Development of DNA aptamers-based biosensors for food safety and medical diagnostics

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Abstract

Antibiotic residues and heavy metals in food and environmental samples, as well as protein biomarkers of neurological disease, are analytes of regulatory or clinical importance that occur at very low concentrations in complex matrices. Although conventional methods are sensitive, they are costly, time-consuming, and poorly suited to on-site applications. This thesis addresses these limitations by designing and characterising DNA aptamer-based biosensors incorporating nanomaterial-functionalised surfaces and antifouling linkers. The food-safety targets were penicillin G (PEN), oxytetracycline (OTC), and mercury, whereas the medical-diagnostics target was myelin basic protein (MBP), a biomarker of demyelination in multiple sclerosis. Electrochemical (DPV, CV, and EIS), acoustic (QCM-D), and optical (LSPR) transduction methods were used.

An Ag-incorporated cobalt-succinate MOF with layer-by-layer immobilised thiol- and amino-modified aptamers detected Hg(II) over the range of 0.7–10 nM, with an LOD of 0.3 nM. All measured values were below the EU limit of 4 ng/mL. A glassy carbon electrode (GCE) modified with an rGO–MWCNT nanocomposite detected OTC with an LOD of 1.72 ng/mL, approximately 58-fold below the EU limit in milk, and was evaluated using spiked milk samples. For PEN, a DPV aptasensor based on an MUA monolayer achieved an LOD of 0.23 ng/mL. A combined QCM-D/LSPR platform, applied to antibiotic detection for the first time, produced nearly identical LODs of 3.0 nM by QCM-D and 3.1 nM by LSPR, with dissociation constants of 50 and 20 nM, respectively. For MBP, a mixed HS-MEG-COOH/DTT antifouling monolayer enabled detection by DPV and QCM-D, yielding comparable LODs of 2.74 and 4.14 ng/mL in buffer and 6.36 and 6.87 ng/mL in artificial cerebrospinal fluid, respectively. In the latter matrix, nonspecific albumin adsorption was the principal matrix effect.

The results indicate that both aptamer affinity and the features of the sensing interface influence sensor effectiveness. These features encompass immobilisation chemistry, nanomaterial support, surface heterogeneity, and antifouling design. By incorporating complementary transduction techniques, sensitive detection was achieved, offering mechanistic insights into the interactions between aptamers and their targets.

Keywords: DNA aptamers, biosensors, electrochemical biosensing, nanomaterials, antifouling surfaces, oxytetracycline, penicillin G, mercury, myelin basic protein, DPV, QCM-D, LSPR, food safety, clinical diagnostics.