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肌红蛋白 - 壳聚糖 - 金胶纳米复合 修饰电极的电催化

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摘要: 在 pH 5.4 的 HAc-NaAc 缓冲溶液中, 肌红蛋白 壳聚糖 金胶薄膜修饰电极 (Mb-Chitosan-Au colloid/GCE) 于 -0.20 V (vs Ag/AgCl) 处有一对可逆的氧化还原峰, 即 Mb 血红素辅基 Fe(II)/Fe(III) 电对的特征峰。本实验条件下, 肌红蛋白与玻碳电极之间的电子传递明显加快, 并考察了扫描速率、溶液 pH 及支持电解质浓度等因素对肌红蛋白电子传递的影响。紫外光谱图表明: 肌红蛋白在壳聚糖 金胶溶液中依然保持其原始构象。该肌红蛋白 壳聚糖 金胶纳米修饰电极还能电催化溶解氧的还原。

关键词: 肌红蛋白; 壳聚糖; 金胶; 电化学; 电催化

中图分类号: O657.1

文献标识码: A

肌红蛋白 (Mb) 是肌肉细胞中的小型血红素类蛋白质分子, 参与呼吸系统的氧化还原过程, 因而其直接电化学研究倍受关注^[1-2]。壳聚糖以其良好的生物相容性、抗菌和可生物降解等特点广泛用于生物医药及废水处理^[3]。近年来壳聚糖作为固定化酶载体的研究引起了不少学者重视^[4-5]。本文以壳聚糖为载体, 研究了壳聚糖 金胶纳米复合薄膜对肌红蛋白电子传递的影响。

1 实验部分

1.1 试剂

肌红蛋白 (myoglobin; Mb, Sigma 公司); 壳聚糖 (Chitosan, 脱乙酰度 > 90%, 上海国药集团化学试剂有限公司); 氯金酸、柠檬酸钠、醋酸、醋酸钠均为分析纯试剂。实验水由 Ultrapure water system (北京历元电子仪器公司) 制得。

1.2 仪器

以玻碳修饰电极为工作电极 ($\phi = 3 \text{ mm}$), 铂丝为对电极, Ag/AgCl (饱和 KCl) 为参比电极组成三电极系统, 电化学测试使用 CHI 660 电化学分析仪 (辰华公司), 实验前电解液通高纯氮气除氧 20

min (室温)。

1.3 肌红蛋白 壳聚糖薄膜电极制备

将 3 mg 壳聚糖溶于 1 mL 0.1 mol/L 的醋酸 / 醋酸钠缓冲溶液 (pH = 5.4), 超声振荡 40 min; 按文献 [6] 制备金胶, 将壳聚糖、金胶溶液等体积均匀混合, 冰箱冷藏备用。用微量注射器取 10 μL 上述壳聚糖 金胶混合液滴于玻碳电极表面, 静置 1 h, 再将 5 μL 2 mg/mL 肌红蛋白溶液滴于金胶 / 壳聚糖薄膜之上, 挥发成膜, 制得 Mb 壳聚糖 金胶纳米复合修饰玻碳电极 (Mb-Chitosan-Au colloid/GCE)。

2 结果与讨论

2.1 Mb-Chitosan-Au colloid/GCE 循环伏安曲线

图 1 示出 Mb-Chitosan-Au colloid/GCE 的循环伏安曲线。如图可见: Mb/GCE (a) 及 Mb-Au Colloid/GCE (b) 在 0.1 mol/L 醋酸 / 醋酸钠缓冲溶液中几乎无氧化还原峰迹象, 而 Mb-Chitosan/GCE 则出现明显的氧化还原峰 (c), 其氧化还原峰电位差 $\Delta E = 70 \text{ mV}$, 而且对 Mb-Chitosan-Au colloid/

GCE, 电流明显增大 (d), 峰电位差进一步减小, $\Delta E = 62 \text{ mV}$, 对应的克式量电位 [$E_0 = (E_{pa} + E_{pc}) / 2$] 为 -0.20 V , 这是 Mb 血红素辅基中氧化还原活性中心 $\text{Fe(III)}/\text{Fe(II)}$ 的氧化还原特征峰^[7], 表明金胶可促进 Mb 壳聚糖体系的电子传递。

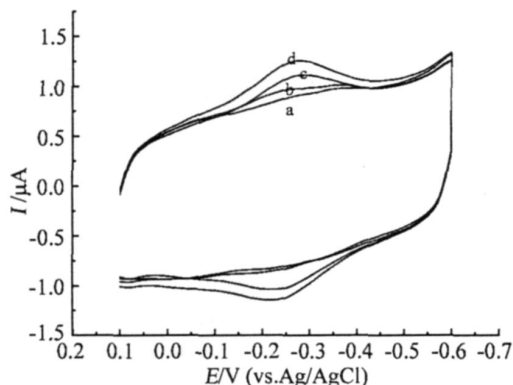


图 1 Mb-Chitosan-Au Colloid/GCE 的循环伏安曲线

Fig 1 CVs of Mb/GCE (a), Mb-Au colloid/GCE (b), Mb-Chitosan/GCE (c), Mb-Chitosan-Au colloid/GCE (d) 0.2 mol/L HAc-NaAc scan rate: 200 mV s^{-1} , $\text{pH} = 5.4$

图 2 示出不同扫描速率下 Mb-Chitosan-Au colloid/GCE 的循环伏安曲线。显然, 随着扫描速率增加, Mb 氧化还原峰电流增大, 且还原峰电位负移。在 $100 \sim 900 \text{ mV/s}$ 范围内, 还原峰电流与扫速成正比关系 (见插图), 表明该电极反应为典型的

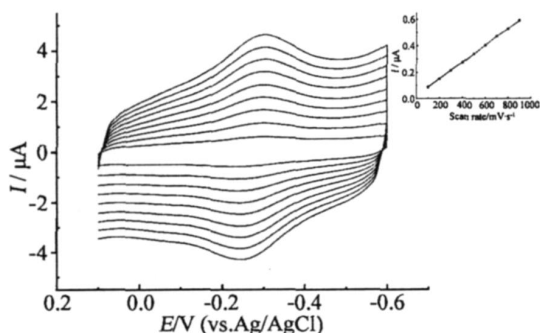


图 2 不同扫描速率下 Mb-Chitosan-Au colloid/GCE 的循环伏安曲线

Fig 2 CVs of the Mb-Chitosan-Au colloid/GCE with different scan rates in 0.2 mol/L HAc-NaAc ($\text{pH} = 5.4$) scan rates from inner to outer/ $\text{mV} \cdot \text{s}^{-1}$: 100, 200, 300, 400, 500, 600, 700, 800 to $900 \text{ mV} \cdot \text{s}^{-1}$, inset: plot of the cathodic peak current vs scan rate

表面控制准可逆过程。计算得出 Mb 的平均表面含量 Γ ($\Gamma = Q/nFA$) 为 $1.6 \times 10^{-7} \text{ mol} \cdot \text{cm}^{-2}$ 。

2.2 支持电解质浓度

图 3 为 Mb-Chitosan-Au Colloid/GCE 在不同浓度支持电解质 HAc-NaAc 中的循环伏安曲线。由图可见, 支持电解质浓度增加, 峰电流增大, 但当电解质浓度增至 0.2 mol/L 后, 氧化还原峰电流反而下降, 这是过量电解质浓度导致 Mb 吸附减弱的缘故。本文选择最佳 HAc-NaAc 浓度为 0.1 mol/L 。

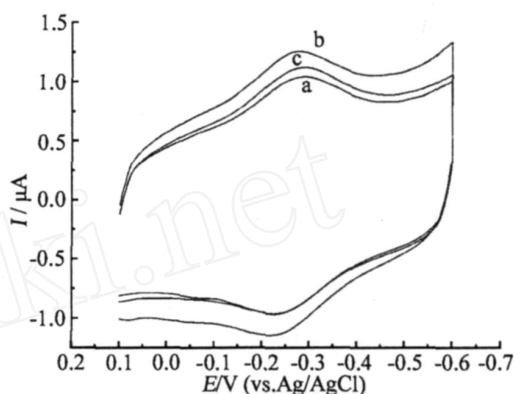


图 3 不同支持电解质浓度 Mb-Chitosan-Au colloid/GCE 的循环伏安曲线

Fig 3 CVs of Mb-Chitosan-Au colloid/GCE with different concentration of buffers the concentration of HAc-NaAc from a to c/ $\text{mol} \cdot \text{L}^{-1}$: 0.02, 0.1, 0.2 other conditions are the same as in Fig 1

2.3 溶液 pH 值

实验表明, 肌红蛋白的阴、阳极峰电位均随溶液 pH 增加而负移, 且 Mb $\text{Fe(III)}/\text{Fe(II)}$ 克式量电位 E_0 与 pH 成线性关系, 线性回归方程为:

$$E_0' = -0.1033 - 0.036 \text{ pH}$$

如上, 可以认为肌红蛋白的血红素辅基 MbFe(III)/MbFe(II) 发生的电子转移反应 (氧化/还原) 伴随有质子转移。

2.4 紫外可见光谱

图 4 示出 Mb 和 Mb-Chitosan-Au colloid 的紫外可见光谱。由图可见: Mb-Chitosan-Au colloid 薄膜在 410 nm 处有一较强的吸收带, 与 Mb 溶液的 Soret 吸收带 (409 nm) 位置近乎一致, 表明在壳聚糖-金胶薄膜上 Mb 仍保持原始二级结构状态^[8]。

2.5 Mb-Chitosan-Au Colloid/GCE对溶解氧的电催化

向除氧后的 HAc-NaAc缓冲溶液 (pH 5.4) 注入一定量的空气, 在 MbFe() 还原电位处的还原峰电流显著增大, 并伴随 MbFe() 氧化峰的消失 (见图 5). 而氧在 Chitosan-Au colloid/GCE 上直接还原的电位为 -0.79 V, 其还原电位负移了约 0.54 V, 表明 Mb-Chitosan-Au colloid/GCE 对溶解氧还原具有一定催化作用.

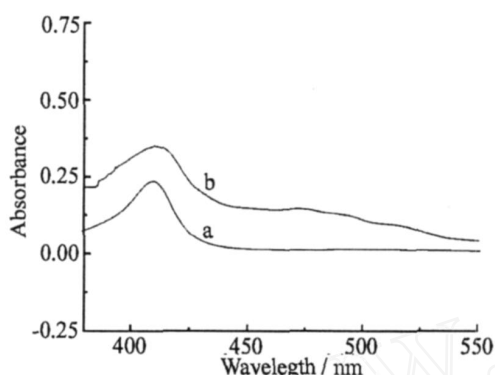


图4 Mb 和 Mb-Chitosan-Au colloid 的紫外可见光谱

Fig.4 Absorption spectra for aqueous of Mb (a) and Mb-Chitosan-Au colloid film (b)

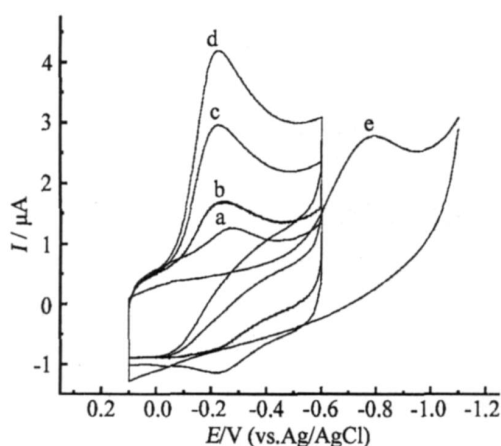


图5 Mb-Chitosan-Au colloid/GCE 的循环伏安曲线

Fig.5 CVs of Mb-Chitosan-Au colloid/GCE

0.2 mol/L HAc-NaAc (pH5.4, 10mL), scan rate: 200 mV/s, Mb-Chitosan-Au colloid/GCE with no oxygen present(a), after 10(b), 20(c), 30 mL(d) of air was injected, Chitosan-Au colloid/GCE after 20 mL of air was injected(e), respectively

2.6 Mb-Chitosan-Au colloid/GCE的稳定性

Mb-Chitosan-Au colloid/GCE 置于 4℃ 冰箱中保存, 30 d 后测其电流值, 电流响应不少于初始电流的 97%.

3 结 论

在 Mb-Chitosan-Au colloid/GCE 上, 壳聚糖表面带正电的氨基吸附金胶后, 使 Mb 发生快速的直接电子转移, 并对溶解氧的还原有电催化响应.

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Electrocatalysis of Myoglobin-chitosan-Au Colloid Film Modified Glass Carbon Electrode

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Abstract: The cyclic voltammogram of Mb/AAO/Au Colloid/GCE shows a pair of well-defined and nearly reversible peaks at about -0.20 V (vs Ag/AgCl) in 0.1 mol/L NaAc-HAc buffer (pH = 5.4), being the characteristic peaks of the Mb heme Fe(II)/Fe(III) redox couples. The electron transfer between Mb and the electrode was greatly enhanced under present testing conditions. The effects of scan rate, pH and buffers concentration on the electrochemical behaviors of Mb were studied in detail. Position of the Soret absorbance band suggested that Mb in chitosan-colloidal gold kept its secondary structure similar to the native states. Oxygen was catalytically reduced by Mb in chitosan-colloidal gold films.

Key words: myoglobin (Mb); chitosan; colloidal gold; direct electrochemistry; electrochemical catalysis