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Myoglobin in polyacrylamide hydrogel films: direct electrochemistry and electrochemical catalysis

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Abstract

Electrochemical behavior of myoglobin (Mb) incorporated in polyacrylamide (PAM) hydrogel films cast on pyrolytic graphite (PG) electrodes were investigated. Mb–PAM film electrodes showed a pair of well-defined and nearly reversible cyclic voltammetric peaks for Mb Fe(III)/Fe(II) redox couple at about -0.27 (vs. SCE) in pH 5.5 buffers. The electron exchange of Mb with PG electrodes was greatly enhanced in PAM films. The apparent heterogeneous electron transfer rate constant (k_s) and formal potential ($E^{\circ\prime}$) were estimated by fitting the data of square wave voltammetry (SWV) with non-linear regression analysis. The formal potential of Mb–PAM films shifted linearly with pH with a slope of -0.52 V, showing the electron transfer was accompanied by a single-proton transportation. Positions of Soret absorbance band of Mb–PAM films suggest that Mb maintains its secondary structure similar to its native state in the films in the medium pH range. Oxygen, trichloroacetic acid (TCA) and nitrite were catalytically reduced by Mb–PAM film electrodes with significant lowering of overpotential. Potential application of Mb–PAM films as biosensors to monitor some substrates was proposed.

Keywords: Myoglobin; Polyacrylamide; Film modified electrode; Electrochemistry; Electrocatalysis

1. Introduction

Study of direct electrochemistry of protein could provide a model for the mechanism study of electron transfer between proteins or enzymes in biological system [1]. It could also establish a foundation for fabricating mediator-free or the third generation biosensors [2]. Myoglobin (Mb) is a kind of heme protein. It contains a single polypeptide chain with an iron heme as its prosthetic group [3]. It is known that Mb stores and transports oxygen in muscle cells in mammals, and does not function physiologically as an electron carrier. However, Mb is considered to be an ideal model protein for the study of electron transfer of heme proteins or enzymes, because of its commercial availability, a known and documented structure, and enzyme-like oxidative and reductive catalytic activity [4,5].

Early electrochemical investigations of Mb focused on its polarographic reduction, showing

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that Mb could be reduced irreversibly on dropping mercury electrodes [6]. It was usually difficult for Mb in solution to transfer electron directly with bare or 'naked' solid electrodes, since unfavorable orientation of the protein on electrode surface may increase the distance between its heme redox center and the electrode surface. Another reason for the difficulty of electron exchange might be the adsorption of impurities in protein solution onto the surface of electrodes. The adsorption of the impurities or denatured protein might block the electron communication between the heme and electrodes. Great efforts have thus been made to enhance the electron transfer of Mb by using mediators or promoters [7–9].

A relatively new avenue to realize direct electrochemistry of protein is to incorporate protein into films modified on electrode surface. Thin films may provide a favorable microenvironment for protein and enhance electron transfer between the protein and electrodes. The protein films may also provide a new method for immobilization of protein on electrode surface, which may be applied more easily to fabricate the corresponding biosensor. Various kinds of Mb films have been developed. Rusling and coworkers reported that in biomembrane-like surfactant films cast on pyrolytic graphite (PG) electrodes, direct electron transfer for Mb heme Fe(III)/Fe(II) redox couple was greatly enhanced [10,11]. Recently, a kind of composite film was developed [12–14]. These polyion-surfactant or clay-surfactant composite films have biomembrane-like structure and fundamental characteristic similar to the surfactant films alone, and may have better stability than the latter because of introduction of polymer or clay backbones in the films. Mb incorporated in these films showed nearly reversible cyclic voltammograms (CV) [12–14]. The third type of film forming materials were amphiphilic polymers [15,16]. These polymer films could absorb a large amount of water from aqueous solution and form hydrogel, but they were water insoluble and very stable in aqueous solution. The hydrogel films might provide a favorable microenvironment for incorporated proteins. For instance, Mb in Eastman AQ films on PG electrodes showed quite reversible CV in blank buffers [15].

Polyacrylamide (PAM) is a kind of non-ionic polymer. The hydrogel formed from PAM was widely used in the field of life science. PAM has a long hydrophobic hydrocarbon backbone with many hydrophilic amide groups. Electrochemistry of proteins in PAM system has been studied. For example, Murray et al reported 'solid-state' voltammetry of cytochrome *c* in PAM gel solvent [17]. Recently, we found that hemoglobin (Hb) in PAM films modified on PG electrodes demonstrated quasi-reversible CV responses for Hb Fe(III)/Fe(II) redox couple [16]. Catalytic reduction of trichloroacetic acid (TCA) at Hb–PAM film electrodes was also studied.

We thus expect that Mb could also be incorporated in PAM films on electrodes and show direct electrochemical responses. In this paper, a quite reversible voltammogram of Mb in PAM films was obtained for its heme Fe(III)/Fe(II) couple, and Mb–PAM films were characterized by electrochemistry and spectroscopy. The catalytic reduction of oxygen, trichloroacetic acid and nitrite at Mb–PAM film electrodes were also studied.

2. Experimental

2.1. Reagents

Horse heart myoglobin (Mb) from Sigma was used as received without further purification. Polyacrylamide (PAM, MW 3 000 000) was from Shanghai Reagent Company. Trichloroacetic acid (TCA) was from Beijing Dongjiao Chemicals and sodium nitrite was from Beijing Sanhuan Chemicals. All other chemicals were reagent grade. The buffer was usually 0.1 M sodium acetate at pH 5.5 containing 0.1 M KCl. Other supporting electrolytes were 0.05 M potassium dihydrogen phosphate, 0.05 M boric acid or 0.05 M citric acid, all containing 0.1 M KCl. The pHs were adjusted with HCl or NaOH solutions. All water used was twice distilled water.

2.2. Preparation of Mb–PAM films

Prior to coating, basal plane pyrolytic graphite (PG, from the Chinese Geological Academy of

Science, geometric area 0.28 cm^2, or from Advanced Ceramics, geometric area 0.16 cm^2) disk electrodes were polished successively with metallographic sand papers (400 grit) and billiard cloth while flushing with water. Electrodes were then ultrasonicated in water for 30 s.

Ten microliters of 1 mg ml^{-1} PAM aqueous solutions were spread evenly onto a freshly abraded PG electrode with a microsyringe. A small tube was fit tightly over the electrode to serve as a closed chamber so that the water was evaporated slowly. After the films stood over night and were dried completely, 10 μl of 68 μM Mb in pH 5.5 buffers were cast onto the surface of the dry PAM films. After being dried in the chamber for over 5 h, the Mb–PAM films were further dried in air overnight.

2.3. Apparatus and procedures

A CHI 660 electrochemical workstation (CH Instruments) was used for cyclic voltammetry (CV) and square wave voltammetry (SWV). A conventional three-electrode cell was used with a saturated calomel electrode (SCE) as reference, a platinum wire as counter electrode, and a PG disk with films as working electrode. All experiments were done at room temperature (20 ± 2 °C).

Voltammetry at electrodes coated with Mb–PAM films was done in buffers containing no Mb. Buffers were purged with highly purified nitrogen for about 30 min before a series of experiments. A nitrogen environment was then kept over solutions in the cell during the experiment.

UV–Vis absorption spectroscopy was done with an UV-250 spectrophotometer (Shimadzu). Films were prepared by first depositing a few tens of microliters of 1 mg ml^{-1} PAM solutions onto surface of glass slides. After the PAM films became dry in an evaporation chamber, an equal volume of 68 μM Mb buffer was put onto the PAM film surface. The Mb–PAM films were then placed in the chamber overnight for being dried.

3. Results and discussion

3.1. Study of direct electrochemistry by CV

Fig. 1b shows the steady state CV of Mb–PAM films in pH 5.5 buffers. When a Mb–PAM film electrode was immersed into a pH 5.5 buffer solution containing no Mb, a pair of well-defined and nearly reversible CV peaks at about -0.27 V (vs. SCE) appeared immediately, characteristic of Mb heme Fe(III)/Fe(II) redox couple [10]. The peak current gradually increased with time at first and then reached the steady state after 12 h of soaking. Plain PAM films containing no Mb gave no CV response at all in this potential range (Fig. 1a). Thus, the following voltammetric experiments, except for those mentioned otherwise, were all performed after Mb–PAM films got to the steady state.

CVs of Mb–PAM films had approximately symmetric peak shapes and nearly equal heights of reduction and oxidation peaks. Reduction peak currents increased linearly with scan rates in the range of 0.02–2 V s^{-1}. Integration of reduction peaks at different scan rates gave nearly constant charge (Q) values. All these are characteristic of surface-control or thin-layer electrochemical behavior [18], indicating that all electroactive MbFe(III) within the films are converted to MbFe(II) on the forward cathodic scan, with full conversion of MbFe(II) back to MbFe(III) on the reverse anodic scan. According to the Γ^*-Q relationship [19], the average surface concentration of electroactive Mb (Γ^*) was estimated to be 1.98 ×

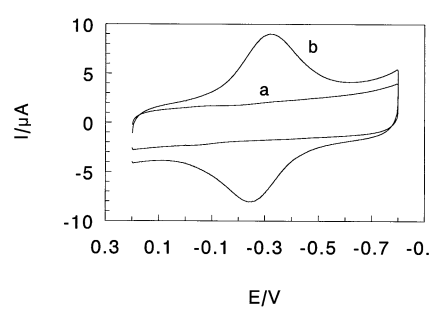

Fig. 1. Cyclic voltammograms at steady state at scan rate of 0.2 V s^{-1} in pH 5.5 buffer solutions containing no Mb for (a) PAM films and (b) Mb–PAM films. PG area 0.28 cm^2.

10^{-10} mol cm^{-2}, which accounted for only about 5% of the total amount of Mb deposited on the electrode surface. This may suggest that only those myoglobin in the inner layers of the films closest to electrodes and with a suitable orientation can exchange electrons with the electrodes and contribute to the observed redox reaction.

It is well known that Mb in solution had very poor CV at bare PG electrodes, showing it was difficult for Mb to transfer electron directly with underlying PG [10,15]. Thus, excellent CV response of Mb–PAM films indicates that PAM films must provide a suitable microenvironment for Mb to exchange electron with PG electrodes. The stability of Mb–PAM films was also investigated with CV. Here, two different methods were used. In the solution studies (method I), a Mb–PAM film electrode was stored in buffers all the time, and CVs were run periodically. Alternatively, with method II, Mb–PAM films were dried in air for most of the storing time and CVs were run periodically after returning the dry electrode into the buffer solution. With method I, CV reduction peak currents had a tendency of increasing with time during the first 12 h of soaking. Afterwards, the reduction peak currents were fairly stable for at least 1 month. In contrast, with method II, Mb–PAM films showed a much slower increasing tendency for CV reduction peak in the first 4 days, and then got to the steady state and displayed a quite stable response during at least 1 month. The different duration before getting to the steady state for different methods may be associated with the influence of water, as discussed later.

To examine the possibility of Mb entering PAM films from its solution, a plain PAM film electrode was immerged into a pH 5.5 buffer solution containing 68 μM Mb, and the CVs were run periodically. The redox peaks at about -0.27 V grew with immersion time (Fig. 2), suggesting that increasing amounts of Mb entered the PAM films. CVs representing the films fully loaded with Mb were obtained in about 5 days. These results cannot be explained by Coulombic attraction as in the situation of Mb–AQ film system [15]. At pH 5.5, with its isoelectric point at pH 6.8 [20], Mb shows positive surface charges, while PAM is

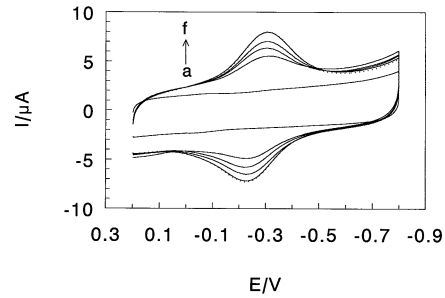

Fig. 2. Cyclic voltammograms at 0.2 V s^{-1} for PAM films immersed in pH 5.5 buffer solutions containing 68 μM Mb for: (a) 1 min; (b) 1 day; (c) 2 days; (d) 3 days; (e) 5 days; and (f) 6 days. PG area 0.28 cm^2.

essentially neutral on the whole with no extra surface charges. Thus, the driving force for Mb to enter PAM films would be mainly hydrophobic interaction between macromolecule Mb and PAM films, in which the long hydrocarbon backbone constitutes the hydrophobic region of the films. This hydrophobic interaction would also be mainly responsible for the retention of Mb in the films and good stability for Mb–PAM films in blank buffers.

Compared with the steady state CV for cast Mb–PAM films, the PAM films fully loaded with Mb had very similar peak positions and a little smaller peak currents. Mb–PAM films can be prepared either by cast or by loading method, but the former is more convenient and quantitative. Thus, in the following experiments, all Mb–PAM films were prepared by the cast method.

3.2. Estimation of parameters by SWV

SWV, basically as a pulse method, is easier to analyze quantitatively and treat theoretically over CV [21], and was used here to estimate the formal potential ($E^{\circ\prime}$) and apparent heterogeneous electron transfer rate constant (k_s). The procedure employed non-linear regression analysis for SWV forward and reverse curves, with combination of the single species thin-layer SWV model [22] and the $E^{\circ\prime}$ dispersion model, as described in detail previously [11,23].

Examples of analysis of SWV data for Mb–PAM films showed goodness of fit onto the mod-

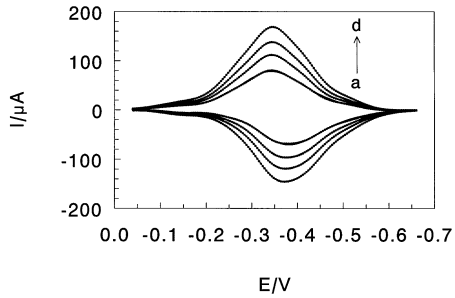

Fig. 3. Square wave forward and reverse current voltammograms for Mb–PAM in pH 7.0 buffer solutions at different frequencies. Points represent the experimental SWVs from which background has been subtracted. The solid lines are the best fit by non-linear regression onto the 5 $E^{\circ\prime}$ dispersion model. SWV conditions: pulse height 60 mV, step height 4 mV, and frequencies (Hz): (a) 152; (b) 179; (c) 200; (d) 225. PG area 0.28 cm^2.

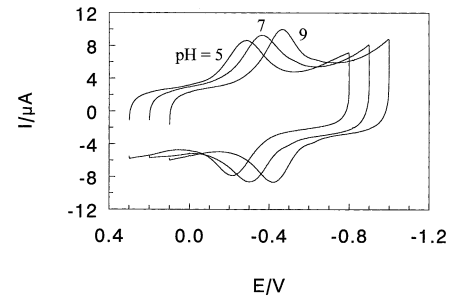

Fig. 4. Cyclic voltammograms for Mb–PAM films at 0.2 $V\,s^{-1}$ in buffers at different pH values. PG area 0.28 cm^2.

els over a range of frequencies (Fig. 3). The average apparent heterogeneous electron transfer rate constant (k_s) obtained from fitting SWV data at pH 7.0 was 86 s^{-1}, and the average $E^{\circ\prime}$ was -0.357 V versus SCE (Table 1). Parameters for other Mb films and Hb–PAM films obtained by the same method are listed in Table 1 for comparison.

For Mb–PAM films, the value of apparent rate constant (k_s) is larger than that of Mb in other films, but in the same magnitude with them. The formal potential ($E^{\circ\prime}$) of Mb Fe(III)/Fe(II) redox couple in PAM films is close to that of Mb–AQ films and more negative than that in other Mb films and Hb–PAM films (Table 1). This confirms a specific influence of film environment on $E^{\circ\prime}$ of heme proteins which had been reported previously [11,23]. Film components may change formal potential through interaction with the protein or by their influence on the electrode double-layer.

3.3. Influence of pH on voltammetry

pH of external solutions had significant influence on CV peak potentials of Mb–PAM films. Both reduction and oxidation peaks shifted negatively with increasing pH (Fig. 4). In general, all changes in CV peak potentials with pH were reversible between pH 5.0 and 9.0. CV data were

Table 1
Apparent heterogeneous electron transfer rate constants and formal potentials for various Mb films and Hb–PAM films on PG electrodes in pH 7.0 buffers

Films[a]	Average k_s (s^{-1})	Average $E^{\circ\prime}$ vs. SCE (V)		Reference[b]
		CV	SWV	
Mb–PAM	86 ± 19	-0.335	-0.357	tw
Mb–DDAB	31 ± 3	-0.228	-0.240	11
Mb–DDAB–PVS	58 ± 7	-0.196	-0.202	12
Mb–DHP–PDDA	27 ± 3	-0.323	-0.326	13
Mb–DDAB–clay	44 ± 8	-0.252	-0.243	14
Mb–AQ	52 ± 6	-0.362	-0.340	15
Hb–PAM	45 ± 8	-0.320	-0.312	16

[a] PAM = Polyacrylamide, DDAB = didodecyldimethylammonium bromide, PVS = polyvinylsulfate, DHP = dihexadecylphosphate, PDDA = polydiallyldimethyl ammonium, AQ = poly(ester sulfonic acid) or Eastman AQ.

[b] tw: this work, reporting average values for analysis of eight SWVs at frequencies of 152–225 Hz, amplitudes of 60–75 mV, and a step height of 4 mV.

used to investigate the pH effect on the formal potential ($E^{\circ\prime}$) for Mb Fe(III)/Fe(II) redox couple, which was estimated as the midpoint of CV reduction and oxidation peak potentials. $E^{\circ\prime}$ had linear relationship with pH in the range of pH 5.0–11 with a slope of -52 mV pH^{-1}. This value is reasonably close to the theoretical value of -58 mV pH^{-1} at 20 °C for reversible one-electron transfer coupled by single-proton transportation [24,25], indicating that a single protonation accompanies the electron transfer of MbFe(III) to electrodes. Thus, the simplified equation for the electrochemical reduction of Mb in PAM films can be expressed as

$$\mathrm{MbFe(III)} + \mathrm{H^+} + \mathrm{e^-} \rightleftharpoons \mathrm{MbFe(II)} \quad (1)$$

An inflection point appeared in the plot at pH 5.0. At pH < 5.0, $E^{\circ\prime}$ values also varied linearly with pH but with a smaller slope of -31 mV pH^{-1}. Similar phenomenon was also observed in Hb–PAM films [16].

3.4. Conformational study by UV − Vis spectroscopy

Positions of the Soret absorption band of heme may provide information about possible denaturation of heme protein, especially that of conformational change in the heme group region [26]. Thus, UV–Vis spectroscopy is a useful tool for conformational study of heme proteins. Both dry films cast from Mb and Mb–PAM on glass slides showed Soret bands at 406 nm (Fig. 5a and b), suggesting that Mb in dry PAM films has a secondary structure nearly the same as the native state of Mb in dry Mb films alone. The position of Soret band depended on pH when Mb–PAM films were immersed into buffer solutions. At pH between 4.0 and 11.0, the Soret band appeared at 402 nm (Fig. 5d–i), same as that of Mb in buffer solutions at medium pH, but different from that of dry Mb or Mb–PAM films. When pH was changed towards more acidic direction, the shape of Soret band showed significant change. For example, the Soret band of Mb–PAM on glass slides tended to become smaller or even disappeared at pH 3.0 (Fig. 5c), indicating Mb in PAM films might denature to a considerable extent in this relatively low pH environment.

3.5. Influence of water

Our previous work showed that both PAM and Hb–PAM films could absorb large amounts of water and form hydrogel in solution [16]. In order to confirm this in the present situation, the weighing method was also used to estimate the relative amount of water absorbed by PAM and Mb–PAM films. Completely dry PAM and Mb–PAM films cast on glass slides were soaked in water for over 2 days so that the films were fully swelled and saturated by water. Both swelled films had much larger volume than the dry one with translucent appearance, indicating the loosening of polymer packing density and forming of hydrogel. The weighing results before and after hydration showed that water accounted for about 95% in fully swelled films for both PAM and Mb–PAM films. Thus, PAM films provided a basically aqueous environment for Mb. That may explain why hydrated Mb–PAM films had the Soret band exact the same as that of Mb in buffers at medium pH, but different than that of dry Mb–PAM films. Hydrogel PAM films also provided a suitable microenvironment for Mb to transfer electron with underlying electrodes. The gradual increase of CV reduction peak current of Mb–PAM films with soaking time in buffers dur-

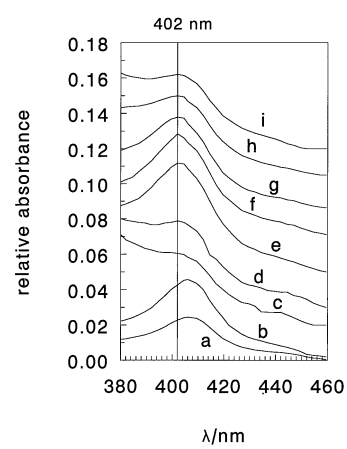

Fig. 5. UV–Vis spectra of Mb and Mb–PAM films on glass slides: (a) Mb dry films, (b) dry Mb–PAM films and Mb–PAM films in different pH buffer solutions: (c) pH 3.0, (d) pH 4.0, (e) pH 5.0, (f) pH 7.0, (g) pH 9.0, (h) pH 10.0 and (i) pH 11.0.

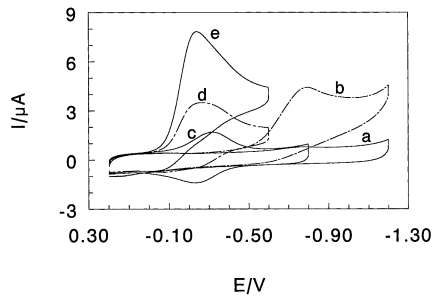

Fig. 6. Cyclic voltammograms at 0.1 V s^{-1} in 10 ml of pH 5.5 buffers for: (a) PAM films with no oxygen present; (b) PAM films after 20 ml of air was injected into a sealed cell; (c) Mb–PAM films with no oxygen present; (d) Mb–PAM films after 10 ml of air was injected; (e) Mb–PAM films after 20 ml of air was injected. PG area 0.16 cm^2.

ing the first 12 h not only suggests the relatively slow hydration process of PAM films, but also indicates that the fully hydrated films are more favorable for the electron exchange of Mb.

3.6. Catalytic reactivity

Electrocatalytic reduction of oxygen by Mb–PAM films was examined with CV (Fig. 6). When a certain amount of air was passed through a pH 5.5 buffer solution by a syringe, a significant increase in reduction peak at about -0.32 V was observed for Mb–PAM films, accompanied by the disappearance of the oxidation peak for MbFe(II). The reduction peak current increased with the amount of oxygen in solution. For PAM films with no Mb incorporated, the peak for direct reduction of oxygen was observed at about -0.8 V, far more negative than the catalytic peak potential. Thus, the overpotential required for reduction of O_2 was lowered by about 0.5 V by Mb–PAM films. Catalytic efficiency expressed as the ratio of reduction peak current of MbFe(III) in the presence (I_c) and absence of oxygen (I_d), I_c/I_d, decreased with increase of scan rate, also characteristic of electrochemical catalysis [27,28].

Organohalides are recognized as widespread environmental pollutants arising from various industrial as well as smaller scale sources [29]. A simple, general-purpose organohalide sensor that could directly detect the organohalides could be of significance. Here, electrocatalytic reduction of trichloroacetic acid (TCA) was tested by CV with Mb–PAM films (Fig. 7). When TCA was added to a buffer at pH 3.0, an increase of MbFe(III) reduction peak at about -0.30 V was observed, accompanied by a decrease of MbFe(II) oxidation peak, since some MbFe(II) had reacted with TCA. The reduction peak current increased with the concentration of TCA. These results indicate chemical reaction of MbFe(II) with TCA in a catalytic cycle, resulting in producing of MbFe(III) again and reductive dechlorination of TCA [30]. Direct reduction of TCA at blank PAM film electrodes showed a very small wave at about -0.45 V. Thus, Mb–PAM films lowered the reduction overpotential for TCA by about 0.15 V. The catalytic efficiency, I_c/I_d, decreased with increase of scan rate, also characteristic of electrochemical catalysis [27,28].

Other organohalides, such as α-bromopropionic acid and monochloroacetic acid, were also tested with Mb–PAM films for electrocatalysis. However, these two organohalides did not show any catalytic property at Mb–PAM films, indicating the films have some selectivity towards the substrates, although the mechanism is not yet clear by now.

The catalytic reduction of TCA by Mb–PAM films can be used to determine TCA in solution. The calibration curve for the system showed that the reduction peak height of TCA on Mb–PAM

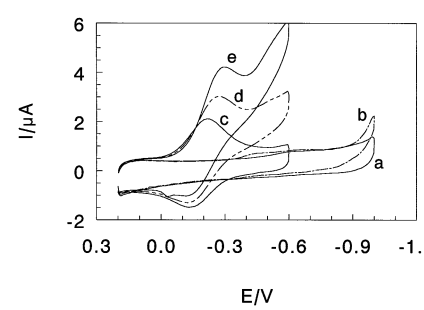

Fig. 7. Cyclic voltammograms at 0.1 V s^{-1} in pH 3.0 buffer solutions for: (a) PAM films with no TCA present; (b) PAM films with 6.45 mM TCA present in solution; (c) Mb–PAM films with no TCA present; (d) Mb–PAM films with 3.28 mM TCA present; (e) Mb–PAM films with 9.52 mM TCA present. PG area 0.16 cm^2.

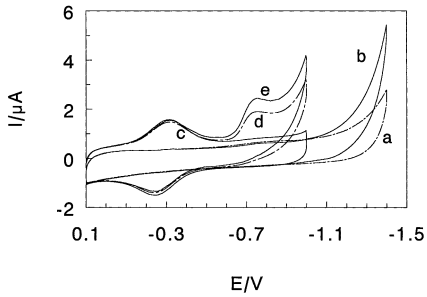

Fig. 8. Cyclic voltammograms at 0.1 V s^{-1} in pH 5.5 buffers for: (a) PAM films with no $NaNO_2$ present; (b) PAM films with 0.5 mM $NaNO_2$ present in solution; (c) Mb–PAM films with no $NaNO_2$ present; (d) Mb–PAM films with 0.3 mM $NaNO_2$ present; (e) Mb–PAM films with 0.5 mM $NaNO_2$ present. PG area 0.16 cm^2.

films had a linear relationship with TCA concentration in the range of 0.3–12.5 mM with correlation coefficient of 0.997 and detection limit of 0.2 mM. The slower increase of peak currents with TCA concentration was observed when the concentration of TCA was above 12.5 mM.

Farmer et al. studied electrocatalytic reduction of nitrite with Mb–didodecyldimethylammonium bromide (DDAB) films modified on PG electrodes [31]. Similar results were found at Mb–PAM film electrodes (Fig. 8). When NO_2^- was added into a pH 5.5 buffer, a new catalytic reduction peak at about -0.75 V was observed while the Mb Fe(III)/Fe(II) peak pair remained intact. The catalytic peak current increased with the concentration of NO_2^- in the linear range of 0.17–3.2 mM with correlation coefficient of 0.996 and detection limit of 0.1 mM. In the higher concentration range of 3.2–10.4 mM, the calibration curve remained linear with a smaller slope ($r = 0.996$). Direct reduction of nitrite at plain PAM film electrodes was found to begin at the potential more negative than -1.1 V. Thus, Mb–PAM films lowered the overpotential for NO_2^- reduction by at least 0.4 V. Although the mechanism of NO_2^- reduction on Mb–PAM films is not yet very clear, it is probably similar to that on Mb–DDAB films [31]. At Mb–DDAB film electrodes, the catalytic reduction product was confirmed to be N_2O, which was detected by mass spectroscopy on electrolysis at -0.895 V in pH 7 buffers.

Mb in PAM films acted here like a reductive enzyme and catalyzed a variety of substrates. The decrease of overpotential required for reduction of O_2, TCA and NO_2^-, the selectivity of the films for different substrates, and the good stability of the films might be used as a foundation for fabricating enzyme-like biosensors in monitoring different substrates such as organohalide pollutants.

4. Conclusions

Fully hydrated PAM films have nearly 95% water contents and form hydrogel, which may provide a favorable microenvironment for electron exchange between Mb and underlying electrodes. Enhanced electron transfer rates were comparable to those for Mb in other films and much faster than those on bare PG electrodes in Mb solutions. The Mb–PAM films are presumably stabilized mainly by hydrophobic interactions between the protein and polymer. Mb essentially retained its native structure in PAM films at medium pH. Mb–PAM films on electrodes can catalyze reduction of oxygen, trichloroacetic acid and nitrite, and may have a potential application in fabricating mediator-free biosensors.

Acknowledgements

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