Journal of Industrial and Engineering Chemistry xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Journal of Industrial and Engineering Chemistry

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

An electrochemical nanofilm sensor for determination of 1-hydroxypyrene using molecularly imprinted receptors

Do-Hyeon Yang[a,b,1], Chang-Soo Lee[c,1], Beung-Hoon Jeon[b], Sung Mook Choi[a], Yong-Dae Kim[d,*], Jae Sup Shin[b,**], Heon Kim[d]

[a] *R&D Lab., PNS Technology, Inc., 301 Yeonsung University, 34, Yanghwa-ro 37 beon-gil, Manan-gu, Anyang-si, Gyeonggi-do 439-730, South Korea*
[b] *Department of Chemistry, Chungbuk National University, 1 Chungdae-ro, Seowon-gu, Cheongju, Chungbuk 28644, South Korea*
[c] *Hazards Monitoring Bionano Research Center, Korea Research Institute of Bioscience & Biotechnology (KRIBB), 125 Gwahak-ro Yuseong-gu, Daejeon 34141, South Korea*
[d] *Department of Preventive Medicine, College of Medicine, Chungbuk National University, 1 Chungdae-ro, Seowon-gu, Cheongju, Chungbuk 28644, South Korea*

ARTICLE INFO

Article history:
Received 12 December 2016
Received in revised form 25 February 2017
Accepted 27 February 2017
Available online xxx

Keywords:
Molecular imprinting
TiO_2 gel matrix
Electrochemical sensor
1-Hydroxypyrene
Polycyclic aromatic hydrocarbon

ABSTRACT

A novel electrochemical nanofilm sensor for 1-hydroxypyrene (1-OHP) was developed based on a molecular imprinted TiO_2 gel matrix, which was prepared with Ti(O-*n*-Bu)$_4$ and 1-OHP using sol–gel technology. Cyclic voltammetry (CV) showed that guest binding on the non-imprinted sensor resulted in nearly the same current changes for all guest molecules, indicating non-specific binding onto the TiO_2 gel matrix. In contrast, binding at the molecular imprinted sensor revealed that the selectivity for 1-OHP relative to structurally related guest molecules was estimated to be 1.8–6.7 times greater at 1×10^{-9} M, reflecting its specific adsorption and binding capacity for the template molecule.

Introduction

Polycyclic aromatic hydrocarbons (PAHs), which are formed during incomplete combustion of plant and animal matter or fossil fuels such as coal or petroleum, are widely distributed in our natural environment [1,2]. Human exposure to PAHs occurs because of breathing ambient and polluted indoor air, smoking cigarettes, and eating certain food items, as well as from occupational contacts. PAHs are highly detrimental to the human health because the reactive metabolites of some PAHs can bind to cellular proteins and DNA. The resulting biochemical disruptions and cell damage can lead to mutations, development malformations, tumors, and cancer. Urinary 1-hydroxypyrene (1-OHP), a kind of metabolites of pyrene, known as a useful biomarker for evaluating PAH, especially in workers with high occupational exposure [3–6]. Various approaches such as high performance liquid chromatography–fluorescence (HPLC–FL) [3,7–9], high performance liquid chromatography–mass spectrometry (HPLC–MS) [10,11], and gas chromatographic–mass spectrometry (GC–MS) [12,13] have been developed and widely used to detect urinary 1-OHP. Although chromatographic methods are greatly effective, these methods are relatively time-consuming, complex, and costly. Therefore, the development of a low cost, quick response and easy manipulation method is desirable.

Molecular imprinting (MI) is one of the most promising synthetic alternatives for bioreceptors and is extensively studied for various applications ranging from sensing to separations [14–19]. MI is based on the formation of a polymer matrix by the polymerization of functional monomers and cross-linkers in the presence of template molecules. When the template molecules are removed from the polymer matrix, imprinted cavities that are complementary to the template molecules in size, shape, and functionality are left, which can function as selective binding sites for specific template molecules.

Several strategies to develop PAH- and/or 1-OHP-imprinted polymer matrices have been reported. Dickert et al. reported PAH-imprinted polymers in which the geometry of imprint molecules, ranging from anthracene to up to 1,12-benzoperylene, determined the selectivity of cross-linked polyurethane matrices using

---

Abbreviations: AMPY, 1-aminopyrene; MIP, molecularly imprinted polymer; NIPY, 1-nitropyrene; 1-OHP, 1-hydroxypyrene; PAH, polycyclic aromatic hydrocarbons; PYCA, 1-pyrenecarboxylic acid.

[*] Corresponding author. Fax: +82 43 274 2965.

[**] Co-Corresponding author.

E-mail addresses: ydkim@chungbuk.ac.kr (Y.-D. Kim), jsshin@chungbuk.ac.kr (J.S. Shin).

[1] The authors contributed equally to this work.

http://dx.doi.org/10.1016/j.jiec.2017.02.020

Please cite this article in press as: D.-H. Yang, et al., An electrochemical nanofilm sensor for determination of 1-hydroxypyrene using molecularly imprinted receptors, J. Ind. Eng. Chem. (2017), http://dx.doi.org/10.1016/j.jiec.2017.02.020

diisocyanatodiphenylmethane and 30% of the respective triisocyanate (isomer mixture) with bisphenol A and phloroglucinol [20,21]. Kirsch et al. reported molecularly imprinted polymer (MIP)-modified screen-printed carbon electrodes (SPCE) for the determination of 1-OHP, using divinylbenzene and styrene as functional monomers, in which 1-OHP binding involved hydrophobic and/or π–π interactions [22,23]. Krupadam et al. reported MIP adsorbents based on a newly developed functional cross-linker—*N*,*O*-bismethacryloyl ethanolamine (NOBE)—for the detection of highly toxic PAHs in seawater. The binding capacity of the resulting MIP for oil spill toxin pyrene was 35 mg/g, nearly 10-fold higher than that (3.65 mg/g) obtained using a non-imprinted polymer [24]. Krupadam et al. also reported an MIP particle adsorbent for carcinogenic PAHs using a non-covalent templating technique. They synthesized MIP particles of 2–5 μm in acetonitrile using a mixture of six PAHs as a template molecule. The MIP particles exhibited remarkable binding affinity toward template molecules [25]. Lai et al. demonstrated that MIPs appeared promising to investigate benzo[a]pyrene enrichment in water and coffee samples [26]. Although organic polymer matrices are dominantly used in this field, imprinted cavities are embedded in bulk polymer matrices, and easy access of target molecules to the imprinted binding sites is often suppressed [27].

The MI of hybrid sol–gel matrices such as silica and titania have attracted considerable attention in the last few decades because of their unique properties [28–31]. In particular, we have demonstrated the advantages of thin TiO_2 gel films as useful matrices for MI, and molecularly imprinted TiO_2 gel matrices have shown good recognition properties with high sensitivity and selectivity for target template molecules [32–35]. Moreover, because the specific binding sites for a template molecule are close to the surface, imprinted TiO_2 gel matrices have been applied to sensor transducers, and ready access of template molecules to binding sites has been reported [36–38]. We recently reported molecularly imprinted TiO_2 gel nanofilms by spin coating [39] and TiO_2 gel-coated microbeads by the surface sol–gel method [40] for the detection of 1-OHP and found some important factors that allow optimal fabrication conditions for improved selectivity and binding. Binding efficiency and imprinting effect of 1-OHP imprinted sites depend on the molar ratio of the template and matrix precursor. The number of binding sites increased with increasing template concentration. However, the template concentration was increased beyond the optimum value, the template molecules overlapped, and their binding sites could be reduced [40].

In our previous study [40], we developed a new method for the extraction of 1-OHP from urine by using a molecularly imprinted TiO_2 gel matrix, and the determination of 1-OHP by HPLC. However, its effectiveness as a practical technique has not been fully examined. The purpose of the present study was to explore the possibility of developing a low cost, quick response, and easy manipulation method for the 1-OHP detection by combining an electrochemical sensor technique and molecular imprinting.

The current approach would provide potential direction for not only creation of novel sensing material but also for on-site environmental monitoring system.

Experimental

Materials

Titanium(IV) *n*-butoxide ($Ti(O\text{-}n\text{-}Bu)_4$) was obtained from Acros Organics, USA. Naphthalene and anthracene were purchased from Tokyo Kasei, Japan. 1-Hydroxypyrene (1-OHP), 1-aminopyrene (AMPY), 1-nitropyrene (NIPY), 1-pyrenecarboxylic acid (PYCA), and pyrene were purchased from Aldrich, USA. All of these chemicals were guaranteed reagents and were used as purchased without further purification. All other chemicals used as solvents were of analytical grade and obtained from commercial sources. Deionized pure water (18.3 MΩ cm) was obtained through the third distillation unit (AquaMax^{TM}-*Ultra*, YOUNGLIN INSTRUMENT, South Korea). Chemical structures of the reagents used in this study are shown in Scheme 1.

Preparation of imprinted TiO_2 gel films on quartz plates

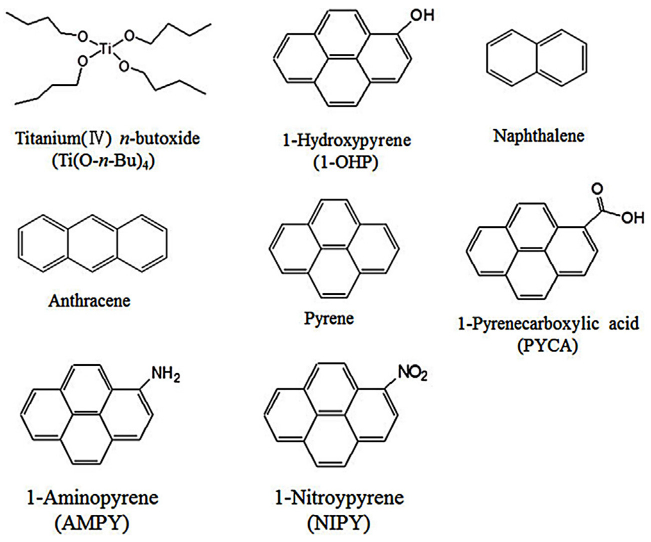
1-OHP-imprinted TiO_2 gel films have been reported [39,40]. Films were prepared by spin-coating using a YS-100D system (Yooil Engineering, South Korea) for 30 s at a speed of 4000 rpm with 10 μL of the film-forming stock solutions on quartz plates. After hydrolysis, template molecules were removed using ethanol [40]. Their removal was confirmed by the disappearance of characteristic UV–vis absorption peaks for the template molecules. UV–vis absorption spectra were acquired using a Lambda 35 UV–vis spectrophotometer (PerkinElmer).

Scanning electron microscopy (SEM) measurements were carried out with a Hitachi S-5200 at an acceleration voltage of 15 kV. A 3-nm-thick platinum layer was deposited on all specimens using a Hitachi E-1030 ion sputter at 15 mA and 10 Pa to prevent the electrical charge-up of the sample by the electron beam.

Fabrication of imprinted sensor and electrochemical measurements

The cleaned gold electrode (GE, ∅ = 2 mm) was hydroxylated by immersion in a 10 mM 2-mercaptoethanol solution in ethanol for 8 h, rinsed with ethanol and pure water, and then dried with nitrogen gas. Imprinted and non-imprinted sensors were prepared by spin-coating the film-forming stock solutions onto the hydroxylated GE, which were then hydrolyzed to remove the template molecules under the experimental conditions mentioned above.

A CompactStat electrochemical analyzer (Ivium Technologies, the Netherlands) was used for cyclic voltammetry (CV) measurements with a typical three-electrode cell. The reference and counter electrodes consisted of a 3-M NaCl Ag/AgCl electrode and a 0.5-mm diameter Pt wire, respectively [41]. The imprinted and non-imprinted sensors were used as the working electrode. CV measurements were collected in a 2 mM solution of potassium



Scheme 1. Chemical structures of templates, guest molecules, and the matrix precursor $Ti(O\text{-}n\text{-}Bu)_4$.

Please cite this article in press as: D.-H. Yang, et al., An electrochemical nanofilm sensor for determination of 1-hydroxypyrene using molecularly imprinted receptors, J. Ind. Eng. Chem. (2017), http://dx.doi.org/10.1016/j.jiec.2017.02.020