Communications

Redox reactions of cytochrome c facilitated by silver-imidazole complex

FAN, Chun-Hai(樊春海) **LI, Gen-Xi***(李根喜) **ZHU, De-Xu**(朱德煦)
Department of Biochemistry and National Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing, Jiangsu 210093, China
ZHU, Jian-Qin(朱剑琴)
Department of Biology and Biological Technology, Nanjing University, Nanjing, Jiangsu 210093, China

An imidazole modified silver electrode is prepared by immersing the substrate silver electrode in a 2% imidazole solution of ethanol at 50℃ for 10 min. The modified electrode is then swept in a cytochrome c solution and the modified layer takes off because the modified electrode is very unstable. Although the amount of the silver-imidazole complex is very small compared with the amount of cytochrome c in the protein solution, it greatly facilitates redox reactions involving the biomacromolecules.

Keywords Chemically modified electrode, cytochrome c, imidazole, silver

Two methods are known to make proteins exhibit current responses in solutions where these species are electrochemically irreversible. It is common that these biological macromolecules are very irreversibly oxidized and reduced. One of these methods employs a mediator or promoter, while the other uses special pretreatment procedures and experimental protocols to allow the use of bare electrodes.[1] In addition, surfactants have drawn our attention for this purpose.[2]

In this paper, we report the redox reactions of cytochrome c facilitated by a silver-imidazole complex. The method to make this protein exhibit an electrochemical response is different from the previously reported methods. The effect on cytochrome c is so striking that the results presented in this paper might have implications for the biological redox processes in nature.

Cyclic voltammetry was performed with a PARC Model 175 Universal Programmer and 173 Protentiostat/Galvanostat (EG&G, U.S.A) and a Yew Type 3036 X-Y Recorder (Chongqing, China), employing a three-electrode system with a saturated calomel reference electrode (SCE) and a platinum auxiliary electrode. The substrate silver electrode was a 2 mm diameter disk electrode.

Horse heart cytochrome c was obtained from Sigma Chemical Company and used without further purification. Silver metal was obtained from the Shanghai Dian Guang Device works, Shanghai, China. Its purity was 99.99%. Other chemicals were of analytical reagent grade.

Previous studies[3] showed that an imidazole modified silver electrode was a good electrode for the electrochemical studies of cytochrome c. The chemically modified electrode (CME) was very stable and long-lived. The optimum experimental protocol for the CME preparation was to immerse the substrate silver electrode in a 2% imidazole solution of ethanol at 50℃ for 30 min.

Further studies revealed an even more interesting result when the electrode was prepared under these same conditions except that the preparation time was only 10 min. It was found that the silver-imidazole complex formed at the electrode surface appeared to be electrochemically stripped by cycling the electrode, but suprisingly, the electrode could be still electroactive towards the redox reactions of cytochrome c. This is described by

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the following observations.

Initial cyclic voltammetric (CV) experiments showed that this CME was able to produce a pair of redox waves even though the electrode was not optimally prepared. The anodic and cathodic peaks were located at about 0.22 V and 0.08 V, respectively in 0.20 mol/L acetate buffer with pH 5.5. However, since the CME was not optimally prepared, it was very unstable. After two cycles no further redox waves could be seen and the CV curve looked the same as from a bare silver electrode as shown in Fig. 1.

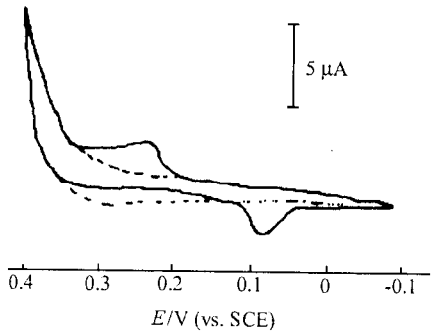

Fig. 1 Cyclic voltammograms obtained at the modified electrode in 0.20 mol/L NaAc-HAc buffer with pH 5.5. (—) the first cycle; (---) the third cycle. Scan rate: 40 mV/s.

On the other hand, if the non-ideally prepared CME was cycled in a cytochrome c solution, redox waves could also be observed and the waves were higher. This might be reasonable since imidazole can be a mediator for cytochrome c.[3,4] However, the modified layer had apparently been removed from the surface during earlier cycling so the CME should have been a bare electrode. Thus, the CME should not have exhibited an electrochemical response towards cytochrome c during further scanning. Fig. 2 shows these data.

However, 30 min later, it was unexpectedly found that redox waves could be once again observed at the CME and the peaks were even higher than before. Even more unexpectedly, the redox waves continued to persist for a hundred or more cycles.

So, what had happened? Was the CME somehow regenerated? Experimental results revealed that this electrode did not have the appearance of a CME. Fig. 3 shows the CV curve of the electrode in the buffer only. It clearly shows only the CV curve for a bare silver electrode. Studies showed that no redox wave could be observed at the electrode for any other solutions except the tested one.

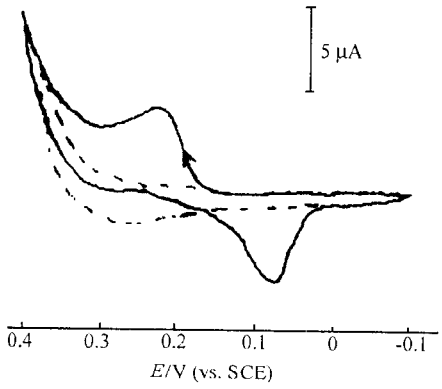

Fig. 2 Cyclic voltammograms obtained at the modified electrode for 0.005 mmol/L cytochrome c. (—) the first cycle; (---) the third cycle. Conditions are the same as those in Fig. 1.

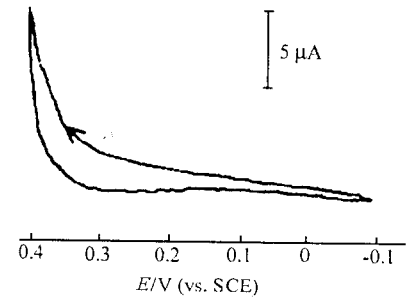

Fig. 3 Cyclic voltammogram obtained at the modified electrode for the buffer only. Conditions are the same as those in Fig. 1.

Our experiments showed the newly developed redox waves were not due to the past history of the CME, for redox waves for cytochrome c could also be obtained at a new bare substrate silver electrode in the test protein solution, as shown in Fig. 4. Most importantly, no redox wave for cytochrome c could not be observed at either the old or new bare electrode in other solutions except one where the original electrode had been cycled during the period when it lost its response.

We hypothesize that the reason the protein exhibited redox waves was because the modifier was removed from the substrate electrode surface and dissolved in the test protein solution during the first few scans when the original response was lost.

Additional experiments were done once the cy-

tochrome c response was regained. The effect of cytochrome c concentration was tested. The more cytochrome c that was added, the higher the redox peaks became. This also helped demonstrate that the redox waves were due to the protein.

We think the CME modifier, which was taken off the electrode surface, dissolved in the solution and facilitated the protein redox reactions.

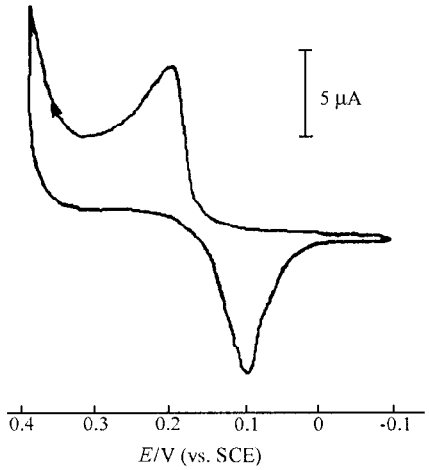

Fig. 4 Cyclic voltammogram obtained at a bare silver electrode for the test protein solution. Conditions are the same as those in Fig. 2.

It is not clear how this can happen because the diameter of the substrate electrode was only 2 mm and the modifier is thus totally insignificant compared with the amount of the protein in the 20 mL of buffer solution (about 2 mg). With such a low ratio of facilitator to protein, how could the species help the redox processes?

We think this means the mechanism to make the protein exhibit electrochemical response in this situation is different from the previously reported ones. We speculate that perhaps the mechanism observed here is close to the redox mechanisms occurring in nature.

We believe the key to the current observation is the short preparation time for the CME. Although the complexes formed from silver and imidazole were polymerized, the polymerization was probably not completed, and therefore, the polymer chain was not very long. These relatively small polymeric chains might have analogs in nature for it is well known that small polymers especially with about 13 monomers often behave unexpectedly. It also seems important to us that the effect could not be observed for 30 min after the initial redox waves disappeared. We do not know how to explain this but we think this is a very important observation.

On the other hand, if the CME was ideally prepared, the CME was very stable and the modified layer could not be stripped from the substrate electrode, as previously reported.[2] Even if the modified layer was mechanically stripped off and dissolved in a cytochrome c solution, the modifier could not have the attribution as the formed species in this work.

Acknowledgments

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(SONG, J.P.; LING, J.)