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Mediated Glucose Biosensor Based on Polyvinylferrocene

AN-LAC NGUYEN AND JOHN H. T. LUONG*

Biotechnology Research Institute, National Research Council Canada, Montreal, Quebec, Canada H4P 2R2

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ABSTRACT

Polyvinylferrocene (PVF) was electrochemically deposited on platinum and carbon electrodes to form a stable and resilient film. During cyclic voltammetry in phosphate buffer, the PVF film deposited on carbon electrodes exhibited anodic and cathodic peaks at 214 and 68 mV, respectively. Both types of electrodes, bearing electrodeposited PVF and crosslinked glucose oxidase, were responsive to glucose, but the carbon electrode appeared to provide a faster response and could determine glucose between 0.1 and 8 m*M*.

When protected by a layer of polymer electrochemically formed from resorcinol and phenylenediamine, the mediated biosensors based on PVF-deposited carbon electrodes were capable of determining glucose up to 25 m*M* with a response time of 1 min, for at least 50 repeated analyses with good reproducibility. The presence of ambient oxygen, ascorbic acid (0.1 m*M*), and uric acid (0.5 m*M*) did not affect their performance. When applied for the determination of the glucose level in reconstituted human serum, the results agreed well with those of the reference hexokinase assay.

Index Entries: Mediated biosensor; polyvinylferrocene; electrodeposition; glucose oxidase; glucose; serum.

*Author to whom all correspondence and reprint requests should be addressed.

INTRODUCTION

Water-insoluble ferrocene [*bis*-(η^5-cyclopentadienyl) iron] and its derivatives appear to be excellent mediators for various FAD-containing oxidases (*1*). Ferrocene-mediated electrodes are generally constructed by applying ferrocene or its derivatives to the hydrophobic surface of a platinum or carbon working electrode. To function as mediator, ferrocene must be first oxidized at the electrode surface to ferricinium in order to react with the reduced oxidase. Since the ferricinium ion is water-soluble, this usually results in the gradual loss of mediator from the electrode surface. Therefore, ferrocene or its derivatives must be anchored to the enzyme (*2*) or some polymer (*3,4*) if reusability is desired, but this approach may lead to diminished mobility and accessibility of the mediating moieties. Attempts to anchor a mediator to a polymer backbone were not satisfactory, since the redox polymer lost its electrocatalytic activity after a relatively short time.

Many polymers containing the ferrocene group have been studied in recent years with respect to their electrical and magnetic properties, and redox or electron-exchange properties (*5*). Vinylferrocene, a relatively efficient mediator for oxidases (*1*), has been reported to undergo free-radical polymerization (*6*) to form polyvinylferrocene (PVF) with a number-average mol wt of 48,600, and PVF is now commercially available. To date, the bioelectrocatalytic characteristic of PVF has not been studied, although there are some reports on the behavior of this polymer after being electrochemically deposited onto a quartz crystal (*7,8*).

The main purpose of this study was to deposit PVF electrochemically onto a platinum or carbon electrode to form a mediated biosensor for glucose. The characterization of such a PVF film with an emphasis on its redox property and applicability as mediator for glucose oxidase is also presented and discussed.

MATERIALS AND METHODS

Polyvinylferrocene (PVF, 50,000 approx mol wt) was purchased from Polysciences (Warrington, PA). Tetrabutylammonium tetrafluoroborate (TBATFB), phenylenediamine (PDA), resorcinol, 2-hydroxypropyl-β-cyclodextrin, and other chemicals were products of Aldrich (Milwaukee, WI). Glucose oxidase type X-S from *Aspergillus niger* (EC 1.1.3.4), hexokinase type VI, glucose-6-phosphate dehydrogenase type IX, β-nicotinamide adenine dinucleotide phosphate (NADP), adenosine 5′-triphosphate (ATP), and lyophilized serum were supplied by Sigma (St. Louis, MO). Carbon rods (99.9995% purity, 3.05 mm diameter, 38.10 mm long) were purchased from AESAR (Brampton, Ontario, Canada).

Equipment

A three-electrode arrangement consisting of a working electrode, an Ag/AgCl reference, and a platinum counter electrode was used in the treatment of carbon rods, electrodeposition of PVF, verification of the electrodeposition, and electroformation of resorcinol-PDA film as well as all studies of electrode performance. The voltammograph and the RXY recorder were products of Bioanalytical Systems (BAS, West Lafayette, IN). Platinum electrodes and glassy carbon electrodes were also purchased from BAS. All voltammetric procedures were conducted with a holding cell of 800 μL capacity equipped with magnetic stirring.

Coating and Treatment of Carbon Electrodes

A commercial product (Plasti Dip, PDI Inc., Circle Pines, MN) of plasticized polyvinyl chloride (PVC) solution was used. The carbon rods were dipped once in the PVC solution, to cover 30 mm of the length, and air-dried. A sharp blade was used to incise around the circumference, 2 mm from the coated end, to allow removal of the PVC coat from the tip. The tip was polished to be flush with the PVC coating, resulting in a circular carbon surface of 3.05 mm diameter. Each coated and polished carbon rod was treated by installing it as the working electrode in a three-electrode arrangement with 0.1*M* NaOH as electrolyte, and 1.2 V potential was applied for 5 min. The treated electrode, herein referred to as PVC-coated electrodes, were rinsed with distilled water and dried before electrodeposition of PVF.

Electrochemical Deposition of Polyvinylferrocene

In a fume hood, a solution (800 μL) of PVF (30 mg/mL) and TBATFB (33 mg/mL) in dichloromethane was placed in the holding cell. The working electrode was platinum (BAS), glassy carbon (BAS), or a PVC-coated electrode. A potential of 800 mV was applied for 2–20 min then the electrode was air-dried and dipped in buffer until required.

Enzyme Deposition

After glucose oxidase (5 or 10 μL, from 5 to 100 mg/mL) was deposited at the tip, the electrode was held tip-down above a solution of glutaraldehyde (25%) for 30 min. Drying was completed in air at room temperature.

Electrochemical Deposition of Resorcinol-Phenylenediamine

The procedure was performed in a glove box that was deaerated and continually flushed with nitrogen. Equal volumes of resorcinol and phenylenediamine (PAB) solutions, separately prepared (3 m*M*) in phosphate buffer, pH 6.5, were placed in the cell without stirring, and the voltage (200 mV) was applied to the working electrode for 1–30 min.

Determination of Glucose Concentration

In most cases, the baseline was noted after placing 200–400 μL of buffer (20 m*M* phosphate, pH 5.8) in the holding cell. Then sufficient solution was added so that glucose in the cell was close to 10 m*M* and the current increase (ΔI) was recorded after 1 min. The solution concentration was calculated by $\Delta I \times D/S$ where D is the appropriate dilution factor and S is the corresponding sensitivity. For solutions of 10 m*M* or less, 200 μL were added to 200 μL of buffer in the cell, and the concentration was calculated similarly.

RESULTS

Verification of the PVF Electrodeposition Process

The PVF-deposited electrode was installed in a three-electrode arrangement with 20 m*M* phosphate buffer in the holding cell, and cyclic voltammograms were performed between −200 and +700 mV, at 10 mV/s scan rate. The first cycle of each electrode exhibited an irregular voltammogram. Regular voltammograms displayed during the second cycle displayed an anodic peak at 214 mV and a cathodic peak at 68 mV with a half-wave potential at about 141 mV (Fig. 1a). PVF in dichloromethane (DCM) solution was reported to exhibit anodic and cathodic peaks at 300 and 130 mV, respectively. However, a platinum electrode electrodeposited with PVF from DCM solution was reported to display anodic and cathodic peaks at 365 and 345 mV, respectively, when immersed in acetonitrile containing 0.1*M* tetrabutylammonium perchlorate (*9*). The quartz crystal microbalance was later applied (*10,11*) to reveal that the electrolyte compositions exerted a great influence on the voltammetric behavior of PVF films.

In this study, the spectrophotometric spectra (between 350 and 700 nm) of vinylferrocene and PVF in dichloromethane solutions showed a single absorbence maximum at 440 nm, and the absorbencies at this wavelength were used to ascertain that the PVF was a homopolymer of vinylferrocene. It was experimentally determined that a solution of vinylferro-

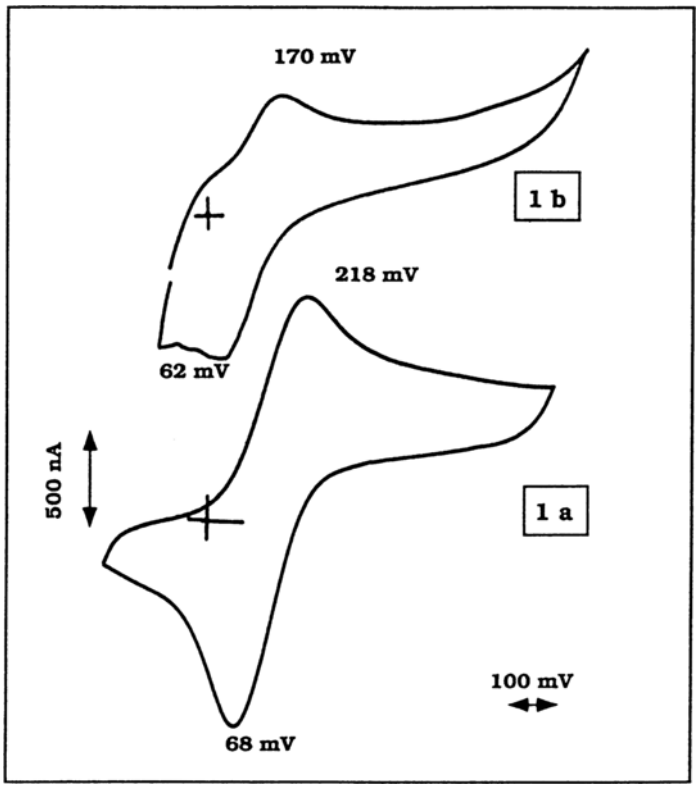

Fig. 1. Cyclic voltammograms at 10 mV/s scan rate, Ag/AgCl reference. (1a) Carbon electrode deposited with PVF, second cycle in phosphate buffer. (1b) Carbon electrode installed in dichloromethane solution of PVF and tetrabutylammonium tetrafluoroborate (0.1 mg/mL each).

cene in phosphate buffer, with 2-hydroxypropyl-β-cyclodextrin added as an inclusion agent, exhibited anodic and cathodic peaks at 317 and 225 mV, respectively (data not shown). It is worth noting that α, β, and γ-cyclodextrin with six, seven, and eight glucose units, respectively, can form inclusion complexes with various water-insoluble organic chemicals by incorporating them into the hydrophobic cavities of their molecules (*10*). In addition, a solution of PVF in dichloromethane containing tetrabutylammonium tetrafluoroborate displayed the peaks at 170 and 62 mV (Fig. 1b). It was thus further indicated that the electrolyte components and the redox moiety interacted closely to change the voltammetric response. Compared with PVF in solution, apparently the electrodeposited PVF film was restricted in mobility and required a higher anodic potential (218 vs 170 mV) for oxidation, but the cathodic peaks were quite close (68 vs 62 mV) for the two situations. It was likely that there was some swelling when the ferrocene moieties were oxidized to become more hydrophilic,

permitting more mobility to the PVF film in the reducing stretch of cyclic voltammetry.

For all of the 30 electrodes prepared, voltammograms became unchanged after 10 cycles, although some electrodes attained unchanged voltammograms after four cycles. The potentials corresponding to the cathodic and the anodic peaks were reproducible within 2 mV, and the peak heights were reproducible within 50 nA.

Stability of the PVF Film

The cyclic voltammogram of the electrodes kept in buffer or in air at room temperature was not affected after up to 30 d. However, when a cycle was allowed to reach 900 mV (note that PVF deposition was performed at 800 mV), the peak heights of the subsequent cycle were significantly reduced. The same effect was observed after an electrode was maintained at 900 mV for 5 min. The PVF-bearing electrode could be maintained at 200 mV vs Ag/AgCl for 3–4 d without any noticeable effect on the voltammogram.

Selection of Electrode Material and Applied Potential

Glucose oxidase (10 μL, 10 mg/mL) was deposited at the tips of a platinum electrode, a glassy carbon electrode, and a PVC-coated electrode. Characteristics of each electrode were obtained at 200 mV applied potential (Table 1). Compared with the platinum electrode, the carbon electrode appeared to provide a faster response. It was clear that the PVC-coated electrode was very satisfactory, although the baseline attainment was somewhat retarded and the baseline variation was slightly greater than the characteristics exhibited by the better-quality glassy carbon electrode.

In general, the severity of interference is often reduced at a lower applied potential. Therefore, another set of electrodes was used to obtain the characteristics corresponding to 150 mV. In every case, the response was very sluggish, continuing to increase even after 5 min of potential application. In light of the foregoing observation, the PVC-coated electrodes were further studied at 200 mV applied potential only.

Influence of PVF Deposition Time

Electrodeposition was performed for a different duration on each of four PVC-coated electrodes. Then the same quantity of glucose oxidase was placed on each resulting electrode. As presented in Table 2, an electrodeposition time of 5 min provided the optimum condition for the response characteristics. Undoubtedly, the amount of deposited PVF was somewhat dependent on the electrodeposition time. A shorter time (2 min) decreased the sensitivity, likely because of an insufficient amount of PVF being deposited. On the contrary, a longer deposition time increased the

Table 1
Characteristics of GO-Bearing Electrodes[a]

	Applied potential					
	200 mV			150 mV		
Electrode	I	II	III	I	II	III
Baseline attainment (min)	5	3	8	8	6	6
Baseline current (nA)	128	153	186	62	65	73
Baseline variation (nA)	8	6	10	2	4	3
Response (nA)	480	510	440	260	240	230
Response time (s)	60	12	14	-	-	-

[a]PVF deposition time: 10 min.
Enzyme amount: 10 μL of 10 mg/mL.
Electrode material:
(I) Pt electrode from BAS.
(II) Carbon electrode from BAS.
(III) Carbon electrode coated with PVC.
Baseline attainment: Time to reach a constant current after voltage application.
Baseline current: Average values from four electrodes.
Baseline variation: Largest absolute difference from average baseline value.
Response: Current increase from baseline after adding glucose to attain 2 m*M*.
Response time: Time required to reach a current response that would not increase more than 5 nA in the following minute.

Table 2
Influence of PVF Deposition Time[a]

	Deposition time, min			
	2	5	10	20
Baseline attainment (min)	3	3	8	15
Baseline current (nA)	168	182	186	184
Baseline variation (nA)	5	5	10	12
Response (nA)	156	427	440	436
Response time (s)	12	10	14	12

[a]*See* notes in Table 1 for definition of terms. After electrodeposition of PVF, 10 μL of glucose oxidase (10 mg/mL) were placed on each electrode.

baseline variation and lengthened the baseline attainment, and this behavior is attributed to the diffusion resistance across a thick film resulting from such a prolonged deposition time.

Effect of Enzyme Quantity

All electrodes were subjected to PVF deposition for 5 min. Then 5 μL of glucose oxidase solution were applied, followed by crosslinking with glutaraldehyde vapor. The introduction of liquid glutaraldehyde into the

Table 3
Effect of Enzyme Quantity[a]

	Enzyme concentration, mg/mL				
	5	10	20	50	100
Baseline attainment (min)	3	3	3	2	4
Baseline current (nA)	178	182	180	173	167
Baseline variation (nA)	5	5	5	7	4
Response (nA)	385	427	418	426	409
Response time (s)	< 10	10	15	< 30	60

[a] *See* notes in Table 1 for definition of terms. PVF was electrodeposited on PVC- coated electrodes for 5 min. In all cases, 5 μL of enzyme solution were used.

enzyme solution resulted in sluggish and low responses. The response was low when the enzyme concentration was < 10 mg/mL, but excessive enzyme quantity prolonged the response time (Table 3). In all cases, the baseline attainment and the baseline current are nearly invariable. It appears that these characteristics are determined by the underlying PVF layer.

Responses at Different Glucose Concentrations

The data in Tables 2 and 3 indicated that 5 min of PVF deposition followed by 5 μL of 20 mg/mL glucose oxidase resulted in very satisfactory electrodes. These conditions were used in preparing four electrodes that were used to obtain the responses to different concentrations of glucose. For each electrode, 500 μL of phosphate buffer (10 m*M*, pH 5.8) were placed in the holding cell. Then 5 μL were replaced with the same volume of 100 m*M* glucose (i.e., an increment of 1 m*M*), and the response was recorded. Successive replacement of the same volume resulted in increasing glucose concentration, but each increment was slightly smaller than 1 m*M*. The record of one electrode response to incremental concentration changes is presented in Fig. 2. The current response as a function of the actual glucose concentration in the cell is presented in Fig. 3, which indicates replicate agreement and linearity for concentrations lower than 8 m*M*. For each of the four electrodes, after the last injection of the above experiment, the glucose solution in the holding cell was replaced with fresh buffer, and three additional exchanges of buffer were required before the baseline was regained. The concentration was increased in 0.1 m*M* steps by replacing 5 μL with the same volume of 10 m*M* glucose solution. As revealed in the inset in Fig. 3, a glucose concentration of 0.1 m*M* could be easily detected. All of the foregoing results were obtained with minimum stirring of the solution in the holding cell. No vigorous stirring could be applied when the glucose solution was replaced with fresh buffer, and therefore several exchanges were required to regain the baseline.

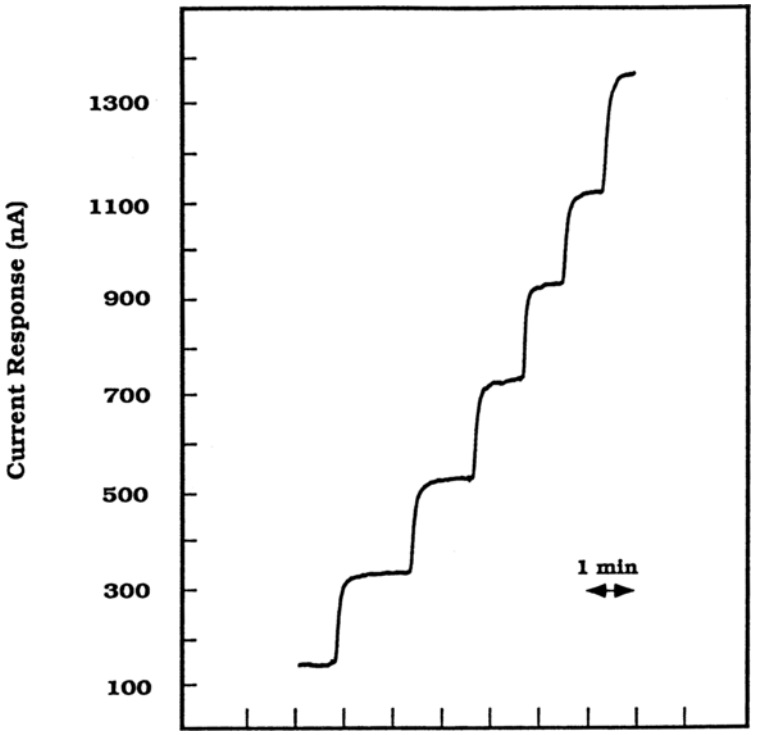

Fig. 2. Current responses to incremental changes in glucose concentration. Carbon electrode deposited with polyvinylferrocene and glucose oxidase.

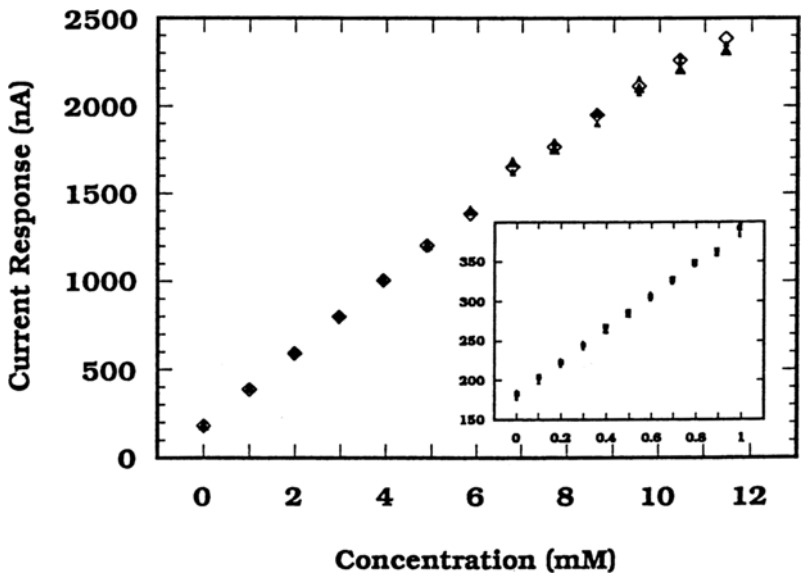

Fig. 3. Response to different glucose concentrations. Different symbols correspond to different electrodes.

Reproducibility of electrode performance was best achieved by allowing partial drying of the enzyme layer after each experiment and preserving the electrodes in a humid container. Storage by dipping electrode tips in buffer was not reliable. In one experiment, two electrodes were dipped in a buffer that was stirred fairly strongly. After such a treatment, the baseline was not affected, but the response to 2 m*M* glucose steadily decreased with increasing time in the stirred buffer. After 1 h of strong stirring, both electrodes did not respond to 2 m*M* glucose. However, after washing, such electrodes still exhibited the typical voltammogram of a freshly prepared PVF film. The PVF film apparently adhered strongly to the carbon, but the slightly crosslinked enzyme could not withstand strong stirring. An earlier experiment indicated that introduction of liquid glutaraldehyde into the enzyme solution resulted in sluggish and low response. This was confirmed in several trials. Thus, some method other than crosslinking must be developed to retain the enzyme at the tip of the electrode if reusability was to be assured.

Retention of the Enzyme Layer

For the BAS carbon electrodes, enzyme was retained by covering the tip with a dialysis membrane (20,000 MWCO), held in place with an O-ring. For PVC-covered carbon rods, enzyme retention was achieved by electroforming a film of resorcinol-phenylenediamine over the enzyme layer. The first attempt of resorcinol-PDA formation was conducted by applying +1V, as commonly used in previously reported studies (*11,12*), but the PVF film delaminated and destroyed the enzyme layer as well. A potential of 200 mV was therefore used in all subsequent electroformation procedures, although a longer time was necessary.

In all cases, covering the electrode did not affect the baseline attainment or the baseline current (Table 4). The response current decreased significantly, whereas the response time increased likely because of the diffusion barrier presented by the membrane or the film. This effect was very pronounced when a long time was used for electroformation. The resorcinol-PDA film resulting from 1 or 2 min of electroformation did not provide adequate enzyme retention as indicated by the loss of response after stirring and storage. The films formed in longer than 5 min offered very good enzyme retention as indicated by a stable response under prolonged stirring and storage. However, a short response time is always desirable for practical applications. Therefore an electroformation time of 5 or 10 min appeared to offer the best compromise. The membrane-covered electrode also displayed good stability, but only film-covered electrodes were further tested.

Response of Film-Covered Electrodes

A film-covered electrode was tested for responses to step changes in glucose concentration. As shown in Fig. 4, a longer time was required to

Table 4
Characteristics of Electrodes Covered with a Membrane or by an Electroformed Film[a]

		PVC-coated carbon Electroformation time, min					
	BAS	1	2	5	10	20	30
Baseline attainment (min)	4	3	4	5	5	5	5
Baseline current (nA)	156	178	186	182	184	179	183
Response (nA)	37	217	126	44	28	25	14
Response time (min)	3	< 0.5	< 1	< 1	1	10	> 30
Residual response (nA)							
After 30 min	38	147	113	43	29	26	13
After 1 h	36	42	86	45	27	24	13
After overnight storage	35	28	57	42	28	25	12
Without membrane or film							
Response time (nA)	510	418					
Response time (min)	0.2	0.25					

[a]PVF deposition time: 5 min.
Enzyme amount: 5 μL of glucose oxidase at 20 mg/mL.
Glucose concentration: 1 m*M*.
BAS electrode covered with a dialysis membrane. PVC-coated carbon electrodes covered by a film electroformed for a specified time.

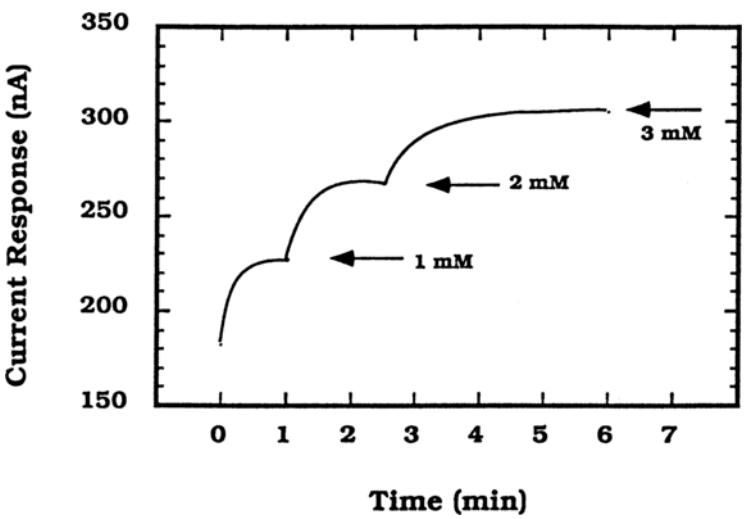

Fig. 4. Response of a film-covered electrode to incremental changes (0–3 mM) in glucose concentration. Electrode preparation: 5 min of PVF deposition, 5 μL of glucose oxidase (20 mg/mL), 5 min of resorcinol–PDA film formation.

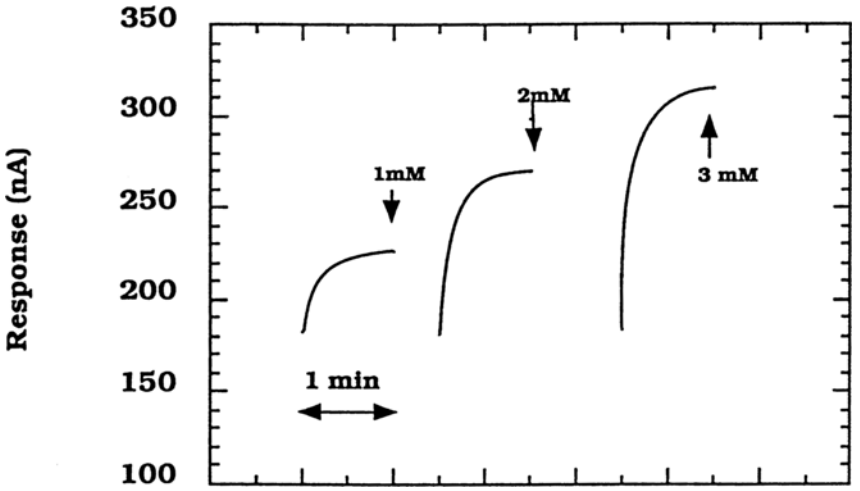

Fig. 5. Response of a film-covered electrode to different concentrations when glucose was added to fresh buffer. Electrode preparation same as in Fig. 5.

obtain a steady response to incremental changes in concentration. After baseline attainment, a steady response was achieved within 1 min of when glucose (1 m*M*) was first introduced, implying a sufficiently high enzyme activity so that any substrate molecule that reached the enzyme layer was quickly catalyzed and induced a rapid diffusion. However, the response time increased to 2 and 4 min, respectively, when the concentration was raised to 2 and then 3 m*M*. Such intriguing behavior is not understood, and requires further investigation to determine whether the mechanism is kinetic or diffusion controlled. The film-covered electrode was also tested for responses to different glucose concentrations, but the holding cell was replaced with fresh buffer after each response. Since the enzyme layer was protected by the film, the buffer solution could be sitrred, and the baseline was regained after 1 min. The response was always obtained in <1 min after glucose introduction (Fig. 5), with a variation of about 10 nA among replicates. As seen in Fig. 6, the linear range was extended to 25 m*M*, but the limit of detection was also increased to about 1 m*M*. This was another manifestation of the diffusion barrier presented by the resorcinol-PDA film, resulting in a concentration gradient across its thickness. Consequently, the glucose level at the enzyme layer was substantially less than the bulk concentration. The electrode response was not affected after being stored overnight in a humid container, and a different electrode was observed to yield essentially the same response. In another experiment, the complete system of voltammetry was installed in a glove box that was continually flushed with nitrogen; the observed responses were more reproducible between replicates (about 5 nA variation), whereas no significant difference from the ambient responses was observed.

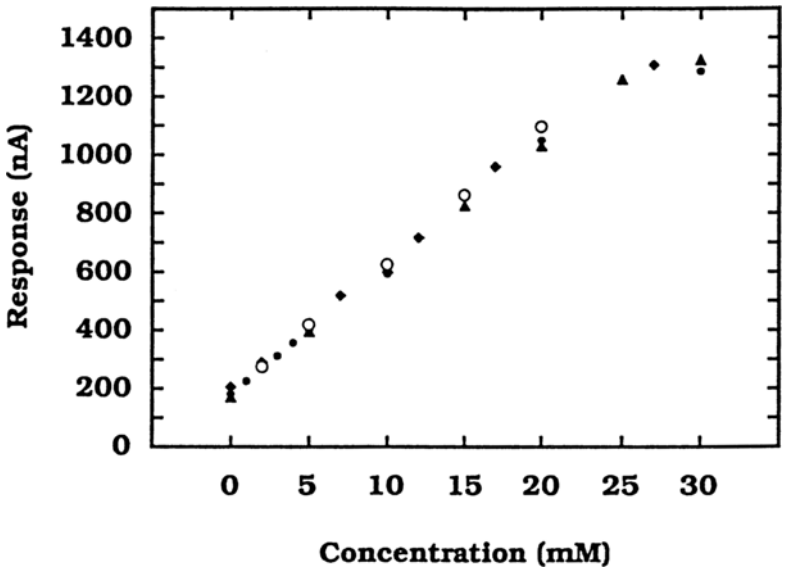

Fig. 6. Responses of film-covered electrodes to glucose. Electrode preparation same as Fig. 5. (■) New electrode, (▲) used electrode after overnight storage, (◆) another electrode.

In some experiments, performed in ambient condition as well as in the glove box, uric acid (0.5 m*M*) and/or ascorbic acid (0.1 m*M*) was added to fresh buffer or a glucose solution in the holding cell. In all cases, the baseline or the current response was not affected. It thus indicated that the resorcinol-PDA film, which in this study was formed at a lower potential, was also effective in screening out some potential interfering species, as have been reported for the film formed at higher potentials (*11*).

Determination of Glucose Concentration

The established procedure could produce electrodes with very good reproducibility. A typical set of four electrodes displayed an average baseline of 180 ± 7 nA and a response sensitivity of 46 ± 3 nA/mM. This set of electrodes was used randomly to determine the concentrations of a series of glucose solutions (2–80 m*M*; *see* Materials and Methods) that were prepared from the same stock of 1*M* glucose. Each solution was analyzed at least in triplicate, and all the average values differed <2% from the expected concentration in every case. However, there was a clear trend of greater deviation among the replicates when the more concentrated solutions were analyzed. For example, 2.01 ± 0.12 and 80.1 ± 2.4 were obtained for the 2- and 80-m*M* solutions, respectively. This could be owing to the inaccuracy in the dilution procedure.

Determination of Glucose in Serum

Lyophilized serum was dissolved in 5 mL water, and the standard hexokinase assay (*13*) was performed in triplicate to yield a glucose content of 4.89 ±0.03 m*M*. The procedure for solutions of < 10 m*M* (*see* Materials and Methods) was then employed to determine the glucose content of reconstituted serum and a fivefold dilution. With four replicates, the results were 5.06 ± 0.19 and 1.00 ± 0.04 m*M* for the undiluted and the fivefold diluted sample, respectively. Apparently the diluted sample yielded a concentration value closer to the spectrophotometric result, but any accuracy gained in diluting the serum was lost in the multiplication process, and the difference between the electrode-determined value and the spectrophotometric value was about 3% in both cases. In many repetitions, the electrode measurements always yielded higher results than the spectrophotometric assays. Such a result was expected, since the serum normally contains many species that are electroactive even at the low applied potentials, although uric acid and ascorbic acid were observed not to interfere (*see above*).

Response of the electrode to glucose-augmented serum was tested by placing 100 μL buffer (100 m*M*, pH 5.8) in the holding cell followed by 100 μL serum, 10–100 μL glucose (10 m*M*), and water to make up 500 μL. The glucose concentration in the holding cell was assessed from the current increase (final response minus the current before adding serum), the known sensitivity (46 nA/m*M*), and the dilution factor. The expected glucose concentration in the holding cell (assuming 4.89/5 = 0.978 m*M* when no glucose was added) and the value assessed from the electrode reading agreed within 3%, in all cases (Fig. 7). The correlation between assessed and expected values yielded a proportionality constant of 0.986 and a correlation coefficient of 0.996.

The ability of the electrode to assess glucose concentrations of undiluted nonbuffered serum was tested by placing 200 μL of reconstituted serum in the holding cell followed by 2–20 μL of 100 m*M* glucose. The glucose concentration was calculated by (I-182)/46, where I was the current response, 182 nA was the baseline current, and 46 nA/mM was the sensitivity. Although a proportionality constant of 0.964 and a correlation coefficient of 0.999 were obtained, the difference between assessed and expected concentrations was up to 9% in some case (Fig. 7), perhaps because of the lack of buffer.

The same set of electrodes was used repeatedly to assess the glucose concentration reconstituted serum samples. On average, each electrode was used more than 50 times without any change in characteristics and sensitivity. The electrodes were effective after 6 wk when stored in buffer at room temperature between tests and in a humid container overnight or longer.

In brief, the ability of electrodeposited PVF in mediating glucose oxidase activity has been demonstrated. The mediated biosensor possessed

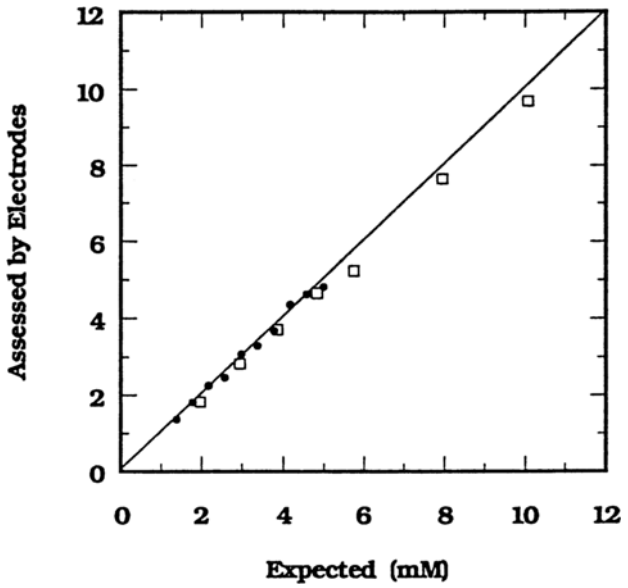

Fig. 7. Correlation between expected and electrode-assessed glucose concentrations in augmented reconstituted serum. (●) Diluted and buffered, (■) nonbuffered, minimum dilution.

a fast response, good reproducibility, and was reusable for at least 50 analyses. The sensitivity of this system (0.5 m*M*) was less satisfactory than that of mediated systems based on ferrocene or its derivatives. However, this sensitivity level may be adequate for several practical applications. Glucose oxidase is very stable, therefore, this enzyme is anticipated to remain intact after several analyses. One may question the integrity of the deposited PVF layer, since this film may be vulnerable to shear effect. Therefore, more studies are required to assess such behavior as well as to develop more stable PVF films. The extended reusability of such electrodes is no longer critical if this type of electrode can be miniaturized and mass produced. Miniaturization of electrodeposited PVF electrodes is conceptually feasible, and this task may be a prerequisite for any attempt to commercialize this type of electrode.

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