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Photoelectrochemical Sensor with Porphyrin-Deposited Electrodes for Determination of Nucleotides in Water

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ABSTRACT

A 5,10,15,20-tetra(4-pyridyl)porphyrin (TPyP)-deposited ITO electrode as a sensor of nucleotides using photocurrent change was prepared. The TPyP-deposited ITO electrode could repeatedly detect nucleotides having concentrations of the μM order by a decrease in the photocurrent.

Much attention continues to be devoted to the development of chemosensory systems using the binding of organic species. Especially, the fixation of a molecular device on a substrate or electrode is important for its reuse as a sensor. Numerous studies have been reported about the development of fluorescence,[1] electrochemical,[2] surface plasmon resonance (SPR),[3] and quartz crystal microbalance (QCM) sensors,[4] because these systems have high sensitivity. On the other hand, the photoelectrochemical method using organic compounds is very limited[5] because organic photocurrent generators are a new topic.[6,7] Among these sensors, we employed the photoelectrochemical sensor for three reasons: (i) a variety of light-harvesting chromophores can be chosen, (ii) high sensitivity is achieved by integration of the photocurrent, and (iii) a small and inexpensive device can be prepared using a single-wavelength laser source and an electrochemical detector. In other words, one might expect the photoelectrochemical sensors have the advantages of both fluorescence and electrochemical sensors. We report a novel method for preparing a new nucleotide sensor that uses changes in photocurrent density. The nucleotide sensor has

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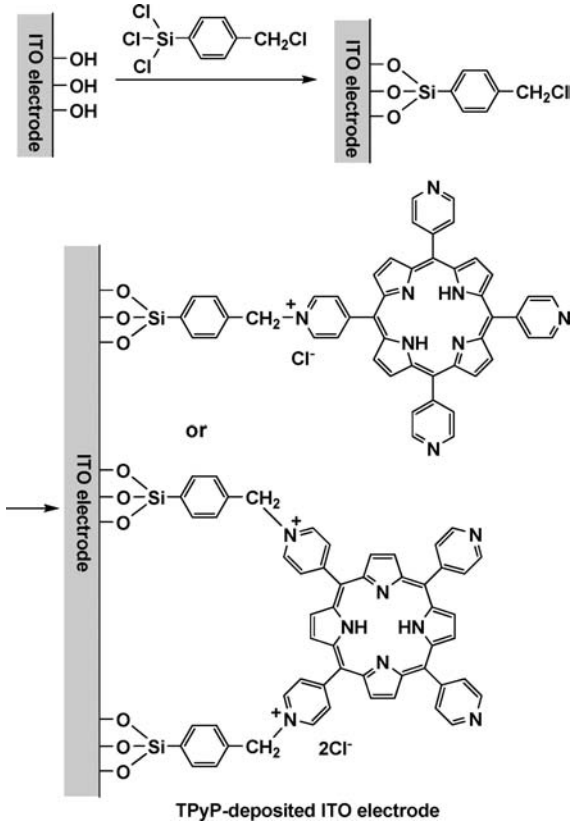
been the main focus of several groups because of not only detection of the contaminants in drinking water but also detection of nucleotides playing important roles in energy transduction in organisms and in metabolic processes by participation in enzymatic reactions.[8] 5,10,15,20-Tetra(4-pyridyl)porphyrin (TPyP) is used as a sensing reagent and is deposited onto an indium−tin oxide (ITO) electrode.

From UV−vis absorption spectral changes of 5,10,15,20-tetrakis(1-methyl-4-pyridinio)porphyrin tetrakis(*p*-toluenesulfonate) (TMPyP) with different concentrations of nucleotides, the isosbestic point was not observed in a basic aqueous solution (Figures S1−S7, Supporting Information).[9] Therefore, association constants (K_{ass}) for the basic aqueous solution could not be determined. Although the reason is not clear now, the formation of not only 1:1 TMPyP•nucleotides complex but also 1:2 complex is suggested. We used association constants (K_{ass}) for a neutral aqueous solution determined using ^{1}H NMR spectra by Pasternack et al. (Table S1, Supporting Information).[10] Although TMPyP is a tetracationic species, Tabata et al. reported that only one of three phosphate ions of ATP interacts with one of four pyridinium ions of TMPyP. Therefore, TMPyP can be used to roughly predict the strength of interactions between nucleotides and cationic porphyrin derivatives in our sensor system.[11]

TPyP-functionalized ITO electrode surfaces were prepared as shown in Scheme 1. The ITO electrodes were exposed to a toluene solution containing 4-(chloromethyl)phenyltrichlorosilane (10.0 mM) at 30 °C for 5 min. After successive washing with toluene and acetone, and then drying with a nitrogen stream, the ITO electrodes were spin coated with a chloroform solution containing TPyP (0.5 mM), and this was followed by brief heating in vacuo at 150 °C and 10 torr for 1 h. The ITO electrodes were successively washed with methanol and chloroform using ultrasonication for 1 min in each case and then dried with a nitrogen stream.

Scheme 1. Two-Step Procedure Used to Anchor TPyP onto the Surface of ITO Electrode



We measured data for a cyclic voltammogram of the TPyP-modified ITO electrode to determine the surface concentration of TPyP. The cyclic voltammogram of the TPyP-modified ITO electrode in a *N*,*N*-dimethylformamide solution showed a redox potential for the TPyP of ca. +600 to +1200 mV (versus Fc/Fc$^+$) (Figure S8A, Supporting Information). From the charge of the anodic peak of TPyP, the surface concentration of TPyP on the ITO electrodes can be estimated as 1.3×10^{-10} mol cm^{-2}, which is comparable to that of a porphyrin-modified ITO electrode produced using a chemical modification method (2.4×10^{-10} mol cm^{-2}).[12] Although a TPyP-modified ITO electrode with a higher concentration of TPyP was prepared using a replicate spin-coating process, the electrode was not suitable for our sensor system because of a reduction in the photocurrent density due to the self-aggregation of TPyP.

Photocurrent measurements were carried out for the TPyP-modified ITO electrode {experimental conditions: the working electrode was the TPyP-modified ITO, counter electrode was Pt wire, reference electrode was Ag/AgCl (3 M NaCl); these electrodes were immersed in a supporting electrolyte phosphate buffer solution ([NaCl] = 0.5 M, [Na_2SO_4] = 0.1 M, [KH_2PO_4] = 0.25 M, pH 10 by the addition of NaOH) containing 0.05 M 2,2′,2″-nitrilotriethanol (TEOA) as an electron sacrificial compound}. Photocurrent waves were observed (80−100 nA cm^{-2}, Figure S9, Supporting Information) when the TPyP-modified ITO electrode was irradiated with 420 nm light at 0.3 V bias voltage. Figure 1 shows the action spectra for the TPyP-

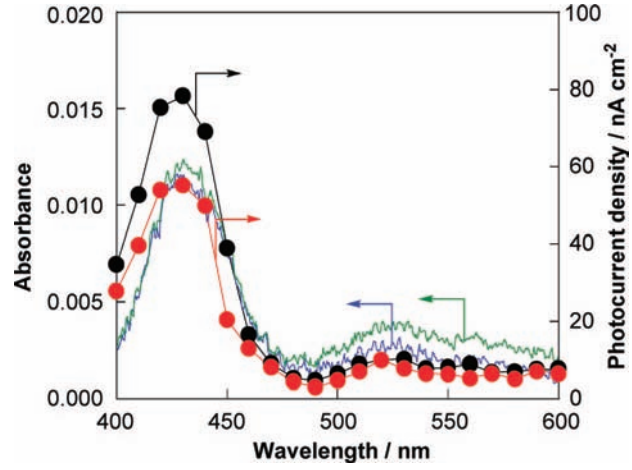


Figure 1. Absorption spectra (blue and green lines) and action spectra (black and red lines with closed circles) of the ITO electrodes coated with TPyP before and after addition of AMP, respectively; applied potential is 0.3 V vs Ag/AgCl; pH 10.0 buffer solution containing 0.05 M TEOA ([NaCl] = 0.5 M, [Na_2SO_4] = 0.1 M, [KH_2PO_4] = 0.25 M, pH 10 by the addition of NaOH).

modified ITO electrode between 400 and 600 nm. The large photocurrent density at 420 nm shows the porphyrin units efficiently act as the photoactive species. Moreover, we examined the influence of the addition of nucleotides {adenosine 5′-monophosphate (AMP), adenosine 5′-diphosphate (ADP), adenosine 5′-triphosphate (ATP), and guanosine 5′-monophosphate (GMP)} on photocurrent values (Figure 2). Plots of

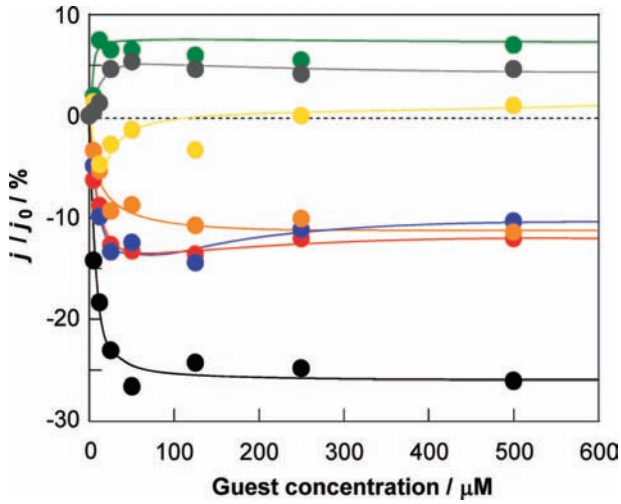
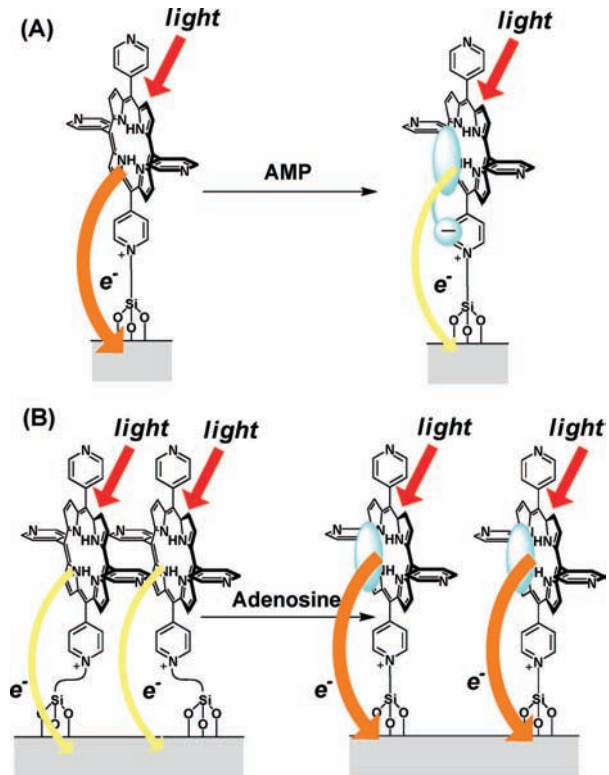


Figure 2. Photocurrent change (j/j_0, where j is the photocurrent density after the addition of nucleotides and j_0 is initial photocurrent density) of the TPyP-deposited ITO electrode with the addition of guest reagents in an aqueous solution: AMP (black line), ADP (blue line), ATP (red line), GMP (orange line), UMP (yellow line), adenosine (gray line), and guanosine (green line).

photocurrent changes (j/j_0, where j is the photocurrent density after the addition of nucleotides and j_0 is the initial photocurrent density) at 420 nm versus nucleotide concentrations (Figure 2) were found to be saturation curves. Figure 2 shows clearly the photocurrent markedly decreased with increasing concentrations of all nucleotides. Concentrations of the μM order could be detected. Although the order is not simply comparable with that of K_{ass} in a homogeneous aqueous solution, the TPyP-modified ITO electrode has stronger affinity for nucleotides. The findings can be attributed to porphyrin interacting with nucleotides in a 2:1 ratio owing to the concentration effect on the ITO electrode in comparison with a 1:1 complex in the aqueous solution. AMP, GMP, and UMP were chosen as guest reagents to estimate the effect of different kinds of bases on photocurrent density. The order of photocurrent changes (j/j_0) was AMP > GMP ≫ UMP. The slight photocurrent change in UMP causes a weak interaction between the uracil moiety and porphyrin, which is comparable with low K_{ass} (220 M^{-1})[10] The difference in photocurrent changes between purin bases such as adenine and guanine and a pyrimidine base such as uracil are attributable to the relative face-to-face π-π stacking abilities between TPyP and natural bases. On the other hand, AMP, ADP, and ATP were chosen as guest reagents to estimate the effects of various phosphates of these adenylic acids on photocurrent density. The order of photocurrent changes was AMP > ADP ≅ ATP. This result is inconsistent with the order of association constants (K_{ass}) between TMPyP and AMP, ADP, or ATP (1400, 2900, and 2900 M^{-1}, respectively)[10] in the aqueous solution. The weakening interaction between TPyP and ADP or ATP on the ITO electrode may contribute to steric hindrances between benzyl residues and diphosphate or the triphosphate moiety of ADP or ATP, which were brought out by the electrostatic interaction between pyridinium and phosphate (Scheme S1, Supporting Information).

The decrease in photocurrent density with the addition of nucleotides can be interpreted in three different ways: (i) the absorbance of TPyP decreased, (ii) the complexation between TPyP and nucleotides prevented the electron transfer from TEOA to TPyP cation radical ($TPyP^{\bullet+}$), or (iii) the phosphate ions of nucleotides prevented the electron transfer from photoactivated TPyP to the ITO electrode. We measured UV−vis absorption spectra of the ITO electrodes coated with TPyP to confirm the surface modification of TPyP and to examine the influence of the addition of AMP (Figure 1). The spectrum of TPyP shows an absorption maximum (λ_{max}) at 432 nm, which corresponds to the Soret band of porphyrin on the ITO electrode. The absorbance of TPyP scarcely changed with the addition of AMP, indicating explanation (i) is incorrect (Figure 1). We can now represent a complex structure on the ITO electrode, as in Scheme 2A. It is known electron transfer is suppressed when an anion exists on a bridge connecting a donor and an acceptor in a

Scheme 2. Schematic Illustrations of (A) the Prevention of the Electron Transfer from Photoactivated TPyP to the ITO Electrode Due to the Phosphate Ions of Nucleotides and (B) the Suppression of the Self-Quenching of Porphyrin Units Due to the Interaction between Adenosine and TPyPs



similar system.[13] On the other hand, because there are phosphate ions between the ITO electrode and the porphyrin moiety in our system, the photocurrent is expected to decrease owing to the complexation between TPyP and nucleotides as shown in Scheme 2A. To clarify whether explanation (ii) or (iii) is correct, we employed adenosine without a phosphate moiety as a guest molecule (Figure 2, gray line). If explanation (ii) is correct, the change in the photocurrent density in adenosine might be similar to that in AMP. On the other hand, if explanation (iii) is correct, the photocurrent density change in adenosine without an anionic moiety may be expected to differ from the behavior in AMP. As shown in Figure 2 (gray line), the photocurrent density slightly increased with the addition of adenosine. This result is distinct from the result for nucleotides. A similar result was obtained for guanosine (Figure 2, green line). The findings are due to the suppression of the self-quenching of porphyrin units due to the interaction between adenosine or guanosine and TPyPs (Scheme 2B). Although the phenomena in Scheme 2B also occurred for nucleotides such as AMP, the effect on the photocurrent density was counteracted by the prevention in explanation (iii). These results support explanation (iii).

The photocurrent densities that decreased with the addition of AMP were almost restored after successive washing of the cell with ultrapure water twice and ethanol once. The j/j_0 values (j is the photocurrent density after the addition of AMP and j_0 is the photocurrent density after washing) for each procedure were approximately constant (−27.0, −25.7, −27.1, and −26.6%) (Figure 3). This result indicates the TMPyP-deposited ITO electrode can be reused.

In summary, this paper demonstrated that the TPyP-functionalized ITO electrode was easily prepared by synthesis on the electrode and the electrode operated as a photoelectrochemical sensor for nucleotides. Both the electrostatic and $\pi-\pi$ interactions between nucleotides and TPyP derivative are important in detecting nucleotides in this system because

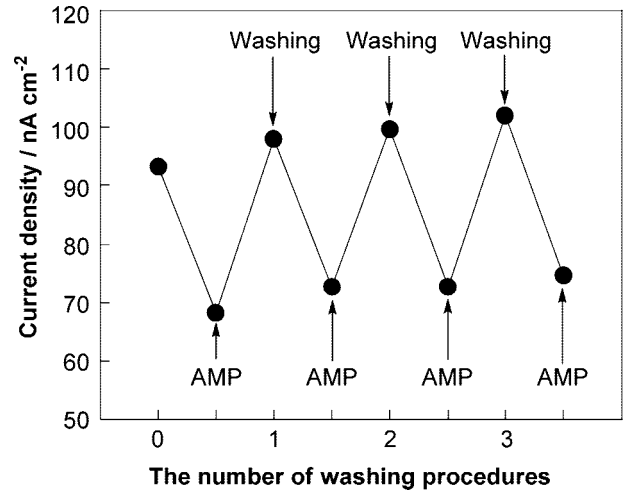


Figure 3. Reversible photocurrent changes after the addition of AMP and after successive washing with water and ethanol.

j/j_0 values for UMP with a phosphate ion but without $\pi-\pi$ stacking capabilities and adenosine with $\pi-\pi$ stacking capabilities but without phosphate ions are smaller than the value for AMP. Furthermore, the photoelectrochemical sensor can be used several times after successive washing with water and ethanol. Because it is possible to control the wavelength of light-irradiation by changing porphyrin derivatives, the photoelectrochemical sensor system can measure without pretreatment of the colored sample solutions.

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Supporting Information Available: Experimental details, Scheme S1, Table S1, and Figures S1−S9. This material is available free of charge via the Internet at http://pubs.acs.org.

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