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Ultra-high frequency piezoelectric aptasensor for the label-free detection of cocaine

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ABSTRACT

This paper describes a label-free and real-time piezoelectric aptasensor for the detection of cocaine. The acoustic wave sensing platform is a quartz substrate functionalized with an adlayer of *S*-(11-trichlorosilyl-undecanyl)-benzenethiosulfonate (BTS) cross-linker onto which the anti-cocaine MN4 DNA aptamer is next immobilized. Preparation of the sensor surface was monitored using X-ray photoelectron spectroscopy (XPS), while the binding of cocaine to surface-attached MN4 was evaluated using the electromagnetic piezoelectric acoustic sensor (EMPAS). The MN4 aptamer, unlike other cocaine aptamer variants, has its secondary structure preformed in the unbound state with only tertiary structure changes occurring during target binding. It is postulated that the highly sensitive EMPAS detected the binding of cocaine through target mass loading coupled to aptamer tertiary structure folding. The sensor achieved an apparent K_d of 45 ± 12 μM, and a limit of detection of 0.9 μM. Repeated regenerability of the sensor platform was also demonstrated. This work constitutes the first application of EMPAS technology in the field of aptasensors. Furthermore, it is so far one of the very few examples of a bulk acoustic wave aptasensor that is able to directly detect the binding interaction between an aptamer and a small molecule in a facile one-step protocol without the use of a complex assay or signal amplification step.

1. Introduction

Currently, medical laboratories rely on a two-tiered approach to drug-of-abuse testing, the first being a qualitative test followed by a confirmatory quantitative test (Wu et al., 2003). Quantitative tests are based on conventional chromatography[1] and spectroscopy[2] techniques. These methods are complex, time-consuming, labor-intensive and expensive. The currently practiced qualitative tests are generally immunoassay-based (Wu et al., 2003). However, immunoassays, although rapid, have limitations associated with sensitivity, cross-reactivity and high cost due to the required monoclonal antibodies. It is therefore beneficial to develop rapid, sensitive and inexpensive drug-of-abuse detection techniques for use in law enforcement and clinical diagnostics. Aptamer-based assays may provide an avenue to alleviate some of the drawbacks of current detection technologies. Aptamer–ligand interactions are often compared to their antibody–antigen counterparts. However, DNA aptamers have some key advantages over antibodies, such as a comparatively smaller size, a cheap synthesis, an *in vitro* selection process, an increased resistance to degradation, and reversible denaturation.

The development of aptamer-based sensors, or aptasensors, is a rapidly expanding area of biochemistry and detection science. Despite their aforementioned advantages, aptasensors have yet to replace conventional immunoassays in analytical and medical laboratories. Perhaps one reason for the lack of aptasensor adoption is the lack of structure conservation in comparison to that of antibodies. Most aptasensors are designed to take advantage of structural differences between the free and complexed state of aptamer receptors (Li et al., 2010; Reinstein et al., 2011; Xie and Walton, 2009). However, unlike antibodies whose structure is mostly conserved (at exception of the variable antigen-binding region) aptamer structures highly vary between different aptamers. Furthermore, many aptasensors require the aptameric probe to be chemically tagged or modified (Baker et al., 2006; Freeman et al., 2010; He et al., 2011, 2013; Shlyahovsky et al., 2007; White et al., 2008). Since there is no universal means of adding a chemical probe or tag to an aptamer, this further complicates sensor

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[1] Brunetto et al. (2010), Buryakov (2004), Clauwaert et al. (1996), Strano-Rossi et al. (2005), Trachta et al. (2004).

[2] Drummond et al. (2003), Du et al. (2010), Fan et al. (2005), Oiye et al. (2009), Xiao et al. (2005).

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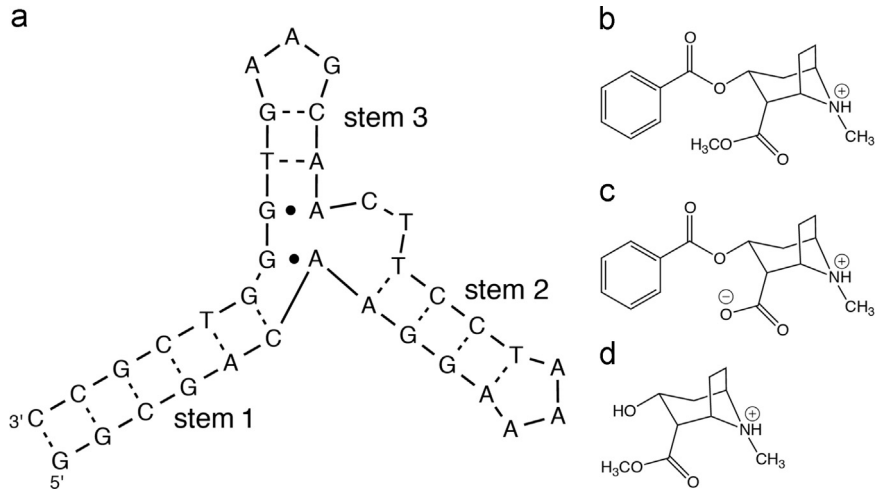

Fig. 1. (a) Secondary structure of the MN4 cocaine binding aptamer. Dashed lines between nucleotides indicate Watson–Crick base pairs, and dots represent non-Watson–Crick base pairs. Chemical structure of (b) cocaine, and its common metabolites (c) benzoyl ecgonine and (d) ecgonine methyl ester.

design as each aptamer must be studied in detail to determine which regions need to be conserved and which can be modified in order to maintain proper function.

The thickness shear mode (TSM) sensor (sometimes referred to as the quartz crystal microbalance or QCM), the most commonly employed bulk acoustic wave sensor, provides a more universal label-free sensor platform. TSM is a powerful technique that allows for the real-time detection of mass loading and viscoelastic changes at the liquid/sensor interface (Keller and Kasemo, 1998; Stavila et al., 2012). This technology has been widely implemented in the study and detection of biological interactions, primarily the detection of DNA oligos and protein; also several aptasensors against protein, DNA and cellular targets have been built on this technology (He et al., 2014; Ozalp et al., 2015; Shan et al., 2014; Song et al., 2014; Wang and Li, 2013). However, the detection of small organic molecules is rarely reported, due to the small mass and viscoelastic changes produced by the adsorption/binding of such small entities to the sensor surface (Chen et al., 2011; Dong and Zhao, 2012; Sheng et al., 2011). Generally, TSM sensors developed for small molecule detection do not directly detect the binding of the small molecule to the surface-immobilized probe but, instead, require the use of complicated competition/displacement assays or other signal amplification methodologies (Sheng et al., 2014; Shi et al., 2013; Zhang et al., 2012a).

The electromagnetic piezoelectric acoustic sensor (EMPAS) is a device developed in our laboratory (Ballantyne and Thompson, 2004; Thompson et al., 2003). Unlike other, more conventional piezoelectric sensors (i.e. TSM) excitation of acoustic resonance occurs remotely within an electrode-free piezoelectric quartz substrate through an external magnetic field generated by a spiral coil placed 30 μm underneath. This arrangement allows for the sensor to operate at ultra-high frequencies (> 1 GHz), and for interfacial phenomena to be studied in a direct manner through the avoidance of intercalated metal contacts (plated electrodes) upon which surface chemistry is carried out (Thompson et al., 2003). The coil and piezoelectric substrate are located in a sample cell, wherein a running buffer is flowed, hence the EMPAS operates in an online and real-time detection format. In practice, the sensor has been successfully applied for the detection of proteins (Sheikh et al., 2011, 2010), and lipopolysaccharide pathogens (Thompson et al., 2015), as well as for the evaluation of surface fouling (Sheikh et al., 2013, 2012). Functionalization of the EMPAS sensor platforms is generally achieved through the use of conditioning organic adlayers. These create an environment favorable to the controlled immobilization of biological probes, and are capable of resisting non-specific adsorption.

The cocaine binding DNA aptamer has become a template for use in a wide range of studies including aptamer small molecule interactions and biosensor applications. The secondary structure of the aptamer is composed of three stem loops that meet in a central three-way junction, that encompasses the cocaine binding pocket (Fig. 1a depicts the secondary structure of the MN4 cocaine aptamer variant). The aptamer has its secondary structure preformed in the absence of ligand, and it is only upon cocaine complexation that tertiary folding occurs. One reason for the widespread use of the cocaine aptamer in sensing technologies is that it can be engineered to undergo a ligand-induced folding mechanism. When stem 1 is shortened to contain three base pairs, the resulting aptamer is unfolded when free, and ligand-binding induces folding (Neves et al., 2010a,b). Another commonly used method to achieve ligand-induced folding is to divide the aptamer into two separate strands that assemble into a single tertiary complex upon cocaine binding (Stojanovic et al., 2000). The vast majority of biosensors based on the cocaine aptamer rely on one of the two ligand-induced folding variants described, rather than the rigid secondary structure variant.[3]

Herein, we describe the EMPAS as the foundation of an ultra-high frequency, label-free acoustic wave aptasensor for the detection of cocaine. The wide operating frequency range offered by the EMPAS system allows for the signal-to-noise ratio to be tuned, hence for the sensitivity of the aptasensor to be maximized. The piezoelectric sensing platform is comprised of a *S*-(11-trichlorosilyl-undecanyl)-benzenethiosulfonate (BTS) adlayer-coated quartz disc to which the cocaine binding DNA aptamer, MN4 (Fig. 1a), is immobilized (Scheme 1). This is termed the MN4 cocaine aptasensor (M4CA). Unlike the structure-switching variants that are utilized in most cocaine aptamer-based biosensors, the MN4 aptamer has its secondary structure formed in the unbound state, and no large-scale secondary structure rearrangements occur during target binding (Neves et al., 2010a,b). Furthermore, the sensitive acoustic wave EMPAS device allows for M4CA to detect the aptamer-cocaine binding interaction directly in a label-free manner using a facile, additive-free, one-step assay. This work

[3] Baker et al. (2006), Das et al. (2012), Deng et al. (2012), Du et al. (2010), Golub et al. (2009), He et al. (2010), Jiang et al. (2012), T. Li et al. (2007), Y. Li et al. (2007), Li et al. (2014, 2011), Nie et al. (2013), Sharma and Heemstra (2011), Shi et al. (2013), Smith et al. (2014), Spiropulos and Heemstra (2012), Stojanovic and Landry (2002), Stojanovic et al. (2001, 2000), White et al. (2008), Yan et al. (2013), Zhang et al. (2012b, 2008), Zhou et al. (2012, 2011), Zou et al. (2009).