Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Journal of Organometallic Chemistry 689 (2004) 4722–4728
Journal of Organo metallic Chemistry
www.elsevier.com/locate/jorganchem

Electrochemical gene detection based on supramolecular complex formation by ferrocenyl-β-cyclodextrin and adamantylnaphthalene diimide bound to double stranded DNA

Shinobu Sato, Takahiko Nojima, Shigeori Takenaka *

Department of Applied Chemistry, Faculty of Engineering, Kyushu University, Fukuoka 812-8581, Japan

Received 27 August 2004; accepted 27 August 2004
Available online 25 September 2004

Abstract

Adamantylnaphthalene diimide **1** was synthesized as a highly selective double stranded DNA (dsDNA) binding reagent. The binding studies with sonicated calf thymus DNA as a model of dsDNA revealed that **1** can bind to dsDNA by the threading mode, where the two adamantyl moieties are located in the major and minor grooves of dsDNA separately and the complex of **1** with DNA duplex was stabilized by capping of the adamantyl moieties of **1** bound to dsDNA by β-cyclodextrin. The adamantyl moieties of **1** could be incorporated also into the cavity of ferrocenyl-β-cyclodextrin (Fc-β-CD), resulting in the formation of a supramolecular complex. When this complex is formed on the DNA probe-immobilized electrode, electrochemical DNA detection was feasible: the electrode hybridized with target DNA gave rise to a current peak corresponding to the ferrocene oxidation upon treatment with **1** and Fc-β-CD.
© 2004 Elsevier B.V. All rights reserved.

Keywords: Adamantylnaphthalene diimide; Ferrocenyl-β-cyclodextrin; Supramolecular complex; Double stranded DNA; Electrochemical detection

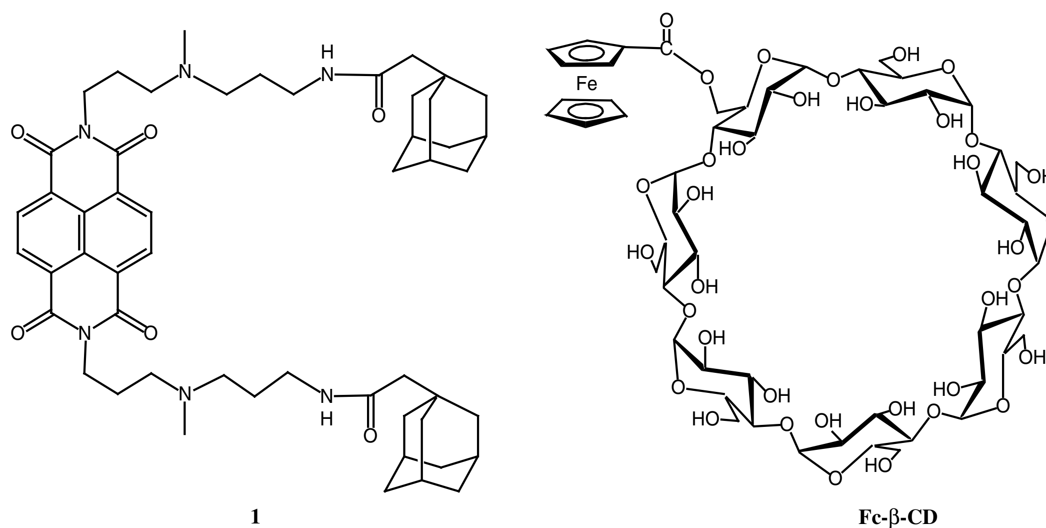
1. Introduction

The research concerning electrochemical detection of DNA is important for the development of gene diagnostic chips of the next generation [1]. Such chips will enable quick and simple gene diagnosis by the electrochemical detecting method [2]. Until now, many researchers have been developing electrochemical DNA detection methods for this purpose [3]. The authors' group established an electrochemical gene detecting method based on ferrocenylnaphthalene diimide as an electrochemical hybridization indicator coupled with a DNA probe-immobilized electrode [4]. Highly sensitive analysis was achieved by the unique property of ferrocenylnaphthalene diimide to bind to double stranded DNA (dsDNA) with high selectivity due to the formation of a threading intercalation complex with dsDNA, where the two ferrocene moieties act as an anchor to prevent it from dissociation from dsDNA.

To extend the electrochemical detection based on naphthalene diimide derivative as a threading intercalator, we designed and synthesized adamantylnaphthalene diimide (**1**) coupled with ferrocenyl-β-cyclodextrin (Fc-β-CD). The chemical structures of **1** and Fc-β-CD are shown in Scheme 1. Adamantylnaphthalene diimide **1** is expected to bind to dsDNA by threading intercalation where the two adamantyl moieties are located in the major and minor grooves of dsDNA. Since it is known that the ferrocene of Fc-β-CD is incorporated into its cavity [5] and adamantane can be incorporated into Fc-β-CD to drive the ferrocene part out [6], Fc-β-CD can bind to **1** bound to dsDNA by its capping of two adamantyl moieties projecting out in the major and minor grooves of DNA (see image structure in Fig. 1). They can act as

* Corresponding author. Tel./fax: +81 926423603.
E-mail address: staketcm@mbox.nc.kyushu-u.ac.jp (S. Takenaka).

0022-328X/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jorganchem.2004.08.039



Scheme 1.

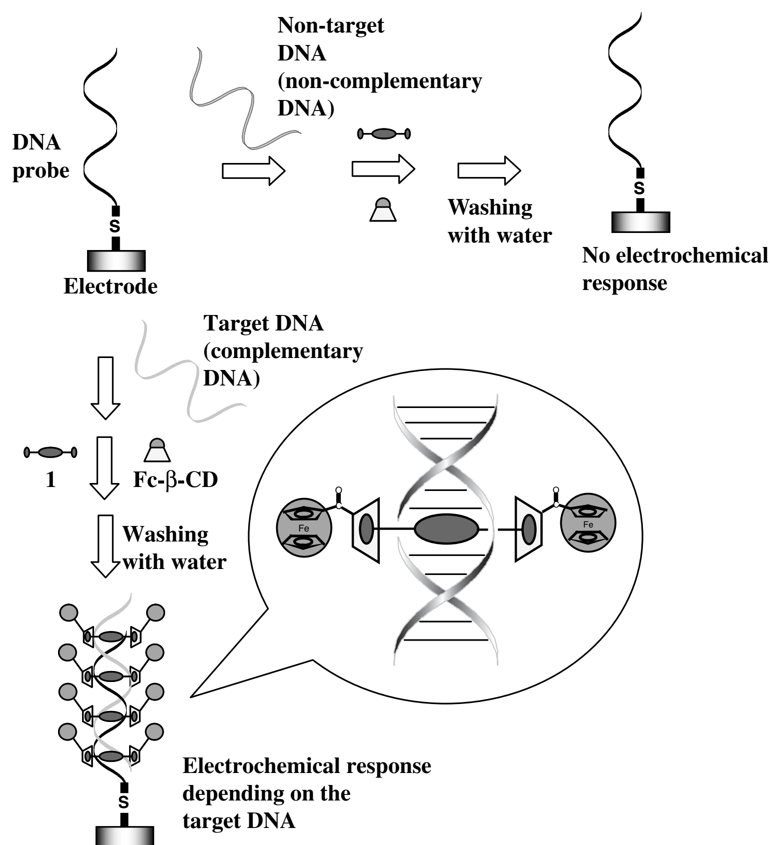


Fig. 1. Stratagem of the new DNA detection system based on Fc-β-CD and **1**. A DNA probe-immobilized electrode is allowed to hybridize with sample DNA. A duplex is formed on the electrode as long as the sample DNA contains a sequence complementary to that of the probe. After immersing Fc-β-CD and **1** and washing with water, the supramolecular complex remains on the electrode in the case of dsDNA on the electrode and one can detect the target DNA from the electrochemical signal of Fc-β-CD. Putative structure of a supramolecular complex of **1**, Fc-β-CD, and dsDNA is also shown in the figure. The ferrocene moiety of Fc-β-CD is incorporated into its cavity in an aqueous solution.

an additional anchor to prevent dissociation of the complex of **1**. Therefore, the supramolecular complex consisting of Fc-β-CD and **1** bound to dsDNA is expected to be stabilized further by capping of the adamantyl moieties of **1** by Fc-β-CD, thereby resulting in the improved discrimination ability for dsDNA. Furthermore,

ferrocene moieties are concentrated on the complex of **1** with dsDNA, which may facilitate electrochemical detection. From this viewpoint, we designed a new DNA detecting system based on **1** and Fc-β-CD as shown in Fig. 1. Target DNA can be trapped on the DNA probe-immobilized electrode by the specific interaction during the hybridization process. When this electrode is treated with a solution containing **1** and Fc-β-CD, the supramolecular complex is formed on the electrode. Since this complex on the electrode is stable to washing with water, one can detect the target DNA by the electrochemical response of Fc-β-CD concentrated on the electrode. This supramolecular complex will never form with the electrode treated with non-target DNA.

This idea was tested in this paper by studying the binding behavior of **1** with dsDNA in the absence and presence of β-CD. Furthermore, we studied the electrochemical behavior of Fc-β-CD alone or bound to **1** bound to a dsDNA-immobilized electrode.

2. Experimental

2.1. General physicochemical methods

Melting points are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Jeol GSX-400 spectrometer operating at 400 and 100 MHz for proton and carbon, respectively, with tetramethylsilane (TMS) as an internal standard. Electronic absorption spectra were recorded with Hitachi 3300 spectrophotometers equipped with an SPR 10 temperature controller. The HPLC system was composed of the following parts: Hitachi L-7300 column oven, L-7450H diode array detector, L-7100 pump, D-7000 interface chromatograph. Circular dichroism (CD) spectra were recorded over the 220–550 nm range on a Jasco J820 spectropolarimeter.

Quartz crystal microbalance (QCM) experiments were performed on AffinixQ (Initium Co., Japan) by using the gold surface (2.5 mm in diameter and 4.9 mm^2 in area) of 27 MHz, and an AT-cut QCM sensor chip (Initium). A QCM is a very sensitive mass-measuring device; its resonance frequency decreases linearly with an increase in the mass on the QCM chip at nanogram levels [7,8].

Viscosity titrations were carried out with a PC-controlled automatic system (Lauda, Germany) equipped with a capillary Ubbelohde-type viscometer, an automatic pump/stop-watch unit and a thermostated water bath at 30 ± 0.1 °C. Aliquots (2 μl) of 1.0 mM solution of **1** were added to a DNA sample solution (0.2 mM) by means of a microsyringe without removing the solution from the viscometer. The relative viscosity ratios of DNA alone and its complex with **1** were calculated using the equation $\eta/\eta_0 = (t-t_0)/(t_{DNA}-t_0)$, where t_0 is the flow time of the buffer; t and t_{DNA} are the flow times of a DNA sample in the presence and absence of **1**, respectively.

2.2. Synthesis

*2.2.1. N,N′-Bis[3-(3-adamantaneacetylaminopropyl)methylaminopropyl]naphthalene-1,4,5,8-tetra-carboxylic acid diimide (**1**)*

A solution of *N,N′*-bis[3-(3-aminopropyl)methylaminopropyl]naphthalene-1,4,5,8-tetracarboxylic acid diimide [4a] (0.39 g, 0.40 mmol), 1-adamantaneacetic acid (0.31 g, 1.60 mmol), BOP reagent (benzotriazol-1-yl-oxytris(dimethylamino)-phosphonium hexafluorophosphate, 1.42 g, 3.2 mmol), HOBt (1-hydroxybenzotriazole, 0.43 g, 3.2 mmol), and triethylamine (2 ml) in DMF (5 ml) was stirred at room temperature for two days. The solvent was removed and the residue was chromatographed on a column of silica gel (Merck 60, $CHCl_3$:diethylamine = 20:1). The solvent was removed from the pooled fractions under reduced pressure. The residue was dissolved in a small amount of $CHCl_3$ and poured into hexane. **1** precipitated was collected by filtration as a purple solid (150 mg, 43%). Homogeneity of the product was confirmed by reversed phase HPLC on Inertsil ODS-3 (inner diameter 5 mm, size 4.6 × 250 mm, GL Science Inc., Japan) in a gradient mode at a flow rate of 1.0 ml/min, where the concentration of acetonitrile was changed linearly to 100% from 10% in water containing 0.1% trifluoroacetic acid over 25 min. Elution was monitored by absorption at 250–400 nm. The retention time of **1** was 18.0 min; m.p. 118–121 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ 1.63 (24H, m), 1.95 (18H, m), 2.25 (6H, s), 2.53–2.46 (8H, m), 3.35 (4H, J = 6.2 Hz, t), 4.27 (4H, J = 7.7 Hz, t), 5.76 (2H, s), and 8.77 (4H, s) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, TMS) δ 25.7, 26.3, 28.7, 32.9, 36.8, 38.5, 39.4, 41.9, 42.7, 51.9, 55.8, 126.7, 131.0, 131.0, 162.8, and 171.0 ppm; MALDI-TOF-MS (time-of-flight mode with α-cyano-4-hydroxycinnamic acid as the matrix) *m/z* [M + H] 875.10 (theory for $C_{52}H_{70}N_6O_6Fe_2 + H^+$ 875.16).

2.2.2. Ferrocene-appended β-cyclodextrin

Ferrocene-appended β-cyclodextrin (Fc-β-CD) was synthesized according to the route previously described [9] from ferrocenecarboxylic acid and 6-*O*-tosyl β-cyclodextrin [10].

2.3. Chemicals

Sonicated calf thymus DNA purchased from Sigma–Aldrich Co. was used as a model of dsDNA. Oligonucleotides used in this study were custom synthesized by Genenet Co., Japan. Their base sequences were as

follows: thiolated or biotinyl DNA probe (HS- or biotinyl-G71(−)): 5′-SH-p(CH_2)$_6$-GTC TTC AAG GTG TAA AAT GCT CCG-3′ or 5′-biotin-GTC TTC AAG GTG TAA AAT GCT CCG-3′, representing part of the UDP-Glucuronosyltransferase gene, which is useful for predicting severe toxicity by irinotecan [11], DNA (G71(+)): 5′-CGG AGC ATTTTA CAC CTT GAA GAC-3′. Their concentrations were estimated from the molar absorptivities at 260 nm [12,13].

2.4. Equilibria and kinetics of binding

The binding affinity of **1** for sonicated calf thymus DNA as a dsDNA in the absence or presence of β-CD was determined by Scatchard analysis using the condition probability method of McGhee and von Hippel shown below [14]: $v/L = K(1-nv)\{(1-nv)/[1-(n-1)v]\}^{n-1}$, where v is the moles of **1** bound per base pair, L is the free **1** concentration, K is the observed binding constant, and site size n is the maximum number of **1** bound per base pair.

Kinetic experiments were performed with an SF-61 DX2 double mixing stopped-flow system (Hi-Tech Scientific Inc., UK) equipped with a temperature controller Lauda RE206. Single wavelength kinetic records of absorbance versus time were collected. Absorbance was collected at 383 nm, the wavelength where absorption of naphthalene diimide derivatives is maximum. The dissociation rate constant (k_d) of **1** from calf thymus DNA in the absence or presence of β-CD was determined by the sodium dodecylsulfate (SDS)-dissociation measurement established by the previous papers [15,16]. Two kinds of reaction solutions (1% SDS and DNA–**1** complex) were mixed instantaneously using a piston, and the change in the absorption spectrum was measured soon after mixing. Thus, when the DNA–**1** complex was mixed with an SDS solution, free **1** was incorporated into the SDS micelle. Since this process is diffusion-controlled, the entire absorption change represents the k_d-dependent process and, therefore, the fitting of kinetic trace provided k_d values.

2.5. Immobilization of DNA on a gold electrode and hybridization

A gold electrode having 2.0 mm^2 in area was polished with 6 μm, 1 μm of diamond slurry, and 0.5 μm of alumina slurry in this order and washed with MilliQ water. The electrode was soaked in boiling 2 M NaOH for 1 h and then washed with MilliQ water. This electrode was then soaked in concentrated nitric acid, washed with MilliQ water, and dried. One ml of 0.25 M NaCl solution containing 1 pmol of a DNA probe was placed on the gold electrode held upside down and kept in a closed container under high humidity for 2 h at room temperature. After the electrode was washed with MilliQ water, 1 μl of 1 mM 6-mercaptohexanol was placed on the electrode for 1 h at 45 °C. The electrode was kept in MilliQ water for 30 min at room temperature. One μl of 2 × SSC (0.03 M sodium citrate buffer containing 0.3 M NaCl) containing 5 pmol of sample DNA was placed on the electrode for 1 h at 37 °C to allow hybridization to proceed.

2.6. Electrochemical measurements

Electrochemical measurements were carried out with an ALS model 600 electrochemical analyzer (CH Instrument Inc., USA). Differential pulse voltammogram (DPV) measurements were carried out at 25 °C with a three-electrode configuration consisting of an Ag/AgCl reference electrode, a Pt counter electrode, and a DNA-immobilized electrode as the working electrode. A DNA-immobilized electrode was immersed in a solution containing 0.05 mM **1** and 0.1 mM Fc-β-CD for 10 min at room temperature and DPV of the electrode was measured in 0.1 M AcOK–AcOH buffer (pH 5.6) containing 0.1 M KCl.

3. Results and discussion

3.1. Effect of β-CD on DNA binding of 1

The absorption spectra of **1** in 10 mM MES and 1 mM EDTA (pH 6.2) containing 0.1 M NaCl showed an absorption maximum at 383 nm based on the naphthalene diimide skeleton (Fig. 2). Large hypochromic and small red shifts were observed in its spectrum upon addition of calf thymus DNA as a dsDNA. Since this change was reminiscent of that of ferrocenylnaphthalene

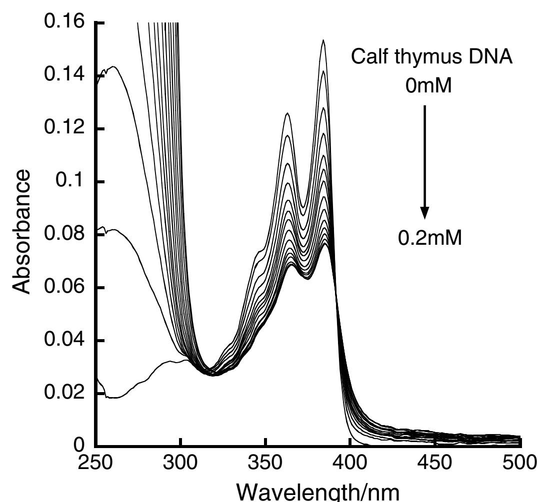


Fig. 2. Spectrophotometric titration of 5 μM **1** with 0–0.2 mM sonicated calf thymus DNA in 10 mM MES (pH 6.2) and 1 mM EDTA.

diimides as a threading-type intercalator [4b], it was inferred that **1** can bind to dsDNA by threading intercalation. The threading intercalation of **1** could be proven by an increase in the viscosity of a dsDNA solution upon addition of **1**. Scatchard analysis based on the absorption change upon addition of a various amount of dsDNA yielded a binding constant $K = 1.0 \times 10^5$ M^{-1}, and site size $n = 3$ in 10 mM MES buffer (pH 6.2), 1 mM EDTA, and 0.1 M NaCl at 25 °C (Fig. 3). Similar experiments in the presence of β-CD (1:β-CD = 1:4) gave $K = 2.0 \times 10^5$ M^{-1}, $n = 3$. Kinetic experiments were also carried out at 25 °C in the same medium. The dissociation rate constants (k_ds) of **1** from its complex with calf thymus DNA were measured by the SDS-driven kinetic experiments in the absence and presence of β-CD [15,16]. The k_d values obtained were 2.8 and 1.4 s^{-1} in the absence and presence of β-CD (**1**:β-CD = 1:4), respectively. The k_d value in the absence of β-CD compares well with those of related ferrocenylnaphthalene diimides [4b] and was reasonable for **1** as a threading intercalator. The decreased k_d value in the presence of β-CD revealed that β-CD could stabilize the complex of **1** with dsDNA.

The binding constant and dissociation rate constant in the presence of β-CD were ≈2- and 1/2-fold greater in the absence of β-CD, respectively, demonstrating that the binding ability of **1** was improved in the presence of β-CD due presumably to the stabilization of the complex by β-CD and give a clue to the design of a hybridization indicator having higher preference for dsDNA.

3.2. Interaction of Fc-β-CD with 1

CD spectra of Fc-β-CD (0.083 mM) were measured in the presence of 20 μM **1** in 0.1 M AcOK–AcOH (pH 5.6) and 0.1 M KCl at 25 °C. A large positive Cotton effect was observed for Fc-β-CD alone at 475 nm (Fig. 4), in agreement with the previous result where the ferrocenyl moiety of Fc-β-CD was incorporated into its cavity and showed a positive Cotton effect in its absorption region [5]. The magnitude of the Cotton effect around 475 nm decreased in the presence of **1**, showing that the adamantane moiety of **1** was incorporated into the cavity of Fc-β-CD and the ferrocene moiety was expelled from the cavity. This result is in agreement with the result described previously for Fc-β-CD and adamantane [6].

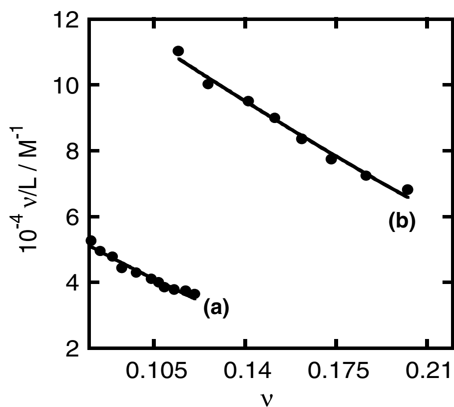


Fig. 3. Scatchard plots for 5 μM **1** with sonicated calf thymus DNA in the absence (a) or presence of 20 μM β-CD (b) in 10 mM MES (pH 6.2) containing 1 mM EDTA and 0.1 M NaCl.

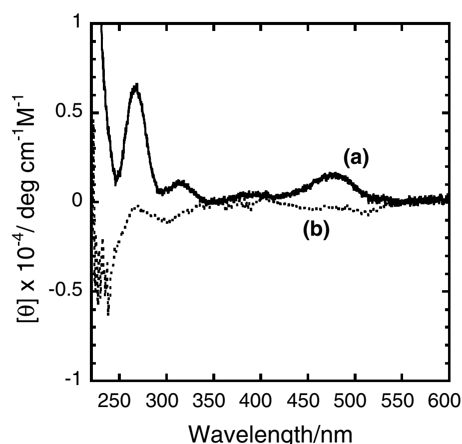


Fig. 4. CD spectra of Fc-β-CD (0.083 mM) in the absence (a) or presence (b) of 20 μM **1** in 0.1 M AcOK–AcOH (pH 5.6) and 0.1 M KCl at 25 °C.

Cyclic voltammograms of Fc-β-CD were measured with a bare gold electrode in 0.1 M AcOK–AcOH (pH 5.6) and 0.1 M KCl at 25 °C in the presence of a varied amount of **1**. Whereas an obscured redox peak was observed in the absence of **1**, the peak became clear with an increase in the amount of **1** with a shift of the peak potential to 0.58 V from 0.62 V, suggesting that the ferrocene moiety of Fc-β-CD was excluded from its cavity by the incorporation of **1** in its place.

3.3. Binding ratio of Fc-β-CD to 1 bound to dsDNA

The supramolecular complex formation of Fc-β-CD with **1** bound to dsDNA was also ascertained by QCM experiments by using a QCM sensor chip [7]. An streptavidin-coated QCM chip prepared by the method previously reported [8] was dipped in a cell containing 8 ml of 0.1 M AcOK–AcOH buffer (pH 5.6) containing 0.1 M KCl. A frequency decrease of 105 Hz was observed after addition of 200 pmol of biotinyl-G71(−) DNA probe, suggesting the immobilization of 0.6 pmol of the probe on the chip surface. This QCM chip was transferred to the same buffer solution and a complementary DNA (G71(+)), **1**, and Fc-β-CD were added in this order. The frequency changes observed after addition of each material are depicted in Fig. 5. When 200 pmol of G71(+) were added, the frequency decreased and then leveled off at 139.8 Hz, indicating that 0.6 pmol of dsDNA were formed on the chip. A

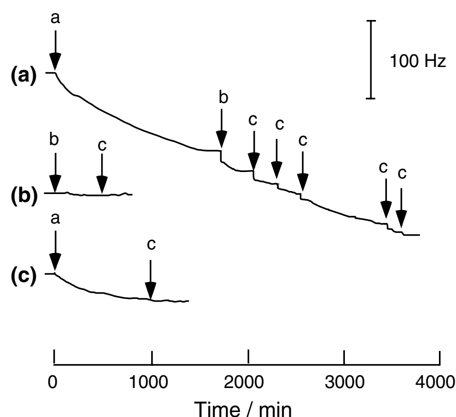


Fig. 5. Time-course of frequency change for a biotin-G71(−)-immobilized QCM chip upon successive addition of G71(+) (addition point (a)) to form dsDNA, **1** (b), and Fc-β-CD (c) in 0.1 M AcOK–AcOH buffer (pH 5.6) containing 0.1 M KCl at 25 °C (a). Time-courses of frequency change for Fc-β-CD with dsDNA (no **1**, c) and for **1** and Fc-β-CD with ssDNA (no G71(+), b) are also shown in the figure.

frequency decrease of 38.3 Hz was observed also upon addition of 10 pmol of **1**, showing that 1.3 pmol of **1** were bound to 0.6 pmol of 24-meric dsDNA. A further frequency decrease was observed which leveled off at 120.3 Hz upon addition of 250 pmol of Fc-β-CD, a magnitude equivalent to 2.7 pmol of Fc-β-CD bound to this chip. In other words, two molecules of Fc-β-CD bound to one molecule of 1 bound to dsDNA. No frequency decrease was observed for Fc-β-CD with dsDNA (no **1**, Fig. 5(c)) and for **1** and Fc-β-CD with ssDNA (no G71(+), Fig. 5(b)), proving that Fc-β-CD could bind to **1** bound to dsDNA specifically. In addition, it is certain from the stoichiometry of binding that Fc-β-CD binds to both of the adamantyl moieties of **1** projecting out in the major and minor grooves of dsDNA. In conclusion, the complex of **1** with dsDNA is stabilized by Fc-β-CD, as the latter can anchor the ferrocenyl moieties on dsDNA through **1** bound to dsDNA. This stable ternary complex was expected to enable facile electrochemical DNA detection.

3.4. Electrochemistry of Fc-β-CD on a dsDNA-immobilized electrode in the absence or presence of **1**

Fig. 6 shows cyclic voltammograms of a dsDNA-immobilized electrode in 0.1 M AcOK–AcOH buffer (pH 5.6) containing 0.1 M KCl and 0.05 mM Fc-β-CD at various scan rates. Plot of peak oxidation currents at 0.6 mV against the square root of the scan rates ($v^{1/2}$) was linear, indicating that the charge of Fc-β-CD is transported by diffusion. Fig. 7 shows cyclic voltammograms under the above conditions in the presence of 0.05 mM **1** at various scan rates. Plot of the peak oxidation current at 0.6 mV versus the scan rate was again linear, indicating the adsorption of Fc-β-CD. In other words, Fc-β-CD can bind to **1** bound to dsDNA immobilized

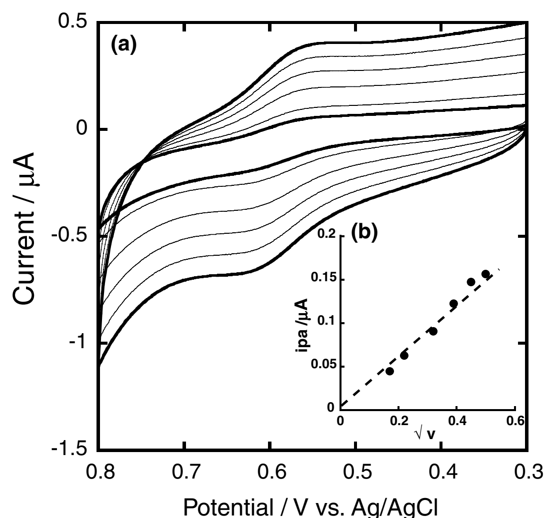


Fig. 6. (a) Cyclic voltammograms of a dsDNA-immobilized electrode in 0.1 M AcOK–AcOH buffer (pH 5.6) containing 0.1 M KCl and 0.05 mM Fc-β-CD at various scan rates and (b) plot of peak oxidation currents against the square root of the scan rate.

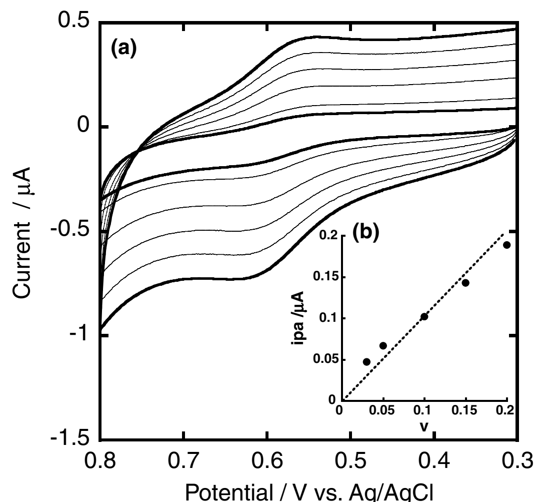


Fig. 7. (a) Cyclic voltammograms of a dsDNA-immobilized electrode in 0.1 M AcOK–AcOH buffer (pH 5.6) containing 0.1 M KCl, 0.05 mM Fc-β-CD, and 0.05 mM **1** at various scan rates and (b) plot of peak oxidation currents against the scan rate.

on the electrode and the ferrocene moieties can be arranged on the dsDNA.

3.5. Electrochemical DNA detection based on supramolecular complex formation

Based on these observations, we applied **1** and Fc-β-CD coupled with a DNA probe-immobilized electrode to electrochemical detection of a DNA fragment from a part of UDP-glucuronosyltransferase gene as a model. Whole manipulations were carried out according to the scheme shown in Fig. 1. Thiolated oligonucleotide,

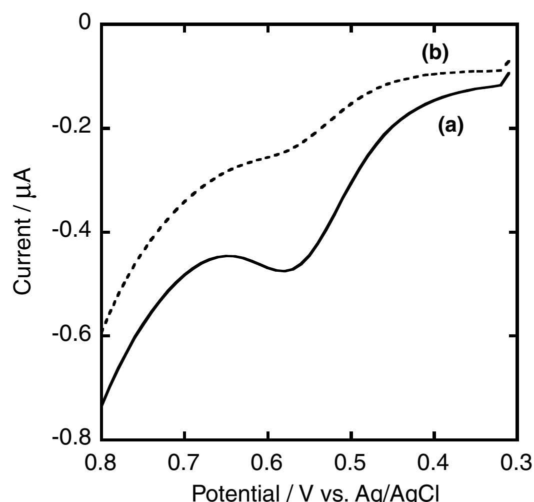


Fig. 8. (a) Differential pulse voltammograms of a HS-G71(−)-immobilized gold electrode in an electrolyte of 0.1 M AcOK–AcOH buffer (pH 5.6) containing 0.1 M KCl after hybridization with G71(+) followed by immersion in a solution containing 0.05 mM **1** and 0.1 mM Fc-β-CD. (b) Differential pulse voltammograms for DNA probe alone and non-complementary DNA (same sequence as that of the DNA probe).

HS-G71(−) was immobilized on the gold surface as a DNA probe and G71(+) was allowed to hybridize on the electrode at 37 °C for 1 h. The hybridized electrode was dipped in a solution containing 0.05 mM **1** and 0.1 mM Fc-β-CD for 10 min at room temperature and was transferred to an electrolyte containing 0.1 M AcOK–AcOH buffer (pH 5.2) and 0.1 M KCl (absence of both **1** and Fc-β-CD). A differential pulse voltammogram (DPV) was measured in this buffer with a three-electrode configuration (DNA probe-immobilized gold electrode as the working electrode, counter electrode Pt wire, and reference electrode Ag/AgCl). A current peak of 0.3 mA was observed at 0.58 V for the hybrid with G71(+), as shown in Fig. 8(a). No DPV signal was observed under the same conditions for DNA probe alone or non-complementary DNA (same sequence as that of the DNA probe) as shown in Fig. 8(b). It is noted that the background current deriving from the ssDNA as a probe is suppressed in this system.

4. Conclusion

In conclusion, **1** and Fc-β-CD can bind to the dsDNA of target DNA with a DNA probe-immobilized electrode as shown in Fig. 1 and an electrochemical signal was generated by its complex formation. This electrochemical gene detection based on the supramolecular complex formation is the first example of such an application of a supramolecular complex.

Acknowledgements

Special thanks are due to Prof. Hiroki Kondo of Kyushu Institute of Technology for reading the manuscript. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan. The authors are also grateful for the financial support from the Japan Society for the Promotion of Science.

References

[1] E. Palecek, M. Fojta, Anal. Chem. 73 (2001) 75A–83A; S. Takenaka, Bull. Chem. Soc. Jpn. 74 (2001) 217–224.
[2] I. Willner, Science 298 (2002) 2407–2408; T.G. Drummond, M.G. Hill, J.K. Barton, Nat. Biotechnol. 21 (2003) 1192–1199.
[3] R.M. Umek, S.W. Lin, J. Vielmetter, R.H. Terbrueggen, B. Irvine, C.J. Yu, J.F. Kayyem, H. Yowanto, G.F. Blackburn, D.H. Farkas, Y.-P. Chen, J. Mol. Diag. 3 (2001) 74–84; S. Brazill, N.E. Hebert, W.G. Kuhr, Electrophoresis 24 (2003) 2749–2757; F. Patolsky, Y. Weizmann, I. Willner, J. Am. Chem. Soc. 124 (2002) 770–772; C. Fan, K.W. Plaxco, A.J. Heeger, Proc. Natl. Acad. Sci. USA 100 (2003) 9134–9137.
[4] (a) S. Sato, S. Fujii, K. Yamashita, M. Takagi, H. Kondo, S. Takenaka, J. Organomet. Chem. 637–639 (2001) 476–483; (b) S. Takenaka, K. Yamashita, M. Takagi, Y. Uto, H. Kondo, Anal. Chem. 72 (2000) 1334–1341; H. Miyahara, K. Yamashita, M. Kanai, K. Uchida, M. Takagi, H. Kondo, S. Takenaka, Talanta 56 (2002) 829–835; K. Yamashita, M. Takagi, H. Kondo, S. Takenaka, Anal. Biochem. 306 (2002) 188–196; K. Yamashita, A. Takagi, M. Takagi, H. Kondo, Y. Ikeda, S. Takenaka, Bioconjugate Chem. 13 (2002) 1193–1199.
[5] A. Ueno, O. Chen, I. Suzuki, T. Osa, Anal. Chem. 64 (1982) 1650–1655.
[6] A.D. Ryabov, E.M. Tyapochkin, S.D. Varfolomeev, A.A. Karyakin, Bioelectrochem. Bioenerg. 24 (1990) 257–262.
[7] Y. Okahata, Y. Matsunobu, K. Ijiro, M. Mukae, A. Murakami, K. Makino, J. Am. Chem. Soc. 114 (1992) 8299–8300.
[8] Y. Okahata, K. Niikura, Y. Sugiura, M. Sawada, T. Morii, Biochemistry 37 (1998) 5666–5672.
[9] A. Ueno, F. Moriwaki, T. Matsue, T. Osa, Makromol. Chem. 6 (1985) 231–233.
[10] K. Takahashi, K. Hattori, F. Toda, Tetrahedron Lett. 25 (1984) 3331–3334.
[11] Y. Ando, H. Saka, M. Ando, T. Sawa, K. Muro, H. Ueoka, A. Yokoyama, S. Saitoh, K. Shimokata, Y. Hasegawa, Cancer Res. 60 (2000) 6921–6926.
[12] W. Muller, D.M. Crothers, Eur. J. Biochem. 54 (1975) 267–277.
[13] R.D. Wells, J.E. Larson, R.C. Grant, B.E. Shortle, C.R. Cantor, J. Mol. Biol. 54 (1970) 465–497.
[14] J.D. McGhee, P.H. von Hippel, J. Mol. Biol. 86 (1974) 469–489.
[15] K.R. Fox, C. Brassett, M.J. Waring, Biochim. Biophys. Acta 840 (1985) 383–392.
[16] F.A. Tanious, S.-F. Yen, W.D. Wilson, Biochemistry 30 (1991) 1813–1819.