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Redox-Labeling of DNA by Photoadduct Conjugate Formation with Ferrocene Derivatized Psoralen

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We describe a ferrocenyl derivative of psoralen which is bound covalently and gives a redox activity to a DNA double-helix. Psoralens are known to react photochemically at their 3,4 and 4',5 double bonds. Results of gel electrophoresis showed that the new compound also formed a DNA photoadduct in the same fashion. Since the reaction occurs selectively with double-stranded DNA, the compund should be useful to discriminate the double-helical DNA from the single-stranded form. Preliminary experiment revealed that the compound was applicable for use as redox-labelling agent in an electrochemical gene sensor applications.

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Nucleic acid recognition processes offer uniquie opportunities for DNA biosensor developments. The DNA hybridization biosensor[1-3] as well as biosensors which target DNA-binding molecules and ions[4] are now almost commonplace and such devices hold a great promise for the clinical purposes. In particular, the use of Watson–Crick base-pair recognition is extremely important for the diagnosis of genetic or infectious diseases including single-base mismatches. A full range of DNA biosensors has been developed, and more recently, significant progress has been accomplished with the assembly of biochips which relies on an optical transduction of the formation of DNA double-strands with particular sequences of interest.[5]

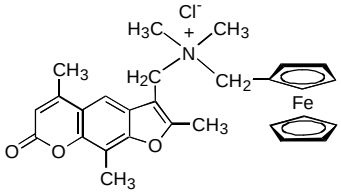
Electrochemistry, which necessarily uses an electrode, is fundamentally compatible to methods using a solid support since simple tailoring of an electrode surface readily gives an interface with sensing/transducing ability. The most electrochemical hybridization DNA biosensors (gene sensors) are taking advantages of a redox-active, indicator molecule; they rely on measuring chages in the faradaic current of the indicator that preferentially binds to the DNA duplex. Since the binding reaction occuors in a reversible fashion, it requires coexisting, free indicator molecule in an equilibrium state. A non-specific, physical adsorption of indicatior molecules is expected to associate, and these factors produce a certain amount of background signal which limits detection sensitivity. In the present paper, we will describe a new redox indicator molecule (**FcPso**) which binds covalently and irreversibly to a DNA doublex.

Experimental

*Chemicals and synthesis of **FcPSo***

4,5',8-trimethylpsoralen (Sigma-Aldrich) was reacted with chloromethyl methyl ether (Sigma-Aldrich) in acetic acid.[6,7] The resulting 4'-chloromethylated form was subsequently reacted with N,N-dimethylaminomethylferrocene (Tokyo Kasei, Co., Ltd.) in chloroform. The final product was purified by HPLC and the fraction which displayed both of the characteristics absorptions of psoralen and ferrocene was collected and was used further experiments

The oligodeoxynucleotides (ODNs) used in the present study were obtained from commercial synthetic services (Takara Shuzo, Co., Ltd.) and there sequences are as follows. Other chemicals were guaranteed reagents and used without further purification.



FcPso

p12 $^{5'}$HS-C_6H_{12}-GCC ACC AGC TCC$^{3'}$

t12 $^{5'}$GGA GCT GGT GGC$^{3'}$ (K-ras12 gene)

*Assay of photoreaction between **FcPso** and DNA by gel electrophoresis*

Plasmid pBR322 DNA was linearized with Sty I, and the DNA was purified by phenol extraction, followed by ethanol precipitation. The purified DNA was dissolved in TE buffer at 400 μg/mL. To 2 μl of the DNA solution in a micro test tube was added a TE solution of **FcPso** then the total volume was made up to 20 μL by adding TE buffer. The final concentration of the DNA was 60 μM (in nucleotide, 1 M = 1 mol dm^{-3}). The concentration of **FcPso** was varied in the range of 0.5 μM and 10 μM. Each solution was irradiated (*ca.* 60 mW/cm^2) on an ice bath with a 500 W ultra-high pressure Hg lamp equipped with a high-pass filter (Toshiba, UV-31) for 10 min.

To a reaction mixture was added 3 μL of aqueous NaOH (1.2 M) in order to denature the DNA, which was then analyzed by gel electrophoresis. For the analysis, the mixture was combined with

3 μL of gel-loading solution consisting of glycerin and water (7:3, v/v), the resulting mixture was loaded on a nondenaturing gel from 1 % agarose, and the gel electrophoresis was performed at 100 V for 30 min in TAE buffer (80 mM Tris-acetate, 1 mM EDTA, pH 7.6). After electrophoresis, the DNA in the gel was stained with ethidium bromide.

Instrumentation and electrode preparation

A one-chamber cell containing a horizontally mounted electrode was used for electrochemical measurement (0.37 cm^2 exposed electrode area). Gold mirror electrodes were purchased from Kinoene Kogaku (Tokyo, Japan). They were placed in the cell in contact with an aqueous 0.1 M H_2SO_4 solution (0.01 M KCl). The electrode was first subjected to anodic potential cycling to give a fresh surface exposed. After rinsing, 0.1 cm^3 of a 10 μM **p12** solution (in TE buffer, pH 7.2) was placed in the cell and the ODN adlayer was developed for 24 h at 5 °C. *In-situ* hybridization was made by treating the electrode with an aqueous solution containing **t12** (10 μM) at 60 ˚C for 1 h. For the photoreaction with FcPso, 0.1 cm3 of 0.2 mM **FcPso** solution was placed in the cell, and the electrode surface was irradiated by UV light (365 nm) for 5 min. Cyclic voltammetry (CV) was performed using EG&G PAR 263 potentiostat.

Results and Discussion

Psoralen is a class of intercalator which binds reversibly, in the most cases, to DNA double strands. However, it forms a photoadduct with the pyrimidine bases upon UV-irradiation. This unique property is expected to be useful in chemical tagging of DNA to give a specific function. Now, psoralens are known to form covalent bonds at their 3,4 and 4',5' double bonds with pyrimidines. If both sites of the psoralen are reacted, the result is an interstrand DNA crosslink; both strands are connected to each other. The ability of **FcPso** to form DNA crosslink was tested according to the literature,[8] by reacting linear double-stranded plasmid DNA.

Fig. 1 shows the result of gel electrophoresis. The photo-reacted DNA was alkali denatured to be the single-stranded form and was loaded onto a nondenaturing agarose gel. Crosslinked DNA immediately renatures in the neutral gel buffer and runs as the double-stranded form, while non-crosslinked DNA remains single-stranded and runs with greater mobility. As seen in Fig. 1, addition of **FcPso** resulted in crosslinking (lane 4-8). Thus, **FcPso** was proved to be bound covalently to DNA double-strands. Shown in Figure 1 is that increasing the amount of **FcPso** resulted in an appreciable retardation of DNA migration (lane 6-8). The retardation should be ascribed to **FcPso**-dependent elongation of the plasmid DNA.

Fig. 2 shows CVs of a DNA-modified electrode. The gold electrode whose surface is modified with the **p12/t12** duplex only gives capacitive currents. Upon treatment with **FcPso**, the electrode turns to give clear faradaic responses. The CV peak currents showed linear dependence to the sweep rate of the electrode potential and this suggests a surface process. On the other hand, almost no voltammetric change was associated for **FcPso** treatment for cases of the gold electrodes without *in-situ* hybridization (**p12** alone).

We attributed the CV response to the redox reaction of the ferrocenyl group bound to the DNA double-strands. As can be seen in Fig. 2, considerable extent of peak broadening is associated and this makes further analysis of CVs difficult. The present system still needs to be investigated in detail. However, we think results in the present study is important for electrochemical sensing of specific genes.

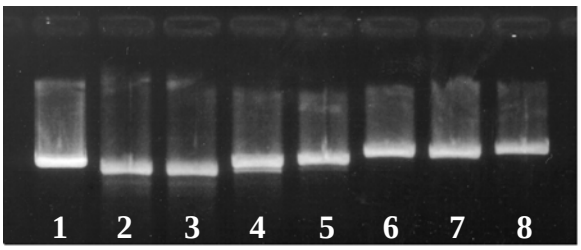


Fig. 1 DNA crosslinking by FcPso. Each sample contains 20 μM DNA (in nucleotide) and samples from lane 2 to 8 were alkaline denatured. Lane 1, native pBR322 DNA; Lane 2, denatured pBR322 DNA; lane 3, [**FcPso**] = 0 M; lane 4, 0.5 μM; lane 5, 1.0 μM; lane 6, 3.0 μM; lane 7, 5.0 μM; lane 8, 10 μM.

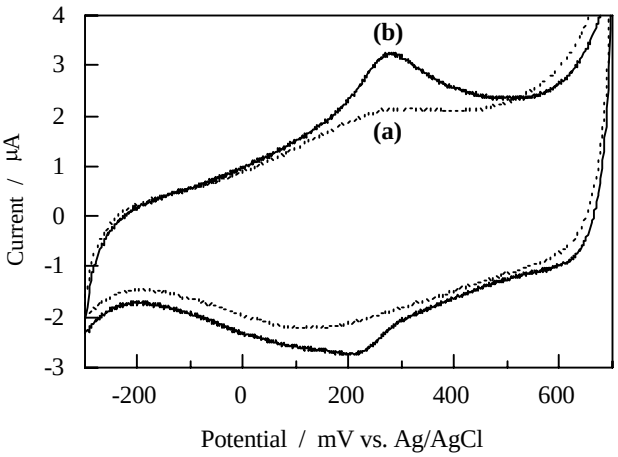


Fig. 2 CVs for the DNA-modified Au electrode (**a**) and after conjugation with **FcPso** (**b**). Measurements were made in 0.2 M KCl solution at 50 mV/s and RT.

Acknowledgments

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