

Effective Electron Transfer of Self-Assembled Redox-Active Peptide with an Affinity for Glucose Oxidase

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We describe the synthesis of a self-assembled redox-active peptide (RAP), which has an affinity for flavin adenine dinucleotide (FAD) as an electron acceptor, as well as its electrochemical characterization as a biosensor. We synthesized N-ferrocene and C-cysteine-terminated RAP (L-H-P-P-M-D-F-H-L-A-M-T-R-εAhx-C) by Fmoc solid-phase peptide synthesis. The C-cysteine-terminated RAP was immobilized on a gold electrode by the self-assembled monolayer method. It exhibited a stable electrochemical response after several potential sweeps. The FAD-captured RAP recognized glucose oxidase on the electrode surface. Current increased as the concentration of glucose increased. The self-assembled RAP-immobilized electrode effectively measures the electron transfer between the substrate and the enzyme; thus, it is suitable as a biosensor material.

1. Introduction

Electrochemical detection biosensors have been widely studied for applications in the medical field,^(1,2) food control,^(3,4) and environmental protection.⁽⁵⁾ By using electrochemical detection biosensors, the experimental setup and devices can be simplified and miniaturized easily with micro-electromechanical systems. These biosensors can be developed as industry-based biosensors. However, previously reported

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electrochemical detection biosensors have insufficient electron transfer efficiency. Because efficient transfer is important, the electrons should be acted on near the electrode surface in the sample solution. To improve the electron transfer efficiency for biosensing applications, better experimental setups using methods such as microfabrication^(6,7) have been reported. Previously, we performed the electrochemical characterization of a biosensor using redox active materials in sample solutions,^(8,9) which increased the electron transfer efficiency, although it was not sufficient for the high-sensitivity detection of target molecules.

In this study, we focused on the bioaffinity between glucose oxidase (GOx) and a redox-active peptide (RAP) to improve electron transfer and chemical immobilization using self-assembled monolayers (SAMs). We selected a peptide sequence with high affinity for flavin adenine dinucleotide (FAD) as an electron acceptor in GOx using Fmoc solid-phase peptide synthesis. The selected peptide redox-active site was also modified with ferrocene on the N-terminal and a cysteine residue on the C-terminal to immobilize it on a gold electrode as a SAM (Fig. 1).

We performed electrochemical characterization to demonstrate the suitability of RAP with an affinity for GOx for biosensing applications. Cyclic voltammetry of the enzyme electrode showed the current dependence on glucose concentration, confirming that RAP allows effective electron transfer to the electrode. This bioaffinity sensing using peptide would be applied to selective analysis in crude samples.

2. Experimental Section

2.1 Synthesis of RAP by Fmoc solid-phase peptide synthesis

Amino acids (AAs), α -aminohexanoic acid (α -hex), ferrocene carboxylic acid and GOx from *Aspergillus niger* (XS, 25000 units/g) were purchased from Sigma-Aldrich (USA). A peptide synthesizer (PSSM-8; Shimadzu, Japan) was used. We synthesized

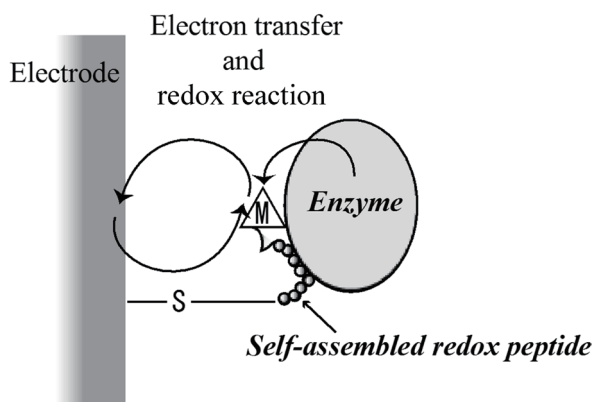


Fig. 1. Schematic illustration of redox peptide biosensor. S: thiol group; M: mediator.

eight RAPs that were selected by a combinatorial chemistry technique. The C-terminus of the resin-immobilized AA sequence and the Fmoc-protected N-terminus of the next AA in the sequence were coupled by 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride and N-hydroxysuccinimide. The Fmoc group was cleaved under mild acidic conditions from the trailing AA. Subsequently, the other Fmoc-AAs were coupled under the same conditions. The N-terminal AA residue was lysine to allow modification with ferrocene carboxylic acid. After the peptide sequence was synthesized, RAP was cleaved from the resin. The crude RAP product was separated on a Shiseido Capcell Pak column using acetonitrile: 0.1% TFA (40:60) by high-performance liquid chromatography. The sequence of RAP was confirmed by mass spectrometry.

2.2 Electrochemical characterization of the RAP SAM

The gold electrode was soaked in phosphate buffer (50 mM, pH 7.0) containing RAP (1 mg/mL) for 60 min to immobilize RAP via SAM formation. A three-electrode configuration was used. A SAM-based RAP electrode, a platinum wire, and Ag/AgCl/KCl were used as the work, auxiliary, and reference electrodes, respectively. Cyclic voltammetry (CV) was carried out in a deoxygenated phosphate buffer (10 mM, pH 7.0) with a computer-controlled potentiostat (CV-50W; BAS, USA) at a scan rate of 1 or 5 mV/s.

3. Results and Discussion

3.1 Synthesis of RAP

The dissociation constants (K_d) vs GOx of these peptides were evaluated by surface plasmon resonance. We chose the sequence L-H-P-P-M-D-F-H-L-A-M-T-R for RAP because it had the smallest K_d ($1.1\text{E}-04$ M) (Table 1). The elution of RAP was detected at m/z 2236.3 as protonated RAP, which was in good agreement with the theoretical value.

Table 1
Redox-active peptide.

Sequence	Anodic peak potential			Dissociation constant	
	E_{pa} (mV)	I_d (μA)	I_k (μA)	I_k/I_d	K_d (M)
(No. 1) FcK(Fc) H P P M D F H K A M T R	519	0.540	11.3	20.9	$1.1\text{E}-04$
(No. 2) FcK(Fc) A P W P S P T H Y L K D	500	0.4698	7.00	14.9	$4.3\text{E}-04$
(No. 3) FcK(Fc) Q I P L M K G P G Y M Y	491	0.140	3.45	24.4	$2.7\text{E}-04$
(No. 4) FcK(Fc) Y S A H A S A H A S A H	514	0.0328	0.223	6.78	$7.8\text{E}-01$
(No. 5) FcK(Fc) G Y T D H G T D H G D T	523	0.491	4.66	10.0	$3.2\text{E}-01$
(No. 6) FcK(Fc) L V D H D T S N I P G S	515	0.526	3.83	7.27	$4.9\text{E}-04$
(No. 7) FcK(Fc) E H K A K H S K H Y H K	520	0.189	5.16	27.3	$3.3\text{E}-04$
(No. 8) FcK(Fc) E S D E S D E S D S S D	485	0.309	0.645	2.08	$2.4\text{E}-04$

All C-termini were added to the lysine residue owing to the modification of ferrocene.

3.2 Electrochemical characteristics of RAP in a homogenous system

RAP showed a typical electrochemical response in a homogeneous system. After 10 CV scans, stable cyclic voltammograms were obtained. The peak-to-peak potential separation (ΔE_p) of RAP was almost 100 mV, and the wave was not reversible because it was a homogenous system. The catalytic electrochemistry of GOx with RAP was also investigated. The RAP-enhanced catalytic electrochemical response in the presence of glucose (10 mM) and GOx in a buffer solution, I_k/I_d , was 6.5 at $E_{pa} = 528$ mV (Fig. 2). E_{pa} was delayed compared with that of ferrocene carboxylic acid, where E_{pa} occurs at 400 mV owing to the homogenous system.

3.3 Electrochemical characteristics of RAP SAM

RAP was immobilized as a SAM on the gold electrode through the thiol group of the cysteine residue. When the electrode was held at a high potential, a physically adsorbed RAP became more hydrophilic because of ferrocene and was removed from the electrode surface, leaving only the RAP SAM. Moreover, the electrode-modified RAP showed a stable response after 10 CV scans. When the RAP is immobilized on an electrode surface via SAM, we would expect to observe a reversible wave, but here we did not observe such a response. The oxidation and reduction peaks were observed at +528 and +450 mV (vs Ag/AgCl), respectively. The α -hex linker between ferrocene and peptide may have created a complex interface; thus, delayed electron transfer occurred.

The catalytic electrochemistry of GOx with a RAP-based electrode and glucose was investigated. A catalytic current was observed upon the addition of glucose at E_p (610 mV), although the current plateaued at 20 mM glucose (Fig. 3). In a control experiment

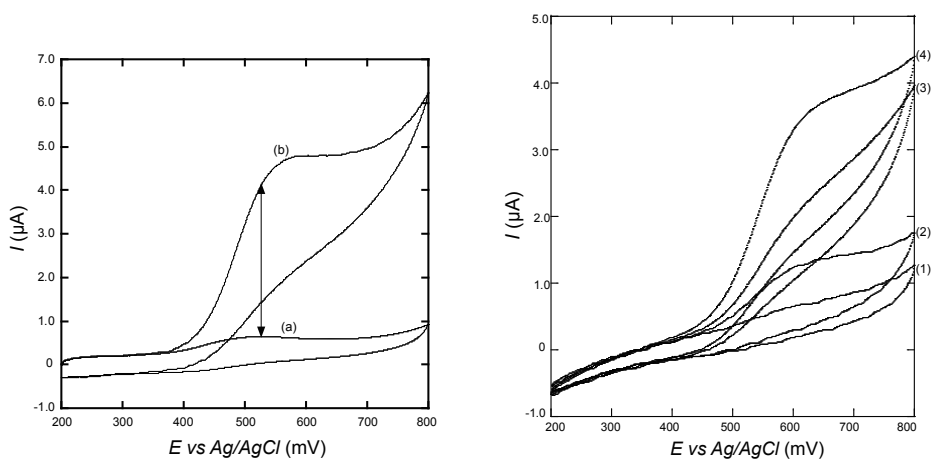


Fig. 2 (left). Catalytic electrochemistry of the redox-active peptide electrode in a homogeneous system. CV was performed at a scan rate of 5 mV/s in 100 mM phosphate buffer (pH 7.0) (a) without and (b) with 10 mM D(+)-glucose.

Fig. 3 (right). Voltammograms of the RAP SAM modified gold electrode in the presence of 1.0 μM GOx. CV was performed at a scan rate of 1 mV/s in 0.1 M phosphate buffer (pH 7.0) and (1) 0, (2) 2.0, (3) 5.0, and (4) 20.0 mM glucose.

using a random peptide sequence, no catalytic response was observed. Thus, the SAM-based RAP, which has bioaffinity to GOx, could retain GOx on the electrode surface and indicated a catalytic response. The peak current for the catalytic response showed a positive shift compared with that without the glucose substrate. For the ΔE_p of the RAP SAM modified gold electrode, the value (610 mV) increased compared with that of the homogeneous system (80 mV) at the same scan rate. The RAP SAM on the electrode showed thin-layer characteristics.⁽¹⁰⁾

4 Conclusions

We have constructed a peptide electrode covalently modified with a redox active compound. RAP has affinity for GOx, indicating the effective electron transfer between GOx and the RAP-immobilized electrode. The catalytic response depended on the glucose concentration from 0 to 20 mM. The RAP-immobilized electrode is a suitable biosensor for GOx and could be used for other enzymes by altering the peptide sequence.

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