Electrochemical Study of Functionalization on the Surface of a Chitin/Platinum-modified Glassy Carbon Paste Electrode

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To functionalize chitin surfaces using proteins, we developed a glucose oxidase (GOD)-chitin/platinum-modified glassy carbon paste electrode (GCPE) as a model. In a weakly acidic solution, negatively charged GOD were immobilized by the protonated acetylamide groups on chitin. When the electrode was immersed in a solution containing GOD, the enzyme was readily immobilized due to the electrostatic interaction. In addition, measurements were performed using electrodes made with powders of different sizes because sensor performance depends on the particle sizes of glassy carbon powder.

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Introduction

Biopolymers are highly biocompatible, and their functional groups allow surface modification with biomolecules. When proteins and carbohydrates with special functions are introduced to the biopolymer, the materials become excellent reaction fields for monitoring the binding between biomolecules. For example, surface modification of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) with collagen has been shown to improve cell compatibility.¹ Skopinska-Wisniewska *et al.* have reported on surface characterization of collagen/elastin for tissue regeneration.² On the other hand, a study on polysialic acid as a biomaterial for cell culture applications was attempted.³ Chitin, which is a polysaccharide, consists of β -1,4-linked *N*-acetylglucosamine residues. Because the chemical and physical properties of chitin are stable, chitin is suitable for the immobilization of enzymes⁴ and plant tissue.⁵ Chitosan with active amino groups is also effective for use as coating material⁶ and for production of composite material.⁷ In the field of electrochemistry, biomaterial contributes to the development of biomolecular sensors. Chitosan-carbon nanomaterial has been used as biosensors for glucose,⁸ laccase⁹ and cytochrome.¹⁰ In addition, covalent immobilization of glucose oxidase onto a carbon sol-gel silicate composite surface has been investigated for creation of a biosensor platform.¹¹ Carbon paste electrodes, which can be conveniently modified with various functional molecules, provide a reproducible electrode response.^{12,13} Recently, carbon paste electrodes have been prepared from glassy carbon powder.¹⁴ Because glassy carbon paste electrodes

(GCPEs) have desirable electrochemical reactivity, GCPEs have been applied to biomolecular monitoring.¹⁵

In the present study, a chitin/platinum-modified GCPE was constructed to functionalize a chitin surface using a protein. Glucose oxidase (GOD), which has an isoelectric point of approximately 4.2, was selected as the model protein. Protonated acetylamide groups on chitin can be used to immobilize negatively charged biomolecules in an acidic solution. The electrostatic interaction confers a molecular-recognition function to chitin. The ionic interaction that permits protein immobilization on the chitin surface is not expected to significantly alter the protein structure. In a previous study, a glucose sensor using a GOD-chitin/platinum carbon paste electrode was reported.¹⁶ A new procedure can improve the sensitivity of glucose sensing using the chitin/platinum GCPE with GOD in comparison with the study mentioned above. Furthermore, the effect of the glassy carbon powder particle size on electrode performance was evaluated because particle size affected both electrode response and preparation of the paste.

Experimental

Reagents and chemicals

Chitin powder was purchased from Funakoshi Co., Ltd., Japan. D(+)-Glucose, glucose oxidase and di-*n*-octyl phthalate were supplied by Wako Pure Industries. Platinum powder (average particle size: 10 μ m) was obtained from Kishida Chemical Co., Ltd. Other reagents were of analytical reagent grade. Glassy carbon powders of different particle sizes were purchased from BAS: 0.4 – 12 μ m (S-12, No. 012090), 10 – 20 μ m (S-20, No. 012091), 20 – 40 μ m (S-50, No. 012092), 50 – 80 μ m (S-80, No. 012093), and 80 – 200 μ m (S-200,

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Fig. 1 Cyclic voltammograms of glucose using a chitin/platinum-modified GCPE with GOD. An electrode containing 10 wt% chitin, 20 wt% platinum and 70 wt% 10–20 μm glassy carbon powder was immersed in 0.1 M acetate buffer (pH 6.2) containing GOD (600 units/ml) for 1 h. Measurements were performed in 0.1 M acetate buffer (pH 6.2) using the electrode. a, Blank; b, 1×10^{-4} M glucose; c, 2.0×10^{-4} M glucose.

No. 012094). A 0.1 M acetate buffer was used as both the solvent during immobilization of GOD and the supporting electrolyte for the electrochemical measurements.

Apparatus

Voltammetric measurements were carried out using a Bioanalytical Systems Inc. (BAS) CV-50W analyzer. The working electrode was a carbon paste electrode (diameter, 3 mm; Model No. 1013, BAS). The counter electrode was a platinum wire, and the reference electrode was Ag/AgCl (NaCl, sat.; Model No. 11-2020, BAS). All potentials were measured against the Ag/AgCl electrode.

Procedure

To immobilize GOD on the GCPE surface, we immersed the electrode in 1 ml of 0.1 M acetate buffer (pH 6.2) containing GOD (600 units) with stirring for 1 h at 4°C. The amount of GOD immobilized on the electrode surface was calculated by subtracting the absorbance at 450 nm of free GOD that remained in solution after immobilization from the absorbance of GOD in solution before immobilization.

A glassy carbon paste was prepared by mixing chitin powder (20 mg), platinum powder (25 mg) and glassy carbon powder (55 mg) with a di-*n*-octyl phthalate binder. A portion of the paste was used to fill the cavity of the carbon paste electrode. The surface of the electrode was smoothed with a wrapper. Measurements were carried out in 0.1 M acetate buffer (pH 6.2). After the solution was stirred, the peak resulting from oxidation of hydrogen peroxide was recorded between 0.2 and 0.8 V using cyclic voltammetry (scan rate: 50 mV s⁻¹). When the platinum powder was not added to the paste, the oxidation current of hydrogen peroxide could not be observed. To detect glucose in sports drinks, a current-time curve was measured at +0.55 V using amperometry.

Results and Discussion

Cyclic voltammetry of glucose using the GCPE

Figure 1 shows the cyclic voltammograms for glucose obtained using the chitin/platinum-modified GCPEs with GOD.

Fig. 2 Effect of the particle size of the glassy carbon powder. An electrode containing 10 wt% chitin, 20 wt% platinum and 70 wt% glassy carbon powder was immersed in 0.1 M acetate buffer (pH 6.2) containing GOD (600 units/ml) for 1 h. Measurements were performed in 0.1 M acetate buffer (pH 6.2) containing 2.0×10^{-4} M glucose using the electrode.

In the range between 0.2 and 0.8 V, no oxidation appeared. On the other hand, when glucose was added to the solution, oxidation peaks of hydrogen peroxide resulting from enzymatic reactions were observed at a potential of 0.55 V. Moreover, peak current increased with increasing glucose concentration. Therefore, immersion of the GCPE resulted in direct immobilization of GOD on the electrode surface. Immobilization took place due to the interaction between the protonated acetyl amide groups on chitin and GOD, which was negatively charged in acetate buffer (pH 6.2). An advantage of this method is that the structural and physical properties of the protein remain relatively unchanged.

Effect of particle size on GCPE performance and preparation

The peak currents for hydrogen peroxide that were obtained using chitin/platinum-modified GCPEs prepared with different glassy carbon powders are shown in Fig. 2. The GCPEs were immersed in 0.1 M acetate buffer (pH 6.2) containing GOD. The peak current for the 10–20 μm powder was greater than that of the 0.4–10 μm glassy carbon powder. The reproducibility of both the 20–40 and 50–80 μm glassy carbon powders decreased because the residual current increased with particle size. A paste could not be prepared using the GCP comprised of the largest of particles, i.e., 80–200 μm in diameter. Therefore, the electron transfer and diffusion of material on the electrode surface were related to the glassy carbon powder surface area. If one judges from the reproducibility and sensitivity, the paste obtained from 10–20 μm powder was suitable to detect hydrogen peroxide. In addition, preparation of the GCP was influenced by the properties of the binder. When nujol was used as a binder, the peak current was smaller than that of an electrode with a di-*n*-octyl phthalate. Consequently, the conductivity of the binder may be related to the peak current of hydrogen peroxide.

Addition of chitin to GCPEs and GOD immobilization on GCPEs

Addition of various amounts of chitin to the glassy carbon powder was investigated. When the platinum powder was 20 wt% of the paste, the peak current increased at 12 wt% of chitin and then decreased at 14 wt% (Fig. 3). The decrease in peak current was attributed to the non-conductivity of chitin.

Fig. 3 Dependence on the amount of chitin. An electrode containing x wt% chitin, 20 wt% platinum and $(80 - x)$ wt% 10–20 μm glassy carbon powder was immersed in 0.1 M acetate buffer (pH 6.2) containing GOD (600 units/ml) for 1 h. Measurements were performed in 0.1 M acetate buffer (pH 6.2) containing 2.0×10^{-4} M glucose using the electrode.

In contrast, the peak current was not observed by using an electrode without chitin. Therefore, adsorption of GOD on the electrode surface did not influence the measurement.

Regarding electrode modification with GOD, the peak current was constant at greater than 500 units of GOD per ml of the solution. The amount of GOD immobilized on the electrode surface was estimated using spectrometry. The absorbance at 450 nm of the solution containing GOD was measured before and after immersion of the electrode. At pH values ranging from pH 5.3 to 6.5, there were 15 units of GOD immobilized on the electrode surface. Although direct immobilization of GOD in solution on the chitin/platinum-modified carbon paste electrode was inadequate, this modification was readily achieved using a GCPE due to the spherical particles.

Amperometric determination of glucose using the GCPE

Figure 4 shows the relationship between peak current measured using the electrode and time. Amperometric measurements at +0.55 V were made after glucose (1×10^{-5} M) was added to the solution several times. The peak current stabilized after approximately 20 s. The electrode response was linear at glucose concentrations ranging from 8×10^{-7} to 4×10^{-4} M. The detection limit for glucose, estimated as three times the standard deviation of the blank, was 5×10^{-7} M. The sensitivity of the GCPE was enhanced four-fold relative to that of the chitin/platinum-modified carbon paste electrode.

To test the GCPE in a practical application, the concentration of glucose in sports drinks was measured using a GCPE (Table 1). In addition to glucose, the drinks contained vitamin B, citric acid, amino acids, Na(I) and Ca(II). The solutions were diluted 500-fold with 0.1 M acetate buffer. The glucose content measured using the method developed in this study agreed with that provided by the manufacturers. Consequently, the method can be applied to the detection of glucose in sports drinks. When the method is applied to other real samples, the influence of coexistence can be reduced by further dilution of the sample solution.

Conclusions

In this study, the surface of a chitin/platinum-modified GCPE

Fig. 4 Typical response curves to glucose. An electrode containing 10 wt% chitin, 20 wt% platinum and 70 wt% 10–20 μm glassy carbon powder was immersed in 0.1 M acetate buffer (pH 6.2) containing GOD (600 units/ml) for 1 h. Measurements were carried out using the electrode containing 10 wt% chitin, 20 wt% platinum and 70 wt% 10–20 μm glassy carbon powder in 0.1 M acetate buffer (pH 6.2) at +0.55 V.

Table 1 Determination of glucose in sports drinks

Sample	Concentration of glucose/M		Concentration of ascorbic acid/M
	Present method	Manufacturer's value	
Sports drink A	0.109 ± 0.005	0.111	0.0005
Sports drink B	0.047 ± 0.003	0.049	0.0003

Number of determinations = 5.

1:500 dilution with 0.1 M acetate buffer (pH 6.2).

was functionalized with GOD. Immobilization of GOD was achieved due to the electrostatic interaction between the protonated acetylamine groups on chitin and negatively charged GOD. Peak current for enzymatic oxidation of hydrogen peroxide was dependent on the particle size of the glassy carbon powder. Thus, particle size influenced electrode performance. The electrode was applied to detection of glucose in sports drinks, and the values obtained using this method agreed with those reported by the manufacturers.

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