

DOI: 10.13208/j.electrochem.131161

Artical ID:1006-3471(2014)04-0386-06

Cite this: *J. Electrochem.* 2014, 20(4): 386-391

Http://electrochem.xmu.edu.cn

基于碳纳米管的高灵敏度免标记 电化学核酸适体传感电极

邓春艳^{1,2}, 范慧敏², 向娟², 李元建^{1*}

(1. 中南大学药学院, 湖南 长沙 410083; 2. 中南大学化学化工学院, 湖南 长沙 410083)

摘要: 以电活性钌化合物 $[\text{Ru}(\text{NH}_3)_6]^{3+}$ 为信号传感源, 借助碳纳米管构建了高灵敏检测腺苷免标记电化学传感电极 (BSA/Apt/CNTs/GC). BSA/Apt/CNTs/GC 电极在最佳实验条件下检测腺苷线性范围为 $5.0 \times 10^{-11} \sim 1.0 \times 10^{-7} \text{ mol} \cdot \text{L}^{-1}$, 检测下限为 $2.7 \times 10^{-11} \text{ mol} \cdot \text{L}^{-1}$. 该传感电极有较高的灵敏度、良好的选择性、重现性和稳定性. 与传统标记型适体传感电极相比, 其制作简便, 也许还适用于其他小分子和蛋白质的检测, 有一定的普适性.

关键词: 核酸适体; 腺苷; 免标记; 碳纳米管

中图分类号: O646

文献标识码: A

腺苷 (Adenosine) 是一种遍布人体细胞的内源性嘌呤核苷, 在生化过程中扮演着重要的角色. 腺苷不仅参与能量代谢, 还是生命遗传物质 DNA 和 RNA 的重要组成部分, 对人体功能具有极其重要的作用.

传统的腺苷检测主要有反相高效液相色谱-质谱联用法 (HPLC-MS 法) 及荧光检测法等^[1-4]. 荧光检测有较高的选择性, 不过腺苷本身并不具有较强的荧光, 其衍生化程序必不可少. HPLC-MS 法灵敏度很高, 但质谱仪价格昂贵, 难以实现常规监测.

核酸适体是可由人工构建的随机寡核苷酸序列库中筛选并能特异性结合生物小分子 (可卡因或腺苷)、蛋白质 (凝血酶或 PDGF) 甚至完整病毒和癌细胞的 DNA 或 RNA 寡核苷酸片断^[5], 因其分子量小、制备简单、性质稳定、易于标记和可修饰在生化研究领域备受关注^[6-8]. 核酸适体用于腺苷检测主要有表面增强拉曼散射法、荧光共振能量转移法、亲和层析法以及比色法等^[9-14]. 这些适体传感电极对靶物质的检测有较高的选择性和灵敏度, 而在构建过程往往需要借助特效试剂或探针分子标记核酸适体, 该标记过程使实验过程变得相对复杂, 一定程度上影响了适体与目标分子之间的

亲和力. 因此, 仍有必要发展一种免标记、低成本高灵敏检测腺苷的适体生物传感电极.

据文献报道, 大部分免标记核酸适体生物传感电极的小分子检测灵敏度不高. 为此, 构建高灵敏度传感电极并识别目标分子就显得十分重要. 高灵敏度的滚环扩增^[15], 链置换扩增^[16]和金纳米粒子^[17-18]等业已被引入核酸适体传感电极的研究, 而其实验条件苛刻、检测步骤繁琐. 故仍需寻找简便、快捷、可靠的适体生物传感电极.

本文借助核酸适体构型与碳纳米管 (CNTs) 构建了一种高灵敏检测腺苷的免标记电化学传感电极. 电活性钌化合物 $[\text{Ru}(\text{NH}_3)_6]^{3+}$ 可静电吸附在带负电荷的 DNA 磷酸盐骨架上, 作为信号传感源. 碳纳米管 CNTs 可吸附大量的 DNA 片段, 以期实现高灵敏检测.

1 实验

1.1 仪器及试剂

由玻碳基底工作电极、饱和甘汞电极 (SCE) 和铂辅助电极组成三电极体系, 采用电化学阻抗法 (EIS)、循环伏安法 (CV) 和计时电量法 (CC) 在 CHI650D 电化学工作站 (上海辰华) 上测试传感电极性能. 在 $0.1 \text{ mol} \cdot \text{L}^{-1} \text{ KCl} + 5 \text{ mmol} \cdot \text{L}^{-1} [\text{Fe}(\text{CN})_6]^{3-/4-}$

收稿日期: 2013-11-26, 修订日期: 2014-04-09 * 通讯作者, Tel: (86-731)82650372, E-mail: yuan_jianli@yahoo.com

国家自然科学基金项目 (No. 21005090)、中国博士后基金项目 (No. 2012M510136) 和中国博士基金特别资助项目 (No. 2013T60774) 资助

(1:1) 溶液中测试电极阻抗谱图, 频率范围0.1 Hz ~ 100 kHz, 振幅5 mV. 在含有 $5.0\text{ }\mu\text{mol}\cdot\text{L}^{-1}$ $[\text{Ru}(\text{NH}_3)_6]^{3+}$ + $20\text{ mmol}\cdot\text{L}^{-1}$ Tris-HCl (pH = 7.4) 缓冲溶液中测试循环伏安曲线, 范围-0.6 ~ 0.2 V, 扫描速率 $50\text{ mV}\cdot\text{s}^{-1}$; 测试计时电量曲线, 范围-0.5 ~ 0.1 V, 脉冲宽度0.25 s. 每次测定缓冲液均需通氮除氧操作, 温度 $(25\pm 2)\text{ }^\circ\text{C}$.

腺苷适体DNA碱基序列: 5'-ACC TGG GGG AGT ATT GCG GAG GAA GGT TTT TTT. 将DNA离心处理, 随后加入Tris-HCl缓冲溶液, 振荡5 ~ 10 min, 配制成浓度 $10\text{ }\mu\text{mol}\cdot\text{L}^{-1}$ 溶液, $4\text{ }^\circ\text{C}$ 冰箱储存备用. 碳纳米管(CNTs, ~95%, 直径20 ~ 30 nm)在浓缩的硫酸-硝酸混合溶液(3:1, V/V)超声降解3 h, 过滤, 二次蒸馏水洗涤, $60\text{ }^\circ\text{C}$ 真空干燥. 将酸化处理的CNTs在二次蒸馏水中超声振荡1 h形成黑色悬浮液(浓度 $1\text{ mg}\cdot\text{mL}^{-1}$). 其它试剂均为分析纯, 未纯化处理. 实验用水均为超纯水.

1.2 BSA/Apt/CNTs/GC电极

玻碳基底经 $0.3\text{ }\mu\text{m}$ 、 $0.05\text{ }\mu\text{m}$ 氧化铝粉打磨至镜面, 并置于新鲜配制的 $\text{H}_2\text{SO}_4\text{-H}_2\text{O}_2$ 混合液 (2:1, V/V)浸泡1 min, 乙醇和超纯水中分别超声清洗2 ~ 3 min. 在 $0.5\text{ mol}\cdot\text{L}^{-1}$ H_2SO_4 溶液电化学扫描 (电位范围-0.15 ~ 1.0 V, 扫速 $50\text{ mV}\cdot\text{s}^{-1}$)直至循环伏安曲线稳定, 超纯水充分洗涤, 氮气吹干.

将 $6\text{ }\mu\text{L}$ CNTs溶液($1\text{ mg}\cdot\text{mL}^{-1}$)滴涂于玻碳基底表面, 室温自然晾干. 而后冲洗电极, 氮气吹干, 即可得CNTs/GC电极. 随后将 $10\text{ }\mu\text{L}$ 核酸适体溶液

滴于CNTs/GC电极表面, 为防止适体溶液挥发, 盖电极帽, 静置120 min, 形成核酸适体分子膜. 再将该电极浸泡于 $5\text{ mmol}\cdot\text{L}^{-1}$ 的牛血清白蛋白(BSA)溶液1 h以封闭电极表面未结合的活性位点, 即得BSA/Apt/CNTs/GC电极, 并置于 $4\text{ }^\circ\text{C}$ 冰箱储存备用. 每步实验操作之后, 均分别用Tris-HCl缓冲溶液和超纯水冲洗电极, 氮气吹干.

1.3 腺苷的检测

将BSA/Apt/CNTs/GC电极浸于含有 $[\text{Ru}(\text{NH}_3)_6]^{3+}$ 的Tris-HCl缓冲液, 因适体DNA带负电荷, $[\text{Ru}(\text{NH}_3)_6]^{3+}$ 可静电吸附于适体DNA修饰的电极表面. 当该电极浸于腺苷溶液, 适体DNA片段会与腺苷结合形成稳定的适体-靶复合物, 并从电极表面脱落释放于溶液中, 使 $[\text{Ru}(\text{NH}_3)_6]^{3+}$ 在DNA修饰电极峰电流信号减小, 其信号减少量与腺苷浓度有关, 故该传感电极可用于腺苷的定量检测. 较高的比表面碳纳米管可吸附更多的适体DNA分子, 也就有更多的阳离子 $[\text{Ru}(\text{NH}_3)_6]^{3+}$ 吸附于修饰电极表面. 一旦加入腺苷, 较大量的DNA链从电极表面脱落, 致使 $[\text{Ru}(\text{NH}_3)_6]^{3+}$ 的电化学信号变化增强(见图1).

2 结果与讨论

2.1 电极的电化学阻抗谱图

图2给出以 $[\text{Fe}(\text{CN})_6]^{3-/4-}$ 为氧化还原探针, 不同修饰电极的电化学阻抗谱图. 玻碳电极的阻抗半圆直径很小($R_{ct} = 80\text{ }\Omega$), 其电子转移非常快(图2a). CNTs/GC电极之后, 阻抗谱图只为一

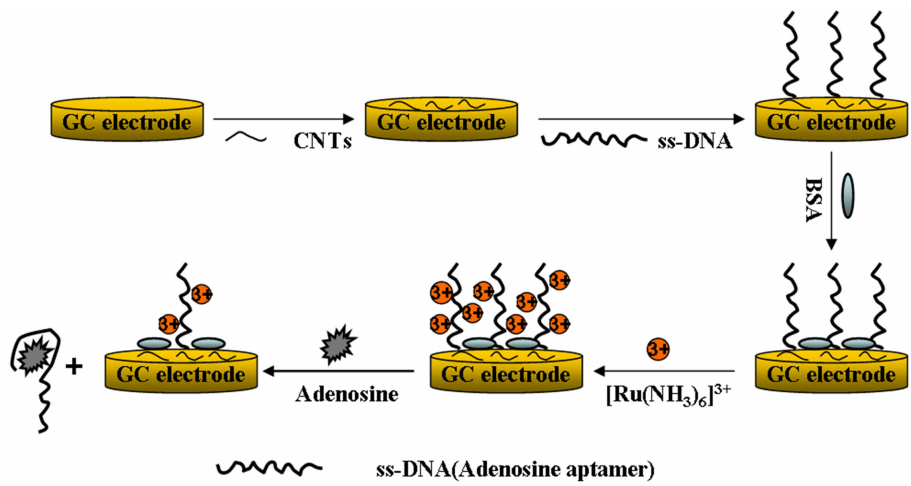


图1 核酸适体传感电极的腺苷检测示意图
Fig. 1 Schematic representation of aptasensor for detection of adenosine

线,其电子转移更快(图2b),这归因于CNTs优良电子传递性能^[19].当适体DNA组装至CNTs/GC电极表面,其 R_{ct} 显著增加(从50 Ω 增至200 Ω ,图2c),因DNA单链核酸的磷酸骨架带负电,静电排斥 $[\text{Fe}(\text{CN})_6]^{3-/4-}$,从而有效阻止了其与电极表面的电子传递.当BSA封闭于电极表面未结合的活性位点消除非特异性吸附, R_{ct} 进一步增大(图2d).将BSA/Apt/CNTs/GC电极浸泡于100 $\text{nmol} \cdot \text{L}^{-1}$ 腺苷溶液2 h后,其 R_{ct} 呈明显下降趋势(图2e,从240 Ω 降至170 Ω).这可能主要归因于适体-腺苷复合物的形成及其从电极表面的脱落,上述实验证明了该电极已成功构筑且CNTs与适体以及适体与腺苷之间可成功复合.

2.2 传感电极的电化学特性

图3示出结合腺苷分子前后BSA/Apt/CNTs/GC电极在5.0 $\mu\text{mol} \cdot \text{L}^{-1}$ $[\text{Ru}(\text{NH}_3)_6]^{3+}$ 溶液中的循环伏安曲线(A)和计时电量曲线(B).如图3A所示,无腺苷时,BSA/Apt/CNTs/GC电极在 $[\text{Ru}(\text{NH}_3)_6]^{3+}$ 溶液有1对明显的氧化还原峰(-0.38V和-0.34 V,图3A-a).而当BSA/Apt/CNTs/GC电极与腺苷分子结合后,该电极在 $[\text{Ru}(\text{NH}_3)_6]^{3+}$ 溶液的电化学特性发生变化,其氧化还原电流明显降低(图3A-b).这主要归因于适体和腺苷之间发生了特异性结合,适体-腺苷复合物从电极表面脱落.图3B为该电极计时电量曲线,引入20 $\text{nmol} \cdot \text{L}^{-1}$ 腺苷,其计时电量信号降低约(15 \pm 1)%.

2.3 腺苷的计时电量曲线

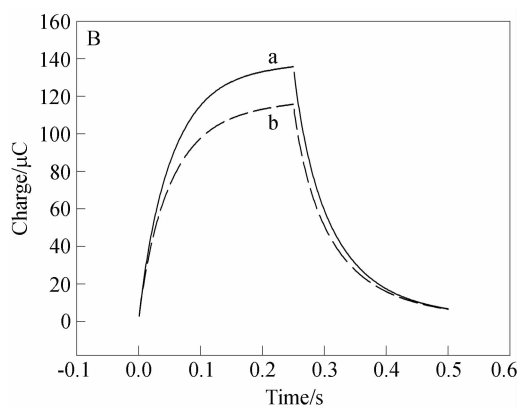
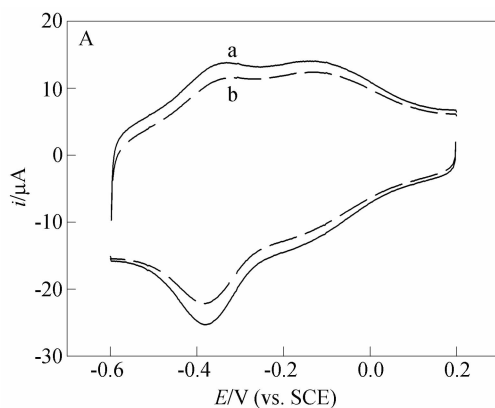


图3 BSA/Apt/CNTs/GC电极结合腺苷分子20 $\text{nmol} \cdot \text{L}^{-1}$ 前(a)、后(b)在5.0 $\mu\text{mol} \cdot \text{L}^{-1}$ $[\text{Ru}(\text{NH}_3)_6]^{3+}$ 溶液的循环伏安曲线(A)和计时电量曲线(B)

Fig. 3 Cyclic voltammograms (A) and Chronocoulometric responses (B) of BSA/Apt/CNTs/GC electrode in the absence (a) and presence (b) of 20 $\text{nmol} \cdot \text{L}^{-1}$ adenosine in 20 $\text{mmol} \cdot \text{L}^{-1}$ Tris-HCl solution (pH = 7.4) containing 5.0 $\mu\text{mol} \cdot \text{L}^{-1}$ $[\text{Ru}(\text{NH}_3)_6]^{3+}$

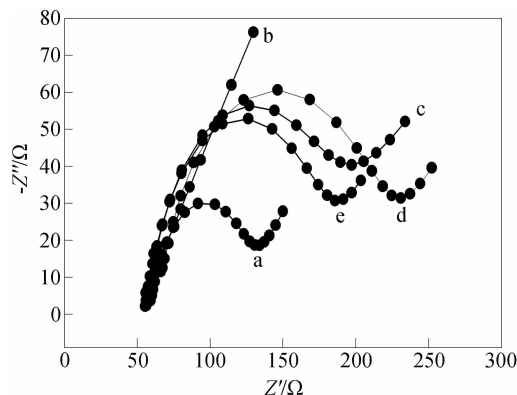


图2 电极电化学阻抗谱图 电解液:0.1 $\text{mol} \cdot \text{L}^{-1}$ KCl + 5 $\text{mmol} \cdot \text{L}^{-1}$ $[\text{Fe}(\text{CN})_6]^{3-/4-}$ (1:1)溶液
a. GC电极;b. CNTs/GC电极;c. Apt/CNTs/GC电极;
d. BSA/Apt/CNTs/GC电极;e. 结合腺苷(20 $\text{nmol} \cdot \text{L}^{-1}$)
的d电极

Fig. 2 Nyquist plots of different electrodes obtained after treatment with 20 $\text{nmol} \cdot \text{L}^{-1}$ adenosine and in 5.0 $\text{mmol} \cdot \text{L}^{-1}$ $[\text{Fe}(\text{CN})_6]^{3-/4-}$ solution containing 0.1 $\text{mol} \cdot \text{L}^{-1}$ KCl a. bare GC electrode; b. CNTs/GC electrode; c. Apt/CNTs/GC electrode; d. BSA/Apt/CNTs/GC electrode; e. BSA/Apt/CNTs/GC electrode

图4为最佳优化条件下不同浓度腺苷BSA/Apt/CNTs/GC电极的计时电量曲线(ΔQ),检测范围0.05 ~ 400 $\text{nmol} \cdot \text{L}^{-1}$.从图可以看出, ΔQ 随腺苷浓度的增加而不断增大,在100 $\text{nmol} \cdot \text{L}^{-1}$ 腺苷浓度时其计时电量信号达到饱和.该传感电极的线性回归方程 $y = 0.2594x + 15.469$ (其中 y 和 x 分别代表

电荷的变化量 $\Delta Q(\mu\text{C})$ 和腺苷浓度 $C(\text{nmol}\cdot\text{L}^{-1})$), 相关系数 $r = 0.9962$, 检出限 $2.7\times 10^{-11} \text{ mol}\cdot\text{L}^{-1}$ (3σ , $n=11$).

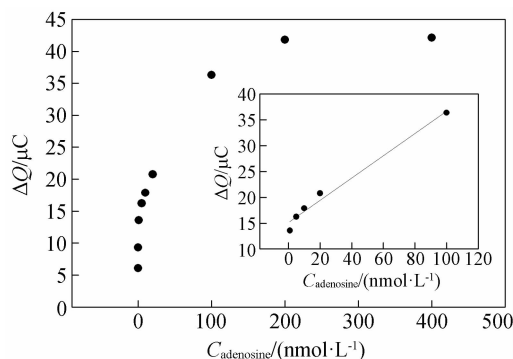


图4 不同浓度的腺苷在BSA/Apt/CNTs/GC电极上的计时电量曲线,插图为低浓度腺苷在BSA/Apt/CNTs/GC电极上的计时电量线性曲线

Fig. 4 Chronocoulometric response of BSA/Apt/CNTs/GC electrode to different concentrations of adenosine (inset shows the chronocoulometric curve at low concentrations)

与其他腺苷检测的DNA酶生物传感电极^[20-21]相比,BSA/Apt/CNTs/GC腺苷传感电极有较高的灵敏度.此外,其检出限远低于之前文献报道,较纳米金团聚的比色法^[22]下降60倍、二茂铁标记核酸适体的传感器下降1000倍、表面等离子体共振(SPR)薄膜传感电极下降至少2个数量级^[23].

BSA/Apt/CNTs/GC传感电极可实现对腺苷的快速检测(约3个小时).一些文献未具体标明腺苷测定所需时间,其他方法的腺苷检测一般均超过9个小时^[24-26].

2.4 传感电极选择性

图5为相同实验条件下传感电极在 $2 \mu\text{mol}\cdot\text{L}^{-1}$ 鸟苷(U)、胞苷(C)和尿苷(G)的电化学响应曲线.尽管这几种干扰物的结构与腺苷非常相似,且其浓度较腺苷($20 \text{ nmol}\cdot\text{L}^{-1}$)高,但与核酸适体和腺苷结合产生的电化学信号相比,其他几种核苷引起的信号却远要小得多.其他核苷(鸟苷、胞苷、尿苷)与腺苷核酸适体之间几乎没有发生非特异性结合.同时,BSA/Apt/CNTs/GC电极在4种核苷混合样品的电化学响应信号变化值与在单一腺苷中相近;其腺苷检测有高度特异性与选择性.

2.5 重现性和稳定性

5支新制备的电极检测 $40 \text{ nmol}\cdot\text{L}^{-1}$ 腺苷,其结果基本相近,相对标准偏差4.6%,重现性好.

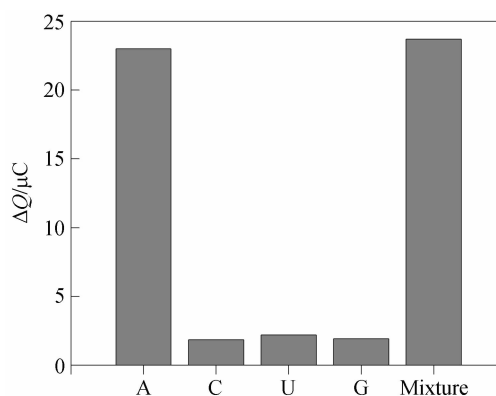


图5 BSA/Apt/CNTs/GC干扰物实验曲线

Fig. 5 Interference of nucleoside analogs ($2 \mu\text{mol}\cdot\text{L}^{-1}$) to the detection system

A. adenosine ($20 \text{ nmol}\cdot\text{L}^{-1}$); C. cytidine; U. uridine; G. guanosine

该电极置于 4°C 冰箱超纯水储存,两周后检测其腺苷电化学信号.与最初信号相比,并无明显变化,有较好的储存稳定性.

3 结论

借助目标分子-适体构型转换与碳纳米管构建了高灵敏度检测腺苷小分子的新型免标记电化学传感电极.电活性钌化合物 $[\text{Ru}(\text{NH}_3)_6]^{3+}$ 因静电作用吸附于适体DNA链表面,且电化学氧化还原有良好的可逆性可作信号传感源,同时碳纳米管可吸附大量的DNA片段,腺苷引入使适体DNA从电极表面脱落致使 $[\text{Ru}(\text{NH}_3)_6]^{3+}$ 氧化还原峰电流降低,其电流下降幅度与腺苷浓度在 $5.0\times 10^{-11} \sim 1.0\times 10^{-7} \text{ mol}\cdot\text{L}^{-1}$ 范围内呈良好线性关系. BSA/Apt/CNTs/GC电极不仅能特异性结合腺苷小分子,且有较低的检出限($2.7\times 10^{-11} \text{ mol}\cdot\text{L}^{-1}$)和较高的灵敏度.也许还可能适用于检测其他小分子和蛋白质,有一定的普适性.

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A Sensitive and Label-Free Electrochemical Aptasensor based on Signal Amplification of Carbon Nanotubes

DENG Chun-yan^{1,2}, FAN Hui-min², XIANG Juan², LI Yuan-jian^{1*}

(1. *College of Pharmacy, Central South University, Changsha 410083, China;*

2. *College of Chemistry and Chemical Engineering, Central South University, Changsha 410083, China*)

Abstract: A label-free electrochemical sensing electrode for highly sensitive detection of adenosine was constructed based on the signal amplification of carbon nanotubes (CNTs). The change in the interfacial feature of the modified electrode was characterized by electrochemical impedance spectroscopy. Using $[\text{Ru}(\text{NH}_3)_6]^{3+}$ as the signaling moiety, adenosine with concentrations as low as $0.027 \text{ nmol} \cdot \text{L}^{-1}$ can be selectively detected. Additionally, the fabrication of this present aptasensor was simple, time-saving and cost-effective. Compared with other reported aptasensors, the proposed aptasensor had advantages of excellent sensitivity, selectivity and simplicity, which plays a potential role in development of aptasensor.

Key words: carbon nanotubes; aptamer; label-free; adenosine