Analytica Chimica Acta 756 (2012) 73–78

Contents lists available at SciVerse ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

An electrochemiluminescence strategy based on aptamers and nanoparticles for the detection of cancer cells

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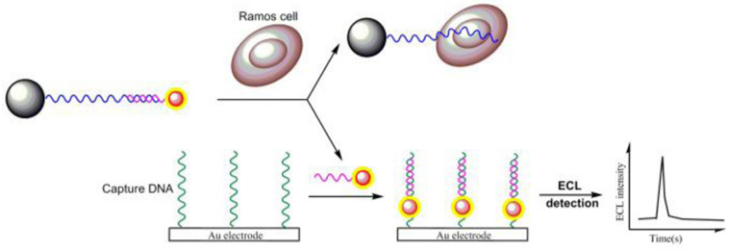
HIGHLIGHTS

- Gold nanoparticles (AuNPs) modified with numerous of ECL signal molecules were used for the amplification of ECL detection.
- Magnetic beads (MBs) were used for the separation tool.
- Under the optimum conditions, a limit of detection as low as 50 Ramos cells per mL could be achieved.

GRAPHICAL ABSTRACT

An electrochemiluminescence (ECL) strategy based on aptamers and ECL nanoprobes was developed for the rapid collection and detection of Ramos cells.



ARTICLE INFO

Article history:
Received 23 August 2012
Received in revised form 10 October 2012
Accepted 14 October 2012
Available online 22 October 2012

Keywords:
Gold nanoparticles (AuNPs)
Electrochemiluminescence (ECL)
Aptamer
Magnetic beads
Cancer cells

ABSTRACT

A PCR (polymerase chain reaction)-free electrochemiluminescence (ECL) strategy based on aptamers and ECL nanoprobes was developed for rapid collection and detection of Ramos cells. The ECL nanoprobes consisted of gold nanoparticles (AuNPs), linker DNA and tris-(2,2′-bipyridyl) ruthenium (TBR)-labeled signal DNA. The linker DNA and signal DNA were modified on the surface of the AuNPs through Au—S bonds. The linker DNA can hybridize partly with the aptamers loaded on the magnetic beads to construct the magnetic biocomplex. In the presence of the cancer cells, the aptamers conjugated with the cancer cells with higher affinity. The ECL nanoprobe released from the biocomplex and subsequently hybridized with the capture DNA modified on the Au electrode. The ECL intensity of the TBR loaded on the nanoprobes directly reflected the amount of the cancer cells. With the use of the developed ECL probe, a limit of detection as low as 50 Ramos cells per mL could be achieved. The proposed methods based on ECL should have wide applications in the diagnosis of cancers due to their high sensitivity, simplicity and low cost.

1. Introduction

In today's society, cancer has greatly threatened people's health [1]. Cancers originate from mutations of human genes. These genetic alterations result in molecular changes which lead to the changes in cell morphology and physiology. So, the noninvasive early detection of cancer and monitoring of its progress are high on the agenda of oncologists [2]. Since polymerase chain reaction (PCR) was introduced in 1985, nearly all assays for DNA detection use PCR to amplify the target. PCR revolutionized the field by providing a reliable method for DNA detection with high sensitivity and stability. However, PCR has been criticized for its complex, expensive, time-consuming and labor-intensive procedures. Furthermore, nonspecific amplification is a major drawback of PCR. In the past decade, various techniques including cytometric methods and cell-enrichment methods have been developed and some of them are commercially available [3]. Although they have high detection rate, many of these methods are expensive and

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http://dx.doi.org/10.1016/j.aca.2012.10.023

Table 1
The sequences of the DNA.

DNA	Sequence
Aptamer for Ramos cells	5′-TAC AGA ACA CCG GGA GGA TAG TTC GGT GGC TGT TCA GGG TCT CCT CCC GGT G- (NH2)-3′
Linker DNA	5′-CGG TGT TCT GTA TTT TTT TTT TTT -SH-3′
Capture DNA	5′-SH-TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT AAA AAA TAC AGA ACA CCG-3′
Signal DNA	5′-NH_2-TTT TTC -SH-3′

time-consuming. Therefore, there is a need for an inexpensive, quick and simple tool with high sensitivity and specificity to detect cancer cells in blood.

Nowadays, a novel class of ligands termed aptamers has been isolated and identified for specific tumor cell recognition. Aptamers were reported for the first time in the year 1990 [4,5]. They are artificially oligonucleic acids in vitro selected through SELEX (systematic evolution of ligands by exponential enrichment). Aptamers have high affinity and specificity to a wide range of target cells. In comparison with molecular probes which are currently available for biomarker recognition, aptamers possess advantages as follows: high specificity, low molecular weight, easy but reproducible production and versatility in application. In view of the above advantages, aptamers have already been applied to bioanalysis, biomedicine and biotechnology [6–8].

At the same time, the rapid development of nanoscience and nanotechnology has brought a variety of novel approaches to the diagnosis and therapy of diseases based on nanoparticles. The unique chemical and physical characteristics of nanoparticles, such as large surface-to-volume ratio, size-dependent optical properties and magnetic properties, hold promise for the development of highly sensitive and selective diagnostic tools for clinical use. The amplification of the DNA–gold nanoparticle assay has shown promise in the development of powerful tools for nucleic acid, protein detection and tumor cells [9–15]. It includes two components, an oligonucleotide-modified gold nanoparticle (AuNP) and a magnetic bead, which can sandwich a specific target. The strategy makes use of nanostructure-based probes to improve sensitivity and it has been testified to be as sensitive as PCR. During the past years, the greatest efforts in cancer cell detection have focused on aptamer-conjugated nanoparticles by Tan's group [16,17]. In one assay, Tan and his team used aptamer-conjugated gold nanoparticles as the basis for colorimetric detection of cancer cells [18]. Moreover, many developed methods using nanoparticles have been reported recently in conjugation with electrochemical [19–21], colorimetric [22], fluorimetric [23] and chemiluminescent [24] techniques. Although these techniques allow the detection without the PCR process, each of them still has shortcomings. For example, some methods mentioned above cost too much or lack simplicity, and some need elaborate instruments and expensive florescent substances. Thus, their clinical application is limited.

Electrochemiluminescence (ECL), which is also called electrogenerated chemiluminescence, refers to the phenomenon that luminescence is produced in a redox reaction of electrogenerated reactants [25–32]. Now it has become a powerfully analytical technique because of its inherent features, such as high sensitivity, low background, simple instrumentation and fast sample analysis. A magnetic bead based on ECL method with tris-(2,2′-bipyridyl) ruthenium and tripropylamine (TPA) reaction has been demonstrated to be a highly sensitive method for biorelated detection [33,34]. Our group has reported the similar work based on ECL method and aptamer with signal DNA-tris-(2,2-bipyridyl) ruthenium (TBR) as ECL probe to detect Ramos cells and the limit of detection reached 89 cells mL^{-1} [35]. To achieve a more sensitive detection, AuNPs were utilized in this assay for further signal amplification. The linker DNA and signal DNA were modified on the surface of the AuNPs through Au—S bonds. The signal DNA was labeled with tris-(2,2′-bipyridyl) ruthenium (TBR). The linker DNA can hybridize partly with the aptamers loaded on the magnetic beads to construct the magnetic biocomplex. In the presence of the cancer cells, the aptamers conjugated with the cancer cells with higher affinity. The ECL nanoprobes consisted of gold nanoparticles (AuNPs), linker DNA and tris-(2,2′-bipyridyl) ruthenium (TBR)-labeled signal DNA released from the biocomplex and subsequently hybridized with the capture DNA modified on the Au electrode. The ECL intensity of the TBR loaded on the ECL nanoprobes directly reflected the amount of the cancer cells.

2. Experimental

All of synthetic oligonucleotides were purchased from SBS Genetech. Co. Ltd. (China). Sequences of the oligonucleotides are listed in Table 1.

2.1. Cells

Ramos cells (CRL-1596, B-cell, human Burkitt's lymphoma) were obtained from Chinese Academy of Medical Sciences. The cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 100 IU mL^{-1} penicillin–streptomycin. The cell density was determined using a hemocytometer, and this was performed prior to any experiment. And then, ~1 million cells dispersed in RPMI 1640 cell media buffer were centrifuged at 3000 rpm for 5 min and redispersed in cell media three times and then redispersed in 1 mL cell media buffer. During all experiments, the cells were kept in an ice bath at 4 °C [36].

2.2. Reagents

Carboxyl-coated MBs (Φ = 3–4 μm) were purchased from BaseLine ChromTech Research Center, China. Mercaptoacetic acid (MAA) was obtained from Yuanhang Chemical Company (China). Tri(2-carboxyethyl) phosphine hydrochloride (TCEP), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), hydrogen tetrachloroaurate(III) trihydrate ($HAuCl_4 \cdot 3H_2O$), $RuCl_3 \cdot xH_2O$, N-hydroxysuccinimide (NHS), 2′,2-bipyridyl-4,4′-dicarboxylic acid (dcbpy), N,N′-dicyclohexyl carbodiimide (DCC), imidazole were purchased from Sigma (USA). Tripropylamine (TPA), 2,2′-bipyridine and 6-mercapto-1-hexanol (MCH) were obtained from First Reagent Corporation of Shanghai (China). All the reagents were analytical grade and used without further purification. 0.1 M PBS buffer (pH 7.4) and 0.1 M phosphate buffer containing 0.1% SDS (pH 7.0) was prepared by standard methods. Deionized and autoclaved water was used to prepare all solutions.

2.3. Apparatus

The electrochemical measurements for stripping voltammetry were carried out on a CHI 660C electrochemical working station (CH Instrument Company, USA) using a three-electrode system consisted of a platinum wire as an auxiliary electrode, an Ag/AgCl electrode as reference electrode, and an Au electrode (Φ = 4 mm) as working electrode. Magnetic separation was carried out with an