ChemComm

Cite this: *Chem. Commun.*, 2011, **47**, 12388–12390

www.rsc.org/chemcomm

COMMUNICATION

Electrochemical signal modulation in homogeneous solutions using the formation of an inclusion complex between ferrocene and β-cyclodextrin on a DNA scaffold†

Toshihiro Ihara,*[ab] Tsugutoshi Wasano,[a] Ryuta Nakatake,[a] Pelin Arslan,[a] Akika Futamura[a] and Akinori Jyo[a]

Received 29th August 2011, Accepted 9th October 2011
DOI: 10.1039/c1cc15365j

Two DNA conjugates modified with ferrocene and β-cyclodextrin were prepared as a pair of probes that work cooperatively for DNA sensing, in which the electrochemical signal of ferrocene on one probe was significantly "quenched" by the formation of an inclusion complex with β-cyclodextrin of the other probe on the DNA templates.

Various analytical platforms based on electrochemical responses have been proposed for studying specific interactions involving nucleic acids and/or proteins.[1,2] Most of these methods, however, require one of the participants in the interactions to be immobilized on the electrode because signal contrasts arise as the result of changes in the distance between the electrochemically active probe and the electrode surface on the targeted events (= change in the local concentration of the probe on the electrode). Generally, the preparation of biomolecule-modified electrodes is time-consuming and it is difficult to obtain reproducible results. We have already reported some electrochemical methods for DNA analysis.[3] In this series of studies, a gene sensor was developed based on the interactions between a ferrocene (Fc)-modified DNA probe and a DNA-modified electrode.[4] Fluorimetry for DNA sensing using β-cyclodextrin (βCyD)-modified DNA has also recently been reported.[5,6] It is known that βCyD binds tightly with Fc to form an inclusion complex, and the electrochemical activity of Fc accommodated in the βCyD cavity is suppressed by being shielded from the bulk solution.[7] βCyD could therefore be regarded as a "quencher" for electrochemical signals, similar to an azo-quencher for FRET-based fluorimetry.[8] This means that specific interactions could be monitored using the electrochemical signals of Fc in homogeneous solutions, if the inclusion complex is formed quantitatively in accordance with the relevant interactions. This would free the preparation of electrochemical molecular sensors from the need to anchor the molecules on the electrodes.[9]

Here we examined the possibility of electrochemical signal modulation in a homogeneous solution using the controlled interaction between two DNA conjugates carrying Fc and βCyD on one end of synthetic oligodeoxyribonucleotides (ODN). All the ODNs used in this study are shown in Fig. 1. The sequences of the two conjugates (**Fc-ODN$_n$** and **CyD-ODN$_n$**) were designed to be complementary to the adjacent sites of the targets (**target27** and **target22N**: a part of the TPMT gene[10]). A pair of the two conjugates forms a tandem duplex with the target, in which both modified units are directed convergently and, hopefully, form a quenched inclusion complex at the center of the duplexes. This could be regarded as a split probe (or binary probe) in electrochemistry.[11]

The system of tandem duplex 1 was subjected to UV melting experiments to examine the interactions between βCyD and Fc on the DNA framework. All the curves showed biphasic features with two inflection temperatures caused by successive meltings (T_{max}).‡[12] The meltings at lower and higher temperatures are

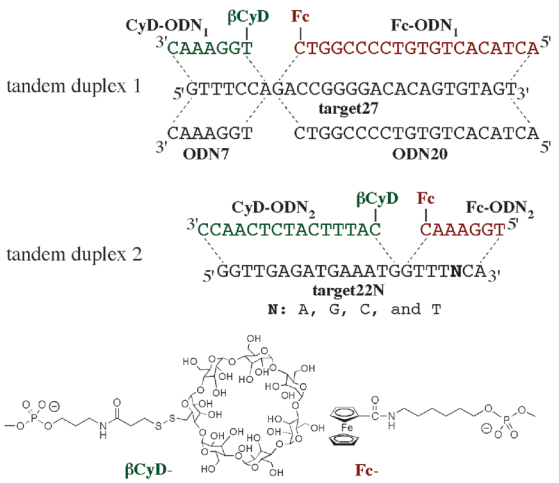

Fig. 1 Sequences of synthetic oligodeoxyribonucleic acids used in this study. Two tandem duplexes (1 and 2) were designed. They consist of **CyD-ODN**, **Fc-ODN**, and the targets (a part of the TPMT gene). **ODN7** and **ODN20** are the unmodified ODNs as references.

[a] *Department of Applied Chemistry and Biochemistry, Kumamoto University, 2-39-1 Kurokami, Kumamoto 860-8555, Japan. E-mail: toshi@chem.kumamoto-u.ac.jp; Tel: +81 96 372-3772*
[b] *CREST, JST, Tokyo 102-0075, Japan*

† Electronic supplementary information (ESI) available: ODN conjugates synthesis and results of UV melting, SWVs, and hydrodynamic voltammograms. See DOI: 10.1039/c1cc15365j

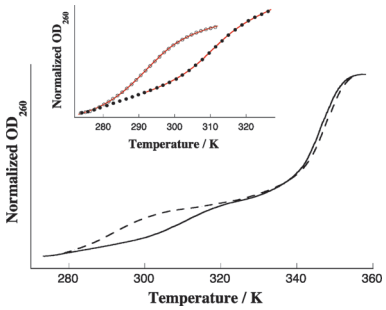

Fig. 2 UV melting curves of tandem duplexes. Solid and broken curves show the meltings of **CyD-ODN$_1$/target27/Fc-ODN$_1$** and **CyD-ODN$_1$/target27/ODN20**, respectively. Melting experiments were carried out in 10 mM phosphate buffer solution (pH 7.0) containing 500 mM KCl. The concentrations of the ODN components were all 1.0 μM. The solutions were heated at a rate of 0.5 °C min^{-1}. Inset shows the fitting of the melting at lower temperature to the theoretical curve. Closed and open circles are the experimental data for each tandem duplex. Red curves are the optimized theoretical curves.

attributed to those of 7-mer and 20-mer ODNs, respectively.[11a] Fig. 2 shows the melting curves of the tandem duplexes **CyD-ODN$_1$/target27/Fc-ODN$_1$** and **CyD-ODN$_1$/target27/ODN20**. The T_{max} of **CyD-ODN$_1$** dissociation from **CyD-ODN$_1$/target27/Fc-ODN$_1$** was higher than that for **CyD-ODN$_1$/target27/ODN20**. The difference in the T_{max} values was almost 20 °C. This could be attributed to the stabilization effect of the interaction between βCyD and Fc on the tandem duplex. The lower temperature regions of both melting curves were fitted with the theoretical equation (Fig. 2, inset).[3a,12] The thermodynamic parameters obtained and the T_{max} values of all the tandem duplexes are summarized in Table 1. The difference in ΔG°_{298} for **CyD-ODN$_1$** binding with **target27/Fc-ODN$_1$** and with **target27/ODN20** ($\Delta\Delta G^{\circ}$) was *ca.* 3.2 kcal mol^{-1}. This is a reasonable value as the free energy comes from the formation of a βCyD–Fc inclusion complex.[7,13] This corresponds to an enhancement by *ca.* 260 times of the binding constant at 25 °C. This means that the βCyD tethered to the ODN end accommodates the Fc tethered to the neighboring end of another ODN on the target DNA to form a tight inclusion complex without significant strain in the structure.

The electrochemical properties of **Fc-ODN$_1$** were investigated by cyclic voltammetry using a dual microelectrode. Fig. 3(a) shows the cyclic voltammograms of **Fc-ODN$_1$** in several states. The current based on the ferrocene/ferrocenium redox couple (Fc/Fc^+) was significantly suppressed only for the ternary tandem duplex **CyD-ODN$_1$/target27/Fc-ODN$_1$**. As we expected, the electrochemical activity of Fc in the DNA conjugate seems to be significantly quenched by shielding with βCyD on the DNA scaffold. βCyD as an intervening aliphatic insulator would prevent the Fc from reaching the electrode directly and would suppress the rate of electron transfer. The difficulty in accessing the counter anion experienced by ferrocenium tightly surrounded by βCyD might be another cause of the current suppression. Recently, several electrochemical methods for DNA analysis have been proposed based on βCyD–Fc interactions.[14] The contrasts in the magnitude of the electric currents obtained in the present study are much higher than those previously reported. This might be the result of differences in the linker chains such as the length, structure, and tethering point on βCyD, which would significantly affect the structure and stability of the inclusion complexes (ESI†).

The effects of the sequence of the target DNAs on the current based on Fc/Fc^+ were examined using the system of tandem duplex 2 with the four targets, *i.e.*, **target22N** carrying one base displacement. Square-wave voltammograms measured at 40 °C are shown in Fig. 3(b). UV melting experiments for these four tandem duplexes, **CyD-ODN$_2$/target22N/Fc-ODN$_2$**, showed that only **target22C** can form a stable tandem duplex at this temperature (ESI†). As we expected, only the signal from the duplex **CyD-ODN$_2$/target22C/Fc-ODN$_2$** almost vanished. The current signal increased with increasing temperature. Then, finally, it gave almost the same magnitude as those of the other three duplexes at 55 °C (ESI†). This result clearly shows that the βCyD–Fc inclusion complex formation is controlled by sequence-specific hybridization of ODNs. Control of the interaction is not difficult by taking advantage of the programmability and the predictable thermal stability of DNA structures.[15]

Table 1 Melting temperatures[a] and thermodynamic parameters[b] of duplex formation

Tandem duplex with **target27**	T_{max}/°C		ΔG°_{298}/ kcal mol^{-1}	K_{298}/mol L^{-1}
	Lower	Higher		
CyD-ODN$_1$//Fc-ODN$_1$	37.4	73.5	−10.6	6.52×10^7
ODN7//Fc-ODN$_1$	19.7	73.4		
CyD-ODN$_1$//ODN20	18.7	73.9	−7.36	2.50×10^5
ODN7//ODN20	24.1	73.5		

[a] The temperatures of biphasic UV inflections. Higher and lower temperatures of two maxima of 1st derivative curves are shown as T_{max}s.‡ [b] ΔH° and ΔS° for duplex formation of **CyD-ODN$_1$** with half duplexes are shown in ESI†. ΔG° and K were calculated from them at 298 K.

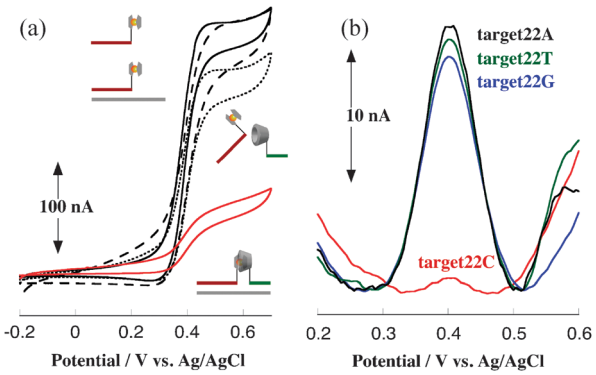

Fig. 3 Electrochemical responses of **Fc-ODN** in several states. (a) Cyclic voltammograms performed with dual-mode techniques using a pair of comb-shaped working electrodes (collector and generator) at 5 °C. A 15 μL solution containing the DNA components (100 μM), 10 mM phosphate buffer (pH 7.0), and 500 mM KCl was dropped on the electrodes. Scan rate of generator: 10 mV s^{-1}; collector potential: −0.2 V (*vs.* Ag/AgCl). Solid curve in black: **Fc-ODN$_1$**; broken curve: **target27/Fc-ODN$_1$**; dotted curve: **CyD-ODN$_1$/Fc-ODN$_1$**; red curve: **CyD-ODN$_1$/target27/Fc-ODN$_1$**. (b) Square-wave voltammograms of **CyD-ODN$_2$/target22N/Fc-ODN$_2$**. A 50 μL solution containing the DNA components (20 μM), 10 mM phosphate buffer (pH 7.0), and 500 mM KCl was subjected to measurements at 40 °C. Amplitude: 25 mV; potential increment: 4 mV; frequency: 15 Hz. Red: **target22C**; blue: **target22G**; green: **target22T**; black: **target22A**.

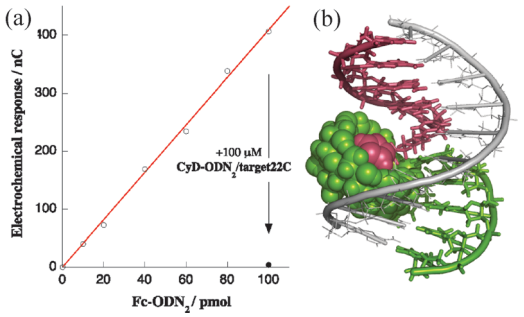

Fig. 4 Switching the electrochemical signal of **Fc-ODN** by specific interaction with **CyD-ODN**. (a) Linear calibration of the electrochemical response of **Fc-ODN$_2$** (open circle) and its quenched signal (closed circle) obtained by a flow system at 25 °C. Twenty μL of standard solutions containing various amounts of **Fc-ODN$_2$**, 10 mM phosphate buffer (pH 7.0), and 500 mM KCl were injected into the HPLC-ECD. To the solution containing 100 pmol **Fc-ODN$_2$**, an equimolar amount of **CyD-ODN$_2$/target22C** was added and its signal was measured as that of the quenched state. Eluent: 100 mM phosphate buffer (pH 7.0), 750 mM NaCl, 5 mg mL^{-1} EDTA; potential: 500 mV (*vs.* Ag/AgCl);§ flow rate: 1.0 mL min^{-1}; column: Inertsil AX (4.6 ϕ × 100 mm). (b) One of the possible structures of the ternary tandem duplex (only the central part of duplex 2). Red: **Fc-ODN$_2$**, green: **CyD-ODN$_2$**, gray: **target22C**. The model was geometry-optimized by AMBER* force field with the GB/SA (generalized Born/surface area) solvent model using MacroModel version 9.1.

The sensitivity of SWV was not sufficient. Therefore, system 2 was subjected to flow analysis to check the applicability of the system for practical use. HPLC equipped with an electrochemical detector (ECD) was used. Fig. 4(a) shows the calibration profile of the electrochemical response of **Fc-ODN$_2$** and its complex form. Without taking any special care, 10 pmol per 20 μL of **Fc-ODN$_2$** were detected with fair reproducibility by amperometry using ordinary ECD. It would not be difficult to improve the sensitivity by reviewing some of the parameters of the flow system such as type of column, flow rate, and eluent. The performance we achieved was reasonable for this concise procedure, in which all we did was mix and inject the components. As observed in the voltammograms of static solutions shown in Fig. 3 and 4, the current signal was almost perfectly suppressed by stoichiometric complexation with **CyD-ODN$_2$** in the flow system. This shows that the tandem duplex is stable, and Fc would be steadily buried in βCyD and shielded from the bulk solution (Fig. 4(b)), even under the conditions of HPLC-ECD. Thus, the present system could be applied to flow analysis without any modification of the system architecture. Good compatibility with flow analyses is one of the advantages of systems that work in homogeneous solutions[9] because, generally, flow analyses enable quick and reproducible measurements to be made, and lead directly to applications in the micro total analyses such as μTAS or lab-on-a-chip.[16]

In conclusion, we have shown that the electrochemical activity of Fc is controlled reversibly by a designed interaction with βCyD on a DNA scaffold. βCyD could be regarded as an effective quencher for Fc. This would free the design of the molecular sensor from the need for electrode modification of one of the counterparts of the specific interactions of interest. The system configuration is general and could be extended as a common design strategy for electrochemical molecular sensing, that is, the targets are not limited to tandem duplexes or even DNA-based molecular systems.

This work was partially supported by a Grant-in-Aid for Scientific Research on Innovative Areas (“Coordinating Programming” Area 2107, No. 22108529) from MEXT (T.I.), Scientific Research (B) (20350038) from JSPS (T.I.).

Notes and references

‡ Melting temperature, T_m, is usually defined as the temperature at which half of the ODN exists in the duplex state and the other half in the single-stranded state.[12] For the sake of convenience, here the temperatures that give maximum of 1st derivative curves were used as the measure for thermal stability of the duplexes.
§ To determine the applied potential, hydrodynamic voltammetries were carried out prior to conducting calibration using the same HPLC-ECD system (ESI†).[3b]

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