hours. The release and accommodation of oxygen in the metmyoglobin molecule and the related conformational change of the metmyoglobin molecule are reversible. Myoglobin molecules are not denatured by bubbling the myoglobin solution with nitrogen for a long time.

It is reported for the first time that although the commercially available myoglobin was used in this work without further purification, the quasi-reversible electrochemical reaction of myoglobin was observed at the indium oxide electrode using cyclic voltammetry technique after removing the oxygen molecules accommodated in metmyoglobin molecules. Therefore, it can be concluded that for observing the quasi-reversible CV response, thoroughly removing the oxygen from the solution and metmyoglobin molecules is an important step, whereas the further purification of commercially available myoglobin may be not a necessary condition.

Key words Myoglobin, Cyclic Voltammetry, Synchronous Fluorescence Spectroscopy

Acknowlegment The authors are grateful for the financial support of National Science Foundation of China.

References

1 Stargardt J F, Hawkridge F M, Landrum H L. *Anal. Chem.*, 1978, 50: 930
2 Schlereth D D, Mantele W. *Biochemstry*, 1992, 31: 7 494
3 Ye J, Baldwin R P. *Anal Chem.*, 1988, 60: 2 263
4 Taniguchi I, Watanabe. Tominaga M et al. *J. Electroanal. Chem.*, 1992, 333:331
5 Heineman W R, Meckstoth M L, Norris B J et al. *Bioelectrochem. Bioenerg.*, 1979, 6: 577
6 King B C, Hawkridge F M. *Talanta*, 1989, 36:331
7 Chou J, Lu T. to be submmited to Spectrochim. Acta.

用循环伏安法和同步荧光光谱技术研究肌红蛋白的电化学行为①

剑　菊　　周海虹　　陆天虹*　　吴　越

(中国科学院长春应用化学研究所电分析开放实验室,长春　130022)

摘要　用循环伏安法和同步荧光光谱技术研究了肌红蛋白的电化学行为,实验结果表明,高铁肌红蛋白分子中至少存在一个可调节分子构象变化的氧分子,而且长时间通入高纯氮气可以除掉高铁肌红蛋白分子内的这个氧,当高铁肌红蛋白分子内的氧被彻底除去后,用循环伏安法可以观察到肌红蛋白在三氧化二铟电极上的准可逆的电学反应. 同步荧光光谱实验表明,高铁肌红蛋白在彻底除氧后,分子构象发生了变化,而且这种构象变化是可逆的.

关键词　肌红蛋白,循环伏安法,同步荧光光谱

① 国家自然科学基金资助项目

Study of Electrochemical Behaviour of Myoglobin Using Cyclic Voltammetry and Synchronous Fluorescence Spectroscopy①

Chou Ju Zhou Haihong Lu Tianhong* Wu Yue

(*Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences Changchun*, 130022, *P. R. China*)

Myoglobin undergoes irreversible heterogeneous electron transfer reactions at bare metal electroeds. Thus, much effort has been devoted to obtain quasi-reversible electrochemical reactions of myoglobin at metal electrodes modified with mediators[1~3]. However, the electron transfer rates at the above modified electrodes is very small so that the spectroelectrochemical techniques should be used in the most of studies. Recently, Taniguchi et al[4]. reported that a quasi-reversible electrochemical reaction of the purified horse heart myoglobin was observed at an indium oxide electrode with cyclic voltammetry technique. It was suggested that high purification of myoglobin may be the main reason for obtaining a quasi-reversible electrochemical reaction of myoglobin in the cyclic voltammetry experiments. In this paper, it was reported for the first time that a pair of well-defined redox peaks were observed in the CV of the commercially available horse heart myoglobin without further purification at an indium oxide electrode after oxygen was eliminated from the solution and the molecules of the oxidized form of myoglobin, i. e. metmyoglobin.

1 Experiment

Horse heart myoglobin was purchased from Sigma Chemical Co. It was used without further purification. The myoglobin solution used for both electrochemical and fluorescence experimetns was 0.15 mM myoglobin with 0.025 M phosphate buffer (pH 7.0) and 0.1 M sodium perchlorate. The synchronous fluorescence spectra of myolobin were collect on a RF-5000 spectrofluorometer with 150 W xenon lamp (Shimadzu Corporation, Japan). The electrochemical experiments were performed using Model 175 Universal Programmer, Model 173 Potentiostat/Galvanostat (Princeton Applied Research, U. S. A.) and a conventional three-electrode electrochemistry cell. The working electrode is an indium oxide flat and was cleaned with ultrasonic washing for 5 minutes each in ethanol and then three times in distilled water. A Pt flat was used as the auxiliary electrode. A saturated calmol electrode (SCE) served as the reference electrode.

① Reveived 5 Oct, 1995

2 Results and discussion

No redox peaks were observed in the cyclic voltammogram (CV) of myoglobin at the indium oxide electrode before bubbling the myoglobin solution with high-purity nitrogen (Fig. 1, curve a). Only the cathodic current increases significantly when the potential was more negative than −0.2 V. The increase in the cathodic current is obviously due to the reduction of oxygen.

After the myoglobin solution was bubbled by nitrogen for 60 minutes, a pair of small redox peaks appeared (Fig. 1, curve b). However, the large cathodic current due to the reduction of oxygen at the potential more negative than −0.2 V was still observed.

When the myoglobin solution was bubbled with high-purity nitrogen for more than two hours, a pair of well-defined redox peaks appeared in the CV of myoglobin (Fig. 1, curve c). The anodic peak is located at −0.15 V and the cathodic peak is at −0.30 V. The peak separation is 150 mV. The midpoint between the anodic and cathodic peak potentials is −0.22 V which is close to the formal potential of myoglobin[5]. The peak current is proportional to the square root of the scan rate in the region of 5～100 mV/s. The ratio of the anodic to the cathodic peak current is approximately unity. In addition, the CV response is stable during the repetitive cycles. These results demonstrated that a quasi-reversible electrochemical reaction occurs at the indium oxide electrode after bubbling the solution with nitrogen for more than two hours, and oxygen plays an important role in the electrochemical reaction of myoglobin.

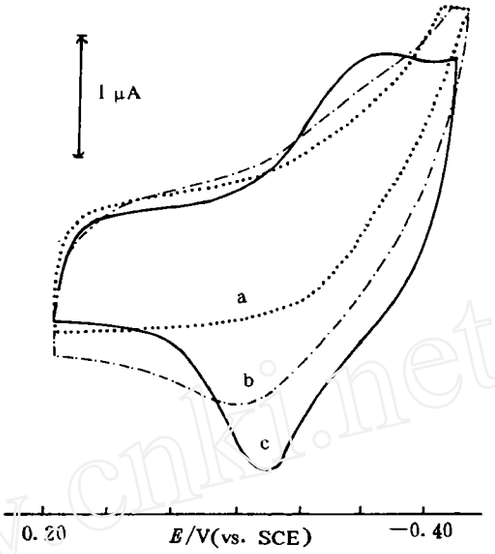

Fig. 1　Cyclic voltammograms of the myoglobin at indium oxide electrodes before(curve a) and after bubbling the myoglobin solution with nitrogen for 60 min (curve b) and 2 h (curve c). Scan rate: 20 mV/s

Usually, the oxygen in the solution can be purged by bubbling the solution with nitrogen for 10～30 min. However, after the myoglobin solution was bubbled with nitrogen for 60 minutes, the reduction current of oxygen was still observed(Fig. 1, curve b). It may indicated that the oxygen can exist not only in the solution, but also in the metmyoglobin molecules. King et al[6]. also indicated that a metmyoglobin molecule may contain at least one oxygen molecule which does not bind to the heme iron in metmyoglobin molecule and can be electrochemically reduced. In addition, the quasi-reversible CV response of myoglobin can be observed at the indium oxide electrode only after bubbling the myoglobin solution with nitrogen for more than two hours. It demonstrated that the oxygen molecules in metmyoglobin molecules can be released thoroughly by bubbling the myoglobin solution with nitrogen for more than two hours and oxygen

palys an important role in the electrochemical reaction of myoglobin.

Fig. 2 is the synchronous fluorescence spectra of myoglobin at wavelength interval 80 mn at which the spectra are mainly contributed by tryptophan residues in myoglobin molecules[7]. Curve a in Fig. 2 is the spectrum before bubbling the myoglobin solution with high-purity nitrogen. The main peak is located at 332.0 nm. After bubbling the solution with high-purity nitrogen for two hours, the peak shifts towards to 347.2 nm(Fig. 2, curve b). If the solution is bubbled with oxygen or exposed to air for a while, such as 10 minutes, the peak position shifts back(Fig. 2, curve c).

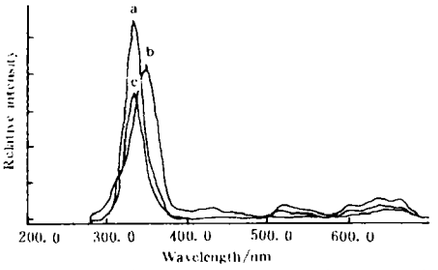

Fig. 2 Synchronous fluorescence spectra of the myoglobin solution at the wavelength interval of 80 mn. a: before, b: after bubbling the myoglobin solution with nitrogen for 2 h, c: after obtaining spectrum b, the solution was exposed to air for 10 min

Fig. 3 is the synchronous fluorescence spectra of myoglobin at wavelength interval 40 nm at which the spectra are contributed mainly by tyrosine residues in myoglobin molecules[7]. Curve a in Fig. 3 is the spectrum before bubbling the myoglobin solution with high-purity nitrogen. There are two peaks in the spectrum. The main peak is located at 322.4 nm and the second peak is at 595.2 nm. After bubbling the solution with high-purity nitrogen for two hours, the peak positions at 322.4 nm were almost the same as that before bubbling, but the peak at 595.2 nm almost disappeared (Fig. 3, curve b). If the solution is bubbled with oxygen or exposed to air for a while, the peak at 595.2 nm appeared again (Fig. 3, curve c).

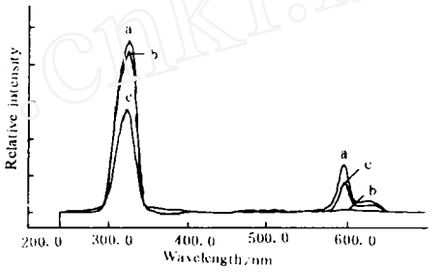

Fig. 3 Synchronous fluorescence spectra of the myoglobin solution at the wavelength interval of 40 nm. a: before, b: after bubbling the myoglobin solution with nitrogen for 2 h, c: after obtaining spectrum b, the solution was exposed to air for 10 min

The above results demonstrated that the peak at 595.2 nm related to the oxygen molecules accommodated in metmyoglobin molecules. In addition, the main peak in the spectra of tryptophan residues shifts towards to the red direction(Fig. 2). It may reflect a conformational change of the metmyoglobin molecule. Thus, the above results further demonstrated that oxygen molecules can be accommodated in the metmyoglobin molecules and can be released by bubbling the myoglobin solution with the high-purity nitrogen for more than two

hours. The release and accommodation of oxygen in the metmyoglobin molecule and the related conformational change of the metmyoglobin molecule are reversible. Myoglobin molecules are not denatured by bubbling the myoglobin solution with nitrogen for a long time.

It is reported for the first time that although the commercially available myoglobin was used in this work without further purification, the quasi-reversible electrochemical reaction of myoglobin was observed at the indium oxide electrode using cyclic voltammetry technique after removing the oxygen molecules accommodated in metmyoglobin molecules. Therefore, it can be concluded that for observing the quasi-reversible CV response, thoroughly removing the oxygen from the solution and metmyoglobin molecules is an important step, whereas the further purification of commercially available myoglobin may be not a necessary condition.

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References

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3 Ye J, Baldwin R P. *Anal Chem.*, 1988, 60: 2 263
4 Taniguchi I, Watanabe. Tominaga M et al. *J. Electroanal. Chem.*, 1992, 333: 331
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用循环伏安法和同步荧光光谱技术研究肌红蛋白的电化学行为①

剑　菊　　周海虹　　陆天虹*　　吴　越
(中国科学院长春应用化学研究所电分析开放实验室,长春　130022)

摘要　用循环伏安法和同步荧光光谱技术研究了肌红蛋白的电化学行为,实验结果表明,高铁肌红蛋白分子中至少存在一个可调节分子构象变化的氧分子,而且长时间通入高纯氮气可以除掉高铁肌红蛋白分子内的这个氧,当高铁肌红蛋白分子内的氧被彻底除去后,用循环伏安法可以观察到肌红蛋白在三氧化二铟电极上的准可逆的电学反应. 同步荧光光谱实验表明,高铁肌红蛋白在彻底除氧后,分子构象发生了变化,而且这种构象变化是可逆的.

关键词　肌红蛋白,循环伏安法,同步荧光光谱

① 国家自然科学基金资助项目