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Impedimetric Cortisol Biosensor with in-Situ Reduced Hexaammineruthenium as Redox-Probe

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Abstract:
Cortisol is an important biomarker to monitor the stress level. In this work, we present a cortisol biosensor using electrochemical impedance spectroscopy as read-out method. It is known that hexacyanoferrat(III/II) can corrode gold electrodes. To overcome this problem, we chose a different path. By applying a DC-bias to the EIS measurement, the redox-pair $[Ru(NH_3)_6]^{3+/2+}$ was generated in-situ by reduction of the hexaammineruthenium(III) on the working electrode. A sensor consisting of a three electrode system (working, counter, reference electrode) was used to ensure the correct application of the bias voltage. The realized impedimetric sensor had a linear range from $1*10^{-11}$ to $1*10^{-7}$ mol/L and a linear correlation coefficient of 0.972.

Key words: Electrochemistry, Impedance Spectroscopy, Biosensor, Cortisol, Hexaammineruthenium.

Introduction

The monitoring of the stress level is important for the prevention of burnout and overworking. The dominant biomarker to give information on the stress levels is Cortisol [1]. To ensure frequent monitoring of this marker, an easy to use point-of-care system is necessary to perform the measurements. Electrochemical impedance spectroscopy (EIS) can provide this measurement platform. The physiological concentration of cortisol in saliva is about 0.50 to 8.25 nmol/L [2]. EIS biosensors are sufficiently sensitive to allow detection of cortisol concentrations in these ranges without the addition of any further enhancement [3]. An EIS biosensor consists of a working electrode (WE) and a counter electrode (CE). Capture molecules are bound to the WE. For the determination of the impedance of the modified working electrode a measurement solution containing a redox-probe, e.g. hexacyano-ferrat(II) and (III) (HCF), is applied to the electrode. An alternating current with a low amplitude is applied with frequencies ranging from 1 MHz to 0.1 Hz and the impedances are calculated. Studies have shown that HCF can corrode gold electrodes [4]. As an alternative redox-probe we applied hexaammine-ruthenium(III), which is reduced in-situ to hexaammineruthenium(II) by applying a direct current (DC) during the EIS measurement. To ensure the correct application of the DC a three-electrode system, consisting of a WE, a CE and a reference electrode (RE), is mandatory.

Materials

Hydrocortisone 98 %, 11-mercapto undecanoic acid 95 % (MUA), N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride 99 % (EDC), ethanolamine hydrochloride 99 % (EA), N-Hydroxysuccinimide 98 % (NHS), 2-(N-Morpholino)ethanesulfonic acid 99% (MES) and Hexa-ammineruthenium (III) chloride 98 % were obtained from Sigma-Aldrich (St. Louis, MO, USA). Cortisol Monoclonal Antibody (F4P1A3) c = 1 mg/ml was obtained from Thermo Fisher Scientific Inc. (Waltham, MA, USA). Sensors of type AC1.W1.R2 were obtained from BVT Technologies (www.bvt.cz). Other chemicals were of analytical quality and were used without further treatment.

Methods: Surface Functionalization

The sensor surface was pre-cleaned by ultrasonication in ultrapure water and ethanol. The thiol-monolayer was formed by immersion of the cleaned sensors in a 10 mmol/L MUA solution with 95 % ethanol and 5 % water as solvent. Next, the carboxylic acids were activated with about 100 µl of a 10 mmol/L EDC

and 10 mmol/L NHS solution with 60 mmol/L MES buffer at pH 4.7 as solvent for 1 h. After this, the sensor was washed and incubated with 90 µl of a cortisol-antibody solution (c = 0.01 mg/ml) for 1 h. The remaining activated carboxylic acids were blocked by immersion in a 10 % EA solution in PBS for 30 min.

Methods: EIS Measurements

The EIS measurements were performed with an Autolab PGSTAT30 with the FRA2 module (www.metrohm-autolab.com). A DC bias voltage of -160mV (vs Ag/AgCl) was applied additionally to the AC voltage with an amplitude of 5mV. The AC frequencies ranged from 120 kHz to 1 Hz. A 10 mmol/L hexaammine-ruthenium(III) chloride in 10 mmol/L PBS buffer at pH 7.4 was used as measurement solution. The curve fitting was performed in Matlab (Natick, MA, USA) using the Randles circuit depicted in Fig. 1 in the upper right corner.

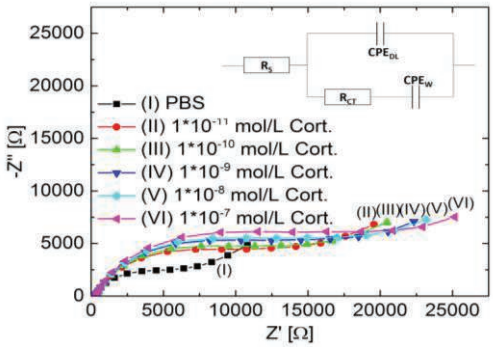

*Fig. 1. Nyquist plots of biosensors with MUA/Cortisol-Ab/EA after 30 minutes of incubation in (I) PBS buffer, (II) 1*10^{-11} mol/L cortisol, (III) 1*10^{-10} mol/L cortisol, (IV) 1*10^{-9} mol/L cortisol, (V) 1*10^{-8} mol/L cortisol and (VI) 1*10^{-7} mol/L cortisol.*

Results and Discussion

First, the blank measurement was performed by incubating the cortisol biosensor 30 minutes with a PBS buffer solution. Figure 1 shows the recorded impedance spectrum as black curve. The blank measurement resulted in very low measured impedances. Next, the sensor was incubated for 30 minutes sequentially with buffered cortisol solutions with concentrations ranging from 1*10^{-11} mol/L to 1*10^{-7} mol/L. After each incubation step, the EIS spectrum was recorded. As shown in Fig. 1, the impedances of the sensor increased with binding of the analyte molecules. The R_{CT} values were determined by curve fitting and are summarized in Table 1. The obtained charge transfer resistances were used to calculate a relative resistance change by subtracting the R_{CT} value of the blind control R_{CT0} and then dividing by this value to decrease the influence of variations in the functionalisation procedure. The functionalized sensor exhibited a linear range with cortisol concentrations over five decades from 1*10^{-11} to 1*10^{-7} mol/L. The correlation coefficient was 0.972. The sensor was measured at each concentration four times. As shown in Fig. 2 the standard deviation was comparatively low for each concentration.

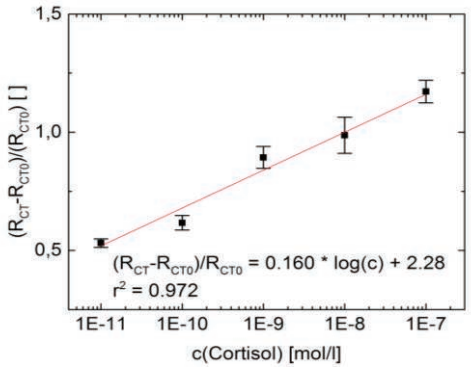

Fig. 2. Calibration curve of buffered cortisol solutions with relative R_{CT} versus the cortisol concentration in semi-logarithmic form, m=4.

Tab. 1: Average R_{CT} and relative R_{CT} of measured cortisol solutions.

c(cortisol) [mol/L]	Average R_{CT} [kΩ]	$(R_{CT}-R_{CT0})/R_{CT0}$
0	9.58	0
1*10^{-11}	14.7	0.531
1*10^{-10}	15.5	0.617
1*10^{-9}	18.2	0.893
1*10^{-8}	19.1	0.987
1*10^{-7}	20.8	1.17

Conclusion

The cortisol immunosensor had a linear range of 1*10^{-11} to 1*10^{-7} mol/L and a linear correlation coefficient of 0.972. Cortisol is usually present in saliva with concentrations ranging from 5*10^{-10} mol/L to ~8*10^{-9} mol/L, which is well within the linear range of the presented sensor.

Acknowledgement

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