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Development of quantum dot aptasensor and its portable analyzer for the detection of di-2-ethylhexyl phthalate

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ABSTRACT

We have developed a quantum dot aptasensor (QD-aptasensor) and its accompanying portable analyzer for the detection of di-2-ethylhexyl phthalate (DEHP). This sensor is based on a newly screened aptamer (60-mer) via SELEX and shows a binding affinity of 213 nmol/L with DEHP. The 60-mer aptamer together with its three shorter truncated aptamers (45, 28, and 22-mer) as well as three different DNA probes (12, 9, and 13-mer) were further investigated to form the best combination for the QD-aptasensor. Using a 22-mer-truncated aptamer and a 12-mer DNA probe combination, the QD-aptasensor demonstrated excellent DEHP sensitivity with an LOQ = 0.5 pg/mL as well as good selectivity in the presence of other phthalate analogs. The binding between the truncated aptamers and DEHP was also characterized. Finally, a QD-aptasensor-based portable analyzer was also developed, and its equivalence to the laboratory protocol was established with a correlation coefficient $r = 0.86$ for DEHP concentrations ranging from 0.0005 to 100 ng/mL.

1. Introduction

Phthalates or phthalic acid esters are one of the most widely used industrial chemicals. This chemical is commonly used as plasticizer to manufacture transparent, flexible and elastic plastics. These plastics include polyvinyl chloride or PVC, food packing materials, adhesives, electronics, pharmaceutical packaging, cosmetics, and toys for children (Earls et al., 2003; Gimeno et al., 2014; Guart et al., 2011; Koniecki et al., 2011; Pecht et al., 2017). The global production rate of phthalates has increased dramatically from approximately 4 million tons/year in the 1990s to well over 8 million tons/year in 2011 (Baril et al., 2013; Kimber and J.Dearman, 2010; Net et al., 2015). Unfortunately, low-molecular weight phthalates such as dibutylphthalate, benzylbutylphthalate, and di-2-ethylhexyl phthalate (DEHP) also behave as endocrine disrupting compounds and also contribute to a myriad of adverse health effects such as cancer, asthma and reproductive disorders (Kimber and J.Dearman, 2010; Ventrice et al., 2013). Of particular concern is DEHP because it behaves as an androgen blocker in the human body (Johnson et al., 2012) and at the same time is also the most widely used plasticizer (Heise and Litz, 2004).

Even though DEHP has already been regulated and its half-life in humans only last a few hours, its ubiquitous presence continues to haunt us (ATSDR, 2002; Meeker et al., 2009). DEHP continues to leach from landfills and find its way into our water supplies. For example, according to previous studies, up to 100 μg/L could be found in surface water (Félix–Cañedo et al., 2013; Net et al., 2014); up to 1 μg/L in drinking water (Ishida et al., 1980; Psillakis and Kalogerakis, 2003) and up to 100 μg/L in the effluent of wastewater (Fromme et al., 2002; Tran et al., 2015). For comparison, the maximum allowable levels of DEHP in surface and drinking water are 1.3 μg/L (European Union) (EU, 2013) and 6 μg/L (United States), respectively (EPA, 2012).

It is an understatement that the *in situ* detection of DEHP in water is vital to safeguarding our water supplies and preventing a major public health crisis. Conventional methods of DEHP detection include gas chromatography, high-performance liquid chromatography or a combination with mass spectrometry (Chang-Liao et al., 2013; Shen et al., 2007; Takatori et al., 2004). These methods are capable of detecting DEHP with high sensitivity and selectivity. However, these techniques are also expensive and time consuming and require bulky equipment. In other words, they are not suitable for the *in situ* detection of DEHP.

To facilitate the eventual *in situ* detection of DEHP in water, we have employed a combination of aptamers, quantum dots and magnetic

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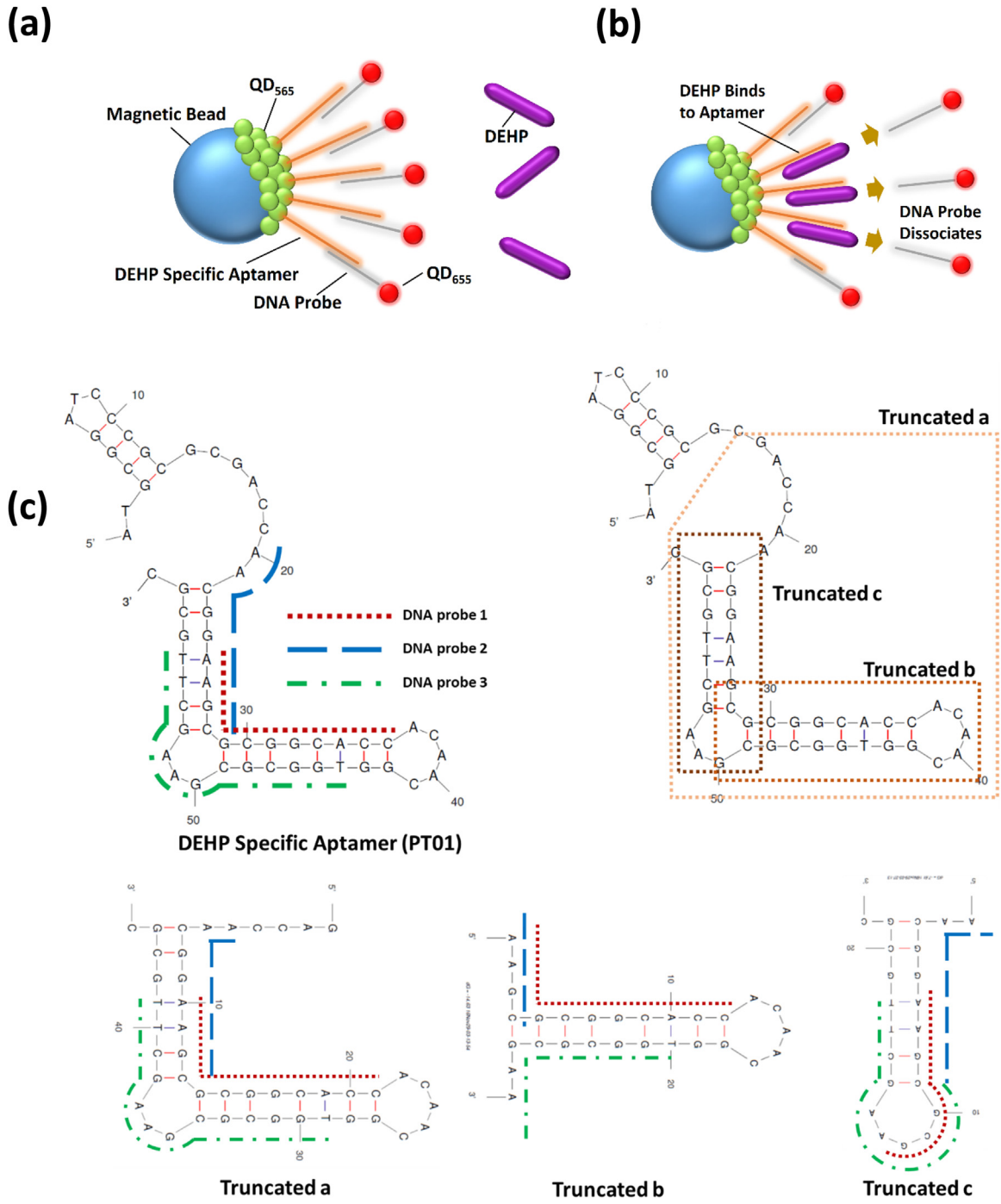

Fig. 1. (a) Schematic of the QD-aptasensor in the absence of DEHP. (b) Schematic of the QD-aptasensor in the presence of DEHP. (c) Designs of the DNA probes 1, 2, and 3 based on the structure of the original PT01 and the truncated a, b, and c aptamers.

beads to develop a quantum dot aptasensor or QD-aptasensor. This particular QD-aptasensor was amenable for implementation in a portable analyzer. Aptamers have been widely used for the molecular level sensing and detection of compounds such as bisphenol A and tetracyclines (Cho et al., 2009; Kim et al., 2010; Lee et al., 2017). However, as far as phthalates are concerned, the only aptamer that could detect phthalates has been the i-motif aptamer designed by Han et al. (2017). However, the binding site of the i-motif aptamer was rather broad and was meant to target the phthalic acid ester group. Therefore, this aptamer is not specific to particular phthalates. Hence, as far as we know, there is no specific aptamer that is designed for DEHP detection.

In this study, a DEHP specific aptamer is designed via the systematic evolution of ligands by exponential enrichment (SELEX) and a reduced graphene oxide (rGO)-based screening method. Leveraging our prior work in bisphenol A detection (Lee et al., 2017), the truncated DEHP specific aptamer and complementary DNA probe are used to further develop the DEHP specific QD-aptasensor. As shown in Fig. 1a, in the absence of DEHP, the DEHP specific aptamer and complementary DNA probe bind to each other. The amount of the DNA probe is given by the fluorescence from the QD_{655} and is normalized to that of QD_{565}. As shown in Fig. 1b, in the presence of DEHP, the DNA probe dissociates from the DEHP specific aptamer. Therefore, the normalized fluorescence of QD_{655}/QD_{565} decreases accordingly. As shown in Fig. 1c, we used 3 truncated DEHP specific aptamers (truncated a, b, and c) and 3