Original Papers

Parameters Important in Fabricating Enzyme Electrodes Using Self-Assembled Monolayers of Alkanethiols

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The fabrication of enzyme electrodes using self-assembled monolayers (SAMs) has attracted considerable interest because of the spatial control over the enzyme immobilization. A model system of glucose oxidase covalently bound to a gold electrode modified with a SAM of 3-mercaptopropionic acid was investigated with regard to the effect of fabrication variables such as the surface topography of the underlying gold electrode, the conditions during covalent attachment of the enzyme and the buffer used. The resultant monolayer enzyme electrodes have excellent sensitivity and dynamic range which can easily be adjusted by controlling the amount of enzyme immobilized. The major drawback of such electrodes is the response which is limited by the kinetics of the enzyme rather than mass transport of substrates. Approaches to bringing such enzyme electrodes into the mass transport limiting regime by exploiting direct electron transfer between the enzyme and the electrode are outlined.

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The immobilization of enzymes on the surface of electrodes modified with self-assembled monolayers (SAMs) provides a number of advantages as a method for the fabrication of enzyme electrodes. Using SAMs has the potential to provide enzyme electrodes with a high degree of reproducibility,[1,2] molecular level control over the spatial distribution of the immobilized enzymes[3-14] and the immobilization of the enzyme close to the electrode thus allowing direct electron transfer to be achieved.[15-24] These advantages have resulted in a recent surge in research into self-assembled monolayers for biosensor applications in general, and enzyme electrodes in particular.[25-27] However, despite the plethora of biosensor research papers using self-assembled monolayers as the base onto which the biomolecule is immobilized, there has been very little fundamental research into what steps are important in the fabrication process or which parameters control the response of the resultant biosensor.

Alkanethiols modifying gold are the most popular SAMs.[25,26] The interaction between the thiol groups and the gold results in a strong pseudocovalent bond[28] which is stable throughout the potential range of 0.8 V to –1.4 V *versus* Ag/AgCl before being oxidatively or reductively desorbed.[29,30] If the alkanethiol has appropriate chemical functionality, such as an amine or carboxylic acid moiety, then once the SAM is formed, enzymes and other biomolecules can be easily covalently bound to the SAM. In this way, a monolayer or submonolayer of enzyme is immobilized. For enzyme electrodes a short alkyl chain alkanethiol, usually three carbons long, is chosen so that the enzyme is as close as possible to the electrode. An additional advantage of the short alkyl chain is that a relatively disordered SAM is formed which means the underlying metal is still electrochemically accessible. In contrast, if a long chain alkanethiol is used the electrode is passivated unless defects are deliberately introduced into the SAM.[31]

There are a number of steps in the fabrication of an enzyme electrode using an alkanethiol. First, the metal surface must be prepared and cleaned. The alkanethiol must self-assemble onto the metal, followed by activation of the chemical functionality of the SAM, leading finally to the exposure of the activated SAM to the enzyme which is when attachment occurs. There are however many unknowns in this fabrication process. Questions that needs answers are: what effect does the roughness of the gold surface have? How long should the metal be exposed to the alkanethiol solution to allow a stable SAM to be formed? What is the optimal procedure for activating the SAM for enzyme attachment? How long should the activated SAM be exposed to enzyme? What should the concentration of the enzyme solution be during enzyme immobilization? Does the buffer used have any effect on subsequent performance? Can the amount of enzyme immobilized be controlled and do different enzyme loadings lead to different electrode activities? What limits the response of the enzyme electrode and how can the response characteristics be varied? We have worked extensively on fabricating enzyme electrodes using SAMs in an attempt to answer some of these questions. The purpose of this paper in the special issue of *Analytical Sciences* for young investigators is to present in a single publication much of this research on the parameters important in fabricating monolayer enzyme electrodes using alkanethiol SAMs.

Experimental

Reagents

Glucose oxidase (EC 1.1.3.4) from *Aspergillus niger* (Type

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VII-S), peroxidase from horseradish, microperoxidase (MP-11) from equine heart cytochrome *c*, 3-mercaptopropionic acid (MPA), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), *p*-benzoquinone were obtained from Sigma Chem. Co. (Sydney, Australia). Reagent grade K_2HPO_4, KH_2PO_4, KCl, NaOH and methanol were purchased from Ajax Chemicals Pty. Ltd. (Sydney, Australia). The biological buffers *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid) (HEPES), 3-(*N*-morpholino)-propanesulfonic acid (MOPS), and 2-(*N*-morpholino)-ethanesulfonic acid (MES) were obtained from Sigma Chem. Co. (Sydney, Australia). The pH of all buffer solutions were adjusted with either dilute NaOH or HNO_3. All reagents were used without further purification. Milli Q grade reagent water was used for all solutions.

Apparatus

Amperometric measurements were performed in a three-electrode cell using a BAS 100B (Bioanalytical Systems Inc., Lafayette, USA) potentiostat. Home-made polycrystalline bulk gold electrodes or evaporated gold films were used as the working electrode, the reference electrode was Ag/AgCl from BAS and a homemade platinum flag was used as the auxiliary electrode. The microbalance used was a home built instrument based upon the circuitry described by Bruckenstein and Shay.[32] The thin gold films were all prepared by thermal vacuum evaporation of approximately 3000 Å gold either onto freshly cleaved muscovite mica or glass (microscopes slides).

Methods

The polycrystalline bulk gold electrodes were polished with a 0.05 μm alumina/water slurry and then cleaned by cycling between the potentials of –0.3 to 1.5 V *versus* Ag/AgCl in 0.05 M sulfuric acid solution at a scan rate of 100 mV s^{-1} until reproducible scans were recorded. The evaporated gold films were used immediately once removed from the vacuum chamber. Cleaned gold electrodes were modified by placing them into a 75:25 (v/v) ethanol:water solution containing 0.01 M MPA overnight. Modified electrodes were then washed in a 75:25 (v/v) ethanol:water solution and dried in a nitrogen stream. The MPA modified electrodes were activated for 1 h in an aqueous solution at pH 5.5 containing 0.002 M EDC, 0.005 M NHS, 0.05 M phosphate and 0.05 M KCl.

With the *reference conditions* for preparing and using the enzyme electrode, polycrystalline bulk gold electrodes were modified with MPA and then activated as above. The activated electrodes were washed in water, and placed in phosphate buffer, pH 5.5, containing 480 μg/mL of the enzyme. The electrodes were left in the enzyme solution for at least 90 min and then washed with copious amounts of water prior to use. For the measurement of analyte calibration curves, the electrodes were allowed to settle to a stable current, over a period of at least 1 h, in the background solution buffered to pH 7.0 with 0.05 M phosphate and 0.05 M KCl. 1 mM *p*-benzoquinone was used as the mediator. The electrode potential was held constant at +0.5 V *versus* an SCE. Once the electrode background current was stable, additions of glucose were made from a stock solution of 1 M glucose in pH 7.0 phosphate buffer. Deviations from this reference system are outlined in the relevant sections of the Results and Discussion.

Results and Discussion

In this paper, the effect of a number of fabrication parameters on the reproducibility and response of monolayer enzyme electrodes fabricated using gold electrodes modified with alkanethiols will be discussed. The parameters which will be discussed in some detail are the role of the underlying gold surface, the amount of enzyme in solution during immobilization, the effect of buffers used during enzyme immobilization, the tuning of the response of the enzyme electrodes and finally the limiting factors in determining the response characteristics of the enzyme electrode once a system is optimized.

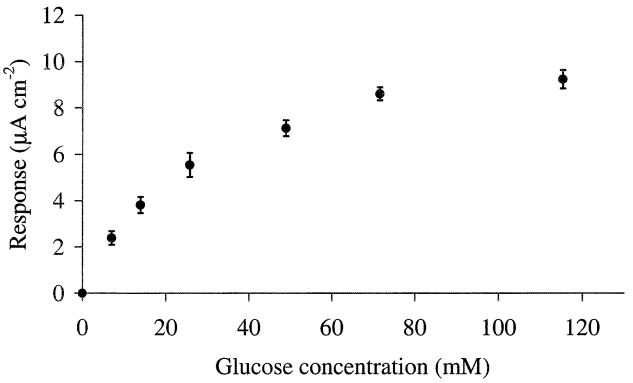

Fig. 1 Glucose calibration curve for an enzyme electrode prepared by modifying a bulk gold electrode with MPA and covalently attaching the enzyme glucose oxidase according to the reference conditions.

A reference enzyme electrode

Studies of the effects of preparation methods will be compared to the reference conditions outlined in the Experimental section. A calibration curve for the reference conditions is shown in Fig. 1. Under these conditions we have shown, using an assay described previously[33] where the flavin adenine dinucleotide redox centers of the GOD are stripped from the enzyme and quantified using fluorescence, that the amount of glucose oxidase immobilized was approximately 1 pmol cm^{-2}.

The calibration curve shown in Fig. 1 has two surprising features. The first is the high sensitivity of the current response, considering that there is only a single monolayer of enzyme immobilized. The sensitivity in the linear range of 170 nA mM^{-1} cm^{-2} is comparable to enzyme electrodes where the enzyme is immobilized throughout a polymer layer, a thick-film enzyme electrode.[34] This similar sensitivity is despite significantly less enzyme being immobilized on a SAM. With glucose oxidase immobilized throughout a polytyramine modified electrode, 15.9 nmol cm^{-2} of the enzyme was found to be immobilized (compared with 1 pmol cm^{-2} for the SAM enzyme electrode). Despite greater than four orders of magnitude more enzyme being immobilized the current sensitivity of the polytyramine enzyme electrode in the linear region of the calibration curve was 2000 nA mM^{-1} cm^{-2}.[34] Therefore, in the monolayer enzyme electrode more than 1000 times the current is being produced per enzyme molecule.

The efficiency of substrate turnover by the enzyme in SAM enzyme electrodes is also reflected in the broad dynamic range. As can be seen from Fig. 1, the reference enzyme electrode is still responding to changes in glucose concentration up to 100 mM (albeit not linearly) despite there being *no* partition barrier between the enzyme layer and the glucose solution. Again, such broad dynamic ranges contrasts with thick-film enzyme

electrodes where the enzyme electrode rarely responds to glucose concentrations above 40 mM.[35] Theoretical studies of the reaction-diffusion system defined by a monolayer enzyme electrode provides an explanation for both the broad dynamic range and the outstanding sensitivity of the SAM enzyme electrode.[2,36] The modelling indicates that the close proximity of the enzyme to the electrode results in little diffusional barrier for the reduced mediator to travel to the electrode where it is oxidized, regenerating *p*-benzoquinone, and thus giving the current signal. The consequence of regenerating *p*-benzoquinone directly adjacent to the enzyme is that there is always a ready supply of mediator to turn the enzyme over. This ready supply of mediator is in direct contrast to thick-film enzyme electrodes which are usually cosubstrate limited.[35,37,38] Hence, with monolayer enzyme electrodes, the ready supply of mediator provides for efficient enzyme turnover, a high sensitivity per enzyme molecule and a broad dynamic range. The disadvantage however, is that the enzyme electrode is kinetically rather than mass-transport limited. This disadvantage will be discussed in more detail below.

Also shown in Fig. 1 is the reproducibility that can be achieved between electrodes made on different days but with the same set of reagents. Under these circumstances variability as low as 10 percent was achieved across the entire concentration range. Note however, that if different reagents are used, in particular, different batches of enzyme, the variability between electrodes can be much greater. The larger variability between reagent batches can be seen by comparing Fig. 1 with the phosphate buffer calibration curve in Fig. 6.

Gold surfaces

One of the conceptual differences between SAM enzyme electrodes and thick-film enzyme electrodes is that in the former case all the enzyme is located close to the electrode surface. When the SAM is a short chain alkanethiol such as MPA, the length of the thiol chains is well within the scale of the surface topography of the underlying gold electrode. As a consequence, the topography and cleanliness of the underlying gold could be expected to have a significant influence on the response of the resultant enzyme electrode. We have studied the effect of six different gold surfaces on the response of enzyme electrodes prepared in all other ways as outlined for the reference system.[39,40] Apart from the bulk gold electrodes all other electrodes were prepared *via* the evaporation of gold which has been shown to give predominantly the Au(111) crystal face.[41–43] The gold surfaces investigated (with the root-mean-square roughness, R_{rms}, measured with a scanning tunnelling microscope in brackets) were bulk gold (5.1 ± 0.5), gold evaporated onto a cold mica surface (7.7 ± 0.3), evaporated onto a mica surface heated to 300°C followed by annealing at this temperature (0.32 ± 0.03), evaporated onto mica followed by removal of the mica to reveal an atomically *flat gold* surface (0.12 ± 0.03), evaporated onto a microscope slide with a titanium adhesion layer (2.1 ± 0.5) and evaporated onto a microscope slide with a 3-mercaptopropylsilane (MPS) adhesion layer (0.95 ± 0.05).

The conclusion from this study was the smoother the gold surface with the fewer the number of grain boundaries, the more reproducible the enzyme electrode and the higher the current sensitivity. Therefore we obtained optimal performance from the enzyme electrodes using the *flat gold*. This higher sensitivity appears to be a result of the enzyme being more accessible to the mediator rather than significantly more enzyme being immobilized. With the rougher gold surfaces it is believed that enzyme molecules are immobilized deep within the crevices of the metal surface where they may not be as readily available to substrate and the mediator.[40]

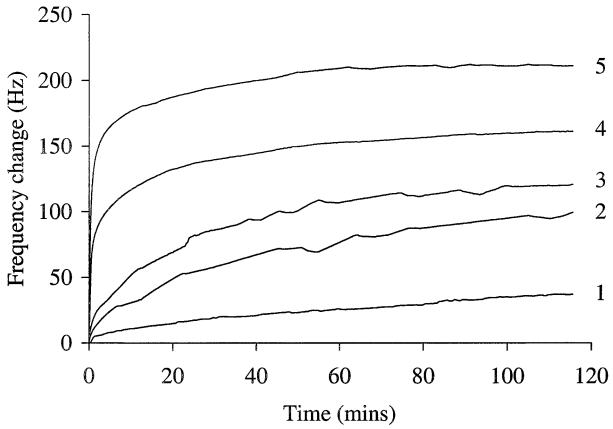

Fig. 2 QCM resonance frequency change as a function of time for the adsorption of GOD on EDC/NHS activated MPA monolayers from pH 5.5 buffer at 25°C. Numbers 1 – 5 correspond to protein concentrations of 2, 8, 20, 120 and 480 μg/mL, respectively.

Immobilization conditions

After preparation of an appropriate gold surface, the next steps in the enzyme electrode fabrication are the SAM formation and subsequent activation to allow covalent attachment of the enzyme. There has been considerable research into both the time required for SAM formation[44,45] and the optimal conditions for activating carboxylic acid terminated SAMs with EDC and NHS.[46-49] Therefore, the conditions of SAM formation and activation were not investigated further in this study. The enzyme immobilization conditions however have not been investigated. In our initial work on fabricating enzyme electrodes using SAMs, the activated SAM was placed into a solution of 120 μg glucose oxidase per ml of buffer.[1,36] Under these conditions the reproducibility of the enzyme electrodes was poor.[50] A quartz crystal microbalance was used to monitor the amount of immobilized glucose oxidase for a given exposure time (Fig. 2). Figure 2 clearly shows that under conditions similar to those used in the preliminary studies, the amount of enzyme being immobilized with time would still be changing after 60 min. Therefore the poor reproducibility was a result of the difficulty in immobilizing the same amount of enzyme onto the SAM surface with each electrode prepared. When the immobilization conditions were changed to 480 μg ml^{-1} of GOD in buffer for 90 min less than 10% variability in calibration curves was achieved. Figure 2 shows that under the optimized conditions, the amount of enzyme immobilized has reached a maximum after 90 min. Therefore minor changes in GOD concentration, immobilization time or temperature will not affect the amount of enzyme immobilized; leading to better reproducibility. The 120 μg ml^{-1} curve shown in Fig. 2 was observed to reach the same maximum change in frequency curve after 6 h (not shown) as observed for the 480 μg ml^{-1} curve. The fact that the same change in frequency of the QCM was observed indicates this frequency change reflects the maximum enzyme loading on the monolayer surface.

The maximum amount of enzyme immobilized corresponds to an enzyme loading of 1×10^{-12} mol cm^{-2}. This loading however, does not correspond to close packing of the enzyme on the SAM surface. Based on the size of glucose oxidase molecule of $7 \times 5.5 \times 8$ nm,[51] the theoretical maximum enzyme

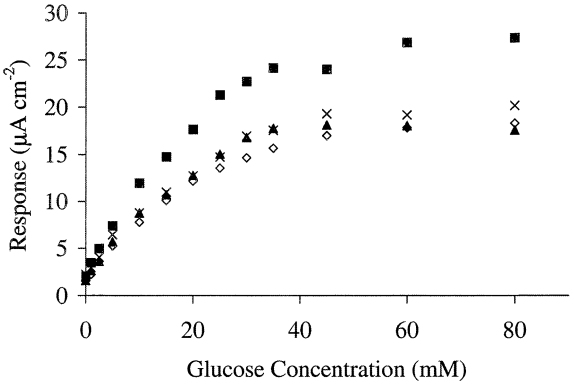

Fig. 3 Calibration curves for GOD-modified MPA electrodes where the following buffer were used during the immobilization step: ◇, HEPES; ■, MES; ▲, phosphate buffer and ×, MOPS.

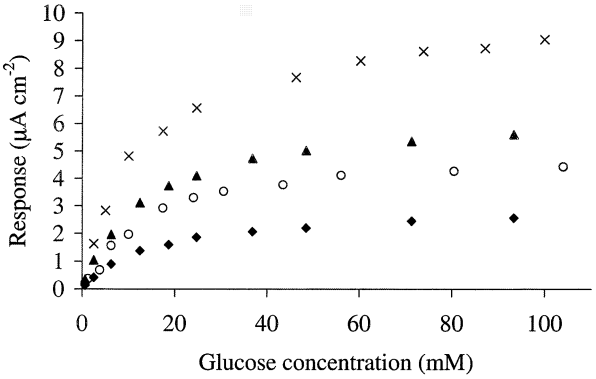

Fig. 5 Glucose sensor response as a function of enzyme loading, where the theoretically predicted enzyme loadings[2] are: ◆, 0.08; ○, 0.13; ▲, 0.18 and ×, 0.30 pmol cm^{-2}.

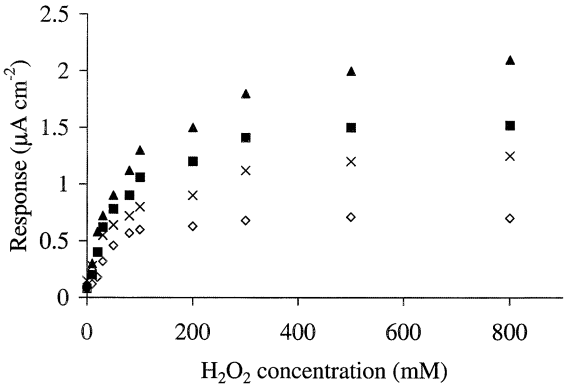

Fig. 4 Calibration curves for the amperometric responses of HRP covalently immobilized onto an MPA modified gold electrode using different buffers (pH 7.0). ◇, HEPES; ■, MES; ▲, phosphate buffer and ×, MOPS.

loading is 2.6 – 3.8 pmol cm^{-2}. The lower density of enzyme packing on the surface is confirmed by tapping mode AFM images of these enzyme-modified surfaces.[52] The AFM pictures show that the surface is covered by rings of what appears to be five or six enzyme molecules in a regular cluster.

The influence of the choice of buffer during immobilization

In the reference system, the buffer used was 0.05 M phosphate in 0.05 M KCl at a pH of 5.5. The current density achieved with this system was considerably lower than that reported by Gooding *et al.*[1] where MES buffer was used during the immobilization of GOD. Therefore, the influence of different biological buffers with regard to the performance of the resultant enzyme electrode was investigated. Figures 3 and 4 show the calibration curves for enzyme electrodes fabricated with glucose oxidase and horseradish peroxidase respectively. For each enzyme, HEPES, MES, MOPS and the phosphate buffer, were used during the immobilization step.

Clearly, there are significant differences in the electrodes' responses when different buffers are used during immobilization. Furthermore, it can be seen that there is a divergence in the variation in current density with different buffers for the two enzymes.

It is clear from these observations that the buffer salts can then be seen to have some sort of "stabilization" effect on the enzyme during the reaction. As the buffers play a role in the conformation of the enzymes during immobilization, which influences the enzymes stability and/or stability,[53,54] this is not too surprising. It has been shown however that each enzyme system is stabilized by different buffers,[55,56] and therefore, as we have observed with GOD and HRP, there is no "ideal" buffer that can be used for all systems, and the choice will be system specific.

Tuning the response of the enzyme electrode

An important issue in any sensing device is the ability to tune the response of the final sensor for a particular application. In the case of thick film enzyme electrodes this can be achieved in a number of ways such as by varying the amount of enzyme, the thickness of the enzyme layer, the partition coefficient or the amount of mediator.[35] With a SAM enzyme electrode we have investigated how to control the output of the sensor *via* varying the amount of enzyme immobilized and the concentration of mediator.

The influence of enzyme loading is shown in Fig. 5. The enzyme loading was controlled by altering the time the activated SAM was exposed to enzyme solution. The relative amount of enzyme immobilized was determined by correlating the exposure time to a change in frequency of the QCM as in Fig. 2. Figure 5 shows that increasing the enzyme loading results in an almost linear increase in current response without any significant reduction in dynamic range. The insensitivity in dynamic range to the enzyme loading is in contrast to thick film enzyme electrodes where an increase in catalytic activity is shown to provide an increase in sensitivity but at the cost of a reduced dynamic range.[35,57]

Increasing mediator concentration has an even more dramatic and positive effect on the response of the SAM enzyme electrodes (Fig. 6). It is apparent from Fig. 6 that an increase in mediator concentration results in increases in both sensitivity and dynamic range. This result implies that the increased concentration of mediator gives an increase in the rate of turnover of the reduced enzyme back to its active oxidized form.

What limits the response of the SAM enzyme electrode?

The variation in electrode response with enzyme loading and mediator concentration indicates that the response of the enzyme electrode is limited by the kinetics of the enzyme reaction rather than mass transport of reactants to the enzyme layer. The enzyme electrode being kinetically limited is confirmed by a previous study where the current response of a SAM enzyme electrode was shown to be independent of the rate

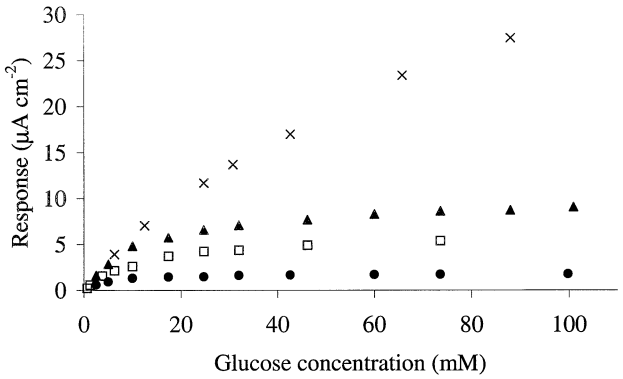

Fig. 6 Glucose sensor response as a function of mediator concentration. The points correspond to: ●, 0.1; □, 0.5; ▲, 1 and ×, 5 mM *p*-benzoquinone in phosphate buffer (pH 7).

of mass transport over a large range of stirring conditions.[2] This kinetic limitation of the SAM enzyme electrode severely limits the utility of monolayer enzyme electrodes as commercial devices as any loss in enzyme activity results in change in the performance of the enzyme electrode.

For a glucose oxidase monolayer enzyme electrode to become transport limited, one of two things is required. Either the amount of enzyme in the monolayer must be increased (our theoretical studies predict 1000 pmol cm^{-2} of enzyme is required) or the rate of enzyme turnover must be increased. Much higher enzyme loadings of 600 pmol cm^{-2} have been achieved with smaller enzyme molecule, microperoxidase MP-11.[20] With glucose oxidase the maximum enzyme loading in a monolayer is limited to less than 5 pmol cm^{-2}. Such high enzyme loadings of glucose oxidase could be achieved with SAM enzyme electrodes if a number of single molecule thick enzyme layers are deposited by exploiting some of the recently developed step-by-step immobilization techniques.[3-14] Such an approach could then lead to a transport limited enzyme electrode being fabricated in a highly controlled manner, thus exploiting the highly controlled immobilization virtues of the SAM approach with the advantages of transport limitation.

The alternative approach of increasing the rate of enzyme turnover could be a more viable solution. The biocatalytic reaction of oxidase enzymes with their substrate involves a number of steps. First the oxidized enzyme binds with the substrate to form an enzyme substrate complex. This complex then dissociates to the product and a reduced form of the enzyme. The reduced enzyme is then converted back to its active oxidized form *via* a reaction with a cosubstrate; oxygen in nature but using the mediator *p*-benzoquinone in our reference system. The theory of the SAM enzyme electrodes suggests that the kinetic limitation is this last step, the turnover of the reduced enzyme to its active oxidized form by the cosubstrate.[2] One way to increase the rate of this step is by exploiting direct electron transfer between the enzyme and the electrode. In this way, the reduced enzyme is converted back to the active oxidized form by applying an appropriately oxidising potential to the underlying electrode. An additional advantage of the direct electron transfer approach is that it obviates the requirement for a cosubstrate. As the rate of electron transfer is highly sensitive to the distance between the redox centre and the electrode,[58] the small distance between the enzyme and the electrode that can be achieved with SAMs is ideal for this purpose. This is the approach that we are currently exploring in more detail.

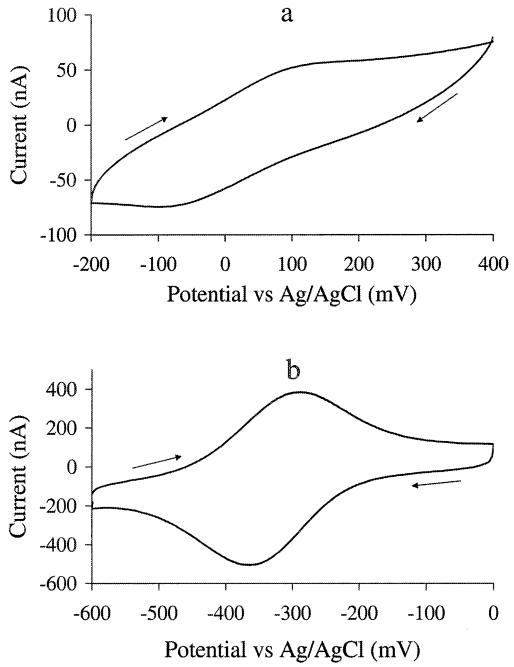

Fig. 7 Typical cyclic voltammograms of MPA-modified gold electrodes with a) HRP and b) MP-11 covalently attached. The enzymes were attached to the SAM using EDC and NHS. Buffer solution was 0.01 M HEPES, pH 7, scan rate, 20 mV s^{-1}.

Direct electron transfer

With some enzymes, most notably the peroxidases, the redox active centers of the enzyme is located sufficiently close to the surface of the glycoprotein to allow electrons to be transferred between the electrode and the enzyme directly.[24,59] The exponential decay in the rate of electron transfer with distance means the closer the enzyme to the electrode the more efficient is the electron transfer. Modifying a gold electrode with MPA and covalently attaching horseradish peroxidase (HRP) using EDC/NHS allows a sufficiently close approach between the enzyme and the electrode for electron transfer to proceed with a rate of 0.287 s^{-1} (see Fig. 7). If microperoxidase MP-11 (a catalytically active fragment of cytochrome *c*) is attached to the electrode rather than the much larger HRP a rate of electron transfer of 4.0 s^{-1} was observed. The higher rate of electron transfer with the smaller peroxidase enzymes is consistent with the observations of Lotzbeyer *et al.*,[19] who used a similar method of fabricating the electrode. However, despite this higher rate of electron transfer for the MP-11 modified electrode, the HRP biosensor gave a more reproducible and higher current response for a given concentration of hydrogen peroxide (Fig. 8). The higher current response despite the slower rate of electron transfer reflects the greater catalytic activity of HRP over MP-11.[20]

The number of enzymes that allow direct electron transfer however is very limited. One approach in the development of useful analytical devices using peroxidase enzymes is to integrate the peroxide sensitive enzyme with another oxidase enzyme which produces H_2O_2 as a product of the enzyme reaction. Such an integrated by enzyme electrode has been demonstrated previously.[59-62] However, the problem of monolayer enzyme electrodes being kinetically limited is not solved. Therefore, we are investigating generic strategies to obtain direct electron transfer to enzymes such as glucose oxidase. With glucose oxidase, the redox active center, flavin

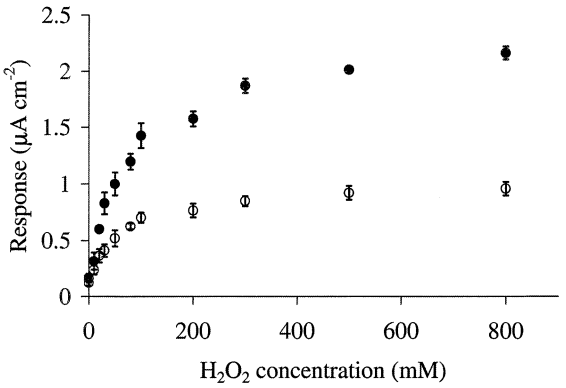

Fig. 8 Comparison of the catalytic reduction of H_2O_2 between ●, HPR and ○, MP-11 modified electrodes. The error bars represent a single standard deviation for 3 electrodes.

adenine dinucleotide (FAD), is embedded deep within the enzyme. Therefore, direct electron transfer is inefficient, although there is one report of it being achieved when glucose oxidase is attached to a SAM.[15] Willner and co-workers have developed a strategy for achieving more efficient communication with glucose oxidase where an electron relay (basically a mediator) is attached to both a SAM modified electrode and the active center of the enzyme. The apo-enzyme is then reconstituted over this FAD modified electrode. Although the electron transfer proceeds *via* a hopping mechanism rather than being direct this approach produces a very efficient monolayer enzyme electrode. This is an elegant and promising approach despite the fact that the shape of the calibration curve does suggest some minor interference from oxygen in the sample. We are developing a similar approach where a molecular wire is attached to the active center and then the enzyme is reconstituted. In this way, it is hoped that true electron transfer to glucose oxidase can be achieved.

Enzyme electrodes fabricated using self-assembled monolayers have considerable potential because of the spatial control over the enzyme immobilization. The covalent attachment of an enzyme to the surface of an alkanethiol modified electrode results in a monolayer of enzyme being immobilized. The reproducibility of fabrication is sensitive to the surface topography of the underlying gold electrode, the conditions during covalent attachment of the enzyme and the buffer used. The resultant monolayer enzyme electrode have excellent sensitivity and dynamic range which can easily be adjusted by controlling the amount of enzyme immobilized. The major drawback of such electrodes is that the response is limited by the kinetics of the enzyme rather than mass transport of substrates. A consequence of this kinetic limitation is any loss of enzyme activity will give a significant change in the biosensors response. Bringing SAM-enzyme electrodes into the mass transport limited regime may be achievable using single layer-by single layer fabrication methods to produce multilayers or by exploiting direct electron transfer between the enzyme and the electrode.

Acknowledgements

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