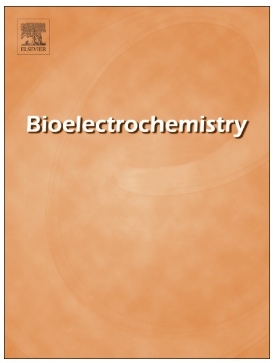

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Development of a Glucose Sensor Employing Quick and Easy Modification Method with Mediator for Altering Electron Acceptor Preference

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Abstract

Enzyme based electrochemical biosensors are divided into three generations according to their type of electron transfer from the cofactors of the enzymes to the electrodes. Although the 3rd generation sensors using direct electron transfer (DET) type enzymes are ideal, the number of enzyme types which possess DET ability is limited. In this study, we report of a glucose sensor fabricated using mediator-modified glucose dehydrogenase (GDH), that was fabricated by a new quick-and-easy method using the pre-functionalized amine reactive phenazine ethosulfate (arPES). Thus mediator-modified glucose dehydrogenase (GDH) obtained the ability to transfer electrons to bulky electron acceptors as well as electrodes. The concentration of glucose was successfully measured using electrodes with immobilized PES-modified GDH, without addition of external electron mediators. Therefore, continuous monitoring systems can be developed based on this "2.5th generation" electron transfer principle utilizing quasi-DET. Furthermore, we successfully modified two other diagnostically relevant enzymes, glucose 3-dehydrogenase and lactate oxidase, with PES. Therefore, various kinds of diagnostic enzymes can achieve quasi-DET ability simply by modification with arPES, suggesting that continuous monitoring systems based on the 2.5th generation principle can be developed for various target molecules.

Keywords

2.5th generation biosensor, electrochemical enzyme sensor, mediator modification of enzymes, glucose dehydrogenase, glucose 3-dehydrogenase, lactate oxidase

1. Introduction

Enzyme based electrochemical biosensors are often used in medical care, a representative being the self-monitoring of blood glucose [1]. The principles of electrochemical enzyme sensors are divided into three generations according to their electron transfer processes. The 1st generation principle uses oxygen as the electron acceptor of the enzyme reaction and the resulting hydrogen peroxide is measured electrochemically [2]. The 2nd generation principle uses artificial electron acceptors (mediators) for the enzyme reaction, and reduced electron acceptors are measured by the electrode [3,4]. This principle is the most popular method in current commercially available sensors, such as sensors for the self-monitoring of blood glucose used for glycemic control of diabetic patients. The 3rd generation principle employs enzymes with the ability to transfer electrons directly to the electrode, without the need for mediators nor oxygen; these enzymes are called direct electron transfer (DET) type enzymes [5]. However, although the 3rd generation principle is ideal, the types of enzymes which have the DET ability are limited. Therefore, it is difficult to develop 3rd generation electrochemical enzyme sensors for various target molecules using naturally DET type enzymes.

In the 1980s, some groups started to attach redox mediators to enzymes in order to improve the electron transfer between the redox center and the electrode. In some reports, ferrocene derivatives were attached covalently via side chains of amino acids to the enzyme after the enzyme was unfolded by urea to expose amino acid residues before modification [6-10]. Other reports describe the covalent

attachment to the enzyme via side chains of amino acids or sugar chains, without the treatment of the enzyme with urea before modification [8,11-15]. Again, others attached ferrocene derivatives to the cofactor of the enzyme [16]. In some reports, ruthenium complexes were attached to the side chains of amino acids of the enzyme [8,17]. However, all of the described methods for the modification of enzymes with mediators included relatively complex procedures [6-17], such as unfolding the enzyme with urea, which leads to a reduced activity of the enzyme; functionalization of the mediators with functional groups to bind it to the side chains of amino acids or sugar chains of the enzyme; or activation of the protein by oxidizing sugar chains of the enzyme to form Schiff bases with amino-functionalized mediators. Furthermore, these reports each focused on a specific enzyme to be functionalized with the mediator. Due to the complexity of the modification procedures, no general mediator modifying method was developed which can be applied to several types of redox enzymes, and the idea of modifying enzymes with mediators was more or less discarded.

With this paper, we are reviving the concept of mediator modification, with the aim of widening the development of ideal 3rd generation type biosensors to various target molecules for which no enzyme with natural DET ability can be found. When an enzyme is modified with mediator, electrons are transferred first from the substrate to the cofactor, then from the reduced cofactor to mediator molecules bound to the enzyme surface, and finally from the reduced mediators to the electrode. This electron transfer type via mediator molecules bound to the

enzyme surface can be described as quasi-direct electron transfer (quasi-DET). Therefore, enzymes can obtain quasi-DET ability by modification with a mediator (Schematic 1). In this paper, we assign biosensors based on the quasi-DET principle as "2.5th generation" based on the definitions for the 2nd (via mediator) and 3rd (DET) generations.

In this study, we used a new artificial electron mediator, N-Hydroxysuccinimidyl ester 1-propoxy-5-ethylphenazinium ethyl sulfate, or amine reactive phenazine ethosulfate (arPES). The structure of arPES is shown in Figure 1. This salt is composed of two parts: 1) PES with phenazine as the mediator and ethosulfate as counterion, and 2) a succinimide group for enzyme modification. PES is a good mediator because of its low redox potential (Em = -0.32 V vs Ag/AgCl), which allows electrochemical detection at low operating potential avoiding the effect of several interferences. Succinimide groups generally react with amine groups of proteins by amine coupling, and therefore, by simply mixing arPES with enzymes harboring lysine residues, PES-modified enzymes can be prepared. This should lead to simple modification of various enzymes with mediator, and consequently the development of continuous monitoring systems without free mediator for various target molecules.

In here, we modified glucose dehydrogenase (GDH) using arPES and developed a 2.5th generation enzyme sensor using PES-modified GDH. Furthermore, lactate oxidase (LOx) and glucoside 3-dehydrogenase (G3DH) were also modified to show the possibility of a general application of this method.

2. Materials and Methods

2.1 Materials

Recombinant flavin adenine dinucleotide- (FAD-) dependent GDH from *Botryotinia fuckeliana* (molecular weight 61.8 kDa, Vmax = 323 U/mg, Km = 20.8 mM for glucose) was kindly supplied by Ultizyme International Ltd. (Tokyo, Japan). G3DH from *Rhizobium radiobacter* (molecular weight 86 kDa, Vmax = 13 U/mg, Km = 3.2 mM for methyl α-D-glucoside) was prepared recombinantly referring Pothukuchy *et al.* [18]. LOx from *Aerococcus viridans* (molecular weight 44 kDa, Vmax = 130 U/mg, Km = 0.72 mM for L-lactate) was prepared recombinantly according to Hiraka *et al.* [19]. arPES was provided by Dojindo Laboratories (Kumamoto, Japan). Phenazine methosulfate (PMS), D-glucose and methyl α-D-glucoside were purchased from Kanto Chemical Co. (Tokyo, Japan). 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) was purchased from Dojindo Laboratories (Kumamoto, Japan). L-lactate was purchased from Sigma-Aldrich (St. Louis, USA). Ultrafiltration filter (Amicon Ultra-0.5 Centrifugal Filter Unit) was purchased from EMD Millipore (Darmstadt, Germany). Ketjen black, ECP600JD, was purchased from Mitsubishi Chemical Corporation (Tokyo, Japan). 25% (w/v) glutaraldehyde solution was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Screen-printed carbon electrodes (SPCE, DEP-EP-P, WE: Carbon, 2.64 mm^2, RE: Ag/AgCl, CE: Carbon) were from Bio Device Technology Ltd. (Ishikawa, Japan). Electrochemical measurements were performed

with PG580RM Potentiostat-Galvanostat (Uniscan Instruments Ltd., Buxton, UK).

2.2 Modification of the enzymes with PES

Mixtures of the enzyme and arPES in 20 mM TAPS buffer (pH8.3) were incubated at 25°C in a thermomixer while shaking (1,200 rpm) for 2 hours. The conditions of the mix ratio of enzyme and arPES for each enzyme were 13.3 μM of enzyme and 2 mM arPES for GDH, 5.3 μM enzyme and 4 mM arPES for G3DH, and 6 μM enzyme and 0.4 mM arPES for LOx. After the incubation, the mixtures were ultrafiltrated 10 times at 4°C at 14,000 *g* for 5 minutes to remove excess arPES. Before each ultrafiltration, 20 mM potassium phosphate buffer (P.P.B.) pH7.0 was added to exchange the buffer.

2.3 Enzyme activity measurement before and after modification

The activities of both unmodified and modified enzymes were measured colorimetrically with two systems, PMS/MTT system and MTT system. For the PMS/MTT system, the enzyme samples were incubated in the presence of 0.6 mM PMS, 1 mM MTT and substrates in 20 mM P.P.B. (pH7.0) at room temperature. The activity was determined by measuring the increasing of absorbance at 570 nm to monitor the formation of formazan dye by the reduction of MTT. For the MTT system, only MTT was used as the electron acceptor. The enzyme samples were incubated in the presence of 1 mM MTT and substrates in 20 mM P.P.B. (pH7.0) at room temperature, and absorbance at 570 nm was monitored. The substrates for

GDH, D3DH and LOx were 100 mM glucose, 40 mM methyl α-D-glucoside and 20 mM L-lactate respectively.

2.4 Preparation of enzyme electrodes with immobilized with GDH

The enzyme electrode with PES-modified GDH (PES-modified Enzyme Electrode) was prepared as follows. Enzyme ink containing 0.91 mg/mL PES-modified GDH, 0.5 % trehalose, 0.8 % Triton X, and 0.4 % ketjenblack in 20 mM P.P.B. (pH7.0) was prepared. 0.2 μL of the enzyme ink was dropped on the working electrode of SPCE, then dried at 25°C for more than 2 hours. After drying, the electrodes were incubated under glutaraldehyde vapor at 25°C for 1 hour. Then the electrodes were stored at 25°C until the measurement. The enzyme electrode with unmodified GDH (Enzyme Electrode) was prepared correspondingly by using the same concentration of unmodified GDH instead of PES-modified GDH.

2.5 Electrochemical measurements

The electrodes were immersed in 100 mM P.P.B. (pH7.0) for 30 minutes before measurement. Cyclic voltammetry measurements were performed in 2 mL of 100 mM P.P.B (pH7.0) in the presence or absence of 33 mM of glucose. The scan rate was 50 mV/sec.

Chronoamperometry measurements were also performed. The electrodes were immersed in 2 mL of 100 mM P.P.B. (pH7.0) and a potential of 0 V vs. Ag/AgCl was applied. Small amount of various concentrations of glucose were added while

stirring so that the final concentrations of glucose in the cell became 0, 0.28, 0.56, 1.1, 2.8, 5.6, 17 and 33 mM. The resulting currents were measured.

3. Results

3.1 Enzyme activity measurements of GDH before and after modification

The activity values of GDH before and after modification with PES measured with PMS/MTT or MTT system are shown in Table 1. The activity values obtained with the PMS/MTT system were almost the same before (10 ± 1.1 U/mg) and after (11 ± 3.3 U/mg) modification. In contrast to this, the activity values obtained with the MTT system showed different results. Since GDH cannot use MTT as the primary electron acceptor, before PES modification, intact GDH did not show activity. After PES modification, however, a definite activity was observed with the MTT system (2.9 U/mg), which was about 30% of the activity of PMS/MTT system.

3.2 Electrochemical measurements

3.2.1 Cyclic voltammetry measurements

The results from CV measurements are shown in Figure 2. At first, CV measurements were carried out in the buffer solution. Line a in figure 2 is the CV for the Enzyme Electrode and line c is for the PES-modified Enzyme Electrode. Redox peaks were observed only with the PES-modified Enzyme Electrode. This redox peak can be attributed to PES bound to the enzyme because the peak

potentials are almost the same as those in the cyclic voltammogram of PES, shown in the inset. Then, CV measurements were carried out in the presence of 33 mM of glucose. Line b in figure 2 is the CV for the Enzyme Electrode and line d is for the PES-modified Enzyme Electrode. No redox peaks were observed with the Enzyme Electrode regardless of the presence of glucose. In contrast, the oxidation peak current from PES was increased with PES-modified Enzyme Electrode in the presence of glucose compared to that in the absence of glucose (line c).

3.2.2 Chronoamperometry measurements

In the chronoamperometry measurements, the currents obtained by applying a potential of 0 V vs. Ag/AgCl were measured because this potential was sufficiently able to re-oxidize the PES as shown in the CV measurements (Figure 2, line c). The response curves of the chronoamperometry measurements using the Enzyme Electrode or the PES-modified Enzyme Electrode are shown in Figure 3a. Each arrow shows a glucose addition point and the concentration values show the final concentrations at that point. Contrary to the Enzyme Electrode which did not show any signal, PES-modified Enzyme Electrode showed a current increase with each addition of glucose. Calibration curves plotting the resulting currents against the glucose concentrations are shown in Figure 3b. An increase of the current depending on the glucose concentration over 0 – 33 mM was observed with PES-modified Enzyme Electrode. The currents increased sharply in the concentration range of 0 – 5 mM and gently at concentration higher than 5 mM.

3.3 Modification of G3DH and LOx with PES and enzyme activity measurements before and after modification

The activity values of G3DH and LOx before and after modification with PES measured with PMS/MTT or MTT system are shown in Table 1. In case of G3DH, the activity values obtained with the PMS/MTT system were almost the same before (1.8 ± 0.090 U/mg) and after (2.2 ± 0.011 U/mg) modification while the activity values obtained with the MTT system increased after PES modification (0.66 ± 0.014 U/mg), and reached 30 % of the activity obtained with the PMS/MTT system compared to before modification. Regarding LOx, the activity values obtained with the PMS/MTT system were almost the same before (16 ± 0.30 U/mg) and after (18 ± 0.48 U/mg) modification as well. Compared to before modification, the activity values obtained with the MTT system drastically increased after PES modification (4.4 ± 0.15 U/mg), and reached 24 % of the activity of PMS/MTT system.

4. Discussion

Electrochemical enzyme sensors have been widely studied for application for medical care. The most common principle among commercialized sensors is that of the 2nd generation, although recently, the number of studies on the development of 3rd generation biosensors has increased sharply [20]. However, the number of enzymes which have DET ability is limited, therefore the number of target

molecules for which 3rd generation biosensors can be developed is also limited. In this paper, we revived the concept of mediator modification of enzymes, which was first investigated in the 1980s. Due to the quasi-DET behavior of the modified enzymes, we assign the electron transfer through mediators covalently bound to the enzyme as the "2.5th generation" principle in this paper. We used arPES for the modification, which is functionalized with a succinimide group and thus spontaneously binds to amine groups of the enzyme. Therefore, a quasi-DET ability can be introduced to enzymes simply by mixing with arPES. To ensure a sufficient number of PES bound to the amine groups of the enzymes, we used a high molar ratio of arPES to enzyme.

As a first example of a 2.5th generation biosensor fabricated by this quick and easy method, we developed a glucose sensor using FAD-dependent GDH. This enzyme catalyzes the oxidation of the C-1 hydroxyl group of pyranoses, and is a representative enzyme for blood glucose monitoring.

One indicator for DET ability of an enzyme is the ability to directly reduce MTT to formazan dye. Enzymes without DET ability usually cannot utilize the bulky MTT as primary electron acceptor and thus do not show activity with the MTT system. In the PMS/MTT system, the small PMS is used as primary electron acceptor and as electron shuttle to MTT. Free PMS can easily access the cofactor of the enzyme and reduced PMS close to the cofactor is rapidly exchanged with another, oxidized, PMS, so that the oxidative half-reaction can restart. Therefore, the electron transfer rate of oxidative half-reaction, in presence of free PMS is high.

The reaction rate of the entire enzyme reaction, therefore, is limited by the reductive half-reaction and thus the substrate concentration. In this case, the activity value follows a Michaelis-Menten-type saturation curve. The activity values in Table 1 were measured with 100 mM glucose, which is sufficiently high to be in the saturated part of the curve. With PES-modified GDH, similar results as with unmodified GDH were obtained, indicating the modification did not affect the activity of the GDH.

When the GDH is modified with PES, electrons can be transferred through the PES to MTT, and thus PES-modified GDH showed activity with the MTT system. In this case, the PES is located at a fixed distance to the cofactor of the enzyme and reduced PES cannot be exchanged with oxidized PES, but has to be re-oxidized. The re-oxidation is generally slower than an exchange, significantly reducing the turn-over rate. Therefore, the reaction rate for the entire enzyme reaction becomes limited by the oxidative half-reaction when the substrate concentration increases. This leads to a saturation signal at lower substrate concentrations than what would be expected from the affinity of the enzyme to the substrate. Therefore, the activity value obtained with the MTT system was lower than that obtained with the PMS/MTT system for the PES-modified GDH.

To demonstrate the possibility to develop disposable sensor strips for medical use based on such PES-modified enzyme, we constructed Enzyme Electrodes (with unmodified GDH) and PES-modified Enzyme Electrodes (with PES-modified GDH) using three-electrode-SPCE-chips. The CV of PES-modified

Enzyme Electrode showed redox peak that can be attributed to PES, indicating that the PES bound to the enzyme was able to react with the electrode. In presence of glucose, the oxidation peak of PES was increased in case of PES-modified Enzyme Electrode. This indicates that electrons were successfully transferred from the cofactor to the electrode via PES bound to the enzyme, meaning that the enzyme obtained quasi-DET ability (Figure 2). The electron probably moves from the cofactor to the PES closest to the cofactor, and then directly to the electrode or through several PESs, like an electron relay, to the electrode. The exact distance of cofactor and side chain of lysine residue required for the transfer of the electron to the PES bound to the lysine residue is unknown. Determination of the specific lysine residue to which the PES is bound which receives the electron directly from the cofactor will be required to evaluate the distance between the cofactor and the important lysine residue for a completely general application of arPES modification.

An advantage of using PES over other common artificial electron acceptors such as ferricyanide, hexaammineruthenium(III), or ferrocene, is the lower redox potential of this mediator. By using PES, we can measure substrates using a potential as low as 0 mV vs. Ag/AgCl and thus avoid the effects of electrochemically active interferences. The apparent Km value for glucose calculated from the calibration curve obtained with PES-modified Enzyme Electrode (Figure 3b) was 2.3 mM, which was about one-tenth of the Km value for glucose of this GDH determined with an enzyme activity assay (21 mM). For the enzyme activity assay, free PMS in the solution is used as primary electron acceptor,

which can easily access the cofactor of the enzyme, and is supplied in excess. Thus, the electron transfer rate of oxidative half-reaction is very high. The reaction rate of the entire enzyme reaction, therefore, is limited by the reductive half-reaction and thus the substrate concentration, and the (apparent) Km is an accurate indicator of the affinity of the enzyme for glucose. In contrast, in the measurements with PES-modified Enzyme Electrode, the mediator is fixed on the surface of the enzyme and thus is located at a distance from the cofactor. Furthermore, the bound reduced PES has to be re-oxidized before the oxidative half-reaction can start again, because it cannot be exchanged with oxidized PES. Therefore, the reaction rate of the oxidative half-reaction for PES-modified Enzyme Electrode is limited by the re-oxidation rate of the PES accepting electrons directly from the cofactor of the enzyme. Since the PES modification scarcely affected the PMS/MTT dependent dye-mediated dehydrogenase activity, and did not affect the reductive half reaction, the large discrepancy of the Km values obtained from the enzyme with free electron acceptor or with enzyme-bound acceptor, would be because the rate limiting step was the oxidative half reaction.

For this method to work, two criteria have to be met: 1) the enzyme has to have an accessible lysine residue close enough to the cofactor for electron transfer to be possible from the cofactor to the PES bound to that lysine residue; 2) the redox potential of the mediator has to be sufficiently high to receive electrons from the cofactor. Further accessible lysine residues might be necessary to attach PES at strategic points for an electron relay to the enzyme surface to enable electron

transfer to the electrode. The PES used here has a redox potential of -320 mV vs Ag/AgCl. Nevertheless, PES has been reported to accept electrons from NAD(P)H and PQQ [21,22], and does accept electrons from FAD in this study. Regarding the lysine residue criterium, the GDH used in this study does seem to have lysine residues at all the necessary positions and thus obtained quasi-DET ability by modification with arPES.

To investigate this possibility of a general applicability, we modified two other enzymes relevant for clinical diagnostics, G3DH and LOx. G3DH contains FAD as the cofactor and catalyzes the oxidation of the C-3 hydroxyl group of pyranoses. G3DH can be used for sensing of 1,5-Anhydroglucitol (1,5-AG), which is a short term glycemic control marker [23]. LOx contains flavin mononucleotide (FMN) as the cofactor and catalyzes the oxidation of L-lactate to pyruvate, and is commonly used for the lactate sensing [24,25]. Lactate is a useful marker for the monitoring of diseases related to metabolism problems [26,27], and the monitoring of exercise in sports medicine [28,29]. The change in activity values of these enzymes before and after PES modification showed the same trend as for GDH. This indicates the possibility to impart quasi-DET ability for different diagnostic enzymes and thus a general applicability of this mediator modification method. Therefore, with this principle, it should be possible to develop continuous monitoring systems for various target molecules without free mediator, which can be operated at a low potential.

However, for the actual construction of enzyme electrodes, factors other

than a possible quasi-DET ability have to be considered. Most importantly, enzyme with sufficient activity has to be immobilized on the electrode surface to achieve a measurable signal. However, although the modification sites of PES on the surface of G3DH might be sufficient to obtain the ability to transfer electrons to the bulky MTT, the specific activity was very low and thus is a poor representative to be subjected to electrochemical evaluation. Therefore, we prepared electrodes with immobilized PES-modified LOx. However, although LOx has modification sites of PES at enough positions to obtain the ability to transfer electrons to the bulky MTT (Table 1), PES-modified LOx electrodes did not show any response currents due to the addition of lactate to the reaction solution (data not shown). It seems that the modified positions might not be accessible for the electrode surface to achieve quasi-DET. Furthermore, since the cross-linking by glutaraldehyde also requires accessible lysine residues, it might be that this immobilization method is not appropiate for PES-modified LOx. Further investigation of the appropriate immobilization method for PES-modified LOx is necessary to construct 2.5th generation biosensors based on this enzyme.

5. Conclusion

In this study, we report of a "2.5th generation" glucose sensor that was constructed using a new quick-and-easy method for the modification of enzymes with mediators. The use of a pre-functionalized mediator such as arPES eliminates complicated chemical procedures, which greatly simplifies modification of enzymes

with mediators and thus the development of 2.5th generation biosensors. In this study, GDH was successfully modified with PES simply by mixing the enzyme with arPES. The modification was confirmed by an altered electron acceptor preference toward the bulky MTT. Furthermore, for PES-modified GDH, quasi-DET ability was also proved by electrochemical evaluation, and the glucose concentration could be measured without addition of an external artificial electron mediator. Two other diagnostical enzymes, G3DH and LOx, were also successfully modified by using arPES and the modified enzymes gained the ability to transfer electrons to the bulky MTT. These results indicate the possibility of the development of continuous monitoring systems for various target molecules using this 2.5th generation principle.

There are several possible approaches to realize the development of such continuous monitoring systems using this quick-and-easy method of modifying enzymes with pre-functionalized mediators. One possible approach is the use of other pre-functionalized mediators, such as ferrocene functionalized with succinimide. However, considering that PES can accept electrons from FAD, FMN, NAD(P)H, and PQQ, as shown in this study and in [21,22], as well as the extremely low redox potential of PES (-320 mV vs Ag/AgCl), arPES might be the most desirable pre-functionalized mediator.

Another possible approach is to engineer enzymes to introduce lysine residues for modification with PES and thus optimize the electron transfer from the cofactor to the electrode via the bound PES. This engineering approach can also be

used for enzymes that do not naturally possess lysine residues at appropriate positions and thus are not suited for this method. For this approach, evaluation of the position of the lysine residues which have to have a bound PES in relation to the cofactor and the enzyme surface will be helpful. Such information should further improve the general applicability of the 2.5th generation principle for an even greater variety of redox enzymes.

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Figure captions

Schematic 1 Principles of electrochemical enzyme sensors according to their generation including 2.5th generation

1st and 2nd generation sensors use oxygen and free artificial electron acceptor as the electron acceptor of enzyme reaction, respectively. 3rd generation sensors use direct electron transfer type enzymes. In the 2.5th generation sensors, a mediator-modified enzyme is used, which has quasi-direct electron transfer ability.

Figure 1 Structure of amine reactive phenazine ethosulfate (arPES)

arPES was used for modification of the enzyme in this study. arPES is a salt composed of two parts: 1) PES, with phenazine as mediator and ethosulfate as counterion, and 2) succinimide group for enzyme modification.

Figure 2 Cyclic voltammograms

Cyclic voltammetry measurements were performed in 100 mM P.P.B. pH7.0 using Enzyme Electrodes in the absence (a) or the presence (b) of 33 mM glucose, or PES-modified Enzyme Electrodes in the absence (c) or the presence (d) of 33 mM glucose. The inset shows the cyclic voltammogram of 1 mM PES in 100 mM P.P.B. pH7.0.

Figure 3

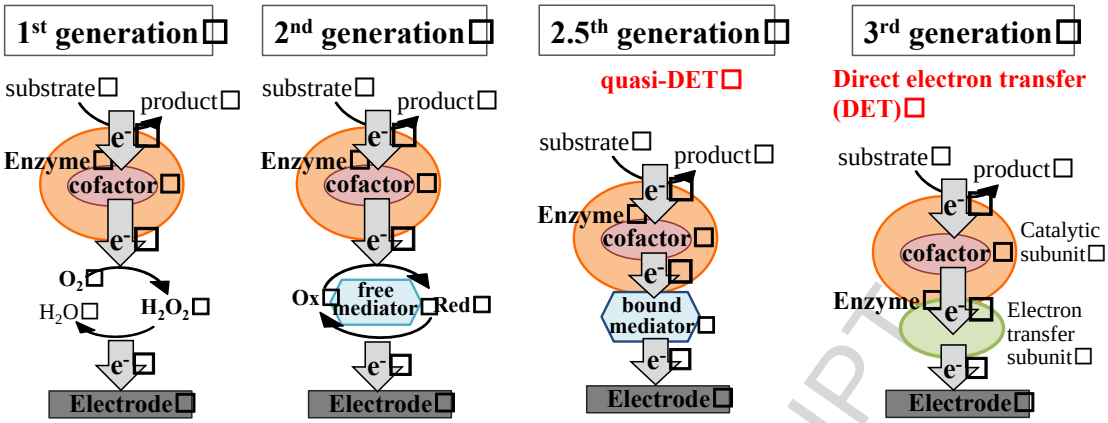
a) Response curve of chronoamperometry measurement of glucose using electrodes with immobilized GDH (light line) or PES-modified GDH (dark line) in 100 mM P.P.B. pH7.0. Arrows show the timing of addition of glucose and the concentrations are final concentrations after the addition. A potential of 0 V vs Ag/AgCl was applied.

b) Calibration curves of glucose measurements using electrodes with immobilized GDH (filled circles) or PES-modified GDH (open circles).

Table

	Activity value (U/mg)					
	GDH		G3DH		LOx	
	Unmodified	PES-modified	Unmodified	PES-modified	Unmodified	PES-modified
PMS/MTT system	10 ± 1.1	11 ± 3.3	1.8 ± 0.090	2.2 ± 0.011	16 ± 0.30	18 ± 0.48
MTT system	n.d.*	2.9 ± 0.84	0.017 ± 0.0069	0.66 ± 0.014	0.098 ± 0.0033	4.4 ± 0.15

Table 1. Enzymatic activity values of unmodified and PES-modified enzymes using PMS/MTT system and MTT system. (*n.d. : not detected)



Schematic 1

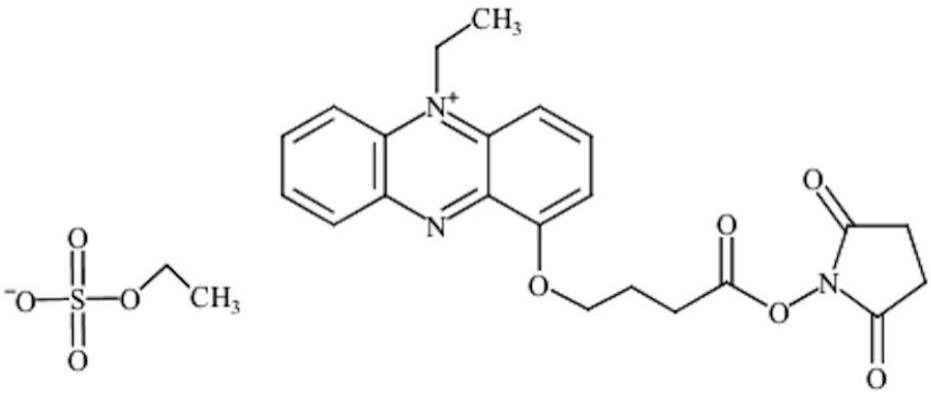


Figure 1

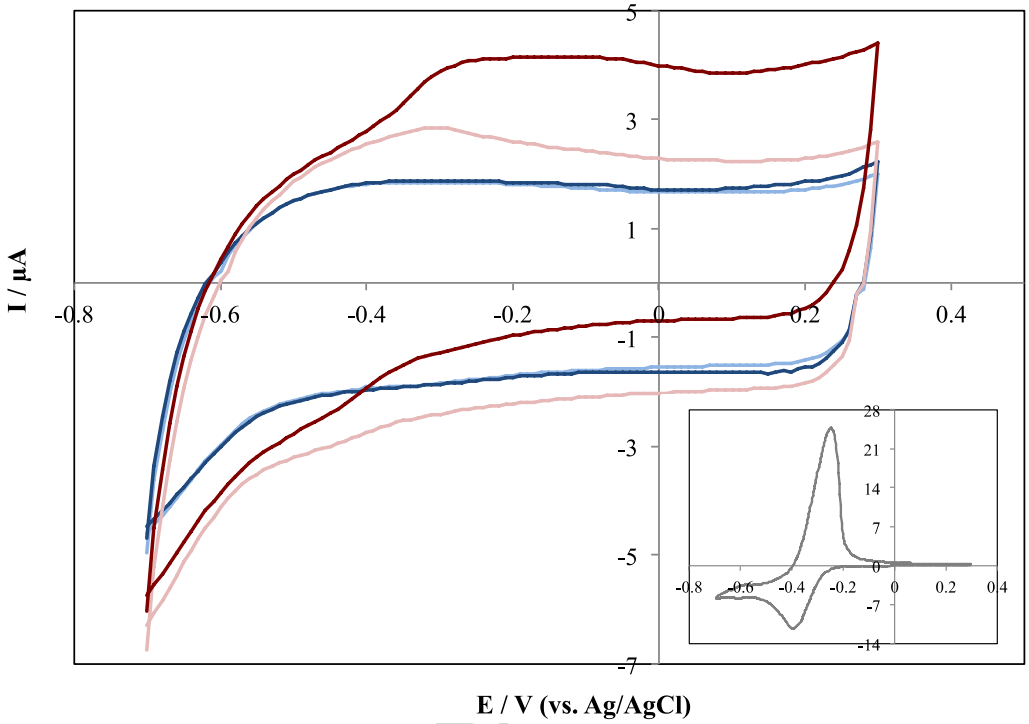


Figure 2

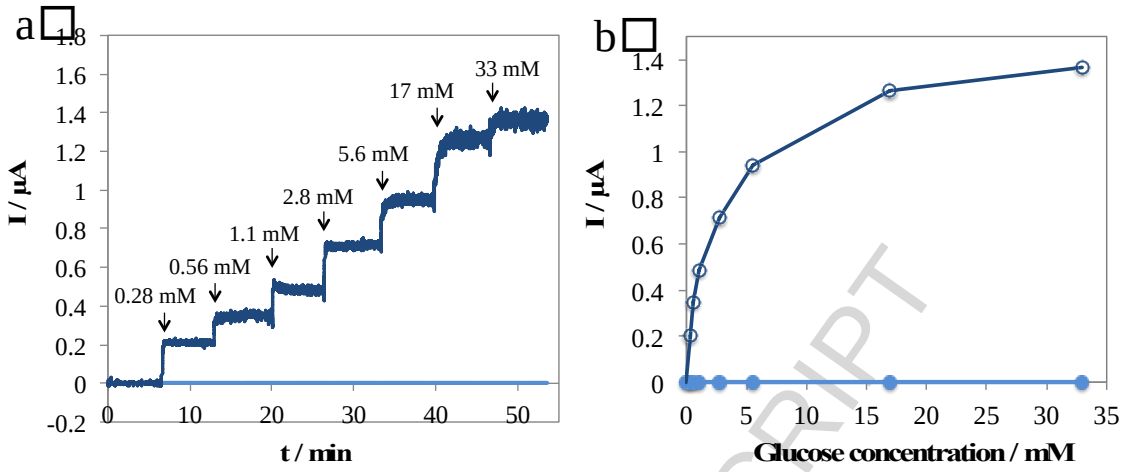


Figure 3

Highlights

- Glucose sensor employing quasi-direct electron transfer ability.
- Quick and easy modification method with mediator for enzymes.
- Pre-functionalized amine reactive phenazine ethosulfate was used as the mediator.
- Glucose concentrations could be measured with 2.5th generation sensor.
- Three types of redox enzymes were successfully modified with the mediators.