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Modification of Functional Group on the Cytochrome *c* Using SPDP Method

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To make a molecular assembly of cytochrome *c* onto the Au substrate, the cytochrome *c* was modified using the 2-succinimidyl-3-(2-pyridyldi-thio)propionate(SPDP), so the functional group on the cytochrome *c* surface was modified. And the modified cytochrome *c* was adsorbed onto the Au substrate. The modified cytochrome c was examined by using UV-visible spectroscopy and FT-IR. Also, the morphology of cytochrome *c* adsorbed onto the Au substrate was analyzed by Atomic Force Microscopy(AFM).

Keywords: Cytochrome *c*; N-succinimidyl-3-(2-pyridyldithio)propionate; Atomic Force Microscopy; SPDP method

INTRODUCTION

Cytochrome *c* takes part in the process of electron transfer in biological system and plays a role as an electron acceptor in bioelectronic device developed by mimicking biological system[1]. To make a molecular assembly onto gold substrate, cytochrome *c* was cross-linked with SPDP for bioelectronic device. The cross-linking reagent, SPDP, was frequently applied for the synthesis of protein-protein conjugates as well as for the synthesis of polymer-protein conjugates[2]. It is known that the NH_2 group in proteins, which binds with these agents to form conjugates, may form

disulfide bridges. The reaction scheme of modification of cytochrome *c* and SPDP is shown in Fig. 1. When the SPDP with a disulfide bonds is exposed to the outer surface of cytochrome *c*, the succinimide group in SPDP is removed. So the amino group on the cytochrome *c* is cross-linked with 2-pyridyldithio-propionamide moieties in the SPDP. And the disulfide bond of 2-pyridyldithio-propionamide moieties in the SPDP can be cleaved with dithiothreitol(DTT). DTT is also a famous reagent for cleavage of the disulfide bond. The amino groups of cytochrome *c* are translated into thiol group, using the SPDP method as first described by Carlsson et al.[3], so cytochrome *c* modified using the SPDP method can be adsorbed onto the Au substrate selectively. In this paper, It is reported a demonstration of the modification of cytochrome *c* using the SPDP method and suggest the feasibility for fabrication of biomolecular electronic device.

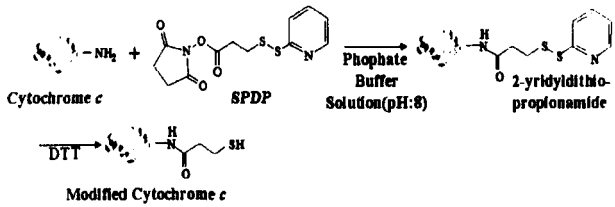


FIGURE 1. Reaction scheme of synthesis of cytochrome *c* and SPDP

EXPERIMENTAL DETAILS

Cytochrome *c* (type IV, Sigma Chemical Co.; 3.2mM, M.W.=12,384) was mixed with phosphate buffer solution (pH:8, 10ml). SPDP (3.2mM, M.W.=312.4) and DTT (25mM) were mixed with deionized distilled water (10ml) repectively. For synthesis of cytochrome *c* was synthesizes with SPDP for 30minites at room temperature, and excess reagent and N-hydroxysuccinimide were removed by gel filtration. The gold was thermally evaporated on cleaned glass substrate.

RESULTS AND DISCUSSION

For comparison of the cytochrome *c* and the modified cytochrome *c* by reaction with SPDP and DTT, UV-visible spectra and FT-IR spectra were measured. As shown in Fig. 2, the maximum absorbance peak of SPDP was 280nm(a) and cytochrome *c* was about 410 nm(c). The maximum absorbance peak of cytochrome *c* cross-linked with 2-pyridyldithio-propionamide moieties in SPDP was 340nm(b). Also, Fig. 3 shows the FT-IR spectra of N-H group in cytochrome *c*(a) and cytochrome *c* having a 2-pyridyldithio-propionamide(b). The amides showed FT-IR bands at 1550.4 and 1639.2 cm^{-1} typically. But FT-IR band at 1639.2 is not found in Fig. 3(a). It might indicate that the amount of NH_2 groups on the cytochrome *c* is relatively reduced as the coupling reaction is preceded, and the amount of NH group is increased relatively. So the modification of functional group on the cytochrome *c* surface was fulfilled by the SPDP method.

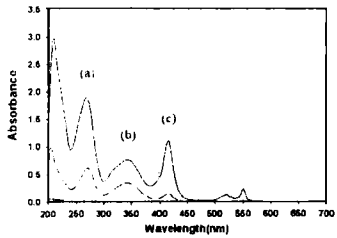


FIGURE 2. UV-visible spectra of (a) SPDP, (b) modified cytochrome *c*, and (c) cytochrome *c*

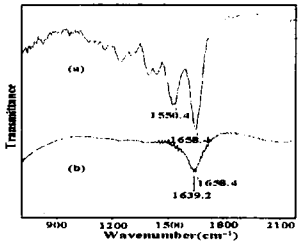
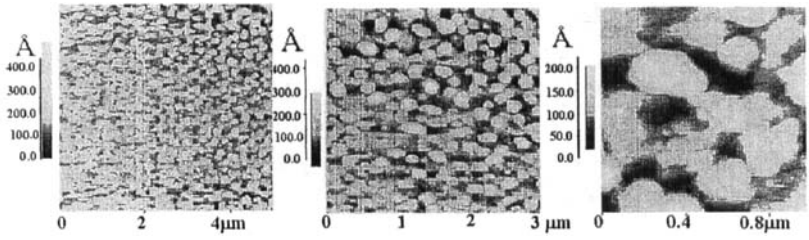


FIGURE 3. FT-IR spectra of (a) cytochrome *c* and (b) modified cytochrome *c*

The cytochrome *c* having a 2-pyridyldithio-propionamide is constructed with 2-pyridyldithio-propionamide and cytochrome *c*. And 2-pyridyldithio-propionamide and cytochrome *c* are linked with disulfide bridge. To cleave this disulfide bridge, DTT was used. Then cytochrome *c* having a 2-pyridyldithio-propionamide has a thiol group. Because thiol group is adsorbed onto the gold substrate, so this modified cytochrome *c* is adsorbed onto the gold substrate. Fig. 4 shows surface morphology of modified cytochrome *c* onto the gold substrate.



(a) (b) (c)

FIGURE 4. AFM image of modified cytochrome *c* onto the gold substrate (a: 5μm, b: 3μm, c: 1μm)

This SPDP method has simple but powerful tool for modification of functional group on the protein surface, because of the easy handling, high resolution and no wholly heating. In this study, the authors mainly focused on the modification of functional group on the cytochrome *c* surface and its potential application to the field of bioelectronics.

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