatives shift the $E_{1/2}$ values of ferrocenyl derivatives to lower potentials relative to that of ferrocene,^{18,25} whereas electron-withdrawing groups, such as carbamyls, shift the redox potentials to higher values.^{26,27} In our case, the four Fe-DNA sequences showed a potential range of 0.4365–0.442 V versus Ag/AgCl in Tris-HCl buffer and electrode potentials (E_p) in the range of 0.071–0.088 V between the anodic and cathodic peaks (Table S2). The current was measured at different scan rates with data proportional to the fast scan rate on the basis of the expectation that the electrochemical reaction would be confined to the surface. However, the measured current was directly proportional to the square root of the scan rate at slower scan rates, indicating a transfer-controlled electro-chemical reaction.²⁸ Our Fe-DNA system was observed with scan rates varying from 1 to 50 mV s⁻¹ and concentrations from 5 to 100 μ M (Figures S11–S14). The electrochemical outputs were monitored for four Fe-DNA sequences and were found to be unchanged after multiple electrochemical cycles and after having been left for 24 h in Tris-HCl buffer (Figure 2). The peak currents of the Fe-DNA sequences were proportional to the square roots of the scan rates, indicating electrochemical reversibility (Figure S15).

Squarewave-Voltammetry Studies of Fe-DNA and cDNA.

Experiments were then designed to identify interactions between the functional Fe-base and cDNA bases through current changes. Squarewave voltammetry (SWV) was explored to study the binding effect of duplex formation. All sequences were evaluated without grafting or immobilization on the electrode to avoid holding an electrode at the terminal part of the DNA sequence. Otherwise, the electrode could potentially gain control over DNA flexibility, thereby skewing the accuracy of the observed charge-transfer dynamics and electron-transfer properties. Over the last two decades, many studies have reported on the electron-transfer rates in DNA, especially with respect to hole transfer, tunneling effects, and hopping.^{29–31} In particular, the coupling or interaction of a ferrocene redox label with DNA bases to monitor the charge-transfer rates in DNA could be achieved in many ways, mainly including π -stacking or dynamic properties.^{32–37} In this system, various concentrations of single-stranded Fe-DNA (Figures 3 and S16) and the addition of complementary targets to a1 (Figure 4) and other Fe-DNA systems (Figures S8–S10 and S16) resulted in a remarkable decrease in the charge-transfer rate in SWV. Interestingly, the change in the transfer rate appeared to be a function of the position of Fe-base in the DNA strand. The effect on the transfer rate clearly displayed the coupling or interactions of the Fe-base with adjacent and complementary bases. Accordingly, four oligomers were prepared with Fe-bases placed in different positions, and each oligomer showed a propensity to electrochemically discriminate the single-nucleobase changes opposite the Fe-base.

Melting Temperature (T_m) Analysis of Fe-Base–DNA-Base Interactions.

Melting-temperature (T_m) experiments were then carried out for oligomer a3, the Fe-base of which was incorporated midsequence in anticipation of duplex-stability studies. T_m data reveal the specific interactions between the Fe-base and natural DNA bases. Furthermore, these data support the SWV results, indicating that Fe–A interactions exhibit lower transfer

kinetics than interactions with other bases (Fe–T, Fe–G, and Fe–C; Table 2). However, when the natural base pair A–T was replaced by Fe–DNA bases, the duplex was slightly destabilized. More specifically, upon addition of the complementary sequence to Fe–DNA, the charge-transfer kinetics to and from the working electrode was reduced. The SWV results (Figure 5) demonstrate that the charge-transfer rates sequentially decreased from oligomers a1 to a4 and that the percentages of the current changes of A, T, G, and C opposite Fe-base fell into the following ranges: a1, 49–56%; a2, 19–40%; a3, 18–28%; and a4, 4–9%. In all cases, the oligomers showed efficient discrimination of single-nucleobase changes; however, the charge-transfer rates of the DNA bases on each oligomer were dependent on the bases adjacent to the Fe-base in a DNA strand. For example, in the case of oligomers a2 and a4, the Fe-bases are positioned near the 5' and 3' termini, respectively, but the results indicate a significant difference in charge-transfer rates of these sequences, clearly pointing to the effect of ferrocene interactions with adjacent bases. In all cases, optimized concentrations (1:1) and conditions were used throughout the experiments to obtain clear signals for Fe-base–DNA-base interactions. The artificial sandwich Fe-base strongly interacts with adjacent and complementary bases during duplex formation, resulting in the discrimination of single-nucleobase changes by signal changes that completely depend on the positional orientation of the Fe-base. We can therefore speculate that the orientation or geometry of the Fe-base, along with interactions with surrounding bases, predisposes the charge transfer in duplex DNA in solution, but this topic is beyond the scope of the present work.

CONCLUSIONS

To summarize, we have designed and synthesized a functional sandwich base, termed Fe-base, inserted at various positions in a DNA strand to determine the nature of its direct interaction with DNA A, T, C, and G bases in the context of quantifiable charge-transfer rates. Indeed, we found that the efficiency of charge-transfer rate upon the addition of cDNA was exclusively based on the interactions between the Fe-base and both the adjacent and complementary bases. Different transfer rates were evident in each base opposite the Fe-base in all positions, suggesting the ability to accurately detect a single nucleobase by such specific signal differences. Therefore, this sandwich Fe-base could be a promising tool for the detection of single-nucleobase changes in any target sequence. Especially, these Fe-base interactions with DNA bases show promise for designing Fe-oligomer chip technology as a tool for DNA sequencing. The unique $1e^-$ redox property of iron is necessary for many biological reactions. As such, with Fe as a molecular base, it may be possible to replace biological iron functions through DNA. Further work will be focused on its applications and specific DNA reactions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

(A) Fe-bases incorporated in different positions along the DNA sequence. Each oligomer showed a propensity to electrochemically detect the single-nucleobase changes opposite the Fe-base. (B) Discrimination of single-nucleobase changes by signal changes that completely depend on the positional orientation of the Fe-base.

Figure 2. Cyclic voltammograms ($1\text{--}50 \text{ mV s}^{-1}$) for four Fe-DNA strands. $[\text{DNA}] = 100 \mu\text{M}$ in pH 6.8 buffer (50 mM Tris-HCl, 50 mM NaCl, 50 mM MgCl_2).

Figure 3.

Squarewave voltammograms for four Fe-DNA strands. $[\text{DNA}] = 1\text{--}200 \mu\text{M}$ at 20 mV s^{-1} in pH 6.8 buffer (50 mM Tris-HCl, 50 mM NaCl, 50 mM MgCl_2).

Figure 4. Squarewave voltammograms of Fe-DNA (a1) alone and after the addition of cDNA with 10–100 μM CS-A (A), CS-C (B), CS-G (C), and CS-T (D).

Figure 5.
Squarewave-voltammetry-current changes for (1:1) ratios of Fe-DNA-cDNA

Scheme 1. Fe-Base Synthesis^a

^a(a) (S)-3-Amino-1,2-propanediol, HBTU, DIPEA, DMF; (b) 4,4'-dimethoxytritylchloride, pyridine; (c) chlorophosphoramidite, DIPEA, DCM.

Table 1.

Predicted and MALDI-TOF-Determined Analyses of Oligonucleotides

strand	sequence (5'-3') ^a	predicted mass (Da)	observed mass (Da)
a1	FeGGACTCTCTCAATG	4603.0	4603.2
a2	TGFeACTCTCTCAATG	4578.0	4578.7
a3	TGGACTCFeCTCAATG	4603.0	4602.9
a4	TGGACTCTCTCAFeTG	4594.0	4594.4

^a5'-TGGACTCXCTCAATG-3', 3'-ACCTGAGYGAGTTAC-5'.

Table 2.Melting Temperatures (T_m) of DNA Duplexes Containing the Fe-Base.

entry	X-Y ^a	T_m (°C)
1	Fe-A	56.8
2	Fe-T	51
3	Fe-G	50
4	Fe-C	52.7
5	Fe-Fe	52
6	A-T	60.9

^a5'-TGGACTCXCTCAATG-3', 3'-ACCTGAGYGAGTTAC-5'.