Research Article

Sensing Estrogen with Electrochemical Impedance Spectroscopy

Jing Li,[1] Byung Kun Kim,[1] Kang-Kyun Wang,[1] Ji-Eun Im,[1] Han Nim Choi,[1] Dong-Hwan Kim,[1] Seong In Cho,[2] Won-Yong Lee,[1] and Yong-Rok Kim[1]

[1]*Department of Chemistry, Yonsei University, Seoul 120-749, Republic of Korea*
[2]*Department of Biosystems and Biomaterials Science and Engineering, Seoul National University, Seoul 151-921, Republic of Korea*

Correspondence should be addressed to Won-Yong Lee; wylee@yonsei.ac.kr and Yong-Rok Kim; yrkim@yonsei.ac.kr

This study demonstrates the application feasibility of electrochemical impedance spectroscopy (EIS) in measuring estrogen (17β-estradiol) in gas phase. The present biosensor gives a linear response ($R^2 = 0.999$) for 17β-estradiol vapor concentration from 3.7 ng/L to 3.7×10^{-4} ng/L with a limit of detection (3.7×10^{-4} ng/L). The results show that the fabricated biosensor demonstrates better detection limit of 17β-estradiol in gas phase than the previous report with GC-MS method. This estrogen biosensor has many potential applications for on-site detection of a variety of endocrine disrupting compounds (EDCs) in the gas phase.

1. Introduction

While rapid industrialization in the 20th century has introduced a significant degree of convenience to human life, the global population is being exposed to a polluted environment that includes contaminated air such as carbon monoxide, sulfur dioxide, dioxin, formaldehyde, chlorine, bisphenol, and estradiol [1]. Among the pollutants, 4-nonylphenol, bisphenol, and estradiol are classified as endocrine disrupting chemicals (EDCs) and these EDCs are known to be related to various diseases of infertility, spontaneous abortions, birth defects, endometriosis, breast cancer, and so forth [2–8]. Several papers have been published on the detection of EDCs in solution phase using methods such as fluorimetry, NMR spectroscopy, chromatography, enzyme linked receptor assays, and linear sweep voltammetry [9–17]. Recently, a few researchers studied the detection of EDCs using gas chromatography-mass spectrometry and found 4~8 ng/L of EDCs in gas phase [18]. Therefore, the development of EDC detection and measurement technique is in a great demand, particularly, at very low concentration (0.2~141 ng/L of 17β-estradiol exists in environment) in gas phase since the extremely low amount of EDCs may cause the fatal diseases to human [19]. Nevertheless, most people are not aware of the seriousness of EDCs in air. Notably, inhalation in an EDC exposed environment is an easy means of being affected by

EDCs. Therefore, one of the main challenges in research is to develop a sensitive and reliable method for determination of EDCs in gas phase.

In the present study, a highly sensitive electrochemical biosensor for the detection of the EDC, 17β-estradiol, in gas phase has been developed using estrogen receptor-α immobilized on a gold electrode. 17β-estradiol in gas phase was detected by electrochemical impedance spectroscopy of $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ at the fabricated biosensor. The present biosensor gives a linear response ($R^2 = 0.999$) for the 17β-estradiol vapor concentration from 3.7 ng/L to 3.7×10^{-4} ng/L. This study is aimed at developing a technique capable of detecting low EDC concentration ($>3.7 \times 10^{-4}$ ng/L) in gas phase.

2. Materials

Estrogen receptor-α (ER-α, >85.0%) was purchased from Calbiochem (San Diego, USA). Estrogen hormone (17β-estradiol, >98%), 3-mercaptopropionic acid (3-MPA, >99%), 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide hydrochloride (EDC, >98%), N-hydroxysuccinimide (NHS, >98%), and bovine serum albumin (BSA, >96%) were purchased from Aldrich. A phosphate buffered saline (PBS) was purchased from Gibco (NY, USA).
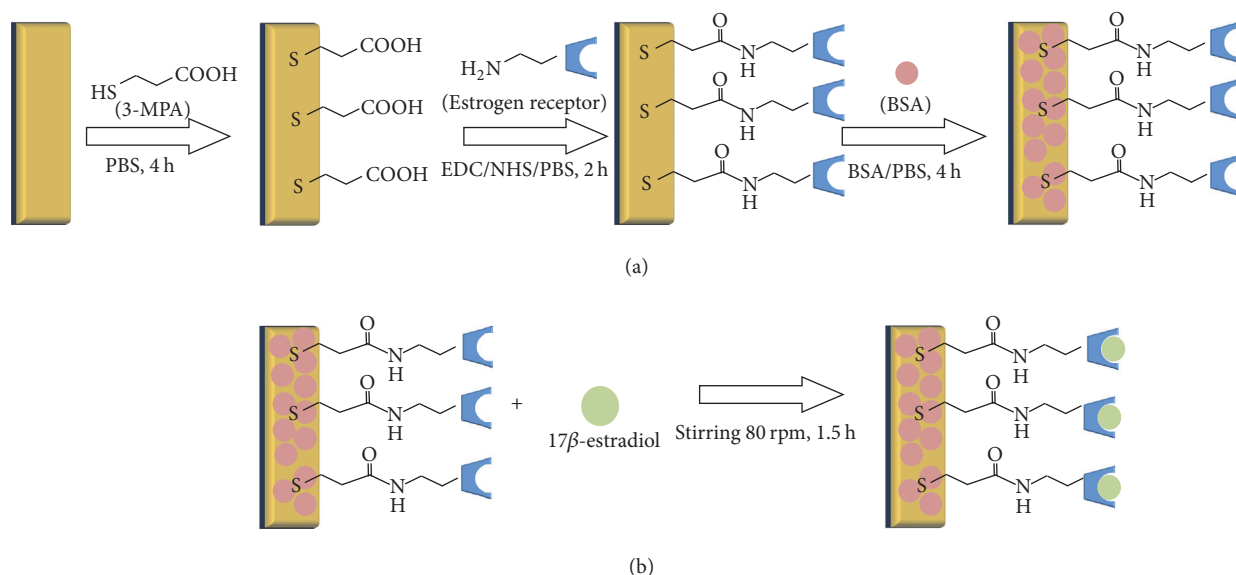
Scheme 1: (a) Fabrication of the estrogen biosensor based on the estrogen receptor-α modified electrode and (b) the binding of estrogen hormone to the biosensor.

For the gas phase experiment, the estrogen biosensor was prepared as previously reported [20, 21]. The fabricated estrogen biosensor was applied to the detection of 17β-estradiol in gas phase. In the experiment, a freshly pretreated gold electrode was incubated in 40 mM 3-MPA solution for 4 h to form a carboxylate terminated self-assembled monolayer (SAM) on the electrode. The electrode was then rinsed using distilled water three times to remove any nonphysically adsorbed 3-MPA molecules before it was stored in distilled water. The 3-MPA modified gold electrode was immersed in a mixture of EDC (1.0 wt.%) and NHS (1.0 wt.%) in 0.05 M PBS (pH 7.4) for 2 h to activate the terminal carboxylic groups, before it was washed using distilled water to remove any nonbonded residual EDC/NHS. Next, a 15 μL aliquot of 0.67 μM estrogen receptor-α solution was applied to the surface of the EDC-NHS/3-MPA modified gold electrode. Any nonbonded estrogen receptor-α on the EDC-NHS/3-MPA modified gold electrode was removed by rinsing using PBS. Finally, the electrode was incubated in 3.5 wt.% BSA for 4 h to block any nonspecific binding sites. Then the biosensor was dried at 25°C for 4 h and then fixed in the empty headspace of a 10 mL vial which contained only 2 mL of 17β-estradiol solution (0.01~100 mM) at the bottom of the vial in order to avoid direct contact between the biosensor and the solution. The vial was stirred at 80 rpm for 90 min (25°C, pH 7). The process was conducted in a sealed vial [22–24]. The schematic diagram of the biosensor fabrication and 17β-estradiol binding is illustrated in Scheme 1.

3. Methods

Electrochemical impedance experiments were performed using an EG&G 263A potentiostat (Princeton, NJ, USA). A 15 mL electrochemical cell that accommodates a platinum wire counter electrode, the estrogen biosensor as the working electrode, and a Ag/AgCl (3 M NaCl) reference electrode was used. AC impedance spectra were recorded in 0.05 M PBS (pH 7.4) containing 5.0 mM $Fe(CN)_6^{3-}$ and 5.0 mM $Fe(CN)_6^{4-}$. The impedance spectra were obtained in the frequency range from 100 MHz to 100 kHz at a bias potential of 0.2 V that was superimposed by an ac potential of 5.0 mV peak-to-peak amplitude. The impedance spectra were plotted in the form of impedance plane plots (Nyquist plots). Zsimpwin program (Princeton Applied Research, Oak Ridge, TN, USA) was used to fit theoretical impedance plots generated based on an equivalent circuit (depicted in Figure 1(c)) to those obtained experimentally. In this way, the diameter of a semicircle in the high frequency range of Nyquist plot was estimated [20, 21].

4. Results and Discussion

For the detection of 17β-estradiol in gas phase, the estrogen biosensor was exposed to 17β-estradiol vapor generated by solutions of concentration between 0.01 and 100 mM for 90 min. Based on Henry's Law, the concentration of 17β-estradiol vapor is proportional to that of aqueous 17β-estradiol. Henry's Law constant (k_H) of 17β-estradiol is known to be 3.64×10^{-11} L·atm/mol [25]. SI unit of Henry's Law constant (k_H) was converted to unit of M/atm and the value is calculated to be 2.7×10^{10} M/atm, and the corresponding concentration of gaseous 17β-estradiol was estimated to be within the range between 0.00037 ng/L and 3.7 ng/L.

As shown in Figure 1(a), the diameter of Nyquist plots, which corresponds to the electron transfer resistance, linearly increases with 17β-estradiol concentration. This is because, with increasing 17β-estradiol concentration, a corresponding increased quantity of 17β-estradiol in the vapor was expected to bind to the immobilized estrogen

TABLE 1: Values of the equivalent circuit elements for 17β-estradiol detection.

17β-estradiol concentration (ng/L)	R_s (Ω)	Q (nF)	R_{et} (Ω)	W (Ω)
0	80	1941	15263	2479
3.7×10^{-4}	80	2019	16982	3034
3.7×10^{-3}	80	1917	19890	3219
3.7×10^{-2}	80	1979	21530	3310
3.7×10^{-1}	80	1197	25603	3895
3.7	80	2041	26067	3068

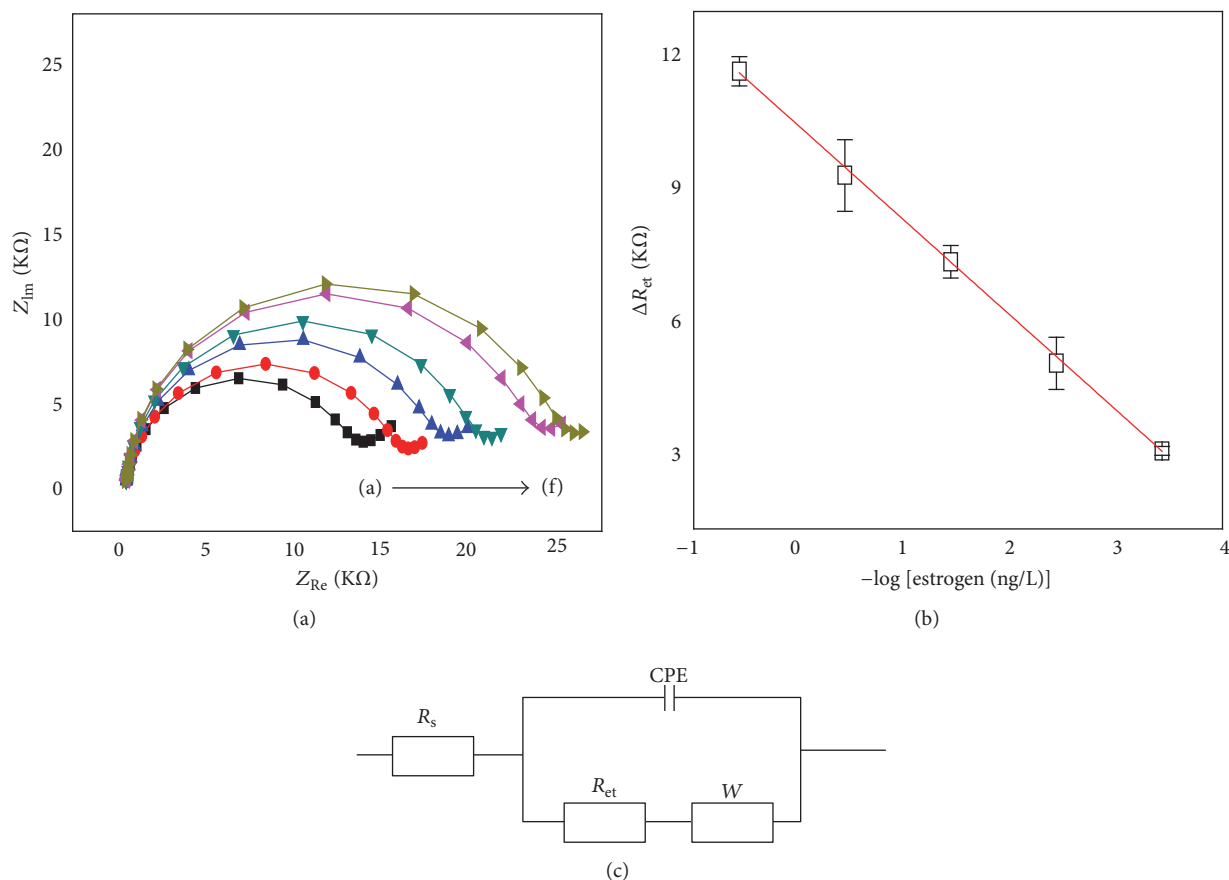
FIGURE 1: (a) Nyquist plots for the faradaic impedance measurements in the presence of 5.0 mM $[Fe(CN)_6]^{3-/4-}$ at the estrogen biosensor before (A) and after the treatment of 3.7×10^{-4} ng/L (B), 3.7×10^{-3} ng/L (C), 3.7×10^{-2} ng/L (D), 3.7×10^{-1} ng/L (E), and 3.7 ng/L (F) of 17β-estradiol in gas phase. (b) Plot of ΔR_{et} versus $-\log[17\beta$-estradiol]. $R_{et}(0)$: electron transfer resistance at the estrogen biosensor before the treatment of 17β-estradiol; $R_{et}(i)$: electron transfer resistance at the estrogen biosensor after the treatment of a certain concentration of 17β-estradiol. $\Delta R_{et} = R_{et}(i) - R_{et}(0)$. (c) Four-component equivalent circuit, R_{et}: resistance of electron transfer, R_s: resistance of solution, W: Warburg impedance, and CPE: constant phase element.

receptor-α, which would in turn gradually hinder the diffusion of $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ to the biosensor surface. We assume that the 17β-estradiol vapor was well bonded to the ER-α on the biosensor surface. In order to normalize the data, the impedance change, $\Delta R_{et} = R_{et}(i) - R_{et}(0)$, was used in the data analysis, where $R_{et}(i)$ and $R_{et}(0)$ represent the electron transfer resistance after and before the binding of 17β-estradiol to the estrogen biosensor, respectively.

As shown in Figure 1(b), the calibration plot was obtained by graphing the impedance change as a function of the 17β-estradiol concentration in gas phase. A linear regression equation was obtained as $\Delta R_{et} = 2195 \log[17\beta$-estradiol] + 4048 ($R^2 = 0.999, S/N = 3, n = 3$). The limit of quantification of the present estrogen biosensor was determined to be 3.7×10^{-4} ng/L of 17β-estradiol.

The resistance of biosensor was measured with a CPE of electrode since the component has incorporated both the Helmholtz double layer and surface roughness or heterogeneity of the electrode. The values of R_s were fixed at 80 ohm as shown in Table 1. CPE and W were determined from the
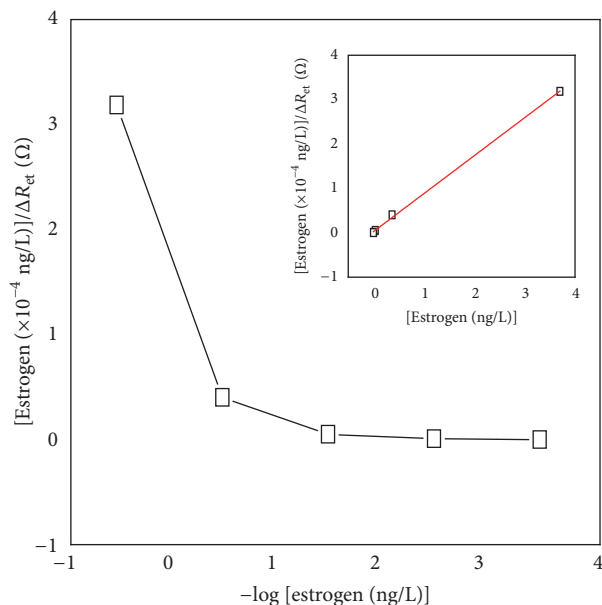
FIGURE 2: Plot of [17β-estradiol]/ΔR_{et} versus $-\log$[17β-estradiol] in 3.7×10^{-4} ng/L to 3.7 ng/L 17β-estradiol. Inset: plot of [17β-estradiol]/ΔR_{et} versus [17β-estradiol] in 3.7×10^{-4} ng/L to 3.7 ng/L 17β-estradiol. Values represent the mean $\pm$ standard deviation from three separate experiments. The experiments were repeated by 3 times.

fitting program (ZsimpWin fitting software). The values of R_{et} were compensated by the fitting software because the semicircle did not reach to the axis of abscissa in the low frequency region. The fitted values were presented in Table 1.

Association constant (K_a) of the binding reaction between 17β-estradiol and estrogen receptor-α was determined using a Langmuir isotherm approach as follows [26]:

$$\theta = \frac{K_a \times C}{1 + K_a \times C}, \tag{1}$$

where θ is the fractional coverage of the surface and C is the concentration of 17β-estradiol vapor. Also, θ is expressed by the following equation between $R_{et}(i)$ and $R_{et}(0)$ [27]:

$$\theta = \frac{R_{et}(i) - R_{et}(0)}{R_{et}(i)} = 1 - \frac{R_{et}(0)}{R_{et}(i)}, \tag{2}$$

where $R_{et}(i)$ and $R_{et}(0)$ represent the electron transfer resistance after and before the binding of 17β-estradiol to the estrogen biosensor, respectively. Above equations are further rearranged to

$$\frac{C}{R_{et}(i) - R_{et}(0)} = \frac{1}{R_{et}(i)}C + \frac{1}{K_a \times R_{et}(i)}. \tag{3}$$

Accordingly, the ordinate intercept of a linear $C/\Delta R_{et}$ versus C plot (as shown in Figure 2) will provide an estimate for $K_a \times R_{et}(i)$, through which K_a can be evaluated.

Figure 2 reveals that $C/\Delta R_{et}$ linearly changes with the concentration, which results in a linear equation of
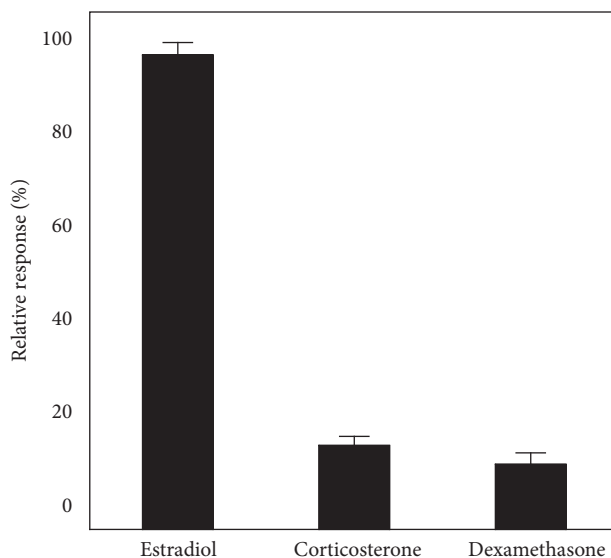

FIGURE 3: Selectivity test of the estrogen biosensor. Values represent the mean $\pm$ standard deviation from three separate experiments.

$C/\Delta R_{et} = 8.56 \times 10^{-5} C + 2.63 \times 10^{-6}$ ($R^2 = 0.999$, $N = 3$). From the fitting of the equation, the association constant was observed to be ca. 32.5 L/ng (8.84×10^{12} M^{-1}). The value of K_a is larger than the reported value of 5.2×10^8 M^{-1} [28]. The result implied that the binding sites on the ER-α were well maintained after the introduction of ER-α to the surface of modified electrode.

The identical biosensing measurements were carried out with corticosterone and dexamethasone that have the similar chemical structures without the bonding characteristics to 17β-estradiol [29]. The selectivity values were obtained by comparing the impedance values at the high concentrations (3.7×10^{-1} ng/L) of corticosterone and dexamethasone with those for 17β-estradiol. As shown in Figure 3, the selectivity values for corticosterone and dexamethasone are $9.44 \pm 2.2\%$ and $5.05 \pm 2.7\%$ due to nonspecific adsorption to the biosensor [30]. The result indicates that the fabricated estrogen biosensor in this study can be used for the selective estimation of 17β-estradiol in gas phase.

5. Conclusion

In this study, the electrochemical impedance biosensor was successfully developed by immobilizing ER-α on a Au electrode and demonstrated for the detection of the gas phase of 17β-estradiol. It is considered that such study may open the possibility of the detector development for the detection of EDCs in gas phase.

Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

Authors' Contributions

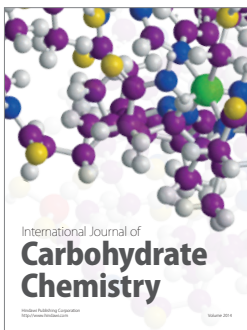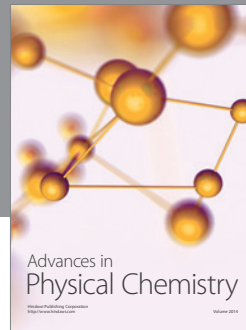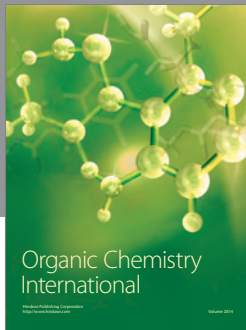Jing Li, Byung Kun Kim, and Kang-Kyun Wang contributed equally to this work.

Acknowledgments

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