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Enzyme-Based DNA Electrochemical Biosensors: Recent Trends

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酶放大 DNA 电化学传感器的研究进展

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摘要: DNA 电化学传感器灵敏度高、选择性好、分析时间短、检测成本低,极大地推动了生物传感器的发展. 结合蛋白质酶、功能核酸酶的高催化效率与特异性,可提高检测灵敏度和选择性. 本文评述了酶放大 DNA 电化学传感器的研究进展,并分析了现存问题,展望了发展趋势.

关键词: DNA; 酶; 生物传感器; 电化学

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生物传感器系将生物敏感物质转换检测方便的信号的器件^[1],主要包括靶标识别元件和信号转换元件. 靶标识别元件是构建生物传感器的关键,必须对靶标有特异性与专一吸附或反应. 根据靶标识别元件的不同,生物传感器可分为酶传感器、免疫传感器、DNA 传感器等^[2]. 信号转换元件是生物传感器的重要组成部分,它对传感器的信号收集、运用、灵敏度等有极大的影响. 根据检测手段的不同,又分为质量敏感型、热敏型、场效应型、光学、电化学等传感器^[3].

DNA 电化学生物传感器以 DNA 为靶标识别元件,以电化学方法为检测手段^[4],具有以下优点: 1)DNA 可人工合成,成本低,易于保存;2)对检测对象,不仅可根据碱基互补配对的原则,检测特定序列的 DNA,还可利用适配体的特点,检测离子、小分子、多肽、蛋白质甚至细胞;3)用量少,易微型化;4)可联用多种检测方式,如质量型、光学、电化学等. 其中电化学传感器由于选择性好、灵敏度高、成本低、操作简便和易于微型化等而备受关注^[5].

酶是指具有生物催化功能的生物大分子,其催化效率很高,对底物专一性好. 酶 DNA 电化学传感器响应快、操作简单,近年发展尤为迅速. 本文结合作者课题组的研究工作介绍酶放大 DNA 电化学传感器的进展.

1 蛋白质酶(聚合酶、连接酶、内切酶、外切酶)

脱氧核糖核酸(DNA)是生物遗传信息的载体,其结构和功能对生物的遗传和变异有重要意义. 1953 年, Watson 和 Crick 提出了 DNA 的反向双螺旋结构,其两条 DNA 链对应的碱基 A-T 以双键形式连接, C-G 以三键形式连接,糖-磷酸-糖主链在螺旋外侧,配对碱基在螺旋内侧. 利用 DNA 链之间碱基互补配对原则,可实现特定序列的 DNA 检测.

聚合酶、连接酶、内切酶、外切酶等蛋白质酶对 DNA 底物有较好的特异性和高效的催化. 利用酶与 DNA 的作用设计的 DNA 电化学传感器已展现出强大的优越性和研究活力^[6]. 通过聚合酶反应的放大作用^[7],实现了目标物(OTA)的超灵敏检测,检测限达 $6.5 \times 10^{-11} \text{ g} \cdot \text{L}^{-1}$. 利用 T4 连接酶将模板 DNA 首尾相接闭合成环并与引物 DNA 相结合,在 DNA 聚合酶的作用下,其引物可沿着环形模板不断扩增,达到滚环放大(RCA)形成多拷贝新链 DNA. 该 DNA 与通过 Au—S 键固定到金电极上的固定探针 DNA 杂交,从而可吸附更多电化学活性物质亚甲基蓝(MB).

单一酶放大方法已可得到令人满意的检测效果,通过有机耦合两种酶放大反应可得到更低的检测限. 鞠焱先课题组通过联用内切酶和聚合酶,实现了特定序列 DNA 的超灵敏检测($1.1 \times 10^{-17} \text{ mol} \cdot \text{L}^{-1}$)^[8]. 他们将生物素标记的捕获 cDNA 固定于亲和素修饰电极. 在目标 tDNA 存在下,与辅助 aDNA 及 cDNA

碱基互补杂交,形成了内切酶活性位点,通过内切酶的作用使目标 tDNA 循环利用,导致了大量的 RCA 引物序列的暴露.又经 RCA 反应形成的长链 DNA 与量子点标记的探针 DNA 杂交,实现了对目标 tDNA 的灵敏检测.通常,聚合酶和内切酶对 DNA 的活性位点只针对某一序列,这给设计通用型 DNA 电化学传感器增添了难度,在一定程度上限制了其运用^[9].外切酶可从核酸一端开始按序列顺序催化水解核酸磷酸酯键,对 DNA 序列无特定要求^[10-11].2010 年,Plaxco 小组率先提出了利用外切酶 III(Exo III)实现目标物循环放大的检测方法^[12].随后报道用 Exo III 酶在电极上反应的灵敏检测各种物质,如 DNA^[13-15]、离子(Hg^{2+})^[10]、蛋白质^[16]等.作者课题组报道了 Exo III 酶放大的免标记通用型检测 tDNA 方法^[11].如图 1 所示,将形成突出 3'端的双链 DNA 探针通过 Au—S 键固定于金电极上,当 tDNA 与双链 DNA 探针杂交时就可得到 Exo III 活性位点(钝的 3'端双链),释放出的 tDNA 又可进行下一轮剪切,最终使含游离鸟嘌呤碱基(G)的探针被大量剪切,使其减弱对电化学活性物质亚甲基蓝(MB)的吸附,实现 tDNA 的灵敏检测.此法利用游离的 G 和 MB 的强烈特异作用,免去了对探针的修饰,提高了灵敏度且降低了检测成本.

2 酶联催化反应(辣根过氧化物酶)

聚合酶、内切酶、外切酶等显示出强大信号放大能力,其作用底物为 DNA,通常需对 DNA 进行电化学活性物质的标记(如二茂铁和亚甲基蓝).在分析过程中,通过构型改变导致电化学活性物质与电极距离变化,从而其电信号改变就可检测

目标物.但这种构型变化引发的电化学信号较弱,其应用受限.酶联免疫分析法利用抗体、抗原的特异性结合酶催化作用,实现蛋白质分析检测^[17-18].该方法在核酸分析领域已引发关注^[19-20].

辣根过氧化物酶(HRP)系常用的标记蛋白质或 DNA 酶,可催化氧化 3,3',5,5'-四甲基联苯胺(TMB)形成蓝色氧化物,已广泛应用于比色传感领域^[21].樊春海课题组^[22]利用 HRP 具有催化氧化 TMB 的性能结合电化学的优势得到了 DNA 的 $\text{fmol} \cdot \text{L}^{-1}$ 级别检测限.他们将修饰 Biotin 和 Dig 的分子信标(MB)固定于 Avidin 电极.这种分子信标的结构阻碍了 Dig 与 Antidig-HRP 的结合.当目标 tDNA 打开分子信标时,同时造成分子构型的改变,促使暴露的 Dig 与 Antidig-HRP 结合,得到 HRP 氧化的 TMB_{ox} 的还原电流.这种方法需分子信标修饰,增加了体系的复杂性和成本.

作者课题组报道了用免标记的功能型分子信标检测特定序列的 tDNA^[23].图 2 设计了含 SA 适配体序列的分子信标探针,其位阻效应阻碍了适配体与 SA 的结合.当目标物 tDNA 与探针相结合时,使适配体序列暴露于溶液中形成可与 SA-HRP 紧密接触的结构,并测得 TMB_{ox} 还原电流,检测达到 $\text{pmol} \cdot \text{L}^{-1}$ 级别的 tDNA.利用 SA 适配体与 SA 的作用,可免去探针繁琐修饰,降低成本,取得优异的检测效果.

3 脱氧核酶(8-17 DNA 酶)

传统观点认为所有的酶均系蛋白质.20 世纪 80 年代初,Cech 和 Altman 发现了核酶(Ribozyme)改变了这一看法^[24],随之人工合成筛选了

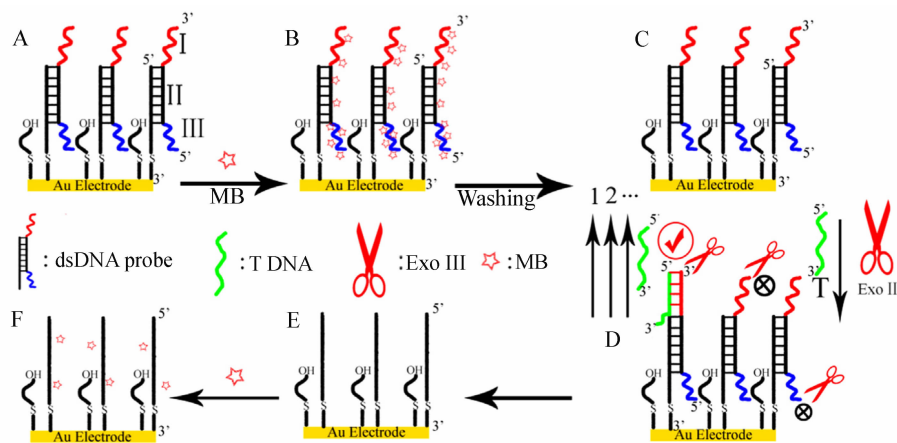


图 1 Exo III 酶放大 DNA 检测的示意图^[11]

Fig. 1 Schematic diagram for the biosensor fabrication based on Exo III^[11]

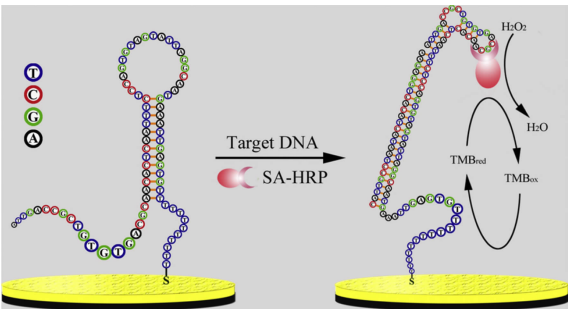


图 2 基于酶联催化反应的 DNA 生物传感器构建示意图^[23]

Fig. 2 HRP-based schematic diagram for the biosensor fabrication^[23]

有 RNA 切割和连接可催化形成肽键、磷酸化等能力的核酶. Breaker 小组也发现有同样催化能力的 DNA 片段,即脱氧核酶(DNA 酶)^[25]. 这一重大的发现为核酸分析领域提供了重要思路,许多 DNA 酶的检测方法已有报道.

在 DNA 酶的催化反应,常需金属离子作为辅酶因子. 因此,DNA 酶的生物传感器常用于金属离子的检测. 目前,已报道 Pb^{2+} ^[26]、 Hg^{2+} ^[27]、 Cu^{2+} ^[28]、 Mg^{2+} ^[29] 等作为辅酶因子的 DNA 酶. Pb^{2+} 作为 8-17 DNA 酶的辅酶,可激发酶的活性并能将一定结构的互补链剪切. Lu 小组率先将其运用于铅离子的荧光分析^[30]. 在 Pb^{2+} 的存在下,8-17 DNA 酶活性被激发,切割其互补链,使修饰于 DNA 链两端的荧光基团和猝灭基团分离,实现了 Pb^{2+} 检测. Xiao 等^[31] 将 8-17 DNA 酶末端修饰电化学活性的亚甲基蓝

并固定于电极上,通过 Pb^{2+} 激发酶活性从而得到电流信号. 作者课题组报道了将 8-17 DNA 酶通过电化学发光(ECL)检测 Pb^{2+} ^[32]. 图 3 将氨基修饰的具有酶活性的 DNA 探针固定于活化羧基的玻璃碳基底,加入功能核酸的底物 DNA 后,在辅酶 Pb^{2+} 的作用下,8-17 DNA 酶将底物 DNA 切断,使该 8-17 DNA 酶通过碱基互补原则与修饰发光材料(Ru)的分子信标杂交,得到发光信号. 这种方法充分利用了 8-17 DNA 酶对底物 Pb^{2+} 的特异选择和碱基互补配对的原则,实现了 Pb^{2+} 灵敏特异检测.

过氧化物酶活性的 DNA 酶在生物分析领域的运用越来越受重视^[33-35]. 这类 DNA 酶对核酸序列的要求不高,一般能形成 G-四分体结构的均能与血红素(Hemin)相作用构成 DNA 酶. G-四分体结构的 DNA 酶有类似于辣根过氧化物酶活性,在 H_2O_2 存在下可使鲁米诺(Luminol)催化发光^[36],也可以使 2,2'-连氮-双 (3-乙基苯并噻唑-6-磺酸) (ABTS)^[37-38]显色,已被广泛运用于比色传感器领域. Liu 等^[13]将 G-四分体结构的 DNA 固定于电极,由于互补链 DNA 以其杂交屏蔽了其活性,当目标在 tDNA 和 Exo III 的作用下,使其恢复催化活性,体现双酶的优势,实现 tDNA $\text{fmol}\cdot\text{L}^{-1}$ 级别的检测.

4 DNA 电化学生物传感器的常见问题与解决方案

国内外研究者一直致力于发展 DNA 电化学生物传感器,结合酶放大方法实现超灵敏、特异的目标物检测,已取得了很好的进展. 目前该方法存

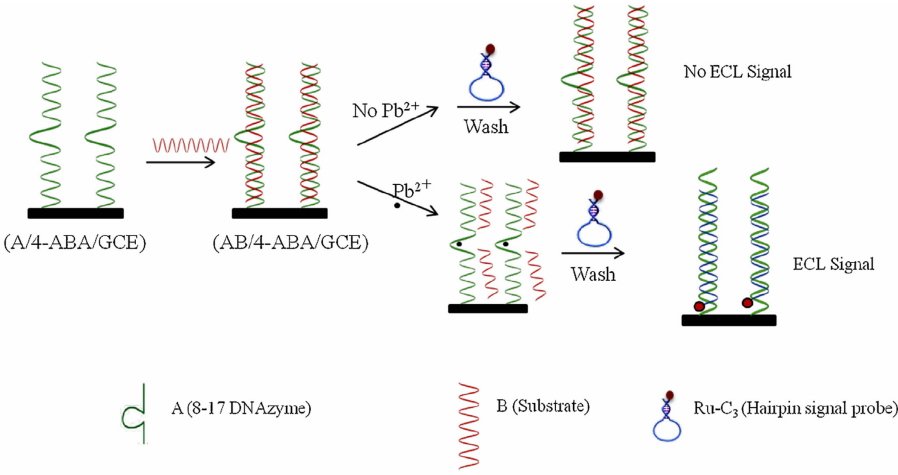


图 3 8-17 DNA 酶电化学发光传感器 Pb^{2+} 离子检测^[32]

Fig. 3 8-17 DNAzyme-based schematic diagram for Pb^{2+} detection^[32]

在的主要问题:1)电极选择:主要有玻碳电极和金电极。玻碳电极需要较为复杂的预处理,固定探针时其露出的羧基与修饰了氨基的探针需经较长时间的孵化^[32]。金电极主要是通过自组装,利用 Au—S 键将探针固定于金电极上^[11,23];2)固定探针的浓度:不同的体系,固定于电极上的探针浓度会极大地影响分析效果,浓度过高造成较大的排斥,降低其与目标物的接触效率,浓度太低则信号较弱。目前主要根据不同体系经优化选取最佳的信噪比^[10,39];3)非特异性吸附:固定探针的同时需防止探针的非特异性吸附,常用巯基乙醇或氨基乙醇。在酶反应的过程中,金电极与酶吸附所造成的背景信号极大地影响其分析效果,通常可用 BSA 封闭金电极^[23];4)杂交效率:非均相反应会不同程度地影响分析效果。Wang 课题组提出了三元单层结构组成的电化学传感界面,其中巯基乙醇(MCH)使固定于电极表面 DNA 探针可直立于电极表面从而得到统一的构型,有利于杂交。二硫苏糖醇(DTT)通过两个巯基固定于电极上,其两个亲水羟基使 DNA 杂交传感器的杂交效率更高,分析效果更好^[40-41]。

5 DNA 电化学传感器的发展趋势

比表面积和导电效果均是影响电化学传感器分析效果的重要因素。碳纳米管、石墨烯、金属纳米粒子等的修饰,结合 DNA 的识别作用越来越受到关注^[42-43]。纳米磁珠的富集能极大地提高信号,已被广泛报道^[5]。免标记的电化学方法简单、快速、方便,已受到极大的重视,特异性好、灵敏度高的免标记 DNA 电化学生物传感器是今后的研究方向。对此,作者在研究免标记 ATP 检测方面进行了一些尝试^[44],研究结果进一步证明了免标记电化学检测的特点和应用潜力。然而,DNA 电化学生物传感器常需将识别探针固定于电极上,这种非均相反应使分析体系受到一定限制,如固定探针过程繁琐、杂交效率低与非特异性吸附。发展均相反应的 DNA 电化学传感器正日益受到重视^[10,45-46]。

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Enzyme-Based DNA Electrochemical Biosensors: Recent Trends

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Abstract: DNA electrochemical sensors have greatly promoted the development of biosensors for their high sensitivity, good selectivity, short analysis time and low cost. To further improve the detection sensitivity and selectivity, protein enzymes, functional nucleic acid enzymes etc. are often used due to the advantages of high catalytic efficiency and specificity. This review demonstrates the research progress of electrochemical sensors based on enzymatic amplification and analyses some existing problems of electrochemical sensors.

Key words: DNA; enzyme; biosensor; electrochemistry