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On the Molecular Mechanism of Electron Transfer of Cytochrome c Modulated by Gold Nanoparticles in Nano-Sandwich Architecture

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纳米金三明治结构调制细胞色素 c 电子传递特性的分子机理研究

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摘要: 以细胞色素 c (Cyt c) 为模型蛋白, 采用表面增强红外吸收光谱监测了三明治结构所吸附的纳米金对氧化还原诱导的 Cyt c 表面增强红外差谱的改变. 研究表明, 在单层 Cyt c 分子表面组装纳米金, 使得血红素的红外差谱特征峰明显增强, 这归因于纳米金和血红素之间的电子传递. 纳米金与 Cyt c 氧化还原活性中心血红素的相互作用加速了蛋白质的电子传递. 这为实现并优化表面吸附蛋白质的直接电化学提供了一种新技术.

关键词: 细胞色素 c; 纳米金; 纳米三明治结构; 表面增强红外吸收光谱; 电子传递

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近年, 基于纳米材料和氧化还原蛋白构建的新型电化学生物传感器因优异的性能而备受关注^[1]. 其中, 纳米金 (Gold Nanoparticles, AuNPs) 是优良的电子导体, 其比表面积大, 制作简单, 易于表面化学修饰^[2-3], 且生物相容性良好, 与生物分子组装可介导生物分子电子传递, 因而具有独特的优势. 纳米金在典型的葡萄糖传感器的构建上已取得良好的成果^[4-8], 但如何进一步提高纳米生物传感器的性能, 已成为该类传感器发展与应用的瓶颈. 若能从分子水平上认识纳米材料介导的氧化还原蛋白的电子传递机理, 对优化生物传感器的性能将起到关键性的推动作用.

细胞色素 c (Cytochrome c, Cyt c) 属线粒体内膜呼吸链上的电子传递蛋白, 它从细胞色素 c 还原酶接受电子传递给细胞色素 c 氧化酶. 因其天然电子传递特性和较小的尺寸, 经常被用于构建生物传感器, 是研究蛋白质电子传递机理的较好的模型蛋白. Cyt c 修饰电极的直接电化学常有报道^[9-12]. 文献曾报道纳米金的修饰可以促进蛋白质的直接电化学, 一定尺寸的纳米金可能会适当渗入到蛋白内部, 降低电极与包埋在蛋白质内部的氧化还原位点的距离^[3, 13-15]. 表面增强红外吸收光

谱 (Surface Enhanced Infrared Absorption Spectroscopy, SEIRAS) 可用于研究界面吸附的单层蛋白的二级结构的微小变化, 并可提供丰富的分子水平信息^[16-19]. 本文用纳米金在基底上构建 AuNPs/Cyt c/Au 三明治模型, 采用 SEIRAS 技术, 从分子水平上探索纳米金介导的 Cyt c 直接电子转移过程机理. 研究表明, 纳米金的组装可影响 Cyt c 电子传递中心血红素的电子传递, 从而显著地改善蛋白的电子传递特性.

1 实验

1.1 试剂与仪器

NaAuCl₄ · 2H₂O (99.99%, Sigma), 马心细胞色素 c (95%, Sigma), 11-巯基十一烷酸 (MUA, 95%, J&K), N-(3-二甲基胺基丙基)-N'-乙基碳二亚胺 (EDC, 99%, J&K), N-羟基琥珀酰亚胺 (NHS, 98%, J&K), D-青霉胺 (DPA, 98%, Sigma), Milli-Q 超纯水 (18.2 MΩ · cm).

傅里叶变换红外光谱仪 (型号 66V/S, BRUKER OPTICS), 自制表面增强红外反射附件. 电化学工作站 (CHI 830A, 上海辰华), 铂网为对电极, 饱和 Ag/AgCl 为参比电极.

1.2 组装与测试

纳米金:在快速搅拌下,向 20 mL 0.01% (by mass) NaAuCl_4 溶液 (100 °C) 中滴入 0.3 mL 1% (by mass) 柠檬酸三钠溶液,继续加热 10 min,冷却^[20].

金膜基底:硅晶体表面用 1 μm 抛光粉打磨抛光,超纯水冲洗 2 次,浸入 NH_4F 溶液 (40%, by volume) 1 min,使其表面疏水.将 0.03 mol · L⁻¹ NaAuCl_4 溶液、0.3 mol · L⁻¹ Na_2SO_4 + 0.1 mol · L⁻¹ $\text{Na}_2\text{S}_2\text{O}_3$ + 0.1 mol · L⁻¹ NH_4Cl 混合液和 HF 溶液 (2.5%, by volume) 各 330 μL 依次混合,滴于硅晶体表面,静置 60 s,超纯水冲洗.该金膜与铂网对电极和饱和 Ag/AgCl 参比电极在 0.1 mol · L⁻¹ H_2SO_4 电解液中,电位范围 0.1 ~ 1.3 V 扫描净化.

Au/Cyt c/AuNPs 电极:将金膜基底浸入 1.5 mmol · L⁻¹ MUA 的乙醇溶液 20 min,超纯水多次冲洗,即得 MUA/Au 电极.将 0.01 g EDC 和 0.007 g NHS 分别溶于 0.5 mL KH_2PO_4 溶液 (10.0 mmol · L⁻¹, pH 4.73),把 MUA/Au 膜浸于 EDC-NHS 混合液中 20 min,超纯水多次冲洗.然后将 MUA/Au 膜浸于 2.0 $\mu\text{mol} \cdot \text{L}^{-1}$ Cyt c 的磷酸缓冲溶液 (PBS, 10.0 mmol · L⁻¹, pH 7.2) 2.5 h,用 PBS 溶液冲洗,将组装 Cyt c 的 MUA/Au 膜浸入 2.0 $\mu\text{mol} \cdot \text{L}^{-1}$ DPA 的 PBS 溶液 (10.0 mmol · L⁻¹, pH 7.2) 5 h.超纯水冲洗电极后,再浸于 1 mL 纳米金溶液中 1.5 h,超纯水多次冲洗后即得 AuNPs/Cyt c/Au 三明治结构电极 (如图 1). 组装过程由表面增强红外吸收仪原位监测,以样品相应溶剂的红外吸收光谱为背景,每条样品谱均 512 次扫描.由 AuNPs/Cyt c/Au 工作电极、铂网对电极、饱和 Ag/AgCl 参比电极和 PBS 电解液 (10.0 mmol · L⁻¹, pH 7.2) 组成三电极体系,在电位范围 -0.2 ~ 0.2 V 进行循环伏安扫描,扫速 100 mV · s⁻¹. 实验前通高纯氮气 15 min 除去溶液的溶解氧.恒电位 -0.2 V,测得 Cyt c 还原的红外吸收光谱 (背景谱),恒电位 0.2 V,测得 Cyt c 氧化的红外吸收光谱 (样品谱),即可得电位诱导表面增强红外差谱.背景谱和样品谱均为 512 次扫描,重复 20 次取平均谱,以提高信噪比.

2 结果与讨论

2.1 循环伏安测试

图 2 给出 Cyt c/MUA/Au 电极在 PBS 溶液 (10 mmol · L⁻¹, pH 7.2) 中的循环伏安 (CV) 曲线.

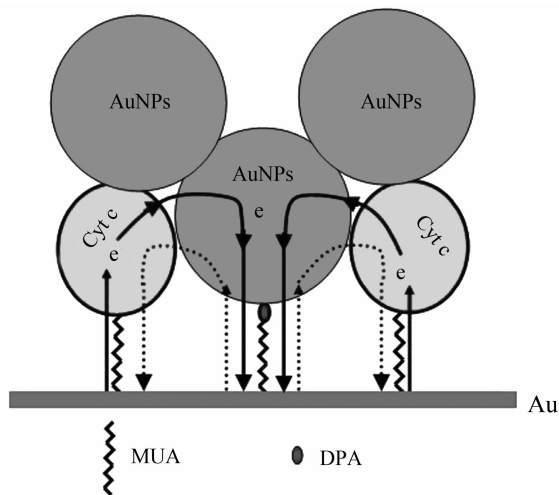


图 1 AuNPs/Cyt c/Au 三明治结构电极示意图

Fig. 1 Schematic representation of AuNPs/Cyt c/Au sandwich architecture

如曲线 a 所示,在 0.0076 V 处出现 Cyt c 氧化电流峰,在 -0.051 V 处出现 Cyt c 还原电流峰,峰电位差 $\Delta E = 0.058$ V. 据公式 $\Gamma = Q/nFA$ (Γ 为电活性物质的表面覆盖度, Q 为反应电量, n 为电子转移数, F 为法拉第常数, A 为电极面积),按粗糙因子为 2.5 计算电极几何面积为 1.77 cm^2 , Cyt c 表面覆盖度为 4 $\text{pmol} \cdot \text{cm}^{-2}$. 此值远小于据 Cyt c 晶体结构计算的理论单层覆盖度 (15 $\text{pmol} \cdot \text{cm}^{-2}$),表明 Cyt c 应以单层形式吸附于电极表面. 纳米金组装后, AuNPs/Cyt c/MUA/Au 电极在 -0.020 V 和 -0.048 V 处出现 Cyt c 氧化和还原峰,峰电位差 $\Delta E = 0.028$ V (曲线 b),小于无纳米金修饰时的峰电位差. 由此可知,纳米金的修饰可较好地促进 Cyt c 电子转移.

2.2 Cyt c 及纳米金组装过程的表面增强红外光谱

图 3 谱线 a 示出金膜浸于 500 μL 无水乙醇中而后加入 500 μL 3.0 mmol · L⁻¹ MUA 乙醇溶液 20 min 后记录的表面增强红外光谱. 从谱线看出, 1709 cm^{-1} 处的吸收峰归属为 MUA 分子中羰基 (C=O) 伸缩振动^[21]. 曲线 b 给出金膜浸于 0.5 mL KH_2PO_4 溶液 (10.0 mmol · L⁻¹, pH 4.73) 中而后又加入 EDC-NHS 的混合溶液 20 min 后记录的表面增强红外光谱. 从谱线看出, 1738 cm^{-1} 和 1782 cm^{-1} 处的吸收峰分别归属为 NHS 头部官能团琥珀酰亚胺环 2 个 C=O 基团的不对称伸缩振动和对称伸缩振动^[22], 1813 cm^{-1} 处的吸收峰则对

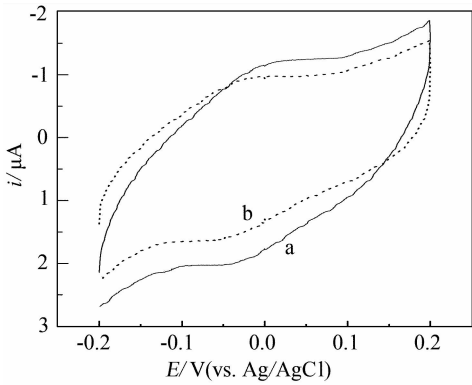


图 2 Cyt c/MUA/Au (a) 和 AuNPs/Cyt c/MUA/Au (b) 在 PBS 溶液 ($10.0 \text{ mmol} \cdot \text{L}^{-1}$, pH 7.2) 中的循环伏安曲线 (扫速 $100 \text{ mV} \cdot \text{s}^{-1}$)

Fig. 2 Cyclic voltammograms of the Cyt c/MUA/Au (a) and AuNPs/Cyt c/MUA/Au (b) in PBS solution ($10.0 \text{ mmol} \cdot \text{L}^{-1}$, pH 7.2) at a scan rate of $100 \text{ mV} \cdot \text{s}^{-1}$

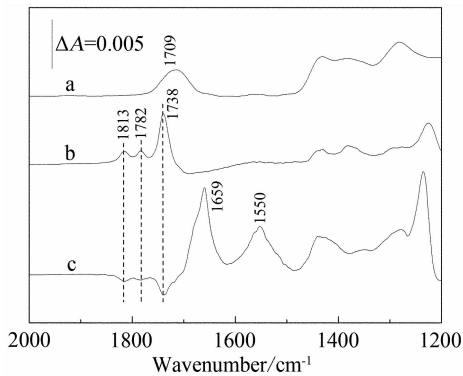


图 3 金膜表面组装 MUA (a)、NHS (b) 和 Cyt c (c) 的表面增强红外吸收光谱图

Fig. 3 Surface-enhanced infrared absorption (SEIRA) spectra of MUA (a), NHS (b) and Cyt c (c) adsorbed on the Au surface

应于酯基中 $\text{C}=\text{O}$ 基团的伸缩振动^[22]. 曲线 c 给出 Cyt c 溶液加入后 Cyt c 与 MUA 通过共价作用连接于金膜表面的表面增强红外光谱. 可以看出 NHS 被消耗, 对应的振动吸收呈现负吸收峰. 对比谱线 b、c, 1738 cm^{-1} 处正负相反的吸收峰强度, 可推断 Cyt c 分子约占据了 $1/3$ 的吸附位点, 并以单层形式吸附于电极表面. 位于 1659 cm^{-1} 和 1550 cm^{-1} 两处正吸收峰归属为细胞色素 c 蛋白质骨架中酰胺 I 带 $\text{C}=\text{O}$ 伸缩振动和酰胺 II 带 $\text{N}-\text{H}$ 面内弯曲振动和 $\text{C}-\text{N}$ 面内拉伸振动^[23-25].

图 4 给出金膜表面组装 DPA (a) 和纳米金 (b) 的表面增强红外光谱谱图. 将 Cyt c 分子修饰的金

膜浸于 PBS 溶液 ($10 \text{ mmol} \cdot \text{L}^{-1}$, pH 7.2), 记录背景光谱, 而后加入 DPA 溶液 5 h 后记录样品谱. 从曲线 a 可以看出 DPA 分子的氨基基团与 NHS 反应, NHS 继续消耗, 在 1813 、 1782 和 1738 cm^{-1} 处呈现负吸收峰. 从吸收峰强度推断, DPA 分子约占据 $2/3$ 的吸附位点. 1665 和 1550 cm^{-1} 处的正吸收峰归属于 DPA 与 MUA 键合生成的酰胺键, 即 $\text{C}=\text{O}$ 伸缩振动和 $\text{N}-\text{H}$ 弯曲振动^[26-28]. 而后金膜又浸于金溶胶中, DPA 分子的巯基与纳米金相互作用, 部分纳米金与 DPA 作用组装到电极上, 部分纳米金与蛋白分子发生非特异性作用而吸附于电极表面, 1719 cm^{-1} 处即是纳米金表面保护剂羧酸的羧基特征吸收峰 (谱线 b)^[21].

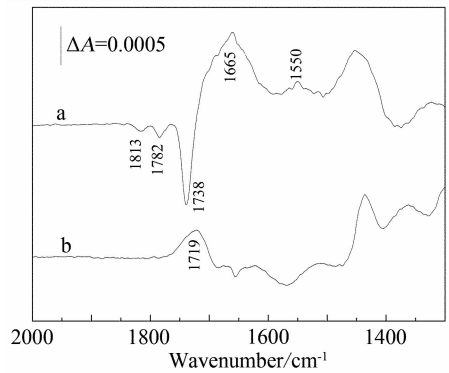


图 4 金膜表面组装 DPA (a) 和纳米金 (b) 的表面增强红外吸收光谱图

Fig. 4 SEIRA spectra of DPA (a) and gold nanoparticles (b) assembled on the Au surface

2.3 电位诱导表面增强红外差谱

图 5 示出 Cyt c 组装纳米金前后电位诱导表面增强红外差谱. Cyt c 的表面增强红外差谱特征与文献报道的基本一致^[29]. 酰胺 I 带位于 1693 cm^{-1} 和 1666 cm^{-1} 处的负峰为还原态 Cyt c 峰, 而 1673 cm^{-1} 和 1660 cm^{-1} 处正峰即为氧化态 Cyt c 峰. 1693 cm^{-1} 和 1673 cm^{-1} 处的差谱峰归属于类型 III β -转角, 由残基 14-17 和 67-70 组成^[29], 1666 cm^{-1} 处吸收峰归属于类型 II β -转角, 由残基 32-35 和 35-38 组成^[29]. 除了酰胺 I 区, 还可以观察到酰胺 II 区 ($1600 \sim 1500 \text{ cm}^{-1}$) 的差谱. $1602 (+)/1595 (-)/1582 (+) \text{ cm}^{-1}$ 处的差谱峰归属于血红素的 ν ($\text{C}_\beta\text{C}_\beta$) 和 ν ($\text{C}_\alpha\text{C}_m$)_{asym} 振动^[26] (各峰归属列于表 1). 1739 、 1789 和 1813 cm^{-1} 处的吸收峰并不是蛋白质氧化还原过程中的差谱特征峰, 而应归属于

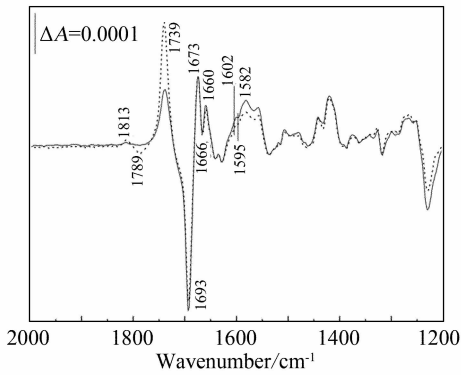


图5 组装纳米金前(虚线)、后(实线)电位诱导细胞色素c的表面增强红外差谱

Fig. 5 SEIRA difference spectra of potential-induced Cyt c without AuNPs (dotted line) and with AuNPs adsorption (solid line)

MUA和NHS氧化还原反应变化. 因组装了DPA分子后, NHS被消耗, 所以1789和1813 cm^{-1} 处的吸收峰消失, 1739 cm^{-1} 处的吸收峰也降低.

值得注意的是, 纳米金组装后血红素的差谱特征峰明显增强, 这可能是由于纳米金和血红素之间的电荷传递的诱导作用. 在入射光的激发下, 电子由纳米金转移到血红素的某一激发态分子轨道(图1虚线路径)或者由血红素已占据的分子轨道向纳米金转移(图1实线路径). 当入射光子的

能量与电子转移的能量差相等时将产生共振, 进而增大分子振动的有效偶极距, 引发红外信号增强. 文献曾报道, 具有适当尺寸和表面修饰的纳米金与酶的氧化还原活性中心相连, 可充当电流收集器和电子释放器, 极大地改善了酶的电子传递特性^[4]. 纳米金与Cyt c氧化还原活性中心血红素的相互作用提高了其电子传递能力.

3 结论

三明治结构中纳米金的组装, 能较好地促进Cyt c的电子传递. 本文采用表面增强红外光谱原位监测三明治结构的组装过程, 并考察了纳米金的组装对氧化还原诱导的Cyt c表面增强红外差谱的改变. 研究发现, 蛋白质的氧化还原活性中心血红素的差谱特征峰明显增强, 这归因于纳米金和血红素环之间的电子传递反应. 固定在修饰电极表面的Cyt c发生氧化还原反应时, 电子不仅可直接与修饰电极进行交换, 还能加速穿过纳米金, 实现与电极间的交换. 总之, 从分子水平上探索界面蛋白质的电子传递机理, 可对优化界面蛋白质的组装条件、实现蛋白质的直接电化学提供指导.

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表1 Cyt c的电位诱导表面增强红外差谱特征峰归属

Tab. 1 Vibrational assignments in the SEIRA difference spectra of redox-induced Cyt c

Reduced state	Oxidized state	Assignment	Designation
1693		Amide I	β -turn type III
	1673	Amide I	β -turn type III
1666		Amide I	β -turn type II
	1660	Amide I	β -turn type II and/or α -helix
1640		Amide I	Unordered
	1633	Amide I	Extended β -strand
1627		Amide I	Extended β -strand
	1602	Heme ν_{37}	$\nu(\text{C}_\beta\text{C}_\beta)$
1595		Heme ν_{37}	$\nu(\text{C}_\beta\text{C}_\beta)$
	1582	Heme ν_{37}	$\nu(\text{C}_\alpha\text{C}_m)_{\text{asym}}$
1564		Amide II	β -turn type III
	1556	Amide II	β -turn type III

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Studies on the Molecular Mechanism of Electron Transfer of Cytochrome c Modulated by Gold Nanoparticles in Nano-Sandwich Architecture

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Abstract: The effects of adsorbed gold nanoparticles on the surface-enhanced infrared absorption (SEIRA) difference spectra of redox-induced cytochrome (Cyt) c in AuNPs/Cyt c/Au sandwich architecture were monitored by SEIRA spectroscopy. The results indicated that the intensity of SEIRA difference spectrum for the vibration of the heme was significantly increased due to the adsorption of gold nanoparticles on the Cyt c. This was induced by the electron transfer between the heme and the gold nanoparticles, which also promoted electron transfer of adsorbed protein. This study suggested a new technique for optimizing the electrochemical property of adsorbed protein.

Key words: Cytochrome c; gold nanoparticles; nano-sandwich architecture; surface-enhanced infrared absorption spectroscopy; electron transfer