Surface & Coatings Technology 205 (2010) S275–S278

Contents lists available at ScienceDirect

Surface & Coatings Technology

journal homepage: www.elsevier.com/locate/surfcoat

Electrochemical detection of estrogen hormone by immobilized estrogen receptor on Au electrode

Ji-Eun Im [a], Jung-Ae Han [a], Byung Kun Kim [a], Jee Hoon Han [a], Tu San Park [b], Seonho Hwang [c], Seong In Cho [b], Won-Yong Lee [a,*], Yong-Rok Kim [a,*]

[a] Department of Chemistry, Yonsei University, Seoul 120-749, Republic of Korea
[b] Department of Biosystems and Biomaterials Science and Engineering, Seoul National University, Seoul 151-921, Republic of Korea
[c] BAEKSEON Communication CO. LTD., Seoul 150-870, Republic of Korea

ARTICLE INFO

Available online 11 August 2010

Keywords:
Biosensor
Estrogen
Electrochemical impedance spectroscopy (EIS)
Self-assembled monolayer

ABSTRACT

Detection of estrogen, a steroid hormone, has intensively been investigated due to the hormone's functionalities of estrus arising and carcinogenic elements. More importantly, it is well known that estrogen contamination in environment disturbs the endocrine system in the ecosystem. In this study, the binding reaction between the hormone and its receptor is applied for the detection of estrogen, which enables high selectivity. Moreover, the electrochemical impedance spectroscopy (EIS) method has been employed for a highly sensitive detection in a wide range of concentration. In order to fabricate the electrode covalently bonded with estrogen receptor, the surface modification was firstly done by the molecules including thiol groups and carboxyl groups. It forms self-assembled monolayers (SAMs) with the carboxylic ends in the open side. The cross linkers are involved to form a solid covalent bonding to the estrogen receptor. The interfacial properties of the modified electrodes have been evaluated in the presence of $Fe(CN)_6^{4-/3-}$ redox couple of the probe by EIS. The accumulation of the treated substances on the electrode surface affects the electrochemical behavior of the redox probe. X-ray photoelectron spectroscopy (XPS) provides intermolecular bonding energy to confirm the surface modifications at each step. Estrogen hormone has been successfully detected in 10^{-6} M concentration.

1. Introduction

Development of biosensor has become important in biotechnology, environmental and industrial monitoring, clinical diagnostics, food safety, pharmaceutical process, and agriculture. Specially, affinity biosensors based on molecular recognition phenomena such as protein–ligand, antigen–antibody, and DNA hybridization have been widely used due to their highly sensitive and specific properties [1–8].

A variety of signal transduction techniques have been used in affinity biosensors, which include surface plasmon resonance (SPR) [9], quartz crystal microbalance (QCM) [10], and cyclic voltammetry (CV) [11]. Especially, electrochemical techniques have the advantages such as low cost, high efficiency and sensitivity. In the electrochemical techniques, electrochemical impedance spectroscopy (EIS) is a powerful method for the study of the interfacial reactions.

Estrogen with estrogenic activity is one of the steroid hormones that are naturally present in mammalian, and it has been reported to bind to the estrogen receptor in responsive cells [12,13]. It is an important bioactive material involved in reproduction in females and the development and maintenance of sexual characteristics. On the other hand, estrogen is known to be a carcinogen due to its tumor initiating and tumor promoting effects [14–16]. Therefore, the development of conventional detection tool with high selectivity and sensitivity is one of the hot issues in the field of biosensor.

In this study, an estrogen sensing electrode is developed for the EIS electrochemical detection of estrogen bindings to estrogen receptors which are covalently bonded to the gold (Au) electrode for the first time. The surface modification of the Au electrode involves the chemicals of 3-mercaptopropionic acid (3-MPA) including thiol groups and carboxyl groups, and 3-(3-Dimethylaminopropyl)-1-ethylcarbodiimide Hydrochloride (EDC), and N-hydroxysuccinimide (NHS). As a last step of modification, the estrogen receptor was covalently bonded to the MPA modified Au electrode.

2. Experiments

2.1. Materials

3-Mercaptopropionic acid (3-MPA, ≥99%), 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide hydrochloride (EDC, >98%), estrogen

* Corresponding authors. Kim is to be contacted at Tel.: +82 2 2123 2646; fax: +82 2 364 7050. Lee, Tel.: +82 2 2123 2649; fax: +82 2 364 7050.
E-mail addresses: wylee@yonsei.ac.kr (W.-Y. Lee), yrkim@yonsei.ac.kr (Y.-R. Kim).

0257-8972/$ – see front matter. Crown Copyright © 2010 Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.surfcoat.2010.08.006

hormone (17β-estradiol, 97%), and N-hydroxysuccinimide (NHS, 98%) were purchased from Aldrich. Estrogen receptor-alpha (ER-α, specific activity: 8700.0 U/mg P, purity by SDS-PAGE: >80%) was purchased from Calbiochem. Distilled water was used throughout the processes. During the preparation of 0.5 M phosphate-buffered saline (PBS) buffer, pH was maintained at 7.4 without the adjustment with acid or base.

2.2. Fabrication of the electrode

Au electrode with 0.1 cm^2 active site area was applied for estrogen receptor immobilization and EIS measurement. Prepared Au plate electrodes were electrochemically polished in PBS by potential scanning between −0.6 and 1.0 V at a rate of 100 mV/s using a potentiostat. The pretreated Au electrode was immersed in an aqueous solution of 40 mM 3-MPA for 8 h to form a carboxylate-terminated self-assembled monolayers (SAMs). The electrode was rinsed with distilled water to remove excess MPA molecules. The MPA modified Au electrode was then placed in a mixture of 1 wt.% EDC and 1 wt.% NHS in PBS solution (pH 7.4) for 1.5 h to convert the terminal carboxylic group to active sites. In order to induce covalent bonding between the estrogen receptor and the MPA modified Au electrode, 25 μL of estrogen receptor in PBS was maintained on the active surface of the EDC–NHS/MPA modified Au electrode for 12 h. Final estrogen receptor-modified Au electrode was obtained after removal of residual estrogen receptor on Au electrode by washing it with the PBS solution.

2.3. Electrochemical measurements

The apparatus used for impedance measurement consists of a frequency response detector model 1025 (Oak Ridge, TN, USA) coupled with an EG&G 263A potentiostat. All experiments were carried out with conventional three-electrode system in electrochemical cell. The working electrode was Au plate electrode (0.1 cm^2) and the counter electrode was platinum wire. All the potentials quoted here were relative to an Ag/AgCl (3 M NaCl) reference electrode.

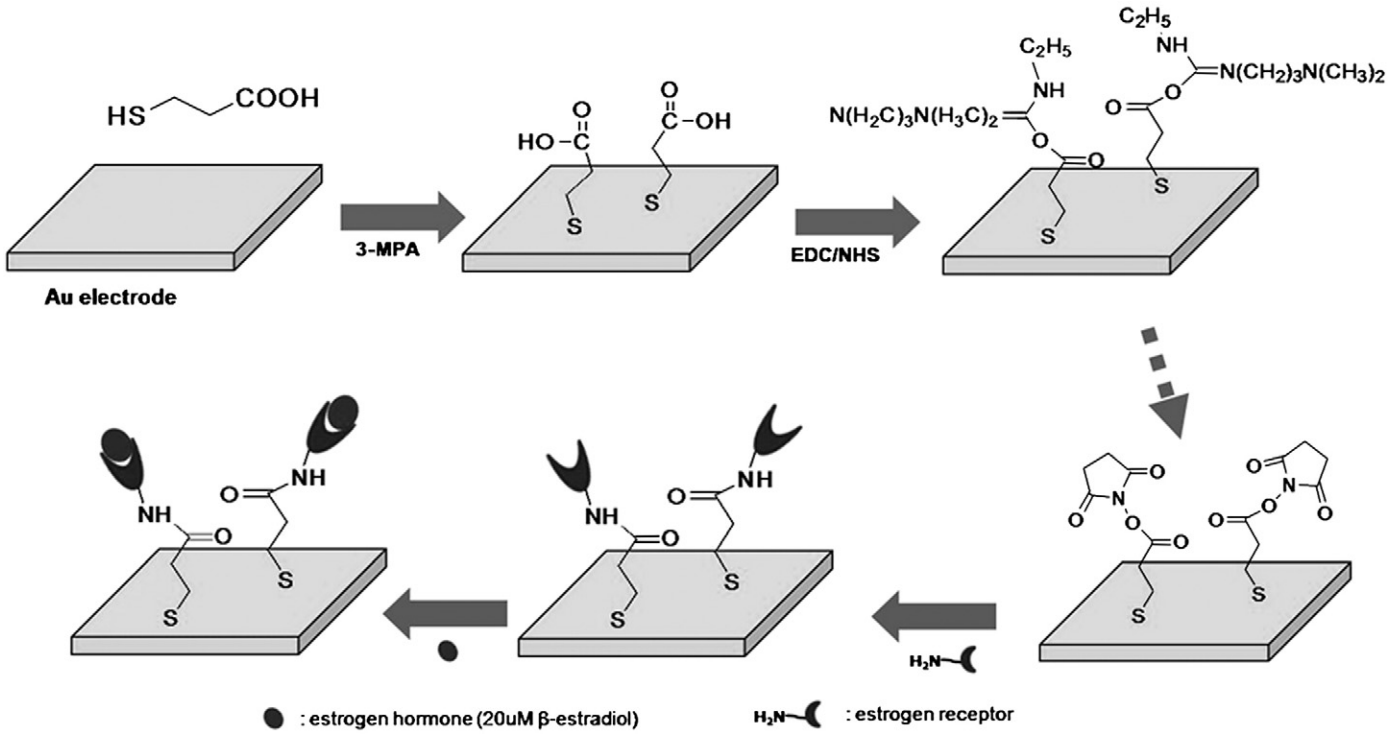
Impedance measurement was carried out in an electrochemical cell containing both 5.0 mL of 5.0 mM potassium ferricyanide ($K_3[Fe_2(CN)_6]$) and 5.0 mL of 5.0 mM potassium ferrocyanide ($K_4[Fe_2(CN)_6]$) at pH 7.0. For impedance measurement, a sine wave potential (AC potential, amplitude sine wave) with 5 mV amplitude superimposed on formal potential of the redox probe of 0.2 V vs. Ag/AgCl (3 M NaCl) was applied. A wide frequency range from 100 MHz to 100 kHz was scanned and the impedances were recorded.

3. Results and discussion

Scheme 1 presents a simplified fabrication procedure of Au electrode for electrochemical detection of estrogen hormone. The resulting modified Au electrodes at each step were investigated by XPS (ThermoVG, U.K) and EIS (EG&G 263A) measurements.

3.1. XPS analysis of modified MPA, EDC–NHS/MPA on Au electrode

XPS was utilized to monitor the completion of each step reaction for the modified electrode. Fig. 1 shows the XPS spectra measured for bare Au electrode, MPA on Au electrode, and EDC–NHS/MPA on Au electrode. After immobilization of 3-MPA molecules on the Au electrode, the XPS spectra present the presence of the sulfur 2p (S-2p) at 162 eV in Fig. 1a. The S-2p spectrum is fitted by the spin–orbit split doublet of S-$2p^{3/2}$ and S-$2p^{1/2}$ [17]. In this spectrum, two binding energies are measured. The first binding energy of 162.1 eV is attributed to gold–sulfur bond (Au–S). The higher second binding energy of 163.5 eV is considered to be the non-covalently adsorbed free thiol groups bonded to the Au electrode [18,19]. Fig. 1b indicates a high-resolution carbon 1s (C-1s) spectrum of the self-assembled MPA monolayer on Au electrode. The binding energy of 285 eV is attributed to the aliphatic carbon (CH_2 group) chains in the MPA component [20]. Moreover, the binding of the MPA molecules on Au electrode is further probed by the peak observed at 288.7 eV that is the characteristic of the carboxyl group of the MPA monolayer on Au electrode. This peak is not observed in the high-resolution C-1s spectrum of the bare Au electrode (inset of Fig. 1b).



Scheme 1. Schematic diagram of the fabrication of the immunosensor for detection of estrogen hormone.

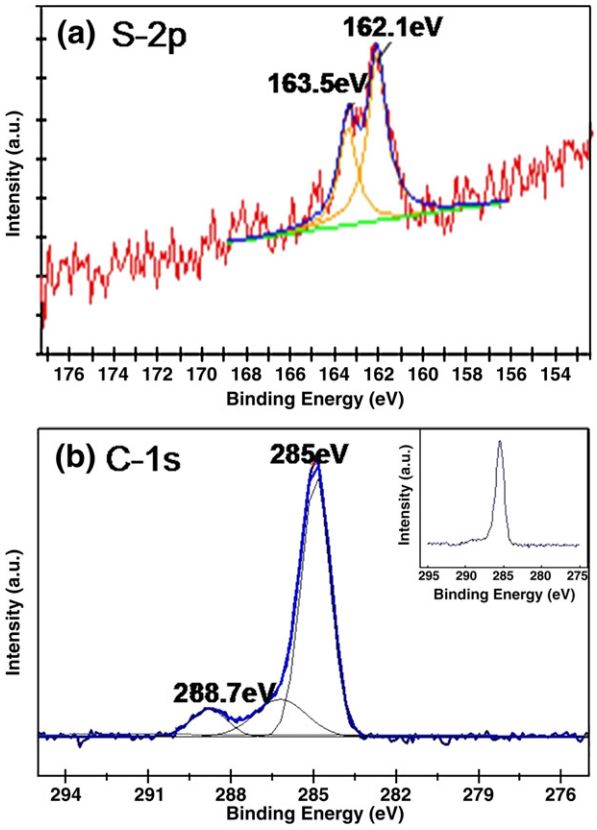


Fig. 1. High resolution XPS (ThermoVG, U.K.) spectra for modified Au electrode. (a) XPS spectrum of the S-2p core level for 3-MPA modified Au electrode. (b) XPS spectrum of the C-1s core level for 3-MPA modified Au electrode (inset: XPS spectrum of the C-1s core level for bare Au electrode).

After the MPA modified electrode surface is activated with EDC and NHS, XPS survey spectrum indicates the presence of the nitrogen 1s (N-1s) peak in Fig. 2. The nitrogen binding energy of the activated surface is found in high-resolution N-1s spectrum, which indicates the presence of the *O*-acylisourea intermediate formed by the reaction of EDC with carboxylic acid parts of MPA [21,22]. There is also NHS ester formed by the reaction of NHS with the *O*-acylisourea intermediate. The binding energy peak at 399.7 eV indicates the presence of the secondary amine and imine of EDC. The peak at 400.7 eV is attributed to the protonated tertiary amine of EDC. The peak at 402.1 eV is to be the characteristic of the nitrogen of the NHS ester, which is shifted to higher binding energy due to the electronegative oxygen bonded to the nitrogen [23–25].

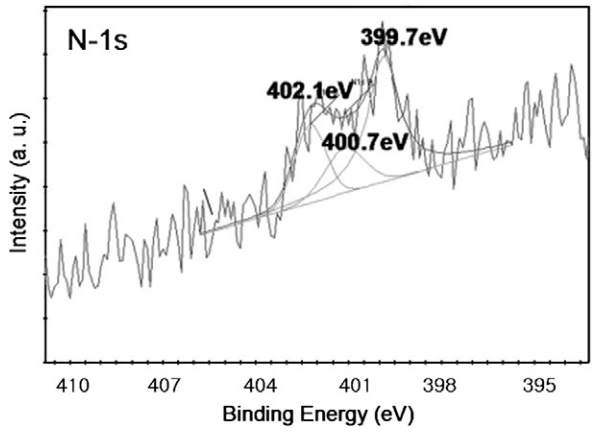


Fig. 2. High resolution XPS spectrum of the N-1s core level for 3-MPA activated with EDC and NHS modified Au electrode.

3.2. Stepwise electrochemical impedance analysis during the modification process on the Au electrode and the EIS detection of estrogen hormone

Electrochemical impedance spectroscopy (EIS) provides useful information on interfacial reaction. The spectrum is typically expressed as a form of Nyquist diagram that is observed as a semicircle corresponding to the electron transfer resistance, R_{et}, followed by a linear portion. The diameter of a semicircle is a reflection of the magnitude R_{et} and the linear portion is the result of the diffusion of redox probes from the solution to the interface.

As shown in Fig. 3, the Nyquist diagrams of the bare Au electrode are obtained at the stepwise modification steps. Important changes are observed in the step-by-step immobilization processes of the modifications of the Au electrode with MPA, EDC and NHS, estrogen receptor, and estrogen hormone. The Nyquist diagram of the bare Au electrode in the presence of the redox couple shows a very small semicircle due to the fast electron transfer reaction. The MPA immobilized Au electrode presents a larger semicircle than that of the bare Au electrode. It is due to the large quantity of negative charges from carboxyl anion ($-COO^-$) groups at pH 7 that inhibits the electron transfer between the MPA modified electrode and the negatively charged redox species in the electrolyte solution [21]. The results indicate that the MPA self-assembled monolayers are densely formed on the Au electrode in the immobilization step above. The MPA modified Au electrode is activated with EDC and, subsequently, by NHS. It results in the sealing of the negative charges of the terminal carboxyl anion of MPA by NHS ester and *O*-acylisourea intermediate. Therefore, the diameter of the semicircle in the Nyquist diagram decreases, implying a decreased R_{et}. Since the NHS ester and the *O*-acylisourea intermediate involve neutral and positive charges, the mass transport of the negatively charged redox couple to the electrode surface is ought to be favorable, leading to a decreased electron transfer resistance. As the estrogen receptor is immobilized on the modified Au electrode, the diameter of the semicircle increases compared to the Au electrode modified by EDC and NHS. It is due to the disturbance of the penetration of the redox couple since the bonded estrogen receptor may work as the insulating layer that

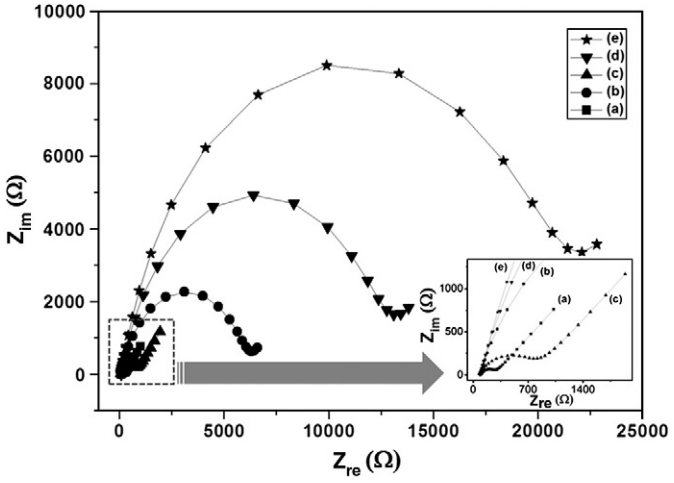


Fig. 3. Nyquist diagram of the EIS (electrochemical impedance spectroscopy) measured for: (a) a bare Au electrode; (b) a bare Au electrode that was modified 3-MPA to form a thiol-Au SAM; (c) a thiol-Au SAM electrode that was activated with EDC/NHS reagent; (d) immobilization of estrogen receptor on thiol-Au SAM electrode; (e) after estrogen hormone(10^{-6} M) binding to estrogen receptor on a thiol-Au SAM electrode.

inhibits the redox couple to reach the Au electrode. Once estrogen hormones bind to the immobilized receptors, the Nyquist diagram presents the largest semicircle as shown in Fig. 3e. Such impedance change in Fig. 3e is obtained by exposing the receptor-modified sensor to the 10^{-6} M concentration of estrogen hormone in PBS buffer solution.

4. Conclusion

EIS is applied to detect the estrogen hormone with receptor-bonded biosensor for the first time. It is achieved by immobilizing estrogen receptor to the modified surface of gold electrode through amide bonding. Each step of the interface reactions is monitored and successfully confirmed by the stepwise EIS and XPS measurements. The detection of estrogen hormone is also accomplished by the relative impedance changes in the EIS measurement. This work demonstrates that the impedance change is observed by exposing the receptor-modified electrode to the 10^{-6} M concentration of estrogen hormone in PBS buffer solution. Currently, the detailed optimization process of all reaction conditions is being performed in order to achieve the highest possible detection limit along with the selectivity. It is highly expected that the present detection method can be applied to the production of potable estrogen sensor with high sensitivity and good selectivity.

Acknowledgements

This study was financially supported by the Technology Development Program of the Ministry of Agriculture and Forestry and a grant from the Korea Healthcare technology R&D Project, Ministry for Health, Welfare and Family Affairs (Grant No. A085136), Republic of Korea.

References

[1] A.R. Chris, B.S. Stephanie, J.F. Mark, P.G. Joel, S.L. Frances, Anal. Chem. 71 (1999) 433.
[2] S.S. Babkina, N.A. Ulakhovich, Anal. Chem. 77 (2005) 5678.
[3] H.T. John, B.M. Keith ale, D.G. Jeremy, Biotechnol. Adv. 26 (2008) 492.
[4] A. Erol, D. Erhan, Biosens. Bioelectron. 20 (2005) 1263.
[5] C. Tlili, N. Jaffrezic-Renault, C. Martelet, J.P. Mahy, S. Lecomte, M.C. Mohamed, H. Korri-Youssoufi, Mater. Sci. Eng., C 28 (2008) 861.
[6] G. Volpe, G. Fares, F. delli Quadri, R. Draisci, G. Ferretti, C. Marchiafava, D. Mosconea, G. Palleschi, Anal. Chim. Acta 572 (2006) 11.
[7] G. Vered, R. Judith, Environ. Sci. Technol. 36 (2002) 1574.
[8] S.J. Ding, B.W. Chang, C.C. Wu, M.F. Lai, H.C. Chang, Electrochim. Acta 50 (2005) 3660.
[9] S.J. Ding, B.W. Chang, C.C. Wu, M.F. Lai, H.C. Chang, Anal. Chim. Acta 554 (2005) 43.
[10] J. Pearson, A. Gill, G.P. Margison, P. Vadgama, A.C. Povey, Sensors and Actuators B 76 (2001) 1.
[11] E. Ruth, S. Kendra, Langmuir 23 (2007) 3880.
[12] V. Granek, J. Rishpon, Environ. Sci. Technol. 36 (2002) 1574.
[13] Z.C. Sanchez-Acevedo, J. Riu, F.X. Rius, Biosens. Bioelectron. 24 (2009) 2842.
[14] L. Xiangqin, L. Yongxin, Biosens. Bioelectron. 22 (2006) 253.
[15] M. Dijksma, B. Kamp, J.C. Hoogvliet, W.P. van Bennekom, Anal. Chem. 73 (2001) 901.
[16] M.B. Byfield, R.A. Abuknesha, Biosens. Bioelectron. 9 (1994) 373.
[17] M.M. Walczak, C.A. Alves, J. Electroanal. Chem. 396 (1995) 103.
[18] K. Uvdal, T.P. Viking, Langmuir 17 (2001) 2008.
[19] J. Huang, J.C. Hemminger, J. Am. Chem. Soc. 115 (1993) 3342.
[20] C. Youngnam, I. Albena, J. Phys. Chem. B 109 (2005) 12731.
[21] G. Ping, Z. Xinai, M. Weiwei, W. Qingjiang, Electrochim. Acta 53 (2008) 4663.
[22] S. Wei, Z. Jianghua, Q. Peng, J. Kui, Anal. Biochem. 377 (2008) 115.
[23] L. Jiang, A. Glidle, Bioelectrochem. Bioenerg. 42 (1997) 15.
[24] T. B¨ocking, M. James, Langmuir 20 (2004) 9227.
[25] E. Delamarche, G. Sundarababu, Langmuir 12 (1996) 1997.