Time-resolved UV-Vis Spectroelectrochemical Studies of the Conformational Rearrangement in the Electron Transfer of Cytochrome c

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The present work used mainly electrochemical in situ time-resolved UV-Vis subtractive reflectance spectroscopy to study the electron transfer process of cytochrome c in the presence of promoter(the equal molal mixture of guanine and cytosine). Three new absorption bands, 420 nm, 450 nm and 500 nm, are observed in the spectra with different time resolutions, as well as two bipolar absorption bands 250 nm and 300 nm. All the results show that the electron transfer of cytochrome c is accompanied by significant conformational rearrangement.

Direct electron transfer reactions between an electrode and redox active groups in biopolymer are of great interest in recent years in order to understand electron transport mechanisms in biological systems. Cytochrome c is the most extensively studied biopolymer so far in this field. However, the mechanism of the electron transport process of cytochrome c has not yet been explicitly clarified and several opinions are exist.[1-5] In spite of the differences among them, most of them agreed that residues surrounding the heme crevice are very important for the electron transfer reaction of cytochrome c. For example, Takano et al[3] suggested an electron transfer pathway consisting of the side chains of Tyr-74, Trp-59, Tyr-67 and heme. On the basis of electronic structure calculation, Nakagawa et al[4] proposed that the electron transfer takes place via the heme, Cys-17 and Phe-82. They also found some significant interactions along the line: heme-iron, Met-80, Tyr-67, Trp-59, and Tyr-74 during the electron transfer, and supposed that the reason for the conservation of these residues is the adjustment of the redox potential of heme-iron. Furthermore, a hypothesis of the conformational rearrangement of cytochrome c during electron transfer process was supposed by Salemme et al,[5] that suggested that conformational changes arise mainly by the position shift of residues Phe-82 and Lys-13, as well as the charges move of residues Met-80 and Tyr-67. Nevertheless, none experimental result to verify the conformational rearrangement has been reported up to now. The present work used mainly electrochemical in situ time-resolved UV-Vis subtractive reflectance spectroscopy[6] to study the electron transfer process of cytochrome c in the presence of promoter(the equal molal mixture of guanine and cytosine), which accelerates the electron transfer between electrodes and the protein but itself is electroinactive at the potentials of interest.

The cyclic voltammograms of cytochrome c at gold electrode in phosphate buffer solution shows that quasi reversible response of cytochrome c can be acquired in the presence of promoter. The following spectral experiments are carried out under the same condition.

The in situ steady state UV-Vis subtractive reflectance spectra (not shown due to limited space) indicate that the absorption bands of oxidized state of cytochrome c (ferricytochrome c) and reduced state of cytochrome c (ferrocytochrome c) are located near 420 nm, 530 nm and 420 nm, 516 nm, 550 nm respectively. They are identical with what reported in references,[7] and are suggested commonly to be produced by the ($\pi \rightarrow \pi^*$) transitions of porphyrin ring in cytochrome c. Then, the electron transfer process of cytochrome c is studied further by the in situ time-resolved UV-Vis subtractive reflectance spectroscopy and a series of spectra with different time resolutions are obtained.

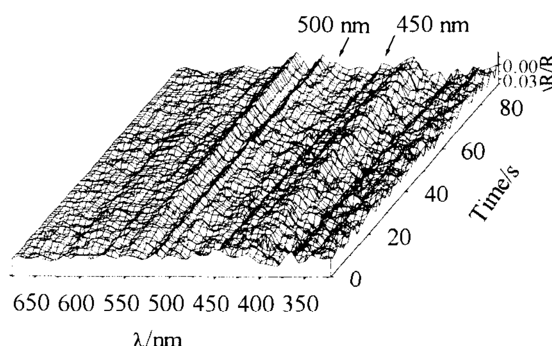

Figure 1. The in situ time-resolved subtractive reflectance spectra in the visible light range of the Au electrode in 0.05 mmol/L cytochrome c + 2.5 mmol/L G + 2.5 mmol/L C + 0.1 mol/L Phosphate buffer solution (pH≈7.0) recorded at potential of -0.15 V(E_2) with reference to +0.25 V(E_1). The time resolution is 1 s.

Figure 1 is the time-resolved spectra with the resolution of 1 s. Two new absorption bands, 450 nm and 500 nm, are observed besides the absorption bands of the steady state of cytochrome c. They are probably produced by the disintegration of the degenerate π energy level in porphyrin ring because of the conformational adjustment of cytochrome c. Then, almost all of the above absorption bands become smaller in the spectra when higher time resolutions(500 ms and 100 ms) are used, even the disappearance of the band 420 nm. However, when the time resolution changes to 20 ms(as shown in Figure 2), the band 420 nm emerges again with considerable intensity. While the other absorption bands are too weak to be observed except the band 450 nm. The intensity change of the band 420 nm with the time resolution is suspected to be aroused by the change of the ($\pi \rightarrow \pi^*$) transition probability of the corresponding absorption band in porphyrin ring of cytochrome c because of the conformational adjustment of cytochrome c. This phenomenon can still be observed when the time resolution is changed to 10 ms and 5 ms. As a result, it is supposed that all the above new absorption bands are arisen by the ($\pi \rightarrow \pi^*$) transitions of porphyrin ring of some intermediate states of cytochrome c that are produced during its

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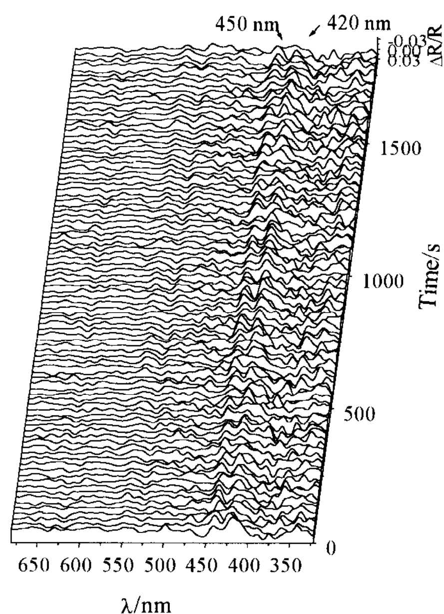

Figure 2. The in situ time-resolved subtractive reflectance spectra in the visible light range of the Au electrode in 0.05 mmol/L cytochrome c + 2.5 mmol/L G + 2.5 mmol/L C + 0.1 mol/L Phosphate buffer solution (pH≈7.0) recorded at potential of -0.15 V(E_2) with reference to +0.25 V(E_1). The time resolution is 20 ms.

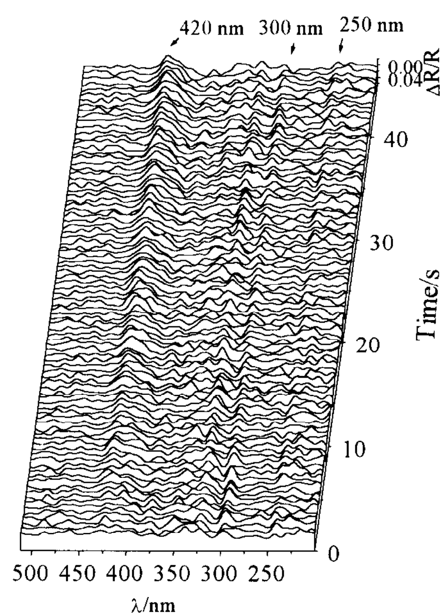

Figure 3. The in situ time-resolved subtractive reflectance spectra in the ultraviolet light range of the Au electrode in 0.05 mmol/L cytochrome c + 2.5 mmol/L G + 2.5 mmol/L C + 0.1 mol/L Phosphate buffer solution (pH≈7.0) recorded at potential of -0.15V (E_2) with reference to +0.25 V(E_1). The time resolution is 500 ms.

electron transfer process. These intermediate states of cytochrome c might correspond to some transient conformational states of cytochrome c. The results indicate that more than one conformational adjustment process exists in the electron transfer process of cytochrome c, and the conformational restructuring can happen within the time shorter than 5 ms.

Meanwhile, two bipolar absorption bands (located near 250 nm and 300 nm respectively) are observed in the ultraviolet light range (Figure 3). It is learned from the reference[8] that the electronic absorption band of cytosine is at 267 nm, and the absorption bands of guanine are at 244 nm and 275 nm in neutral buffer solution. The absorption band of the equal molal mixture of cytosine and guanine is measured too and only the band near 260 nm can be observed. Cytosine and guanine form an base pair, which has rich π conjugate electron, when the mixture of them uses as the promoter for the electron transfer process of cytochrome c. Therefore, the bipolar band near 250 nm is suggested to be produced by the shift of energy level in the promoter for the coulomb interaction between electron rich promoter and cytochrome c, which has positively charged lysine residues, in the electron transfer process of cytochrome c.

While another bipolar band near 300 nm is supposed to be produced by the amino acid residue Tyr-67 in the cytochrome c because of the conformational adjustment. In general, none absorption bands of the amino acid residues can be observed in the spectra that obtain by subtractive method despite that almost all of the amino acids have absorption bands in the ultraviolet range. However, if the position of the absorption band of some amino acid residues changes during the electron transfer process of cytochrome c, new absorption bands(especially bipolar band locates near the original absorption position of the corresponding residue) will emerge. On the other hand, as what have mentioned in paragraph one that some residues may change their position or charge in the electron transfer process of cytochrome c. The absorption bands of the relevant amino acids are compared and find that the absorption band of tyrosine locate near 290 nm under neutral condition.[9] Moreover, some investigators[5] agree that residue Tyr-67 will possess negative charge on its hydroxyl oxygen because of electrostatic effort in the electron transfer process of cytochrome c, which will give rise to the red shift of its absorption band. Therefore, the bipolar band 300 nm is proposed to be produced by Tyr-67 in the cytochrome c because of the conformational adjustment.

The above results show that the electron transfer of cytochrome c is accompanied by significant conformational rearrangement. Further investigation is being carried out.

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