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PREPARATION AND PROPERTIES OF THE POLY (NEUTRAL RED) HORSERADISH PEROXIDASE ENZYME ELECTRODE

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Abstract: A new hydrogen peroxide biosensor was constructed by immobilization of horseradish peroxidase (**HRP**) in poly (neutral red) (**PNR**) film. The conditions for the electropolymerization of neutral red at glassy carbon electrode to form the PNR membrane have been carefully studied with orthogonal design. On this basis, HRP was added to the electrolyte, and with the electropolymerization of neutral red, HRP was immobilized in the PNR membrane to construct poly (neutral red) horseradish peroxidase enzyme electrode, which was designed as PNR/HRP. The bioelectrocatalytic reduction of H_2O_2 at the enzyme electrode was studied. The electrode exhibits an excellent activity to catalyze the reduction of H_2O_2 in the absence of any electron transfer mediator, and the response current is proportional to H_2O_2 concentration in the range of $5.0 \times 10^{-5} \sim 1.0 \times 10^{-3}$ mol/L^{-1}, with the correlation coefficient $r = 0.9995$ ($n = 6$). Moreover, the PNR/HRP enzyme electrode can be easily fabricated, and its reproducibility and lifetime are perfect. So the sensor can be used for the conventional detection of H_2O_2.

1 Introduction

The most important work in constructing enzyme biosensors is the immobilization of enzymes. In order to obtain the sensitive, stable and practical biosensors, different kinds of immobilization techniques have been developed. Electropolymerization is one of the most advanced and simple methods. Immobilization of enzyme molecules into electrochemically polymerized conducting or non-conducting polymer matrices has become an attractive approach for preparation of enzyme-based biosensors over the past decade. The advantages and applications have also been reviewed [1,2]. Up to present, the fabrication of enzyme electrodes by immobilization of some kinds of enzymes in electropolymerized films has been reported [3-9]. Among them, the monomers used to form the polymer membrane by electropolymerization are pyrrole [3-5], phenol [6,7], o-phenylenediamine [8] and quinone [9] et al. However, there are few reports with organic dyes such as phenazines, phenothiazines and phenoxazines as monomers to construct enzyme-based biosensors. These dyes which have a big heterocycle conjugate system have similar molecule structure, and fine electrochemical activity. They contain electron-donating pendant amine groups or hydroxyl groups, and at least one ortho or para unsubstituted position. So they can be electropolymerized at electrode to form conducting polymer films [10, 11]. If these dyes can be used to immobilize enzymes by

electropolymerization and construct biosensors, the sensors will be of good electric conductivity and a high rate of electron transfer without any electron transfer mediator, and accelerate the reaction and minimize the interference. The biosensors studied before are nearly all monocycle substances, while these dyes all have no less than three cycles in their structures. The previous investigation [12] has manifested that the electrocatalytic activity and stability of substances with more conjugate cycles are better than those of the substances with less cycles. Therefore, the phenazine, phenothiazine and phenoxazine dyes are likely to be ideal monomers for constructing biosensors with electropolymerization. If that could be put into practice, large kinds of available monomers, would be found and the space of fabricating applicable biosensors with electropolymerization would be greatly broadened.

In this work, neutral red, which belongs to phenazine dyes, was chosen as the monomer for electropolymerization to immobilize HRP. Karyakin et al [13] once used the PNR film electropolymerized on electrode to construct an alcoholdehydrogenase biosensor, but their method was putting a layer of Nafion® membrane which contains enzyme onto the PNR film, and the enzyme immobilization mode should be ascribed to entrapment [14].

In this paper, HRP was immobilized during the electropolymerization of neutral red on glassy carbon electrode, and the PNR/HRP enzyme electrode was fabricated for the first time. This new HRP biosensor possesses good bioelectrocatalytic reduction to H_2O_2, and the electron transfer (**ET**) of HRP is direct ET mechanism [15]. The catalytic current is linearly related to H_2O_2 concentration in the range from 5.0×10^{-5} to $1.0 \times \times 10^{-3}$ mol L^{-1} with the correlation coefficient r = 0.9995 (n = 6). The fabrication of PNR/HRP enzyme electrode is simple and its stability is good. The electrode can be stored for more than 70 days and is suitable for the conventional detection of H_2O_2. This success of constructing enzyme electrode with electropolymerization of neutral red gives light to the studies on other phenazines, phenothiazines and phenoxazines dyes in the similar usage.

2 Experimental

2.1 Reagents

Horseradish peroxidase (EC 1.11.1.7, 250 U/mg, R.Z.>3.0) was produced by Shanghai Lizhu Dongfeng Biochemical Technique CO., LTD. and neutral red (reagent grade) by Shanghai No. 3 Reagent Factory; 30 % hydrogen peroxide (analytical grade) was purchased from Qingdao Huanghai Hydrogen Peroxide Factory, and the exact concentration of the 30 % hydrogen peroxide was determined by titrating with potassium permanganate. H_2O_2 was freshly diluted from the 30 % solution prior to use. All the other chemicals were of analytical grade, and all solutions were prepared with distilled water. All the experiments were carried out without deaeration and at 15 °C if not mentioned.

2.2 Apparatus

A model CHI 832 electrochemical analyzer (CH Instruments, Shanghai) was employed, and an IBM 586 computer was connected with model CHI 832 for controlling measurements and treating data. All electrochemical experiments were carried out with a conventional three-electrode system comprising of a platinum foil counter electrode, a saturated calomel electrode (SCE) and a glassy carbon electrode or a modified electrode as working electrode.

2.3 Preparation of the enzyme electrode

Prior to use, the glassy carbon electrode was polished with diamond paper followed by 0.05 μm Al_2O_3 slurry on a microcloth, and then sonicated for 5 min in an ultra-sonic cleaner containing distilled water. The electrode was then transferred to a 0.2 mol L^{-1} phosphate buffer (pH 7.0) and oxidized at potential of + 0.2 V for 100 s, followed by a cathodization of the electrode at – 1.1 V for 50 s. The electrode was further activated by successively scanning the potential between – 1.1 V and 0.9 V at a scan rate of 200 mV s^{-1} until the cyclic voltammetric curves (CV) were stable.

The immobilization of HRP on glassy carbon electrode was performed by scanning the potential between –1.2 and 1.8 V at a scan rate of 100 mV s^{-1} for two cycles in an unstirred phosphate buffer (pH 7.0) containing 5.0×10^{-4} mol L^{-1} neutral red and 0.1 mol L^{-1} $NaNO_3$, then 2.0 g L^{-1} HRP was added to the electrolyte and continued scanning by successively scanning the potential between – 0.8 and 0.6 V at a scan rate of 50 mV s^{-1} for 10 cycles. The enzyme electrode was thoroughly rinsed with distilled water and stored in pH 7.0 phosphate buffer at 4 °C when not in use. The enzyme-free PNR film modified electrode was prepared in a similar way, but in the absence of HRP.

2.4 Measurement of the enzyme electrode performance

The responses of the PNR/HRP enzyme electrode to H_2O_2 were measured by CV between the potential –0.8 V and 0.2 V at a scan rate of 50 mV s^{-1}. The reduction currents at – 0.70 V on the CV curves were recorded, and the difference of measured values between the sample containing a known concentration of H_2O_2 and the blank was taken as the response value of the enzyme electrode.

3 Results and discussion

3.1 Electropolymerization of neutral red on glassy carbon electrode

The electropolymerization of neutral red has been studied before [11, 13, 16], but the conditions for polymerization in this work are somewhat different from those in these literatures. The conditions that influence the formation of PNR membrane include the concentrations of neutral red and the supporting electrolyte, which is $NaNO_3$ in this study, the pH value of buffer solution, the scanning rate and the time of electropolymerization when employing CV. These conditions were investigated with orthogonal design. The factors and levels chosen are listed in table 1. Ignoring the interactions among the factors, the experiments were planed according to orthogonal layout $L_{16}(4^5)$. In these experiments, neutral red was electropolymerized on glassy carbon electrode to construct PNR film modified electrodes, and then the modified electrodes were used to detect ascorbic acid employing CV between potential – 0.6 and 1.0 V at a scan rate of 50 mV s^{-1}. The oxidation peak currents and potentials were recorded as the designation of the properties of the formed PNR films: the larger current and lower potential the electrode possessed, the better the film was. The experimental plan, the experimental data and analysis of the results are shown in table 2. According to the characteristics of orthogonal design, we could infer from table 2 that the optimum conditions should be $A_3B_4C_4D_3E_4$, that is, when the electropolymerization is performed in the electrolyte that composed of phosphate buffer (pH 6.0) containing $5.0 \times \times 10^{-5}$ mol L^{-1} neutral red and 1.0 mol L^{-1} $NaNO_3$ by successive scanning potential between – 0.8 and 0.6 V at the scan rate of 50 mV s^{-1} for 10 cycles, it produces a more stable and conductive film, and this was testified by further experiment.

Table 1

Factors and levels of orthogonal design

factor / level	A	B	C	D	E
	pH	C_{NR}, mmol L^{-1}	C_{NaNO3}, mol L^{-1}	cycle	scan rate, mV s^{-1}
1	6.5	0.1	0.2	20	100
2	7.5	1.0	0.5	25	20
3	6.0	0.5	0.1	10	10
4	7.0	0.05	1.0	15	50

Table 2

Experimental plan for electropolymerization of neutral red on glassy carbon electrode, and the records and analysis of the results

factor / No.	A	B	C	D	E	results	
	pH	C_{NR}, mmol L^{-1}	C_{NaNO3}, mol L^{-1}	cycle	scan rate, mV s^{-1}	current, μA	potential, V
1	1 (6.5)	1 (0.1)	1 (0.2)	1 (20)	1 (100)	121.0	0.408
2	1 (6.5)	2 (1.0)	2 (0.5)	2 (25)	2 (20)	110.7	0.474
3	1 (6.5)	3 (0.5)	3 (0.1)	3 (10)	3 (10)	122.1	0.486
4	1 (6.5)	4 (0.05)	4 (0.1)	4 (15)	4 (50)	197.0	0.340
5	2 (7.5)	1 (0.1)	2 (0.5)	3 (10)	4 (50)	185.4	0.353
6	2 (7.5)	2 (1.0)	1 (0.2)	4 (15)	3 (10)	104.4	0.466
7	2 (7.5)	3 (0.5)	4 (1.0)	1 (20)	2 (20)	124.8	0.499
8	2 (7.5)	4 (0.05)	3 (0.1)	2 (25)	1 (100)	159.8	0.392
9	3 (6.0)	1 (0.1)	3 (0.1)	4 (15)	2 (20)	159.5	0.425
10	3 (6.0)	2 (1.0)	4 (1.0)	3 (10)	1 (100)	166.4	0.361
11	3 (6.0)	3 (0.5)	1 (1.2)	2 (25)	4 (50)	122.6	0.485
12	3 (6.0)	4 (0.05)	2 (0.5)	1 (20)	3 (10)	133.3	0.128
13	4 (7.0)	1 (0.1)	4 (1.0)	2 (25)	3 (10)	146.3	0.432
14	4 (7.0)	2 (1.0)	3 (0.1)	1 (20)	4 (50)	99.16	0.488
15	4 (7.0)	3 (0.5)	2 (0.5)	4 (15)	1 (100)	146.7	0.398
16	4 (7.0)	4 (0.05)	1 (0.2)	3 (10)	2 (20)	149.2	0.402
K_1	550.8	612.2	497.2	478.26	593.9		
K_2	574.4	480.66	571.6	539.4	544.2		
K_3	581.8	516.2	540.56	623.1	506.1		
K_4	541.36	639.3	634.5	607.6	604.16		
k_1	137.7	153.05	124.3	119.56	148.48		
k_2	143.6	120.16	144.02	134.85	136.05		
k_3	145.45	129.05	135.14	155.78	126.52		
k_4	135.34	159.82	158.62	151.9	151.04		
range	40.44	158.64	137.3	144.84	98.06		

3.2 Electrochemical immobilization of HRP

The most important procedure in constructing an enzyme electrode is the immobilization of enzyme. In order to immobilize HRP in the PNR film during the electropolymerization and ensure that HRP keeps its biologic activity, the conditions for electropolymerization are different from the enzyme-free ones apparently. The electropolymerization of neutral red without HRP is more suitable under conditions of weak acid, low neutral red concentration and high $NaNO_3$ concentration, as we can see from the above orthogonal design. However, after the addition of HRP to the solution, the influence of pH value and the ionic strength on the activity of HRP should be taken into the consideration. The results of experiments illustrate that the immobilization of HRP should perform at neutral solution and lower $NaNO_3$ concentration (0.1 mol L^{-1}). This conclusion was in accord with references [3, 17]. Moreover, the electropolymerization of neutral red need to be initiated at a high potential [11] while the high potential may cause the decrease of HRP activity. Thus, we added HRP to the electrolyte after two cycles at a higher potential range and then performed the experiment at a lower potential range. On the same conditions, the higher neutral red concentration leads to the thicker PNR film [10]. By altering the concentration of neutral red and the cycles of electropolymerization, the thickness of the film and the amount of HRP immobilized on the electrode can be controlled.

3.3 Characteristics of the PNR/HRP enzyme electrode

3.3.1 Catalytic reduction of H_2O_2 at the PNR/HRP enzyme electrode

Fig. 1 shows the CV curves of the reduction of H_2O_2 in phosphate buffer (pH 7.5) at glassy carbon electrode, PNR film modified electrode and PNR/HRP enzyme electrode. The reduction currents increased with the potential shift negatively and the reduction process is irreversible. On the same conditions, the reduction current at PNR/HRP enzyme electrode is bigger than that on PNR film modified electrode, and the latter is bigger than that on glassy carbon electrode. While the reduction current at PNR film modified electrode is bigger than that at glassy carbon electrode indicates that the PNR film can accelerate the reduction of H_2O_2, which should be ascribed to the conductivity of the PNR film.

The enzymatic reduction of HRP takes part in three steps [18-21]:

$$HRP + H_2O_2 \rightarrow \text{Compound} - I \quad (1)$$

$$\text{Compound} - I + AH_2\,(\text{or } e^-) \rightarrow \text{Compound} - II + AH\bullet \quad (2)$$

$$\text{Compound} - II + AH_2\,(\text{or } e^-) \rightarrow HRP + AH\bullet + H_2O \quad (3)$$

Where Compound – I and Compound – II are oxidized enzyme intermediates, AH_2 refers to organic substances such as phenols and aromatic amines. Since the PNR/HRP enzyme electrode and the experimental solution contain no other substrates, we can infer that the catalytic reduction of H_2O_2 by HRP is performed through the first reaction and produced Compound – I, and then Compound – I (subsequently Compound – II) was directly reduced back to native HRP on the electrode surface in a direct electron transfer way.

3.3.2 Effect of pH on the response current of the PNR/HRP enzyme electrode

The response current of the PNR/HRP enzyme electrode in 0.2 mol L^{-1} phosphate buffer containing 5.0×10^{-4} mol L^{-1} H_2O_2 is shown as a function of pH in Fig. 2. The maximum response current was obtained at pH 7.5, i.e. the optimum pH of HRP immobilized in the PNR film is about 7.5. The optimum pH of native HRP is pH 7.0 [22, 23]. Comparing the results in Fig. 2 with the optimum pH of native HRP, we can see that the optimum pH of immobilized HRP is close to that of native HRP. This means that the activity of HRP is almost not affected after immobilization.

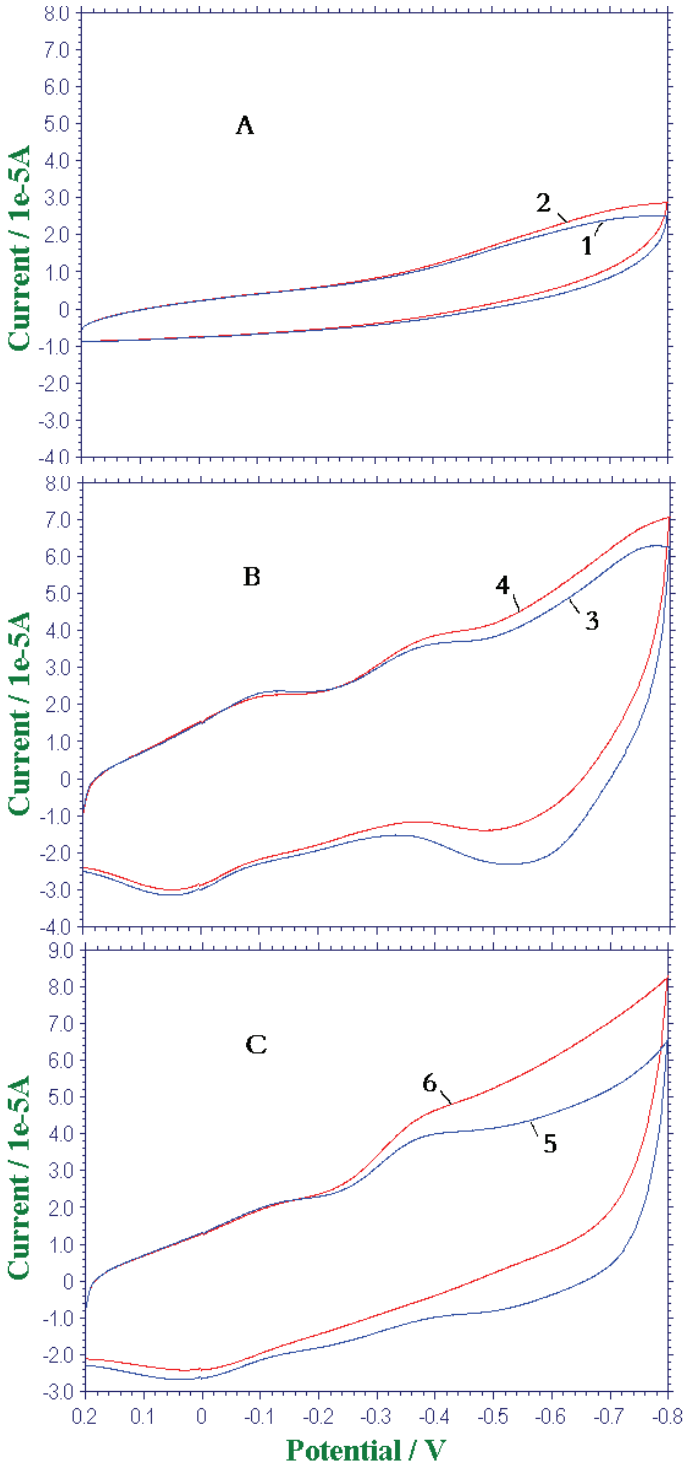

Fig. 1 The CV curves of different electrodes in the pH 7.5 phosphate buffer with (curve *1*, *3*, *5*) and without (curve *2*, *4*, *6*) 1.0 × 10^{-3} mol L^{-1} H_2O_2; A: glassy carbon electrode; B: PNR film modified electrode; C: PNR/HRP enzyme electrode. Scanning rate; 50 mV s^{-1}

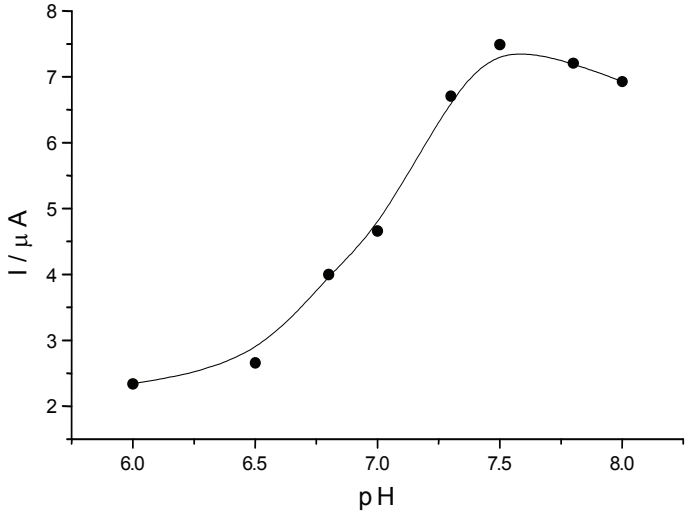

Fig. 2 The relationship between the pH value and the response current of the PNR/HRP enzyme electrode in 0.2 mol L^{-1} phosphate buffer containing 5.0×10^{-4} mol L^{-1} H_2O_2, at 15°C

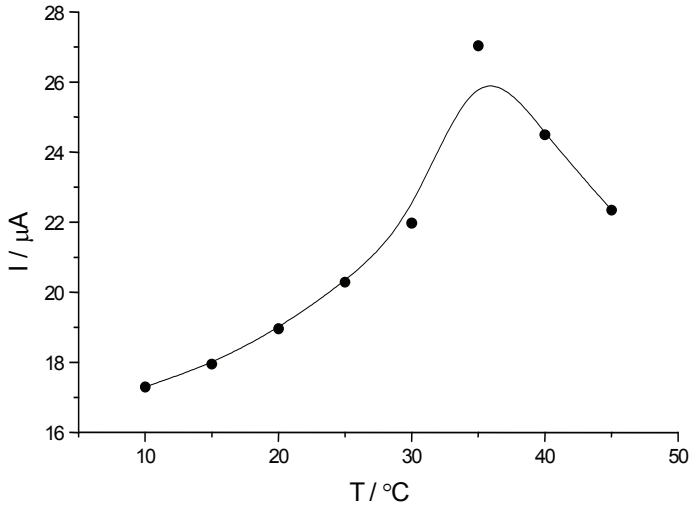

Fig. 3 The relationship between temperature and the response current of the PNR/HRP enzyme electrode in 0.2 mol L^{-1} phosphate buffer containing 1.0×10^{-3} mol L^{-1} H_2O_2, pH 7.5

3.3.3 Effect of temperature on the response current of the PNR/HRP enzyme electrode

The relationship between temperature and the response current of the PNR/HRP enzyme electrode in the phosphate buffer containing 1.0×10^{-3} mol L^{-1} H_2O_2 with pH 7.5 is shown in Fig. 3. The response current increases with increasing temperature between 10 and 35 °C, and then decreases as the temperature increases further. The maximum response current appears at 35 °C. This result is near to that of the polyaniline horseradish peroxidase electrode fabricated by Y. Yang et al [24] with glassy carbon electrode.

3.3.4 Effect of H_2O_2 concentration on the response current of the PNR/HRP enzyme electrode

The relationship between the response current of the PNR/HRP enzyme electrode and the H_2O_2 concentration at pH 7.5 is shown in Fig. 4. The response current increases linearly with increasing H_2O_2 concentration below 2.0×10^{-3} mol L^{-1}, and then increases more slowly with further increase of H_2O_2 concentration. The linear response currents to H_2O_2 concentration is in the range of 5.0×10^{-5} to 1.0×10^{-3} mol L^{-1}, and the regression equation is: I(μA) = – 0.53 + 18.13 C (mmol L^{-1}) with the correlation coefficient $r = 0.9995$ ($n = 6$). The detection limit was 3.0×10^{-5} mol L^{-1}.

3.3.5 Reproducibility and lifetime of the PNR/HRP enzyme electrode

The reproducibility of the PNR/HRP enzyme electrode was tested by measuring the response current of 1.0×10^{-3} mol L^{-1} H_2O_2 in phosphate buffer (pH 7.5) for 7 times, and the results are listed in table 3. The enzyme electrode possessed good reproducibility.

The lifetime of the PNR/HRP enzyme electrode was investigated by measuring the response current of 2.0×10^{-3} mol L^{-1} H_2O_2 in phosphate buffer (pH 7.5) at intervals of 2~3 days. The enzyme electrode was stored in 0.2 mol L^{-1} phosphate buffer (pH 7.0) at 4 °C between the measurements. The results are shown in Fig. 5. The enzyme electrode exhibited very good long-time stability, and there was no apparent loss of sensitivity after 70 days storage.

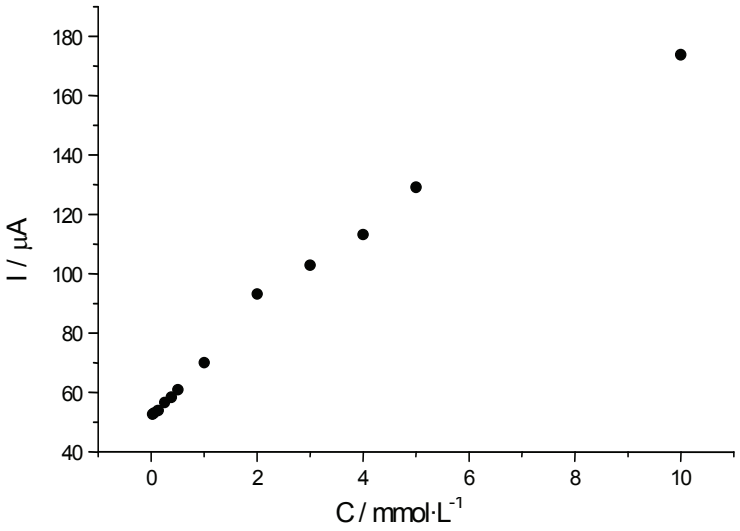

Fig. 4 The relationship between the response current of the PNR/HRP enzyme electrode and the concentration of H_2O_2 in 0.2 mol L^{-1} phosphate buffer (pH 7.5) at 15 °C

Table 3

The reproducibility and result of the detection of sample with PNR/HRP electrode

Sample	detected I (μA)	average I (μA)	RSD	detected concentration	recovery
H_2O_2 (1.0 mmol L^{-1})	18.08, 17.43, 17.89, 17.19, 17.12,	17.55	0.03 %	0.997 mmol L^{-1}	99.7 %

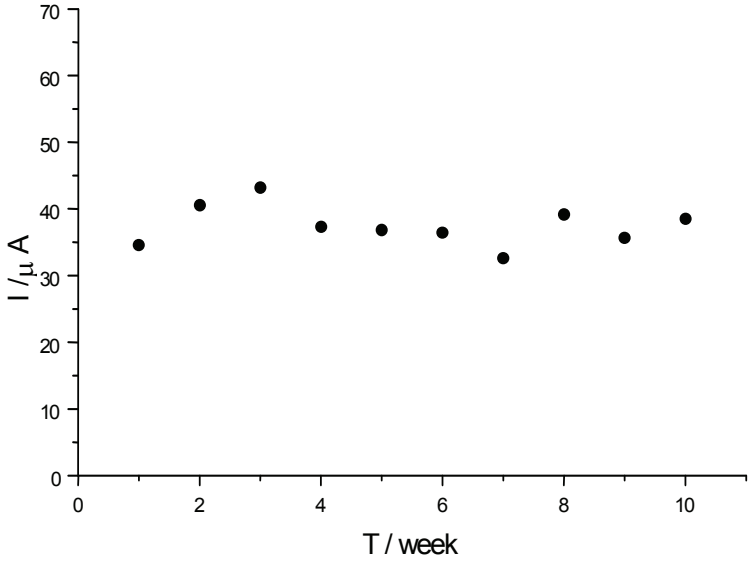

Fig. 5 The lifetime of the PNR/HRP enzyme electrode. The responses were measured in 0.2 mol L^{-1} phosphate buffer (pH 7.5) containing 2.0×10^{-3} mol L^{-1} H_2O_2 at 15 °C. The enzyme electrode was stored in pH 7.0 phosphate buffer at 4 °C between measurements

4 Conclusion

With the electropolymerization of neutral red, HRP was immobilized in PNR film and the PNR/HRP enzyme electrode was constructed. The enzyme electrode possesses good bioelectrocatalytic reduction to H_2O_2 in the absence of any mediator. The activity of HRP was not affected after immobilization, and the enzyme electrode exhibited perfect reproducibility and long-time stability, which can be used for the conventional detection of H_2O_2.

HRP is an ordinary enzyme in biochemistry. According to the successful immobilization of HRP, it is obvious that the method can also be used to immobilize other enzymes. Furthermore, phenazines, phenothiazines and phenoxazines are similar in molecule structure and chemical properties. Since neutral red, one of the phenazines, is suitable for the immobilization of HRP by electropolymerization, it can be inferred that other chemicals which belong to phenazines, phenothiazines or phenoxazines, may also be possible for this usage, provided that they can be electropolymerized. The further studies are continuing in our laboratory.

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References

[1] P. N. Bartlett and J. M. Cooper, J. Electroanal. Chem., 362 (1993) 1.

[2] Y. Fu, J. Ma and B. He, J. Functional Polymers, 3 (1997) 443.

[3] F. Yang, S. Xu, H. Wu and S. Zhou, Chem. J. Chinese University, 7 (1994) 1042.

[4] S. Uchiyama and H. Sakamoto, Talanta, 44 (1997) 1435.
[5] T. Tatsuma, M. Gondaira and T. Watanabe, Anal. Chem., 64 (1992) 1183.
[6] Z. Zhang, C. Lei, W. Sun, H. Liu and J. Deng, J. Electroanal. Chem., 419 (1996) 85.
[7] E. Csöregi, L. Gorton and G. Marko-Varga, Anal. Chim. Acta, 273 (1993) 59.
[8] B. Chen, K. Zhu, H. Wu and Y. Zhang, Chem. J. Chinese University, 4 (1997) 615.
[9] G. Arai, S. Takahashi and I. Yasumori, J. Electroanal. Chem., 410 (1996) 173.
[10] J. M. Bauldreay and M. D. Archer, Electrochim. Acta, 28 (1983) 1515.
[11] Y. Sun, B. Ye and X. Zhou, Chinese J. Anal. Chem., 2 (1998) 166.
[12] B. Persson and L. Gorton, J. Electroanal. Chem., 292 (1990) 115.
[13] A. A. Karyakin, O. A. Bobrona and E. E. Karyakina, J. Electroanal. Chem., 399 (1995) 179.
[14] K. Jiao, X. Luo and W. Sun, Qingdao Huagong Xueyuan Xuebao, 1 (2001) 1.
[15] T. Ruzgas, E. Csöregi, J. Emnéus, L. Gorton and G. Marko-Varga, Anal. Chim. Acta, 330 (1996) 123.
[16] Y. Sun, B. Ye, W. Zhang and X. Zhou, Anal. Chim. Acta, 363 (1998) 75.
[17] D. Wei and H. Wu, Electrochemistry (Chinese), 2 (1995) 186.
[18] A. Lindgren, J. Emnéus, T. Ruzgas, L. Gorton and G. Marko-Varga, Anal. Chim. Acta, 347 (1997) 51.
[19] T. Ruzgas, J. Emnéus, L. Gorton and G. Marko-Varga, Anal. Chim. Acta, 311 (1995) 245.
[20] A. Lindgren, M. Tanaka, T. Ruzgas, L. Gorton, I. Gazaryan, K. Ishimori and I. Morishima, Electrochem. Commun., 1 (1999) 171.
[21] Y. Liu, J. Qian, X. Fu, H. Liu, J. Deng and T. Yu, Enzyme Microb. Technol., 21 (1997) 154.
[22] The compilation committee of Encyclopedia of Chinese Chemical Products. Encyclopedia of Chinese Chemical Products. 2.Edition, Vol. 3. Beijing: Chemical Industry Press, 1998, p. 58.
[23] P. Ouyang, Biochemical Products. Beijing: Chemical Industry Press, 1999, p. 115.
[24] Y. Yang and S. Mu, J. Electroanal. Chem., 432 (1997) 71.

Подготовка и свойства ферментного электрода поли(нейтральной красной) пероксидазы хрена

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Ключевые слова и фразы: электрополимеризация; ферментный электрод; пероксидаза хрена; пероксид водорода; поли(нейтральный красный).

Аннотация: Новый пероксидноводородный биосенсор был получен при иммобилизации пероксидазы хрена (**ПОХ**) в поли(нейтральной красной) (**ПНК**) плёнке. С помощью ортогонального планирования были тщательно изучены условия электрополимеризации нейтрального красного на стеклоуглеродном электроде для формирования ПНК мембраны. На основе этого исследования разработан следующий способ изготовления ферментного электрода ПОХ/ПНК:

пероксидаза хрена добавляется к электролиту и вместе с электрополимеризацией нейтрального красного иммобилизуется в ПНК мембрану. Изучено биоэлектрокаталитическое восстановление H_2O_2 на ферментном электроде. Установлено, что данный электрод проявляет отличную каталитическую активность в процессе восстановления H_2O_2 в отсутствие любого медиатора – переносчика электронов, а отклик тока – пропорционален концентрации H_2O_2 в пределах $5{,}0 \cdot 10^{-5}$ … $1{,}0 \cdot 10^{-3}$ моль/л с корреляционным коэффициентом r = = 0,9995 (n = 6). Кроме того, ПОХ/ПНК ферментный электрод может быть легко изготовлен, его характеристики превосходно воспроизводятся. Срок службы достаточно длителен. Таким образом, этот сенсор может быть использован для аналитического обнаружения H_2O_2 в контролируемых средах.

Vorbereitung und Eigenschaften der Fermentenelektrode von Poly (neutral rot) Meerrettichperoxydase

Zusammenfassung: Es wurde der neue wasserstoffperoxidische Biosensor bei der Immobilisation der Meerrettichperoxydase (MRP) in der Poly (neutral rot) (PNR) Umhüllung erhalten. Die Bedingungen für die Elektropolymerisation des Neutralrotes auf der Glaskohleelektrode für die Formierung der PNR Menbrane wurden mit Hilfe der Orthogonalplanierung genau erlernt. Auf diesem Grund wurde MRP zum Elektrolyt zugesetzt und zusammen mit der Elektropolymerisation des Neutralrotes wurde MRP in die PNR Membrane für die Erhaltung der Fermentenelektrode von Poly (neutral rot) Meerrettichperoxydase immobilisiert. Es wurde die bioelektrokatalytische Wiedererstehung von H_2O_2 beim Fehlen jedes elektroneübergebenden Mediators zu katalysieren. Dabei ist der Gegenstrom zur H_2O_2 Konzentration in den Grenzen $5.0 \cdot 10^{-5} \sim 1.0 \cdot 10^{-3}$ mol/L mit dem Korrelationskoeffizienten r = 0/9995 (n = 6) proportional. Außerdem kann MRP/PNR Fermentenelektrode leicht hergestellt werden, und ihre Reproduzierung und Lebenszeit sind perfekt. So kann dieser Sensor für das analytischen Auffinden von H_2O_2 benutzt werden.

Préparation et propriétés de l'électrode enzyme de la poly (rouge neutre) peroxydase du raifort

Résumé: Le nouveau biosenseur hydrogéné peroxyde a été reçu par l'immobilisation de la peroxydase du raifort (**PRF**) dans un film poly (rouge neutre) (**PRN**). Les conditions pour l'électropolymérisation de l'électrode de charbon et de verre (rouge neutre) pour la PRN-membrane ont été étudiées à l'aide du dessin orthogonal. Sur cette base, la PRF a été ajoutée à l'électrolyte et, par l'électropolymérisation du rouge neutre, la PRF fut immobilsée dans PRN pour la construction de l'électrode enzyme de la poly (rouge neutre) peroxydase du raifort. La réduction bioélectrocatalytique de H_2O_2 dans l'électrode enzyme a été étudiée. L'électrode a montré une activité excellente de catalyser la réduction de H_2O_2 dans l'absence du médiateur du transfert de l'électrode le courant de réponse étant proportionnel à la concentration de H_2O_2 dans le rang $5{,}0 \cdot 10^{-5}$ … $1{,}0 \cdot 10^{-3}$ mol/L avec le coefficient de corrélation r = 0/9995 (n = 6). L'électrode enzyme PRN/PRF peut être facilement fabriqué; sa reproductibilité et son cycle vital sont parfaits. Donc, un tel senseur peut être utilisé pour la détection conventionnelle de H_2O_2.