Sol−Gel Derived Composite Materials for the Construction of Oxidase/Peroxidase Mediatorless Biosensors

L. Coche-Guérente,* S. Cosnier, and P. Labbé*

Laboratoire d'Electrochimie Organique et de Photochimie Rédox, UMR CNRS 5630, Université Joseph Fourier Grenoble 1, BP 53, 38041 Grenoble Cedex 9, France

Received September 26, 1996. Revised Manuscript Received February 25, 1997[⊗]

A new bienzymatic electrode for glucose sensing has been constructed with the aid of the sol−gel process. This mediatorless sensor was made by the simultaneous immobilization of graphite microparticles and two enzymes (horseradish peroxidase (HRP) and glucose oxidase (GOD)) in a silica gel matrix. The amperometric detection of glucose was assayed by potentiostating the biosensor at 0 V vs SCE in order to reduce the enzymically generated H_2O_2. The active graphite particles evenly dispersed within the silica gel matrix establish an efficient electrical communication between the electrode surface and the HRP active center. The effect of graphite and HRP amounts have been investigated in order to improve the analytical characteristics of the amperometric H_2O_2 biosensor. For determination of glucose, a bilayer structure where HRP and GOD were immobilized in two separated silica films exhibited the higher analytical performances.

Introduction

The application of the sol−gel process for trapping enzymes is now widely demonstrated,[1] and it is well recognized that under adequate synthesis conditions, porous silica xerogel preserves the catalytic activity of the immobilized protein molecules. Optical biochemical sensors based on the hydrolytic polycondensation of alkoxysilane precursors (principally tetramethoxysilane or tetraethoxysilane) in the presence of enzyme and a chromogenic dye are the subject of active research and were the first examples[2] of sol−gel host matrixes for protein molecules exhibiting catalytic activity. Such materials have been used in a range of sensor configurations[3] and have been recently reviewed.[1c] These include monolithic glasses, fibers, powders, etc. The application of the sol−gel process in the design of enzymic sensors has not been limited to optical devices. Indeed, as far as the enzymatic reactions involve electron transfer as well as the appearance or the disappearance of electroactive species, electrochemical transducers have been used.[1c,4] The success of such devices is partly inherent in the possibility of producing thin films which can be adsorbed onto an electrode surface.

A sol−gel glucose flow injection analyzer using amperometric detection has been described by a Japanese group.[5] It was based on a sensor made of silica−GOD film attached to an oxygen electrode by a nylon net and cellulose membrane.

Lev et al. have reported[6] the construction of a new class of amperometric biosensors prepared by the sol−gel doping procedure. In this last example, the authors have immobilized GOD as a model enzyme in a porous electrically conducting vanadium pentaoxide matrix. Following this prototype, other approaches have involved the sol−gel method for the development of enzyme electrode with the entrapment of GOD in wet gels[7,8] or undergoing xerogels such as carbon ceramic composite matrix[9,10] (comprising carbon powder percolation through sol−gel derived silica).

The fundamental problem arising in the construction of an amperometric glucose biosensor concerns the selectivity of the substrate detection. The detection mode involved in such sensors is often based on electrochemical oxidation of the hydrogen peroxide produced by the enzymatic reaction and requires a relative high working potential. At such a potential, endogeneous or exogeneous compounds commonly present in biological samples (as for example urate, ascorbate, or paracetamol) can be electrochemically oxidized leading to a high level of interference in the quantitation of glucose concentration. To circumvent this problem, it numerous systems have been described based on the use of electrochemical mediators which were more or less bound to the matrix. In this biosensor design, the oxidized form Med_{ox} of the mediator replaces molecular oxygen and must be able to accept electrons from the $FADH_2$ prosthetic group of GOD. The electrode function consists to regenerate Med_{ox} by electrooxidation of the reduced form Med_{red}. The redox mediator is chosen so that the potential of

[⊗] Abstract published in *Advance ACS Abstracts,* April 1, 1997.

(1) (a) Avnir, D.; Braun, S.; Lev, O.; Ottolenghi, M. *Chem. Mater.* **1994**, *6*, 1605. (b) Zink, J. I. Valentine, J. S.; Dunn, B. *New. J. Chem*, **1994**, *18*, 1109. (c) Dave, B. C.; Dunn, B.; Valentine, J. S.; Zink, J. I. *Anal. Chem.* **1994**, *66*, 1120A.

(2) (a) Braun, S. Rappoport, S.; Zusman, R.; Avnir, D.; Ottolenghi, M. *Mater. Lett* **1990**, *10*, 1. (b) Ellerby, L. M. Nishida, C. R.; Nishida, F.; Yamanaka, S. A.; Dunn, B.; Valentine, J. S.; Zink, J. I. *Science* **1992**, *255*, 1113.

(3) Maccraith, B. D.; Mcdonagh, C. M.; O'Keefe, G.; Mcevoy, A. K.; Butler, T.; Sheridan, F. R. *Sensors Actuators* **1995**, *B29*, 51.

(4) Livage, J. *C.R. Acad. Sci. Paris, Ser. IIb* **1996**, *322*, 417.

(5) Tatsu, Y. Yamashita, K.; Yamaguchi, M.; Yamamura, S.; Yamamoto, H.; Yoshikawa, Y. *Chem. Lett.* **1992**, 1615.

(6) Glezer, V.; Lev, O. *J. Am. Chem. Soc.* **1993**, *115*, 2533.

(7) (a) Audebert, P. Demaille, C.; Sanchez, C. *Chem. Mater.* **1993**, *5*, 911. (b) Audebert, P.; Sanchez, C. *J. Sol-Gel Sci. Technol.* **1994**, *2*, 809.

(8) Narang, U. Prasad, P. N.; Bright, F. V.; Ramanathan, K.; Kumar, N. D.; Malhotra, B. D.; Kamalasanan, M. N.; Chandra, S. *Anal. Chem.* **1994**, *66*, 3139.

(9) Tsionsky, M. Gun, G.; Glezer, V.; Lev, O. *Anal. Chem.* **1994**, *66*, 1747.

(10) Pankratov, I.; Lev, O. *J. Electroanal. Chem.* **1995**, *393*, 35.

S0897-4756(96)00498-X CCC: $14.00

Med_{red} electrooxidation is sufficiently low (generally below 0.2 V vs SCE) in order to avoid interferents electrooxidation. Another alternative has been developed by Wang[11] and Lev,[12] who used the catalytic properties of palladium-modified carbon particles in order to detect glucose at lower potentials (+0.3, +0.5 V). In both cases, GOD was entrapped within a sol−gel matrix containing palladium-modified carbon particles, via a screen-printing process[11] or by molding the porous organically modified silica in a glass capillary.[12]

In the past few years, another approach has been developed and several articles reported on the construction of bienzymatic peroxidase/hydrogen peroxide-producing oxidase amperometric biosensors based on a direct electron transfer between the electrode and the active site of immobilized peroxidases.[13] The role of such "electrically connected" peroxidases is to catalyze the reduction of the enzymically produced H_2O_2 in the presence of dioxygen and oxidase substrate. If an efficient electrical wiring is operative between the electrode surface and the peroxidase active site, it becomes then possible to monitor amperometrically the reduction of H_2O_2 within the optimal potential range −200 to 0 mV vs SCE where potential interferents are electroinactive.

The aim of the present work is to take advantage of the sol−gel process for the preparation of composite graphite−silica matrixes coimmobilizing a peroxidase/oxidase bienzymatic system. Coating this electronically conducting composite biomaterial onto a glassy carbon (GC) electrode surface allows the construction of a mediatorless bienzymatic sensor as examplified by the use of horseradish peroxidase (HRP)/glucose oxidase (GOD) model system for the detection of glucose. Investigation of the characteristic functionning parameters as well as their optimization will be presented.

Experimental Section

Chemicals. Horseradish peroxidase (E.C. 1.11.1.7. type II, 180 purpurogallin units/mg denoted HRP), glucose oxidase (E.C.1.1.3.4. type VII-S from Aspergillus niger, 150 Sigma Units/mg, denoted GOD), and β-D glucose were obtained from Sigma.

Stock solutions of glucose were allowed to mutarotate overnight before use. Tetramethoxysilane (TMOS) was obtained from ABCR Chemicals Industries. All other chemicals were from analytical grade. Aqueous solutions were doubly distilled in a quartz apparatus. Graphite powder was purchased from Aldrich (ref 28286.3).

Apparatus. The electrochemical equipment has been described elsewhere.[14] All electrochemical studies were carried out with a conventional three-electrode potentiostatic system. Potentials are reported versus a saturated calomel reference electrode (SCE). The working electrode is a glassy carbon disk electrode (5 mm diameter) previously polished with 1 μm diamond paste. The electrochemical experiments were carried out in a 10 mL electrochemical cell.

Spectrophotometric assays were performed with a Varian Cary 1 UV−visible.

Sol−Gel Film Preparation. Hydrogen peroxide sensor performances were studied using two different film preparation protocols. TMOS gels were made from an adaptated procedure previously described.[7a] Typically a homogeneous sol is prepared by vigorously stirring for 10 min at 4 °C 300 μL of TMOS, 100 μL of bidistilled H_2O, and 3 μL of HCl 0.04 M. Then 3.9 mg of graphite powder is adducted to 84 μL of this sol and stirred at 4 °C for 5 min (sol I). Separately a solution of HRP 0.9 mg in 900 μL phosphate buffer 0.01 M pH 8 is prepared. This solution is also cooled to 4 °C (sol II). Then a casting solution is prepared by mixing for 5 min at 4 °C, 219 μL of sol II with 84 μL of sol I. This resulting sol (12 μL) is deposited onto a glassy carbon electrode surface. The film was then allowed to stand at 4 °C (in a refrigerator) during 20 min for the gelation step.

The electrodes (*GC*/TMOS−HRP−graphite) are then stored at 4 °C for 1 h in a 0.1 M aqueous phosphate buffer pH 8, after which most of the methanol resulting from the polymerization of the TMOS has been washed out.[7a] After this film washing, the electrode is equilibrated for 2 h in a 0.1 M phosphate buffer pH 7, to desorb the enzyme molecules not efficiently bind in the pores of the gel before being transferred to the electrochemical cell.

The second film preparation protocol involves an overlying film of TMOS gel prepared from the procedure previously described. Following the gelation of the active film (TMOS−HRP−graphite), 20 μL of a sol solution (sol III) is deposited to recover the first film. Sol III is prepared by mixing for 1 min 75 μL of sol I (without graphite powder) with 197 μL of phosphate buffer 0.01 M pH 8, at 4 °C. The film is then gelled at 4 °C during 45 min before undergoing the soaking steps, giving rise to an electrode GC/[TMOS−HRP−graphite]−[TMOS].

Glucose sensors are prepared as follows: 135 μL of sol I containing 12.6 mg of graphite powder are mixed for 2 min with 355 μL of phosphate buffer (0.01 M, pH 8) containing 0.71 mg of HRP at 4 °C. This sol (12 μL) is deposited onto a glassy carbon electrode, and gelification is performed for this film at 4 °C during 20 min.

Separately TMOS/GOD solution is prepared by mixing for 2 min 150 μL of sol I (without graphite powder) and 394 μL of a phosphate buffer (0.01 M, pH 8) containing 0.79 mg of GOD (sol IV). 12 μL of sol IV are deposited so as to recover the (TMOS−HRP−graphite) film. Gelation occurred at 4 °C during 20 min and gives rise to an electrode GC/[TMOS−HRP−graphite]−[TMOS−GOD]. The material appears as a massive film with a thickness of about 2−3 mm.

Indirect Determination of the Amount of Entrapped Enzyme. To determine the amount of enzyme efficiently binded in the pores of TMOS−composite gel, the amount of enzyme lost in the two soaking solutions (pH 8 and 7) was estimated from the residual enzymatic activity of these two solutions by spectrophotometric procedures. HRP activity is determined by following the absorbance at 420 nm of purpurogallin produced from pyrogallol. GOD activity is determined by an assay procedure of hydrogen peroxide based on the absorbance increase at 425 nm generated by the *o*-tolidine−HRP system.

Results and Discussion

Peroxidases have been intensively studied for the construction of amperometric biosensors for sensing hydrogen peroxide and small organic peroxides. It is now well established[15] that immobilized peroxidases on carbon electrode materials could undergo a direct electron transfer with various kinds of graphite (powder, pyrolytic, etc.) This direct electron transfer is attributed to the existence of different *o*-quinone functionalities on the electrode surface. This redox function is capable of

(11) Wang, J. Pamidi, P. V. A.; Su Park, D. *Anal. Chem*. **1996**, *68*, 2705.

(12) Sampath, S.; Lev, O. *Anal. Chem*. **1996**, *68*, 2015.

(13) Csöregi, E. Gorton, L.; Marko-Varga, G. *Electroanalysis* **1994**, *61*, 925 and references therein.

(14) Coche−Guerente, L. Deronzier, A.; Mailley, P.; Moutet, J.-C. *Anal. Chim. Acta* **1994**, *289*, 143.

(15) (a) Gorton, A.; Jönsson-Pettersson, G.; Csöregi, E.; Johansson, K.; Dominguez, E.; Marko-Varga, G. *Analyst* **1992**, *117*, 1235. (b) Johansson, E. Marko-Varga, G.; Gorton, L. *J. Biomater. Appl.* **1993**, *8*, 146.

mediating the electron transfer (eq 2) between the electrode to the oxidized form of HRP (HRP_{ox}).

$$H_2O_2 + HRP_{red} \rightarrow H_2O + HRP_{ox} \quad (1)$$

Native form

$$HRP_{ox} + 2e \rightarrow HRP_{red} \quad (2)$$

On such a biosensing system, the electroenzymatic reduction of hydrogen peroxide occurs within the optimal potential range between − 150 and 0 mV vs Ag/AgCl. Within this range, easily electrooxidizable interferents and electroreducible molecular oxygen do not contribute to the response current. As mentioned in the Experimental Section, upon gelation onto a glassy carbon (GC) electrode surface of a sol obtained by mixing the methoxy precursor tetramethyl orthosilicate (TMOS) containing graphite powder with a buffered solution of horseradish peroxidase (HRP), it resulted in a dark film which remained strongly held and in contact with the electrode. To overcome the possible denaturation of biomolecules from the solvent, alcohol was not used in the synthetic procedure. Otherwise, the addition of a buffer solution of proteins to the sol raises the pH to biologically compatible values and thus prevents acid denaturation or aggregation of the proteins.

The sol−gel process applied to peroxidase/graphite particles system allows us to maintain in a close contact HRP and the graphite particles. The electron percolation between the particles consecutive to the high amount of graphite in the gel is expected and appeared necessary both to a sufficient electronic conductivity of the gel and to an efficient HRP electrical connection. Figure 1A shows a typical amperometric response of GC/TMOS−HRP−graphite electrode for successive increments of H_2O_2 at 0 V vs SCE. The time required to obtain 95% of maximum response is about 1 min. It can be seen in Figure 1 that sensitive responses to changes in the H_2O_2 concentration were achieved. The bioelectrode exhibits linear calibration up to 7.1×10^{-5} M with curvature at higher concentrations. The biosensor sensitivity determined as the slope of the linear calibration curve is 14.9 mA M^{-1} cm^{-2}.

Figure 2 demonstrates the effectiveness of the electrocatalytic system based on the electrical connection of HRP by the active sites of the graphite particles. For the concentration range investigated, electrochemical reduction of H_2O_2 on glassy carbon gives no response (curve C, Figure 2). The increase in the conductive surface for GC/TMOS−graphite gives detectable currents (curve B, Figure 2) which remain significantly lower than those measured on GC/TMOS−HRP−graphite (curve A, Figure 2). It shows that without the enzyme in the composite silica gel matrix no catalytic current can be measured. Two components are necessary in this system: HRP and graphite particles. To confirm this conclusion, the influence of biomolecules and graphite amount in the coatings onto the biosensor performances have been investigated.

Two successive experiments have been achieved with respectively 17.4 μg of HRP and 43.5 μg of HRP in the coatings. Since the sol−gel procedure would be a suitable technique to encapsulate biomolecules because the host matrix is formed around the protein molecule, a very low enzyme leakage out of the film could be

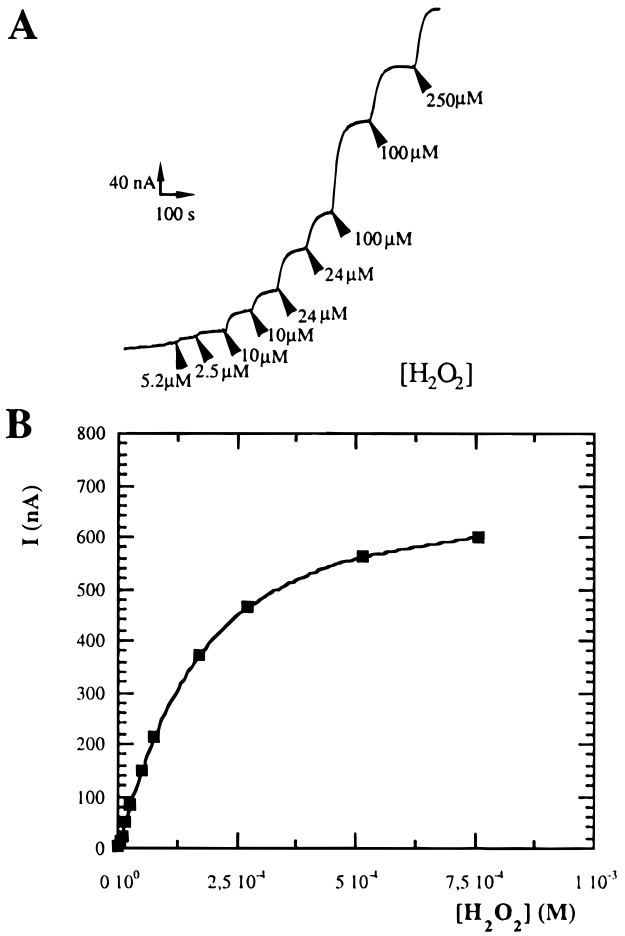

Figure 1. (A) Typical current time plot for successive increase of H_2O_2 in 0.1 M phosphate buffer (pH 7) and (**B**) calibration plot for a bioelectrode GC/TMOS (2.55 mg)−HRP (8.7 μg)−graphite (154 mg). Applied potential of 0 Vvs SCE.

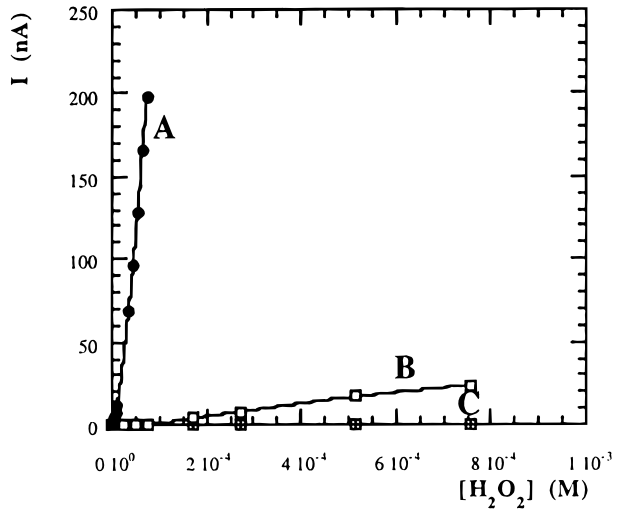

Figure 2. Calibration plots I_c versus $[H_2O_2]$ characterizing the reduction of H_2O_2 on three different modified electrodes, in phosphate buffer 0.1 M pH 7. (A) GC/TMOS (2.55 mg)−HRP (8.7 μg)−graphite (154 μg). (B**)** GC/TMOS (2.55 mg)−graphite (154 μg). (C) GC/TMOS (2.55 mg)−HRP (8.7 μg).

expected during the soaking steps at pH 8 and then at pH 7. In contrast, spectrophotometric determination of HRP activity in the two successive soaking solutions indicates a leaching of enzyme comprised between 25 and 65% of the initially deposited amount. This loss of immobilized enzyme could be related to the weak

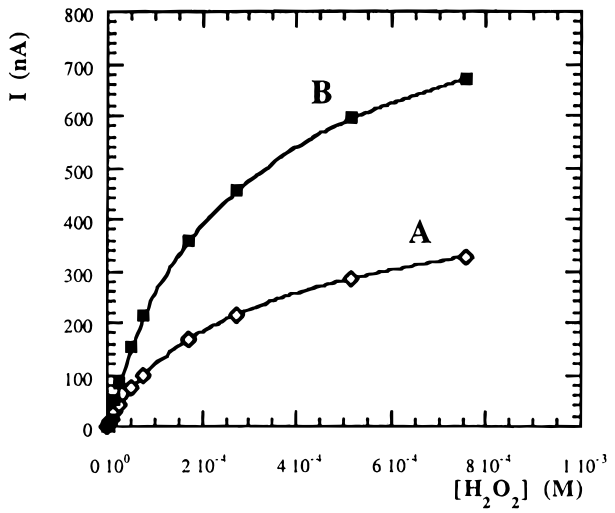

Figure 3. Steady-state amperometric measurements of current vs peroxide concentration in pH 7 phosphate buffer 0.1 M with increasing amount of HRP in the coatings TMOS (2.55 mg)−HRP − graphite (154 μg). The amount of HRP is (A) 17.4 μg and (B) 43.5 μg.

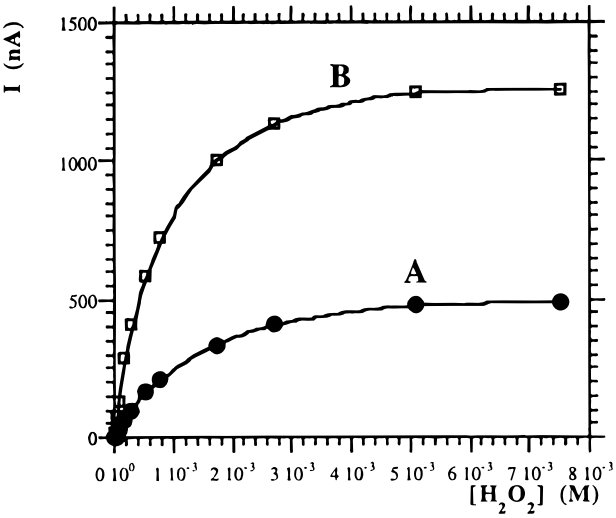

Figure 4. Calibration curves of hydrogen peroxide biosensors recorded for GC/[TMOS−HRP−graphite]−[TMOS] in phosphate buffer (pH 7, 0.1 M), at 0 V vs SCE. (A) Film composition [2.55 mg−17.4 μg−154 μg]−[4.1 mg]. (B) Film composition [2.55 mg−17.4 μg−309 μg]−[4.1 mg].

molecular weight of the HRP molecule. Not only is the enzyme leakage for this film configuration is relatively high but there was a certain lack of reproducibility on the amount of enzyme lost in solution. For the two experiments reported on Figure 3, the amount of entrapped enzyme have been determined to be 37% for coating A and 57% for coating B (i.e., respectively 6.4 and 24.8 μg truly trapped in the gel). The sensitivity of these biosensors for increasing amounts of trapped HRP increases from 8.1 mA M^{-1} cm^{-2} (curve A, Figure 3) to 14.9 mA M^{-1} cm^{-2} (curve B, Figure 3).

Figure 4 shows the calibration curves of novel series of bioelectrodes involving the presence of an additional overlayer of pure TMOS gel (4.1 mg) onto the surface of the first TMOS−HRP−graphite layer. This additional porous material exempt of HRP would exhibit pores of limited sizes through which the proteins could not go away. As it can be seen in Figure 4 (curve A) the overlying gel of pure TMOS is responsible for a lower sensitivity as compared to that observed for coating A in Figure 2 and an increase in the linear part of the calibration curve, up to 0.161mM instead of 0.037 mM without the overlayer of TMOS. The additional overlayer film causes a subsequent increase in diffusional constraints through the sol−gel network, thus lowering the sensitivity to the enzyme substrate and increasing the dynamic range as currently observed in amperometric biosensors. Otherwise, as expected, the addition of pure TMOS gel onto the active layer leads to a significant reduction of the enzyme leakage to the soaking solutions. Indeed, the amount of enzyme lost in solution with this multilayer configuration does not exceed 5% (instead of 65%, the maximum value observed in the absence of the additional TMOS overlayer). In addition, this result is accompanied by a better reproducibility in amount of entrapped enzyme.

The striking feature of Figure 4 (curve B) is the substantial growing of the sensitivity inherent to the increase in amount of graphite dispersed in the gel (corresponding to 2-fold the amount of film A). The increase in catalytic sites would facilitate the regeneration of the HRP active form and lead to higher current responses relative to the reduction of H_2O_2. With this bilayer configuration the sensitivity reaches the value of 9.25 mA M^{-1} cm^{-2}.

The refinement of the electroenzymatic system for the reduction of hydrogen peroxide with a bilayer structure has allowed us to immobilize GOD inside the overlayer film for the construction of a bienzymatic glucose biosensor. GOD and HRP were coimmobilized in a molar ratio of 1:8 based on previous studies on solid graphite electrodes reported by Jösson-Pettersson[16] and Csöregi et al.[17] The high amount of HRP was motivated by the competition between the wanted H_2O_2 uptake by HRP and its expected disappearance away from the electrode by diffusion. A higher amount of HRP was therefore expected to give a higher conversion of the hydrogen peroxide produced by GOD. Previously, we have investigated the monolayer structure constructed from the bienzymatic system (HRP/GOD) entrapped in the host matrix simultaneously with graphite particles during the gelation of the TMOS sol (film composition: 4.1 mg of TMOS−14.5 μg of HRP−14.5 μg of GOD−257 μg of graphite). The performances of such glucose biosensor are not striking. The calibration curve plotted from amperometric measurements at 0 V vs SCE exhibits a relative low sensitivity of 1.85 mA M^{-1} cm^{-2} with a dynamic range up to 6.4 $\times$ 10^{-5} M and a maximum current of 74 nA. The reasons for these low characteristics could be related to the film arrangement. The close proximity between HRP and GOD due to their simultaneous immobilization constitutes an advantage for the recognition of enzymically produced H_2O_2. However, the presence of the bulky GOD molecules in the vicinity of HRP could isolate by steric constraints HRP active center from the graphite particles and hence decrease the efficiency of the electrical communication.

To improve the biosensor performances HRP and GOD were immobilized in separated TMOS gel matrix and the amperometric detection of glucose was assayed at 0 V. Higher cathodic current arises after glucose addition giving evidence that H_2O_2 produced during

(16) Jönsson-Pettersson, G. *Electroanalysis* **1991**, *3*, 741.
(17) Csöregi, E. Gorton, L.; Marko-Varga, G. *Electroanalysis* **1994**, *6*, 925.

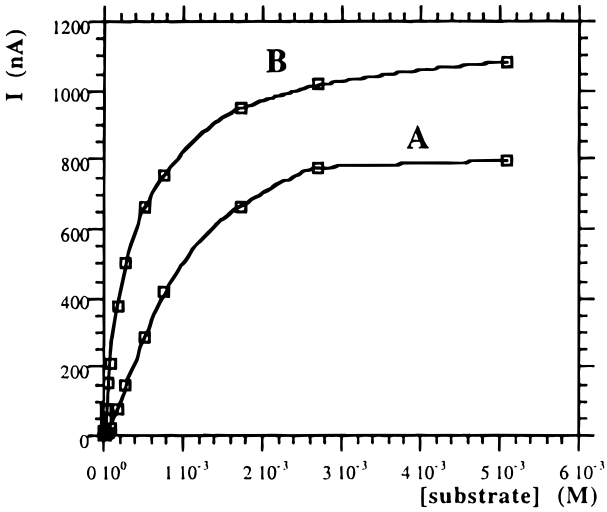

Figure 5. Calibration graph for GC/[TMOS−HRP−graphite]−[TMOS−GOD] in phosphate buffer 0.1 M pH 7. Electrode potential is 0 V vs SCE (film composition: [2.42 mg−17.1 μg−304 μg]−[2.46 mg−17.4 μg]). (A) As a glucose sensor. (B) As a H_2O_2 sensor.

glucose oxidation is introduced more efficiently into the electroenzymatic cycle of HRP. In this configuration, glucose is converted in the GOD layer producing H_2O_2 which diffuses both into the measuring cell and to the HRP underlayer containing active graphite particles with succeeding bioelectrocatalytic reduction at 0 V vs SCE. As a consequence of this sophisticated system the response time for glucose is close to 2 min. The calibration curves for glucose and hydrogen peroxide over the concentration range 5.0×10^{-5}−5.0×10^{-3} M are presented on Figure 5 (curves A and B, respectively). For glucose substrate, the bioelectrode exhibits linear calibration up to 0.70 mM while a curvature is observed at higher concentrations with a maximum current close to 800 nA and a sensitivity determined from the slope of the initial linear range of 2.95 mA M^{-1} cm^{-2}. In a second experiment, the bioelectrode has been studied as hydrogen peroxide sensor. Since the permeability to H_2O_2 substrate is higher than that of glucose substrate and since during glucose sensing an important fraction of H_2O_2 produced by the enzymatic oxidation of glucose is lost in the measuring cell, the sensitivity of the bioelectrode toward H_2O_2 as a substrate rises to a higher value: 14.60 mA M^{-1} cm^{-2}. The linear region extends up to 0.112 mM while the cathodic current was almost saturated at above 6 mM and reaches a maximum current of 1.2 μA. To evaluate the extent of enzymes (HRP and GOD) lost in the soaking solution before the investigation of the amperometric measurements, a spectrophotometric determination of enzyme activity has been performed. 5.6% of HRP and 7.5% of GOD have been lost in the soaking solution (pH 7 and 8). Since the thickness of the protecting overlayer of TMOS gel is lower (by about 2-fold) than that involved in the experiment reported in Figure 4 (curve B) it would be expected a sensitivity superior to 9.25 mA M^{-1} cm^{-2} as is observed. At the same time and for the same reasons the extent of the linear region is weaker (from 0.161 to 0.112 mM).

A preliminary storage stability test of the GC/[TMOS−HRP−graphite]−[TMOS−GOD] was realized by storing the electrode at 4 °C in phosphate buffer pH 7 and measuring each day over a week the dynamic response for glucose. We observed that 100% of the initial sensitivity was maintained after 3 days and 74% after one week.

Conclusion

The preceding results demonstrate the possibility of immobilizing and connecting simultaneously HRP in a sol−gel host matrix containing graphite particles. The potentialities of this new system have been successfully examplified by the fabrication of a glucose biosensor based on the coimmobilization of HRP and GOD in a bilayer sol−gel host matrix. The electroenzymatic system reported in this paper involves the direct electron transfer from the electrode surface to HRP via the surface functionnalities of graphite particles dispersed in the gel. Work is underway to increase the catalytic activity of graphite particles and to conceive a novel electrode design allowing to increase the current responses.

Acknowledgment. The authors thank D. Limosin for technical assistance and Dr. A. Deronzier for his interest in this work.

CM960498X