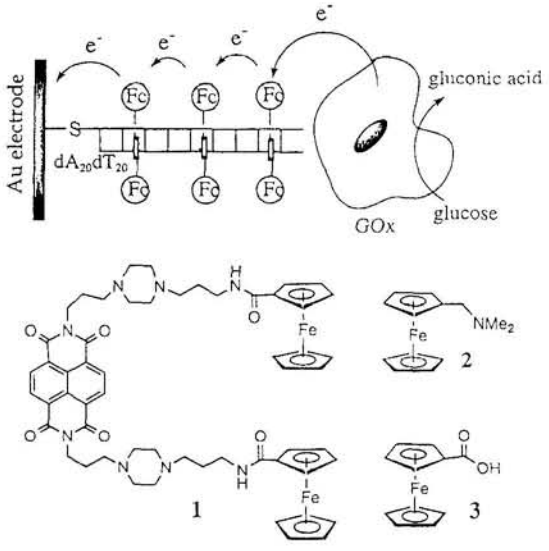
Enhanced Electron Transfer from Glucose Oxidase to DNA-Immobilized Electrode Aided by Ferrocenyl Naphthalene Diimide, a Threading Intercalator

Shigeori Takenaka,* Yoshihiro Uto, Makoto Takagi, and Hiroki Kondo†
Department of Chemical Systems and Engineering, Kyushu University, Fukuoka 812-8581
†*Department of Biochemical Engineering and Science, Kyushu Institute of Technology, Iizuka 820-8502*

(Received June 10, 1998; CL-980441)

A ferrocene-modified 1,4,5,8-naphthalenetetracarboxyldiimide (**1**), bound to double stranded DNA by a threading intercalation mode, enhanced the electron transfer between glucose oxidase and a DNA-immobilized electrode.

We recently reported a DNA sensor consisting of an oligonucleotide-modified gold electrode and ferrocenyl naphthalene diimide (**1**).[1] The probe DNA on the electrode undergoes hybridization with a sample DNA having the complementary sequence, and the diimide intercalates into the resulting double stranded (ds) DNA specifically. Since the intercalating ligand carries electrochemically active ferrocene moiety, it undergoes a redox reaction on the electrode surface. With a high binding affinity of **1** to dsDNA (see below), **1** becomes concentrated on the DNA double helix on the electrode even under low concentration of **1** in the bulk solution. Moreover, because of a peculiar stereochemical configuration of **1** when it "thread"-intercalates into dsDNA, the ferrocene moieties of the bound **1** are placed in arrays along each groove of dsDNA. The resultant architecture of the ferrocenyl ligand-dsDNA complex may be regarded as a pseudo-polyferrocene polymer extending along a one-dimensional DNA strand. Such an arrangement may be expected to be capable of efficiently mediating electron transfer between the electrode and the chemical species in the bulk solution away from the electrode. In this paper, we show that this seems to be the case; the electron transfer between reduced glucose oxidase (GOx) in solution and a DNA-immobilized gold electrode is enhanced in the presence of ferrocenyl ligand **1**.



Ferrocenyl naphthalene diimide (**1**) undergoes hypochromic and bathochromic shifts of the absorption band of the naphthalene diimide chromophore upon binding to sonicated calf thymus DNA, which is indicative of DNA intercalation.[2] Spectrophotometric titration was carried out for **1** with calf thymus DNA in 10 mM morpholinoethanesulfonate and 1 mM EDTA at pH 6.25 and 25 °C. The data obtained (Scatchard plots) were compared with the theoretical curves generated by the binding equation of McGhee and von Hippel[3] to give a binding constant of 1.3 x 10^5 M^{-1} with a site size of 2 and a cooperativity parameter of 0.4. The site size of 2 implies that at binding saturation, **1** is placed on double stranded DNA every two base pairs.

Intercalation of **1** to dsDNA was confirmed by viscometric titration of supercoiled plasmid DNA with **1**. Addition of increasing amount of **1** to DNA caused first a supercoil relaxation and then a reversed supercoil induction.

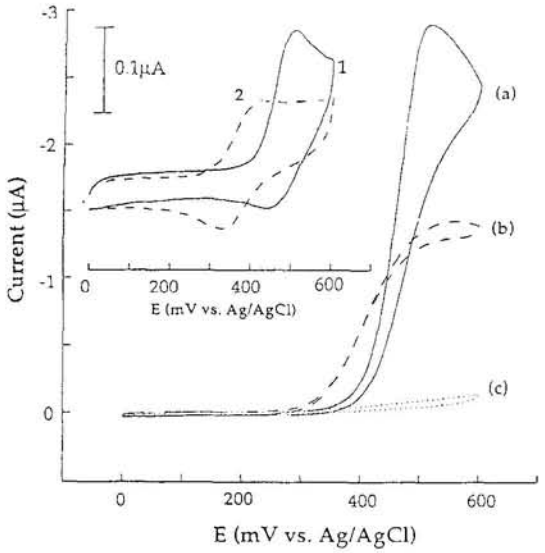


Figure 1. Cyclic voltammograms of systems consisting of GOx (200 units) and glucose (0.01 M) in the presence of 50 μM **1** (a), **2** (b) or **3** (c) in 0.1 M phosphate buffer (pH 7.0) and 1 mM KCl. $dA_{20}dT_{20}$-immobilized Au electrode, Pt wire counter electrode, and Ag/AgCl reference electrode were used. The scan rates were 10 mV/s. Insertion : Cyclic voltammograms of **1** and **2** on a $dA_{20}dT_{20}$-immobilized Au electrode in the absence of GOx and glucose. Other conditions were the same as above.

Cyclic voltammetry (CV) was carried out at 10 mV/s in 0.1 M phosphate buffer (pH 7.0) containing 1 mM KCl. A normal three-electrode configuration was adopted which consisted of a Ag/AgCl reference electrode, a Pt wire counter electrode and either an un-modified or a DNA-immobilized gold electrode. The dsDNA-immobilized electrode was prepared by chemisorption on a gold electrode of dT_{20} carrying a mercaptohexyl group at the 5'-phosphate end, followed by hybridization with dA_{20}. HPLC analysis of the solution before and after chemisorption indicated that the amount of the immobilized dT_{20} was 10 pmol/mm^2.

Cyclic voltammograms were studied for the solution containing 200 units of GOx from *Aspergillus niger* (Wako, Osaka) and 0.05 M glucose in the presence of 50 μM of **1**, dimethylamino-

ferrcene **2**, or ferrocenecarboxylic acid **3** in 0.1 M phosphate buffer (pH 7.0) and 1 mM KCl. Compounds **1** and **2** are cationic, whereas **3** is anionic under the experimental conditions. Figure 1 shows electrocatalytic anodic currents obtained for the $dA_{20}dT_{20}$-immobilized gold electrode. As the currents indicate, ferrocenyl ligands **1** and **2** acted as an electron transfer mediator, whereas **3** gave only a ca. 25-fold smaller electrocatalytic anodic current (I_{max} = 0.1 μA). Since GOx has an isoelectric point of pI=3.6,[4] the access of anionic **3** to the negatively charged GOx appears to be hampered. The access of **3** to the negatively charged DNA-modified electrode could also involve some barrier.

The electrocatalytic anodic current of **1** was twice larger than that of **2** (2.8 μA for **1**, 1.4 μA for **2**). Since DNA is a poly-anionic polymer, the cationic mediators, **1** and **2**, should be concentrated on the surface of the DNA-immobilized electrode. We estimated the amount of **1** and **2** bound within the diffusion layer on this electrode. The calculation from the area under the normal CV peak (insertion in Figure 1)[5] gave the value of 10 pmol for each ligand. This indicates that for the same number of ligands effectively bound on the electrode surface, **1** mediates the electron transfer from GOx to the electrode twice more efficiently than **2** does. This observation seems to be very much contradictory if one simply looks at the difference in the binding mode of the two ferrocene-derived ligands. **2** becomes bound only electrostatically around the negatively charged DNA strand without involving any specific stereochemical requirement. In other words, the diffusion of **2** should takes place freely except only for electrostatic interaction. On the other hand, **1** is thread-intercalated, and its binding and dissociation on dsDNA is kinetically hindered to a considerable extent. This along with a greater molecular size of **1** leads one to expect a reduced diffusion-controlled electron transfer rate for **1** on the DNA-modified electrode. As mentioned carlier, the reverse is the experimental observation.

The salt effect on the electrocatalytic anodic current was found to be opposite for **1** and **2**. Thus, the current for **1**-mediated electron transfer decreased by 10% in the presence of 0.1 M sodium chloride, while that for **2**-mediated reaction increased by 28%. An increased salt concentration weakens electrostatic interaction of electrolytes, and the DNA-bound fractions of **1** and **2** molecules decrease in 0.1 M sodium chloride as is usually the case for all the positively charged DNA ligands. However, the observed effect on the catalytic current was in the opposite direction between **1** and **2**. This suggests that we were handling two types of electric current here: (i) an ionic current carried by molecularly diffusing electroactive ligand and (ii) an electronic current mediated by an electron exchange among the DNA-fixed ligands, the summation of the two representing the observed total CV current. It may be deemed that the contribution from process (i) is important for ligand **2** while process (ii) prevails for **1**. However, actually, there should be many other factors that can affect the present catalytic current such as detailed kinetics of ligand - DNA binding and ligand - GOx electron exchange, which are yet to be studied to clarify the exact event taking place.[6]

Whatever the exact mechanism, the observed catalytic current is the first observation of enhanced electron transfer between a proteic redox factor and a metal electrode that is mediated by the array of redox ligands arranged along a one-dimensional dsDNA molecule. Its greater efficiency of mediation as compared with a similar ligand - DNA combination but lacking a defined stereochemical configuration of the ligand molecules implies that there can be some stereochemically favorable disposition for the collected electroactive molecules to effect an efficient electron conduction through a molecular scaled wire, in this case, a dsDNA. Recently, Braun et al.[7] reported the construction of a silver wire by reduction of silver chloride followed by the aggregation of the resulting metallic silver on a ds DNA skeleton. Our finding could be some alternative to this approach in utilizing a DNA matrix to construct an electron conduction network which takes advantage of all the peculiar supramolecular architecture that should be attained with DNA.

Authors are grateful to the financial support from the Japan Society for the Promotion of Science.

References and Notes

1 S. Takenaka, Y. Uto, H. Saita, M. Yokoyama, H. Kondo, and W. D. Wilson, *J. Chem. Soc., Chem. Commun.*, 1111 (1998).

2 W. D. Wilson and F. A. Tanious, in Molecular Aspects of Anticancer Drug-DNA Interactions, ed by S. Neidle and M. J. Waring, Macmillan Press, Riverside, NJ (1994), p. 243.

3 J. D. McGhee and P. H. von Hippel, *J. Mol. Biol.*, **86**, 469 (1974).

4 J. G. Voet, J. Coe, J. Epstein, V. Matossian, and T. Shipley, *Biochemistry*, **20**, 7182 (1981).

5 The CV wave for **2** is reversible, while that of **1** is not as is inferred by its reduced cathodic CV peak. However, the reproducibility is quite good. The CV behavior of the ferrocene part of **1** is also connected to the behavior of the naphthalene diimide part of **1**, the details of which is to be published elsewhere.

6 The following observations should further help understand the nature of the catalytic current facilitated by **1**.

a) Comparison between ssDNA- and dsDNA-modified electrodes: The two electrodes were incubated in 10 mM solution of **1** and then washed with pure water for 5 s at 20 °C. The CV measurement was then made in the solution containing 0.05 M glucose and 200 units glucose oxidase (pH 7.0 with phosphate buffer, 0.1 M KCl). The catalytic current I_{max} on dsDNA electrode (0.2 μA) was twice as great as that on ssDNA electrode (0.1 μA). This reconfirms the greater kinetic stability of **1**-dsDNA complex observed in the previous study, and suggests that **1** intercalated to dsDNA can play an important role in the present electron transfer reaction through the DNA layer on the electrode.

b) Comparison between unmodified and dsDNA-modified electrodes: The redox reaction of **1** on a bare Au electrode was not quite reversible;[5] and the comparison with the modified electrode did not give much information. However, the Imax values were nearly the same for the two electrode in the same GOx-glucose sample solution, typically 2.4 μA for bare Au electrode and 2.8 μA for modified electrode. This indicates that **1** did not reduce its mediating ability even though it is complexed to DNA and thus ankered to the electorde suface. This is contarary to ordinary observations that the mediators reduce its ability when they are chemically firmly ankered on the electrode.

7 E. Braun, Y. Eichen, U. Sivan, and G. Ben-Yoseph, *Nature*, **391**,775 (1998).