Biosensors and Bioelectronics 54 (2014) 78–84

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Carbon nanotubes based electrochemical aptasensing platform for the detection of hydroxylated polychlorinated biphenyl in human blood serum

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ARTICLE INFO

Article history:
Received 28 June 2013
Received in revised form
8 October 2013
Accepted 9 October 2013
Available online 1 November 2013

Keywords:
Multi-walled carbon nanotubes (MWCNTs)
Hydroxylated PCB (OH-PCBs)
Electrochemical aptasensor
(APT-MWCNT/GCE)
Serum analysis

ABSTRACT

A novel strategy to sense target molecules in human blood serum is achieved by immobilizing aptamers (APTs) on multi-walled carbon nanotubes (MWCNT) modified electrodes. In this work, the aminated aptamer selected for hydroxylated polychlorinated biphenyl (OH-PCB) was covalently immobilized on the surface of the MWCNT-COOH modified glassy carbon electrode through amide linkage. The aptamers function as recognition probes for OH-PCB by the binding induced folding of the aptamer. The developed aptasensing device was characterized by electrochemical impedance spectroscopy (EIS), atomic force microscopy (AFM) and Fourier transform infrared spectroscopy (FTIR). The aptasensor displayed excellent performance for OH-PCB detection with a linear range from 0.16 to 7.5 μM. The sensitivity of the developed aptasensing platform is improved (1×10^{-8} M) compared to the published report (1×10^{-6} M) for the determination of OH-PCB (Turner et al., 2007). The better performance of the sensor is due to the unique platform, i.e. the presence of APTs onto electrodes and the combination with nanomaterials. The aptamer density on the electrode surface was estimated by chronocoulometry and was found to be 1.4×10^{13} molecules cm^{-2}. The validity of the method and applicability of the aptasensor was successfully evaluated by the detection of OH-PCB in a blood serum sample. The described approach for aptasensing opens up new perspectives in the field of biomonitoring providing a device with acceptable stability, high sensitivity, good accuracy and precision.

1. Introduction

Aptamers are specific recognition elements which were isolated from a random oligonucleotide library, followed by an iterative selection procedure, known as the systematic evolution of ligands by exponential enrichment (SELEX) (Guo et al., 2007; Tuerk and Gold, 1990). Due to significant advantages of aptamers such as small sizes, chemical synthesis, stability, specific binding capabilities and modification, aptamers are becoming ideal recognition elements for biosensing applications, including mechanical (Savran et al., 2004), optical (Pavlov et al., 2004) and electrochemical biosensors (Radi et al., 2006). Electrochemical aptasensors are a class of biosensors (Radi, 2011) in which the APTs function as biorecognition elements to electrochemically sense the target molecules, resulting in portable, low cost, simple to operate, robust, easily miniaturized and stable analytical devices (Mairal et al., 2008; Pilehvar et al., 2012; Cheng et al., 2009). The combination of APTs with nanomaterials (NMs) offers a new hybrid sensing platform for specific and sensitive target recognition (Yang et al., 2011; Cai et al., 2013; Chen et al., 2011). NMs are promising candidates for the fabrication of biosensors due to a large surface area, unique size, shape and composition-dependent physical and chemical properties. Among different NMs, carbon nanotubes (CNTs) are considered as an important group of nanostructures with attractive electronic, chemical and mechanical properties (Khezrian et al., 2013; Gooding et al., 2003; So et al., 2005; Li et al., 2008) and therefore attracted great interest in biosensor development. CNTs possess high chemical stability (Balasubramanian and Burghard, 2006), high surface area, unique electrical conductivity (Gooding, 2005), metallic and structural characteristics (Baughman et al., 2002), high mechanical strength and elasticity (Kratz and Ferraro (2004)). In addition, aptamers can be readily adsorbed on the surface of CNTs through π–π stacking (Khezrian et al., 2013; So et al., 2005). Here, we propose the

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http://dx.doi.org/10.1016/j.bios.2013.10.018

immobilization of aptamers (ssDNA-APT) on the surface of CNTs through covalent attachment between carboxylated CNTs and amine functionalized aptamers. The fabricated aptasensor is designed for the detection of OH-PCB, an environmentally harmful pollutant.

Polychlorinated biphenyls (PCBs) are a broad class of synthetic organic compounds classified as persistent organic pollutants. High persistence and chemical stability result in their bioaccumulation which leads to the transfer to higher levels of the food chain. Several studies have shown that PCBs can be detected in products such as fish, eggs, meat, poultry and dairy products, all consumed by humans (Ricci et al., 2007; Laschi et al., 2003). In humans and wildlife animals, PCBs can get metabolized into hydroxylated PCBs (OH-PCBs) by cytochrome P450 enzyme-mediated phase oxidation mechanism (Olsson et al., 2000). There is a growing interest in the detection of OH-PCB as contaminants due to their presence as residues in aquatic and marine vertebrates. Sewage treatment plants and byproducts of industrial-scale reactions including biphenyl and biphenynol are also possible sources of OH-PCB (Sinjari and Darnerud, 1998). These compounds are transferred and stored in both humans and animal tissues including blood, liver and lung. The hydrophilic nature of the hydroxyl group in OH-PCB suggests that these chemicals may be readily excreted from the body. However, the possibility of retention rather than excretion also exists due to the increased hydrophobicity contributed by the physicochemical properties of chlorines attached to the biphenyl backbone. Moreover, their high lipophilicity and affinity to certain proteins such as transthyretin (TTR) lead to the retention of OH-PCB in different body compartments, mainly in blood (Malmberg et al., 2004). The OH-PCBs are known to interfere with the transport of thyroid hormones by competing for binding with TTR possibly explaining their bioaccumulation and toxicity (Turner et al., 2007). In addition, it has been shown that some OH-PCBs have estrogenic and anti-estrogenic activities and inhibit intercellular communication. They may also activate aryl hydrocarbon receptors and may cause damage to DNA (Park et al., 2009). Therefore, the development of a fast, low cost and sensitive analytical tool for the detection of OH-PCB in serum samples, as a biomarker of PCB exposure, is essential for toxicological studies and remediation purposes.

The methods currently used for the analysis of OH-PCB are based on chromatographic techniques coupled with mass spectrometry. Recently, serum levels of both PCBs and OH-PCBs in humans were explored using GC/MS and GC/ECD (Bergman et al., 1994): total PCB levels were 3.6 ± 1.6 ppm (on a lipid weight basis) and 2,2′,4,4′,5,5′-hexa CB was the dominant congener (2.2 ± 0.9 ppm). Sample extraction cleanup and derivatization steps are required before analysis (Ueno et al., 2007; Tetcher et al., 2005; Vincy et al., 1997; Nomiyama et al., 2010). These factors combined with the expenses associated with instrumentation and the need for trained laboratory technicians quickly drive up the cost and time required for analysis. On the other hand, electrochemical biosensor technology is characterized by a highly sensitive and selective sensing platform based on simple instrumentation. In addition, electrochemical biosensors based on aptamers are suitable for designing reusable analytical devices (Sassolas et al., 2009). Until now, most of the biosensing studies were carried out for detection of PCBs and very limited studies focused on the detection of OH-PCBs. While, the levels of OH-PCBs in human blood correspond to approximately 10–20% of the PCB concentrations but the toxic effects are comparable to PCBs. In this way, Turner et al. (2007) have developed a whole-cell sensing system for the detection of a group of OH-PCBs by employing the strain *Pseudomonas azelaica HBP1*. This bacterium comprises the hbpCAD genes, which are responsible for the degradation of hydroxylated biphenyls. A detection limit of 1×10^{-6} M was achieved for the detection of 2-hydroxy-2′,3′,4′,5,5′-pentachlorobiphenyl (OH-PCB) using this system. However, bioreporter systems lose their favor in terms of sensitivity, quantification and identification of unknown molecular structures.

Therefore, the present work is focused on designing a novel and fast electrochemical aptasensor based on aptamers immobilized on the surface of MWCNTs through covalent linkage. Integrating the unique properties of OH-PCB specific aptamers with MWCNTs allowed an accurate and sensitive determination of OH-PCB which can be applied in human blood serum samples.

2. Experimental

2.1. Chemicals and reagents

The oligonucleotide used in this study was purchased from Eurogentec (Belgium) with the following sequences: 5′ NH_2-AGC-AGC-ACA-GAG-GTC-AGA-TGC-ACT-CGG-ACC-CCA-TTC-TCC-TTC-CAT-CCC-TCA-TCC-GTC-CAC-CCT-ATG-CGT-GCT-ACC-GTG-AA. The compound 2-hydroxy-2′,3′,4′,5,5′-pentachlorobiphenyl (OH-PCB) was obtained from Accustan-136 dard (Da Vinci Europe Laboratory Solutions, The Netherlands). Multi-walled carbon nanotubes (MWCNTs – with a 99% purity, i.d.=2–15 nm and 1–10 μm tube length), 1-ethyl-3-(3-(dimethylamino)propyl) carbodiimide hydrochloride (EDC) (~98%) and N-hydroxysuccinimide (NHS) (98%) were obtained from Sigma-Aldrich, Belgium. $K_4[Fe(CN)_6]$ was purchased from Merck and $K_3[Fe(CN)_6]$ was obtained from Sigma-Aldrich, Belgium. A stock solution (0.05 M) of OH-PCB was prepared in 1,4-dioxane. Potassium chloride KCl (0.1 M) solution was prepared in double-distilled water and used as a supporting electrolyte.

2.2. Instrumentation

Electrochemical measurements were performed using a μ-Autolab Potentiostat/Galvanostat PGSTAT from Metrohm (The Netherlands), operated with a PC equipped by GPES 4.2 and FRA software. A three-electrode system consisting of aptamer/multi-walled carbon nanotubes modified glassy carbon electrode (APT-MWCNT/GCE) as working electrode, Ag/AgCl as reference electrode and a graphite rod as auxiliary electrode were used. All the solutions examined by electrochemistry were initially purged with purified nitrogen gas for 10 min. All pH-metric measurements were made on a Decibel DB-1011 digital pH meter fitted with a glass electrode and a saturated calomel reference electrode, which was previously calibrated using buffers of known pH. Fourier Infrared spectroscopy was performed by using a Nicolet Model 20DXB FTIR Spectrometer. Topography images were recorded using a Park XE 100 (Suwon, South-Korea) AFM equipped with a 100 μm × 100 μm XY scanner and 12 μm Z-scanner. Images were acquired from a field-of-view between 4 μm × 4 μm and 50 μm × 50 μm with a resolution of 256 × 256 pixels. The N-type Si tip (AppNano, Santa Clara, USA) had a radius < 10 nm and height of 12–16 μm. The cantilever (Al-coated on the backside) had a length of 125 μm, a width of 40 μm and thickness of 4 μm. All images have been recorded in intermittent contact mode.

2.3. Preparation of MWCNT-COOH

The carboxyl functionalized MWCNTs (MWCNT-COOH) were prepared as reported in the literature (Niu et al., 2008). Briefly, 100 mg of MWCNTs was dispersed in 100 mL of a nitric acid and sulfuric acid (1:3) solution. The mixture was ultrasonically agitated for 30 min and then refluxed for 4 h at 80 °C. After that, the mixture solution was filtered and rinsed with distilled water until