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Detection of Fish Hormones by Electrochemical Impedance Spectroscopy and Quartz Crystal Microbalance

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ABSTRACT

Detection of three fish hormones, cortisol, insulin-like growth factor 1 (IGF-1), and vitellogenin is reported using both electrochemical impedance spectroscopy (EIS) and quartz crystal microbalance (QCM). For cortisol, IGF-1, and vitellogenin, the EIS (QCM) detection limits are 7.9 (0.50) μM, 3.0 (2.4) nM, and 43 (13) pM, respectively, in PBS buffer. Thus the two detection methods, EIS and QCM, have similar sensitivity, but QCM is ~4× more sensitive, which is consistent with the published literature. The molecular weights of cortisol, IGF-1, and vitellogenin are 362.5 Da, 7.3 kDa, and 440.5 kDa, respectively. For both EIS and QCM, the sensitivity and detection limit improve dramatically with increasing molecular weight, reflecting the larger change in polymer-protein film thickness at the Au electrode upon recognition and binding of larger analytes. This is the first time that such a trend has been reported for EIS biosensing, since the published literature does not show any clear trend with molecular weight. Potential applications to understanding of fish physiology are discussed, including hormone detection in fish blood plasma, and ambient water.

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1. Introduction

Fish hormones are widely studied as biomarkers of fish physiology and metabolism, with applications to understanding fish reproduction, migration, and the impact of exposure to environmental stressors [1–4]. For protein and steroid hormones, concentration assays typically employ methods such as enzyme linked immunoassay (ELISA), lateral flow immunoassay, or radioimmunoassay. These methods require trained personnel and are time consuming, difficult to automate and miniaturize, and difficult to standardize [5]. ELISA techniques suffer chemical interference from matrix effects and cross-reactivity [6], in part due to the complexity of the solid stationary phase. Perhaps the biggest shortcoming of ELISA and related methods is that they are difficult to multiplex, so users employ a separate test for each analyte [7]. Recent trends in ecophysiology suggest that monitoring and study of a suite of biomarkers is necessary for complete understanding of an organism's physiological state [8–10] The use of multiple biomarkers is in part useful for identifying the most important biomarker(s), but also reflects the complexity of organisms' physiological response to environmental change and anthropogenic disturbance, which may vary with physiological and environmental variables.

Among fish physiologists, common practice is to collect blood or other biological samples in the field, then store them for later analysis at a central facility. For field assays, rapid, portable and all-electrical measurements are available for glucose and lactate measurement [11–14], but not for quantification of protein and steroid hormones. However, field testing requires that organisms be captured, handled, measured, and released back into the field, so gathering information from individuals is time-consuming and expensive. In addition, blood sampling has possible limitations due to the time lag between sampling and testing, possible effects of tissue trauma, and the small volumes of plasma involved, which often requires post-treatment to increase analyte concentrations to measurable levels [15]. For these reasons, alternatives to blood sampling have been developed, including sampling of ambient water, into which fish excrete steroid hormones [16,17].

For protein and steroid fish hormones, rapid, quantitative and user-friendly assays for concentration would offer significant advantages relative to current methods. The current report discusses detection of three fish hormones, cortisol, insulin-like growth factor 1 (IGF-1), and vitellogenin by both electrochemical impedance spectroscopy (EIS) and

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quartz crystal microbalance (QCM). These three hormones have a wide range of molecular weights and therefore hydrodynamic diameters, so the highest sensitivity and lowest detection limit are expected for the largest analyte, vitellogenin. The usefulness of these methods for monitoring of fish hormones in blood plasma and ambient water will also be discussed in detail.

2. Experimental

Glass slides with a 100-nm Au film and 5-nm Ti adhesion layer were purchased from EMF Corp. (Ithaca, NY), and Au-coated quartz crystal microbalance (QCM) electrodes were purchased from Gamry Instruments. IgG-free bovine serum albumin (BSA), 11-mercaptoundecanoic acid (11-MUA), *N*-(3-dimethylaminopropyl)-*N′*-(ethylcarbodiimide hydrochloride) (EDC), potassium dihydrogen phosphate, and dipotassium dihydrogen phosphate were purchased from Sigma Aldrich. *N*-hydroxysulfosuccinimide sodium salt (NHSS) was purchased from Pierce biotechnology, and potassium ferri/ferrocyanide was purchased from Acros Organics. *Cyprinus carpio* vitellogenin and its mouse monoclonal IgG_1 antibody were purchased from Cayman Chemical (Ann Arbor, MI), *Lates calcarifer* insulin-like growth factor 1 (IGF-1) and its rabbit polyclonal antibody were purchased from GroPep (Thebarton, Australia), and cortisol and its rabbit polyclonal antibody were purchased from Sigma Aldrich.

For both electrochemical impedance spectroscopy EIS) and quartz crystal microbalance (QCM) measurements, an electrochemical cell purchased from Gamry Instruments was employed with an Au electrode area of ~0.19 cm^2 and a cell volume of ~5–6 ml. Au electrodes were first cleaned with ethanol and dried. The electrode was then modified by immersion for 17 h into 1.0 mM 11-MUA and 50 mM phosphate buffer solution (pH = 10) to form a self-assembled monolayer (SAM) with carboxylate termination. Alkaline pH was employed to increase the solubility of 11-MUA in PBS buffer. The terminal carboxylate groups were then activated for 1 h in 75 mM EDC and 15 mM NHSS in 50 mM phosphate buffer solution (pH = 7.3). The antibody-coated electrode is then created by immersion for 1 h into a solution containing 50 μg/ml antibody and 50 mM PBS at pH 7.3, forming amide bonds to the protein surface.[1] For some experiments, this was followed by immersion in 0.1% IgG-free BSA for an hour to reduce the non-specific adsorption. For both EIS and QCM detection of fish hormones, increasing antigen concentrations were added to the background electrolyte, 50 mM PBS and 5 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ at pH 7.3. For testing in fish blood plasma, non-stress channel catfish plasma was collected within 5 min of capture, so the cortisol concentration should be <5 ng/ml. This was then diluted by 10× in the electrolyte above.

All electrochemistry measurements were performed with a three-electrode configuration using a Pt spiral counter electrode and an Ag/AgCl reference electrode. Impedance measurements were performed at the open circuit potential using a Gamry Instruments Reference 600 over the frequency range from 0.05 Hz to 15 kHz, with an AC probe amplitude of 5 mV. QCM measurements were taken using a Gamry Instruments eQCM 10 MHz quartz crystal microbalance. Each impedance spectrum takes about 3 to 4 min to acquire. Both EIS and QCM experiments were performed at the open circuit potential (OCP). Following gentle electrolyte stirring, successive impedance spectra were identical, confirming that the reported impedance results correspond to steady state conditions.

3. Results and discussion

Figs. 1–3 show Nyquist plots of the impedance response at antibody- and BSA-coated electrodes to increasing concentrations of cortisol, IGF-1, and vitellogenin, respectively. The test solutions also contain 50 mM PBS and 5 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ at pH 7.3. The concentration ranges studied in Figs. 1–3 reflect the inverse relationship between the sensitivity of impedance biosensing and the analyte molecular weight. For the largest species studied (vitellogenin), concentrations up to 0.19 μg/ml (4.3×10^{-10} M) are studied. In this case, analyte binding causes a much larger increase in the polymer-protein film thickness atop the Au electrode than the other two analytes. On the other hand, for the smallest species studied (cortisol), much higher concentrations up to 38.0 μg/ml (1.0×10^{-4} M) are studied. For all three analytes, the impedance response eventually saturates at high analyte concentrations due to saturation of the surface antibody film, suggesting that binding can be described by the Langmuir adsorption isotherm [21].

The impedance spectra in Figs. 1–3 approximate semicircular behavior at moderate to high frequencies. These results can be fit with a Randles equivalent circuit, which is shown in Fig. 4, with the differential capacitance (C_d) replaced with a constant phase element (CPE). In Fig. 4, R_s corresponds to the solution resistance, R_{ct} to the charge transfer

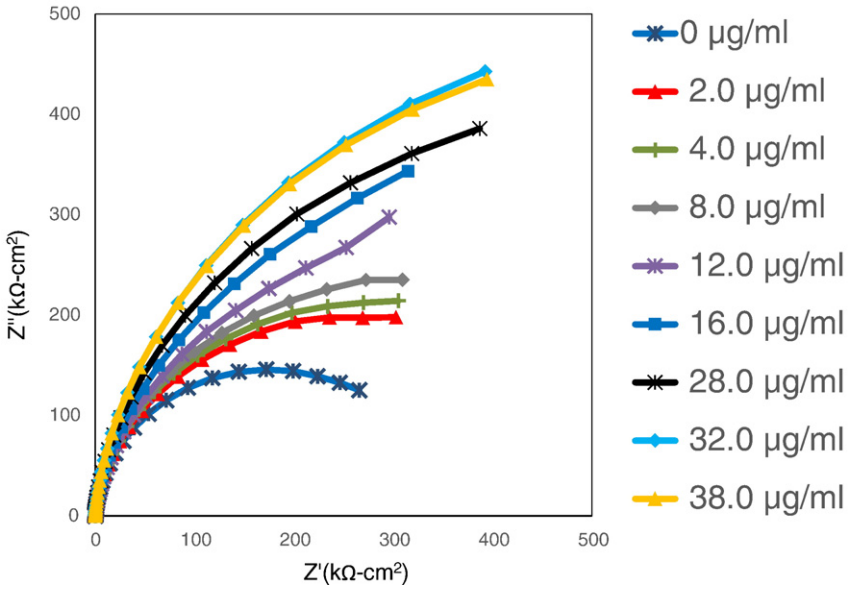

Fig. 1. Nyquist plot of the interfacial impedance of the antibody-coated electrode after exposure to increasing cortisol concentrations in 50 mM PBS buffer + 5 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ at pH 7.3.

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