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纳米粒子-细胞色素 c 复合体系的  
光谱和光电化学研究

Spectroscopic and Photoelectrochemical Study of  
Nanoparticles-Cytochrome c Hybrid Systems

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## 摘要

近年来, 纳米粒子与生物大分子, 尤其是与蛋白质的相互结合作用及应用成为化学, 生物学和医学领域的研究热点。DNA、蛋白质(抗原/抗体、酶)等生物大分子由于和纳米粒子尺寸相近, 两者可以很好地构筑纳米粒子-生物大分子复合体系。生物分子具有生物独特的催化性能和独特的分子识别能力, 而纳米粒子具有特殊的光学、电学、催化性质, 复合体系结合两者的独特优势, 而被广泛应用在生物传感器、微型电路、纳米设备等等各个方面, 同时对生物学研究和医学诊断也有一定的指导意义。

本文的工作围绕着纳米粒子-细胞色素 c 复合体系的光谱性质, 两者内部的相互作用及应用展开, 主要进行了以下两方面的工作:

1. 构筑半导体纳米粒子-细胞色素 c 复合体系, 通过该复合体系的光谱性质和光电化学性质研究, 讨论了细胞色素 c 与不同类型的半导体纳米粒子之间的光诱导电子传递过程。
2. 构筑金纳米粒子-细胞色素 c 复合体系, 研究该复合体系的光谱性质, 利用金纳米粒子比色测定不同情况下细胞色素 c 的构象变化, 并将该复合体系应用作检测金属离子的光学传感器和 pH 值传感器。

研究取得以下主要结果:

1. 构筑了半导体纳米粒子-细胞色素 c 的复合体系, 得到了细胞色素 c 与两类半导体纳米粒子和一种半导体纳米棒之间的光诱导电子传递过程的光谱和光电化学证据。

- (1) 以  $\text{Na}_2\text{P}_4\text{O}_7$  为稳定剂的 CdS 和 ZnS 纳米粒子与细胞色素 c 间发生了光诱导电子传递过程, 导致半导体纳米粒子的荧光猝灭, 氧化态细胞色素 c 被还原。

- (2) 以 DNA 为模板兼稳定剂的 CdS 和 ZnS 纳米粒子与细胞色素 c 间的光诱导电子传递过程受阻, 氧化态细胞色素 c 没有被还原, 而细胞色素 c 的吸附作用导致 CdS 和 ZnS 纳米粒子的荧光发生不同程度的猝灭。

- (3) 通过光电流实验证实: 单纯的 ZnO 纳米棒与细胞色素 c 间的光诱导电子传递过程难以发生; Nafion 膜在 ZnO 纳米棒的表面修饰使得 ZnO 纳米棒与细胞

色素 c 间的光诱导电子传递过程容易发生。

2. 构筑了不同情况下的金纳米粒子-细胞色素 c 复合体系, 利用金纳米粒子比色测定了不同情况下细胞色素 c 的构象变化, 并将该复合体系应用为检测金属离子和 pH 值的光学传感器。

(1) 构筑不同 pH 值(pH=1-13)的金纳米粒子-细胞色素 c 复合体系, 利用金纳米粒子比色检测了不同浓度  $H^+$  引起的细胞色素 c 的构象变化。

(2) 构筑不同浓度的金纳米粒子-(L-半胱氨酸-细胞色素 c)复合体系, 利用金纳米粒子比色测定了不同浓度的 L-半胱氨酸引起的细胞色素 c 的构象变化。

(3) 构筑了金纳米粒子-(不同种类和不同浓度的金属离子-细胞色素 c)复合体系, 利用金纳米粒子实现了对  $Cu^{2+}$ ,  $Fe^{3+}$  的比色测定, 同时实现了比色测定不同种类和不同浓度金属离子引起的细胞色素 c 的构象变化。

(4) 利用金纳米粒子-( $Cu^{2+}/Fe^{3+}$ -细胞色素 c)复合体系对 pH 值的高灵敏度, 探索分别将两者应用为弱碱性和强酸性条件下的 pH 值传感器。

**关键词:** 细胞色素 c; 半导体纳米粒子; 金纳米粒子; 光谱; 光电化学

## Abstract

In recent years, the integration between nanoparticles and biomacromolecules, especially proteins has attracted a great many concerns in chemistry, biology and medicine field. Many biomaterials such as DNA and proteins (antigens/antibodies, enzyme) possess nanometric dimensions comparable to those of nanoparticles, and the size comparability paves a way to integrate nanoparticles and biomacromolecules into hybrid systems. As a result of these integrations, the unique electronic, photonic and catalytic properties of nanoparticles can be combined with biocatalytic functions and specific recognitions of biomacromolecules. Recently, substantial investigations have been carried out on nanoparticles–biomacromolecule hybrid systems to fabricate novel composites that might be applied to biosensors, nanoscale electronic circuitry and even nanodevices. Besides, the investigations of nanoparticles–biomacromolecule hybrid systems play an important role in biological and medicinal fields.

Accordingly, the emphasis of this thesis is focused on investigating spectroscopic properties of nanoparticles–biomacromolecules hybrid systems, the interaction of biomacromolecules and nanoparticles in hybrid systems and the application of them. These works can be divided into two parts:

1. Hybrid systems of cytochrome c and different kinds of semiconductor nanoparticles were constructed, and the photo-induced electron transfer between cytochrome c and semiconductor nanoparticles were investigated by spectroscopic photoelectrochemical properties of the systems.
2. gold nanoparticles-Cytochrome c hybrid systems were constructed and the spectroscopic properties of them were studied. These systems were applied for colorimetric detection conformational changes of cytochrome c, colorimetric detection of metal ions, and applied as pH sensors.

The main results are summarized as follows:

1. Hybrid systems of cytochrome c and two kinds of semiconductor nanoparticles

were constructed, and the photo-induced electron transfer between cytochrome c and semiconductor nanoparticles were investigated by spectroscopic and photoelectrochemical properties.

(1) In  $\text{Na}_4\text{P}_2\text{O}_7$  stabilized Cd(Zn)S nanoparticles-cytochrome c hybrid systems, the electron transfer between nanoparticles and cytochrome c occurred. As a result, the fluorescence of these nanoparticles quenched, and oxidized state cytochrome c was reduced.

(2) In DNA stabilized Cd(Zn)S nanoparticles-cytochrome c hybrid systems, the electron transfer between nanoparticles and cytochrome c was hindered along with oxidized state cytochrome c was not reduced. The different fluorescence quenching intensities of these nanoparticles were caused by the absorption of cytochrome c.

(3) Photocurrent responses approved: photo-induced electron transfer between pure ZnO nanorod and Cytochrome c did not happen, while Nafion film modification caused this electron transfer process happen.

2. Hybrid systems of cytochrome c in different conditions and gold nanoparticles were constructed, in which gold nanoparticles applied for colorimetric detection conformational changes of cytochrome c, colorimetric detection of metal ions, and applied as pH sensors.

(1) Hybrid systems of cytochrome c in different pH values (pH=1-13) and gold nanoparticles were constructed, in which gold nanoparticles were applied for colorimetric detection of  $\text{H}^+$  induced conformational changes of cytochrome c.

(2) Hybrid systems of different concentrations of L-cysteine-cytochrome c and gold nanoparticles were constructed, in which gold nanoparticles were applied for colorimetric detection of L-cysteine induced conformational changes of cytochrome c.

(3) Hybrid systems of different kinds and concentrations metal ions-cytochrome c and gold nanoparticles were constructed, in which gold nanoparticles were applied for colorimetric detection of  $\text{Cu}^{2+}$ ,  $\text{Fe}^{3+}$  and of different kinds and concentrations metal ions induced conformational changes of cytochrome c.

(4) As gold nanoparticles-( $\text{Cu}^{2+}/\text{Fe}^{3+}$ -cytochrome c) hybrid systems are quite sensitive to the values of pH, they were applied as pH sensors for weak base and



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