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Serrs and Electrochemical Study of Yeast Iso-1-Cytochrome c Mutant F82H

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Abstract: The redox properties of iso-1-yeast cytochrome c and its mutant F82H were studied by surface-enhanced Raman spectroscopy and cyclic voltammetry. The results showed that the replacement of phenylalanine-82 with histidine led to a more stable global structure of the protein. A negative shift in the redox potential of the mutant relative to that of wild type protein is ascribed to a ligand switching reaction during the redox processes.

Key words: Cytochrome c, Mutant, Surface-enhanced Raman spectroscopy

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Homogeneous and heterogeneous electron transfer processes associated with the c-type cytochromes have been widely studied because such studies can yield important information not only about thermodynamic, kinetic and structural properties of the proteins, but also provide novel insights into the electron transfer mechanism of the proteins in vivo[1~3]. The emergency of molecular genetic techniques provided an opportunity to study a variety of spectroscopic properties on the structure basis and the correlation between the electron transfer and structure of the protein in such a designed way that the structure of the proteins can be specifically modified[4]. In this paper, attention was mainly focused on the effect of this mutation on the stability and redox properties of yeast iso-1-cytochrome c (WT) and its mutant F82H.

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The Surface-enhanced Resonance Raman Scattering (SERS) spectra of WT measured at bare roughened silver electrodes at 0.2 and - 0.5 V, respectively, reveal that mixed states of WT, corresponding to five- and six-coordinate state of heme iron, appeared in both oxidized and reduced forms. This result indicates that WT was partially denatured or unfolded, due to the direct contact of the protein with metal surface. The spectrum of F82H, however, was mainly characteristic of six-coordinate, low-spin state of heme, implying that the global structure of F82H was well preserved under the same experimental conditions. Thus, the replacement of Phe-82 in WT with His-82 in F82H led to a more stable global structure of the protein.

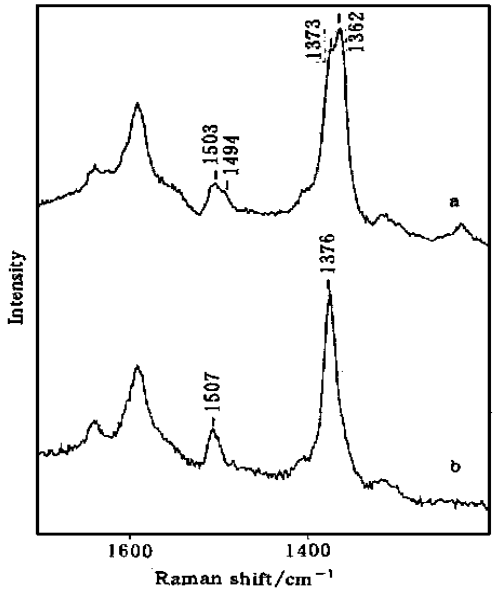

Fig. 1 SERRS of WT (a) and F82H (b) at MUA Modified Ag electrodes

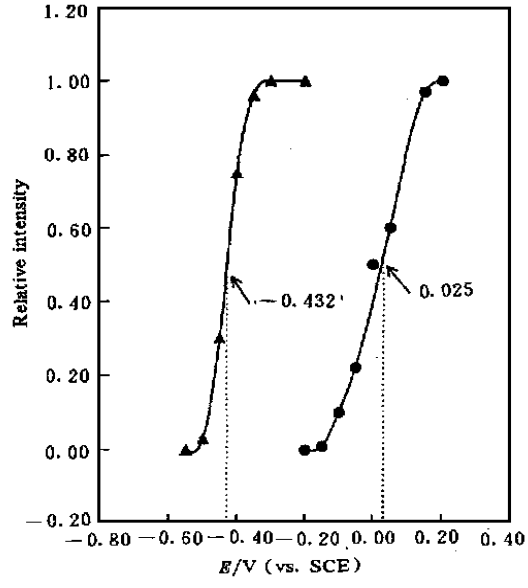

Fig. 2 Plots of relative Raman intensity as a function of the applied potential

The SERRS measurements were further performed on 11-mercaptoundecanic acid (MUA) modified roughened silver surface to prevent the proteins from being denatured (Fig. 1). Under the open circuit conditions, in addition to the band at 1 373 cm^{-1} for the oxidized form of the protein, the characteristic band for the reduced form was also observed at 1 362 cm^{-1}. The corresponding coordination and spin state mark bands appeared at 1 494 cm^{-1}, along with the band at 1 503 cm^{-1} for the oxidized form. The appearance of reduced form in the spectrum of WT demonstrates that WT adsorbed at the MUA-modified roughened silver electrode surface was partially reduced under the experimental conditions. This may be ascribed to the photoinduced electron transfer between the nano-structured silver particles at the surface and the adsorbed species as we described previously for cytochrome c adsorbed on silver colloid with idiodine ions. Only the oxidation state marker band at 1 376 cm^{-1} and the spin and coordinate state marker band at 1 507

cm^{-1} were observed in the spectrum of F82H. Thus, the mutant has less ability to accept photoexcited electrons. The potential dependence of the SERRS spectra was measured to estimate the redox potentials for both proteins. The midpoints of the plot of relative intensities of oxidation state marker bands as a function of applied potential were 0.025 V for WT, and - 0.432 V for F82H, respectively. Obviously, the redox reaction of F82H occurred at more negative potential than that of WT.

The electrochemistry of mitochondrial c has been extensively studies on various electrodes[5]. The reduction potential of mitochondrial cytochrome c is usual about 0 V. Cyclic voltammetry (CV) was further performed to confirm the redox potentials for the proteins adsorbed on MUA modified Ag electrodes. In the case of mutant, for an instance, the oxidation and reduction peaks were located at - 0.39 and - 0.51 V, respectively. The formal redox potentials estimated from the CVs are in good agreement with those obtained in SERRS. The negative shift of the redox potential of the mutant is unlikely to be the result of the protein conformational change due to the adsorption. Considering the results of our previous Resonance Raman spectroscopic study as well as the studies of NMR and CD spectroscopy, a ligand switching process during the redox reaction of the mutant is proposed.. The electrochemical reactions of F82H could be expressed as followings:

For the reduction process,

$$\text{His(82)} - \text{Fe(III)} + e \rightarrow \text{His(82)} - \text{Fe(II)} \quad (1)$$

$$\text{His(82)} - \text{Fe(II)} + \text{Met(80)} \rightarrow \text{Met(80)} - \text{Fe(II)} + \text{His(82)} \quad (2)$$

For the oxidation process,

$$\text{Met(80)} - \text{Fe(II)} - e \rightarrow \text{Met(80)} - \text{Fe(III)} \quad (3)$$

$$\text{Met(80)} - \text{Fe(III)} + \text{His(82)} \rightarrow \text{His(82)} - \text{Fe(III)} + \text{Met(80)} \quad (4)$$

where His(82) - Fe(III) etc. represent the sixth ligand and the oxidation states of F82H.

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细胞色素 c 突变体 F82H 的表面增强拉曼和电化学研究

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摘要: 应用表面增强拉曼光谱和循环伏安法研究了细胞色素 c 及其突变体 F82H 的氧化还原性质. 结果表明苯丙氨酸对组氨酸的取代使得蛋白质结构更为稳定. 相对于原体蛋白质,突变体的氧化还原电位向负电位方向移动,这被归因于由氧化还原过程中伴随有配体转换反应影响所致.

关键词: 细胞色素 c;突变体;表面增强拉曼