

Glucose Sensor Strip Using Flavin Adenine Dinucleotide-dependent Glucose Dehydrogenase with Quinones as Redox Mediators

Yue Gong and Seiya Tsujimura*

Faculty of Pure and Applied Sciences, University of Tsukuba,
1-1-1 Tennodai, Tsukuba, Ibaraki 305-8573, Japan

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In this study, a glucose sensor strip that has an extended dynamic range and low mediator loading was developed. Flavin adenine dinucleotide-dependent glucose dehydrogenase (FAD-GDH) was used as the enzyme, with quinone as the electron transfer mediator. When 10 U of FAD-GDH and 10 nmol of 1,2-naphthoquinone were loaded onto the electrode, the glucose calibration curve showed high linearity up to a concentration of 1000 mg/dL. The curve was also highly linear when 2-methyl-1,4-naphthoquinone was used as the mediator. Furthermore, the addition of a surfactant improved the linearity. However, 9,10-phenanthrenequinone could not be used to measure glucose at high concentrations. The rate constant of the FAD-GDH reaction and the water solubility of the mediator (actual mediator concentration near the electrode) were determined to be important parameters.

1. Introduction

Diabetes mellitus, also known as diabetes, is a very common lifestyle-related disease. The number of people with diabetes exceeds 537 million, and this number is growing annually. Diabetes is difficult to cure and can lead to complications if left untreated. Complications such as retinopathy, nephropathy, and neuropathy can significantly impact quality of life. Therefore, self-monitoring of blood glucose (SMBG) is important for those with diabetes.⁽¹⁻⁴⁾ Glucose levels in small-volume blood samples (~1 μ L) can be measured with high accuracy using electrochemical self-monitoring devices. Recently, flavin adenine dinucleotide (FAD)-dependent glucose dehydrogenase (FAD-GDH) has been widely used as a glucose-oxidizing enzyme instead of glucose oxidase (GOx).⁽⁵⁻⁹⁾ GOx uses O₂ as an electron acceptor, which can lead to measurement errors depending on the blood O₂ concentration. However, FAD-GDH does not employ O₂ as an electron acceptor. Fungus-derived FAD-GDH generally requires the use of a mediator; potassium ferricyanide has been utilized to date.^(1,2,4) The principle of the sensor strip is that blood glucose is oxidized by the enzyme, and an equivalent amount of ferricyanide is reduced to ferrocyanide, and the resulting ferrocyanide is electrochemically oxidized at the

*Corresponding author: e-mail: seiya@ims.tsukuba.ac.jp
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electrode.^(1,2) The amount of glucose can be determined from the generated oxidation current using a calibration curve. The oxidation current is determined by the diffusion of ferrocyanide to the electrode, that is, the concentration gradient of ferrocyanide at the electrode surface. The reason why ferricyanide has been used is that it shows high solubility in water; when blood reaches the working electrode, the enzymes and mediators deposited on the electrode are dissolved immediately. However, the high redox potential of potassium ferricyanide may affect the current response in the presence of high concentrations of electrochemically active interferents such as ascorbic acid and uric acid. The calibration curve and upper limit of detectable glucose concentration depend on the amount of potassium ferricyanide loaded on the electrode. A sufficiently large number of mediators must be loaded to detect high concentrations of glucose, which leads to technical difficulties and instability, including noise.

Our group has recently developed sensor strips using quinolinedions [quinoline-5,8-dione (QD) and isoquinoline-5,8-dione (IQD)] as mediators.⁽¹⁰⁾ These mediators exhibit low redox potential [-0.07 V vs Ag|AgCl|KCl(sat.)], high reaction rates with enzymes (the bimolecular reaction rate constants of QD and IQD can reach $10^{7.7}$ and $10^{7.4}$ $\text{M}^{-1} \text{s}^{-1}$, respectively), and high solubility in water (the water solubility of QD is 88 mM). In addition, the redox reaction of quinone compounds involves two electrons per molecule; thus, a mediated electron transfer (MET) reaction is anticipated to occur with a relatively low amount of loading.

In this study, we investigated the performance of disposable biosensors using three quinone compounds: 1,2-naphthoquinone (12NQ), 2-methyl-1,4-naphthoquinone (MeNQ), and 9,10-phenanthrenequinone (PQ). The rate constants of 12NQ and PQ are higher than those of QD and IQD; the bimolecular rate constants of 12NQ, MeNQ, and PQ toward FAD-GDH are $10^{8.1}$, $10^{5.7}$, and $10^{8.2}$ $\text{M}^{-1} \text{s}^{-1}$, respectively.⁽¹¹⁾ The solubilities of these quinones are much lower than that of QD; specifically, the solubilities of 12NQ, MeNQ, and PQ in water are 1.1, 9.3×10^{-1} , and 2.0×10^{-3} mM, respectively. The effects of water solubility, the reaction rate with FAD-GDH, the amount of electrode loading, and the addition of a surfactant on the sensor response were investigated.

2. Materials and Methods

In this study, 12NQ, MeNQ, and PQ were purchased from Tokyo Chemical Industry Co., Ltd., and used without further purification. FAD-GDH (25 mg/mL, 2 kU/mg) was purchased from Ikeda Tohka (Japan). Polyoxyethylene sorbitan monolaurate (= Tween 20) was purchased from Fujifilm Wako Pure Chemical Corp. (Japan). Disposable test strips comprising a three-electrode system using Au as the working (area is 1 mm²) and counter electrodes and Ag|AgCl as the reference electrode were donated by Arkray Inc. (Japan).⁽¹⁰⁾ Here, 0.2 μL of mediator acetonitrile solution was added dropwise onto the working electrode of the sensor chip and allowed to dry for 20 min at 25 °C, followed by the dropwise addition of 0.2 μL of FAD-GDH (10 U/strip), which was also allowed to dry for 20 min at 25 °C. A spacer was placed on the electrode section, and 4 μL of glucose solution was injected into the electrode through the capillary channel. The sensor chip was connected to a potentiostat (CHI 1020, BAS), and the response current was evaluated by chronoamperometry at 0.5 V for 50 s.

3. Results and Discussion

3.1 Electrochemical response of sensor strip using quinone as a redox mediator

The current responses of the sensor strips loaded with 10 nmol of 12NQ as a mediator for up to 50 s at various glucose concentrations are shown in Fig. 1. The numbers of moles of glucose in the reaction cavities of the chips were 22, 44, 88, 133, and 222 nmol at 100, 200, 400, 600, and 1000 mg/dL, respectively. Therefore, the loading of the mediator was lower than the number of moles of glucose introduced into the sensor. The current increased with the glucose concentration. This finding indicates that some of the mediator previously dried on the electrode was dissolved when the glucose solution reached the reagent/FAD-GDH area and functioned as a mediator for FAD-GDH. The amount of mediator loaded on the electrode was much lower than that of glucose, so not all of the introduced glucose reacted. The rate-limiting step in generating the oxidation current would be the diffusion of glucose. This result is consistent with those of our previous studies using quinolinedions (QD and IQD).⁽¹⁰⁾ Although the current values for QD and IQD decay according to the Cottrell equation, depending on the diffusion rate of glucose, in the case of quinones, some of the current-time curves are time-independent.

Figure 2 shows the dependence of the response current at 5 s on glucose concentration for the four mediators. Here, 12NQ exhibits the highest linearity, with a highly linear response up to approximately 600 mg/dL, and the response current increases even at a concentration of 1000 mg/dL. For the other mediators, the slope of the calibration curve below 400 mg/dL of glucose decreases in the order 12NQ, MeNQ, and PQ. This finding suggests that at low glucose concentrations, the glucose at the electrode surface was consumed and a sufficient glucose concentration gradient formed near the electrode surface. However, at high glucose

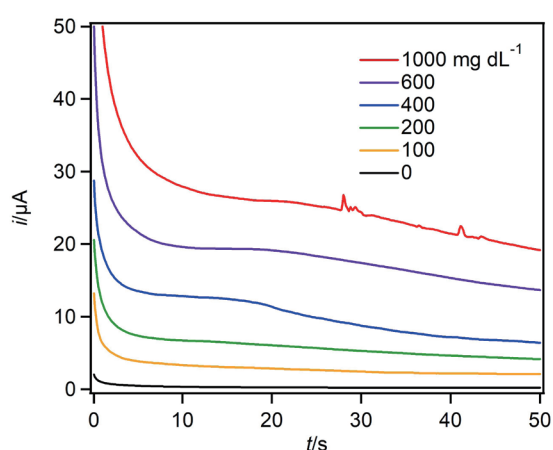


Fig. 1. (Color online) Amperometric response curve at different glucose concentrations [0 (black), 100 (gold), 200 (green), 400 (blue), 600 (purple), and 1000 (red) mg/dL] of test strip modified with 12NQ (10 nmol) and 10 U of FAD-GDH at an applied potential of 0.5 V vs Ag/AgCl.

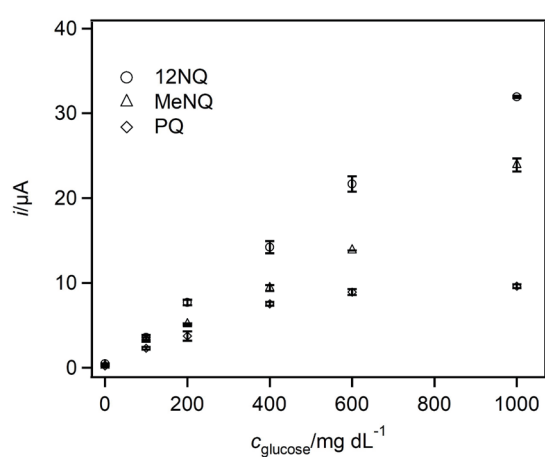


Fig. 2. Dependence of glucose oxidation current on glucose concentration (0, 100, 200, 400, 600, and 1000 mg/dL) at 5 s with FAD-GDH (10 U)-modified test strip with different mediators: 12NQ (10 nmol, open circle), MeNQ (10 nmol, open triangle), and PQ (4 nmol, open diamond) at an applied potential of 0.5 V vs Ag/AgCl.

concentrations, the rate of glucose consumption did not increase further because of the slow electrochemical regeneration of the enzyme by the mediator. Furthermore, there was no change in glucose concentration gradient and no increase in current.

Figure 2 suggests that the slope of the calibration curve depends on the enzyme reaction rate at the electrode. As the enzyme concentration is constant, the bimolecular rate constant and solubility of the mediator affect the slope. For the reaction rate constants between FAD-GDH and the mediator, the order of the reaction rate constants was $12\text{NQ} > \text{MeNQ}$ among the naphthoquinones, which agrees with the slopes of observed results. Although PQ has a higher rate constant than MeNQ, the response current of the PQ-based sensor is lower. This low response current is due to the low solubility of PQ. The solubilities of 12NQ, MeNQ, and PQ in water are 1.1, 9.3×10^{-1} , and 2.0×10^{-3} mM, respectively. The solubility of PQ is very low, which leads to the low response current.

3.2 Effect of mediator loading

Figure 3 shows the dependence of the current response on the glucose concentration when the loading of the mediator was decreased. For the naphthoquinones, the linearity of the current response as a function of glucose concentration extends to 600 mg/dL for 12NQ and 400 mg/dL for MeNQ. The linear regions are narrower than those for higher mediator loadings, as shown in Fig. 2. However, no significant effect on PQ was observed. In the case of 12NQ, the reaction rate (including the rate constant and solubility) was sufficiently high to consume glucose, and a glucose concentration gradient was formed near the electrode surface. However, for MeNQ, which has lower reaction rate constants than 12NQ, the electrochemical regeneration rate of the enzyme decreased because of its lower surface concentration, and the glucose consumption rate was affected. In the case of PQ, the concentration of the mediator near the surface of the electrode was independent of the loading amount because of the very low solubility of PQ in water, which means that it may not have an effect on the reaction rate.

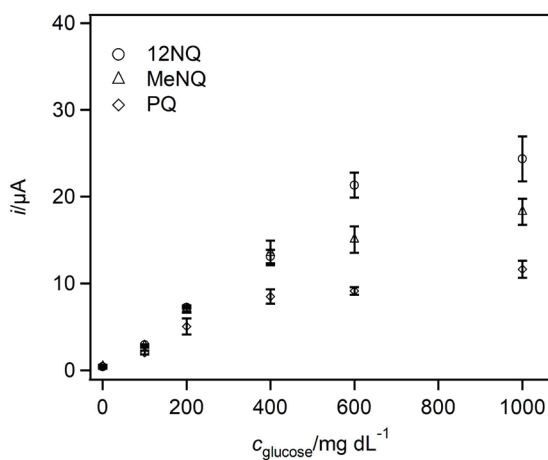


Fig. 3. Dependence of glucose oxidation current on glucose concentration (0, 100, 200, 400, 600, and 1000 mg/dL) at 5 s with FAD-GDH (10 U)-modified test strip with different mediators: 12NQ (1 nmol, open circle), MeNQ (1 nmol, open triangle), and PQ (1 nmol, open diamond) at an applied potential of 0.5 V vs Ag|AgCl.

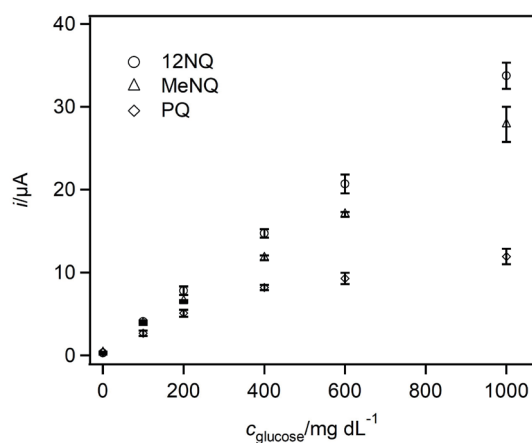


Fig. 4. Dependence of glucose oxidation current on glucose concentration (0, 100, 200, 400, 600, and 1000 mg/dL) at 5 s with FAD-GDH (10 U)-modified test strip and different mediators: 12NQ (10 nmol, open circle), MeNQ (10 nmol, open triangle), and PQ (4 nmol, open diamond) with Tween 20 at an applied potential of 0.5 V vs Ag|AgCl.

3.3 Effect of surfactant

As an alternative to increasing the loading of the mediator, we investigated the use of surfactants to improve elution from the electrode and to increase the effective concentration of the mediator near the electrode. Tween 20 (0.01 wt% in buffer solution, 0.2 μL), a nonionic surfactant, was added to the sensor chip, along with the enzyme. The results are presented in Fig. 4. The addition of a surfactant did not affect the response of 12NQ, and the calibration curve exhibits high linearity, as shown in Fig. 2. The surfactant significantly improved the linearity of the curves for MeNQ. However, no improvement was observed in the response currents of the PQ-based sensor strips. This finding suggests that Tween 20 does not improve the solubility (or elution) of PQ and that a stronger surfactant with a higher concentration is required. However, a stronger surfactant may have a negative effect on the enzyme by concentrating it during the drying process.

4. Conclusions

Historically, mediators have been selected for GOx-based SMBG sensor strips on the basis of their stability and solubility. In this study, we used quinone as the mediator for the sensor strips. Quinone has a low solubility in water but exhibits a very high electron-exchange reaction rate constant with FAD-GDH. A high reaction rate even with small amounts of quinone enables the generation of a glucose concentration gradient by electro-enzymatically consuming glucose. The current response was found to depend on the glucose supply to the electrode surface from the bulk. The quinone was dried and deposited on the electrode surface; however, when in solution, some of the quinone dissolved and reacted with the enzyme. The solubility also affected the current response. We found that 12NQ was a good mediator that worked effectively even with a very small amount of loading. However, it was difficult to measure high concentrations of glucose when PQ was employed as a mediator, owing to its very low solubility and resulting low effective concentration.

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