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Analytical Methods

Photocontrolled solid-phase extraction of guanine from complex samples using a novel photoresponsive molecularly imprinted polymer

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ABSTRACT

A novel photoresponsive molecularly imprinted polymer (MIP) was developed for the selective extraction of guanine from complex samples. The photoresponsive MIP was fabricated using guanine as the template, water-soluble 5-[(4-(methacryloyloxy)phenyl)diazenyl]isophthalic acid as the functional monomer, and water-soluble triethanolamine trimethacrylate as the cross-linker. The MIP displayed good selectivity toward guanine with a dissociation constant of $(2.70 \pm 0.16) \times 10^{-5}$ mol L^{-1} in aqueous media. The density of the guanine-specific receptor sites in the MIP material was (4.49 ± 0.22) μmol g^{-1}. Quantitatively release and uptake of guanine by the MIP occured with irradiation at 365 and 440 nm, respectively. The MIP could efficiently extract guanine from beer and then release it into aqueous media under photocontrol. This method could be used for selective separation and subsequent determination of a specific analytes from complex samples.

1. Introduction

The purine bases contain guanine and adenine are involved in a variety of biochemical processes (Schmidt, Lara, & Souza, 2007; Schwarzschild, Agnati, Fuxe, Chen, & Morelli, 2006) such as storage of genetic information, protein biosynthesis, and cell metabolism. Base changes in DNA or RNA can affect seriously the structure and function of products of gene expression-protein, which is considered to be a leading cause of inherited disease and cancer (Bont & Larebeke, 2004; Maynard, Schurman, Harboe, Souza-Pinto, & Bohr, 2009). Therefore, determination and separation of purine bases is important. Various analytical methods such as capillary electrophoresis (Klampfl, Himmelsbach, Buchberger, & Klein, 2002; Wang & Ren, 2004), micellar electrokinetic capillary electrophoresis (Cortacero-Ramírez, Segura-Carretero, Cruces-Blanco, Hernáinz-Bermúdez de Castro, & Fernández-Gutiérrez, 2004), and high-performance liquid chromatography (HPLC) (Burdett et al., 2013; Nikitas, Pappa-Louisi, Agrafiotou, & Mansour, 2011) have been used for separation and determination of purine bases. However, quantitation of purine bases (Cheng, He, Huang, Huang, & Zhou, 2014; Cheng et al., 2014; He et al., 2014; Kiianitsa & Maizels, 2014; Nelson, Strachan, Sloane, Li, & Landers, 2014) is affected by the complexity of the sample matrix. Therefore, an alternative affinity material for simple, rapid, inexpensive, and highly selective separation of purine bases from complex samples is highly desirable (Huang, Lin, & Liu, 2004).

Molecular imprinting creates specific recognition sites in synthetic polymers, and these sites are suitable for selective extraction of specific materials from complex matrices (Wulff, 1995). Molecularly imprinted polymers (MIPs) are synthesized from a reaction mixture of a cross-linker, template molecules, and functional monomers in a solvent. After polymerisation, the functional groups are "frozen" in the cross-linked polymeric network. Subsequent removal of the template molecule leaves empty cavities in the polymer matrix, which are complementary in size, shape and functionality to the template. This allows for selective recognition of the template by the MIP. MIPs are used extensively in chemical sensors (Chuang, Rick, & Chou, 2009), catalysis (Cheng, Zhang, & Li, 2004), controlled release of drugs (Shi et al., 2012), and solid-phase extraction (Chen, Xie, & Shi, 2013; Prasad, Sharma, & Lakshmi, 2007; Qu, Zhang, Gao, & Yang, 2012). In molecularly imprinted solid-phase extraction (MISPE), a MIP is packed into a HPLC column and used as a sorbent. A solution containing the analyte is then loaded into this column, and the MIP selectively

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extracts the analyte. The analyte is then eluted for quantitative analysis via HPLC. The application of MISPE is limited by the following issues: (1) MISPE is often used in cartridge mode, which usually involves a tedious column-packing procedure, high back-pressure, and is time consuming; (2) template release use large volume of solvent and is time consuming; and (3) MISPE is only applicable for extraction and concentration of small amounts of sample because of the limited amount of MIP in the column. MIPs that can realise uptake and release of the analyte in response to external stimuli have received widespread attention in recent years (Xu, Lu, Zheng, & Chen, 2013). Stimuli-responsive MIPs undergo large conformational changes in response to specific external stimuli such as temperature (Li, Pilla, & Gong, 2009), pH (Lynn, Amiji, & Langerp, 2001), magnetic fields (Li et al., 2006; Lin et al., 2012), and photoirradiation (Fang, Chen, Zhang, & Zhang, 2011; Gong, Lam, & Yu, 2006; Wang, Xie, Shi, Sun, & Zhao, 2013; Yamada et al., 2008). Photoirradiation is frequently used for responsive materials because light is 'clean energy' and can be manipulated precisely and rapidly. Photoresponsive MIPs containing azobenzene as the chromophore are popular because they exhibit excellent photoinduced fast and reversible isomerization between the *trans*- and *cis*-isomers of the azo moieties on exposure to ultraviolet or visible light. This can trigger a large change in the strength of host–guest interactions. Although remarkable advances have been made in the field of photoresponsive MIPs, some challenges remain. For example, most reported MIPs only work in organic media (Xu et al., 2013) and fail to show specific binding in aqueous media. So far, two research groups have reported preparation of water-compatible MIPs by different methods. Zhang's research group reported preparation of water-compatible MIP microspheres by grafting poly(NIPAAm) brushes to MIP microspheres (Fang et al., 2011). Our group reported preparation of photoresponsive water-compatible MIPs using a water-soluble azobenzene-containing functional monomer (Gong, Wong, & Lam, 2008; Tang et al., 2012; Yang et al., 2014). To the best of our knowledge, there has been little research on photoresponsive MIPs for guanine. In this work, we developed a photoresponsive MIP for guanine using a new water-soluble azobenzene derivative containing two carboxylic acid groups (–COOH), 5-[(4-(methacryloyloxy)phenyl)diazenyl]isophthalic acid (MAPDIA), as the functional monomer, water-soluble triethanolamine trimethacrylate (TEAMA) as the cross-linker, and guanine as the template. After removal of guanine via Soxhlet extraction, the MIP displayed photocontrolled specific affinity to guanine in aqueous media. The MIP was successfully applied to the photocontrolled solid-phase extraction of guanine from complex real samples (beer) with good recovery. Subsequent photocontrolled release of guanine in aqueous media allowed for convenient quantitative analysis with high efficiency.

2. Experimental

2.1. Materials

Guanine, adenine, uric acid, *N*,*N*-dimethylaminopyridine, triethanolamine, triethylamine, phenol, 5-aminoisophthalic acid, azodiisobutyronitrile (AIBN), methanol, ethanol, methacrylic acid, tetrahydrofuran (THF), dichloromethane, dimethylformamide, acetic acid, NaH_2PO_4, NaOH, $SOCl_2$, $NaNO_2$, HCl, Na_2SO_4, $NaHCO_3$, and methacrylic anhydride were purchased from Aladdin Co. Ltd. (Shanghai, China). Methacrylic chloride (colourless oil, b.p. 96–98 °C) was synthesized from methacrylic acid and $SOCl_2$. A phosphate buffer solution (0.10 mol L^{-1}, pH = 7.0) was prepared by dissolving 15.6 g of NaH_2PO_4 in 1000 mL of water–methanol (95:5, v/v), and adjusting the pH of the solution to 7.0 with aqueous NaOH. This buffer was used throughout the experiment.

2.2. Instrumentation and apparatus

^1H NMR and ^{13}C NMR were recorded on a Bruker AV-300 ((Bruker, Billerica, MA, USA) NMR instrument at ambient temperature using tetramethylsilane as the internal standard. Ultraviolet–Visible (UV–Vis) spectra were obtained on a UV-4802 spectrophotometer (UNICO [Shanghai] Instruments Co. Ltd., China). A CEL S-500 Xe light (500 W) with 365 and 440 nm quartz filters was used as the light source (Beijing Zhongjiao Jinyuan Keji Co. Ltd., China). HPLC analysis was performed with an LC2000 series system equipped with a quaternary pump (LC2130) and variable wavelength detector (LC2030) (TECHCOMP [Shanghai] Instruments Co. Ltd., China). HPLC-mass spectra were recorded on a Bruker Esquire 2000 HCT LC/MS system.

2.3. Preparation of photoresponsive MIP

Synthesis details for MAPDIA and TEAMA are given in the Supplementary Electronic Information. In a 250 mL round bottom flask, MAPDIA (0.50 g, 1.40 mmol) and TEAMA (1.50 g, 4.20 mmol) were dissolved in 50 mL of dimethylformamide. Guanine (42.70 mg, 0.28 mmol) was dissolved in 50 mL of deionized water, which contained a trace amount of HCl to increase the solubility of the guanine. The mixture was sonicated for 30 min and stirred in the dark at room temperature for 12 h. After addition of AIBN (0.10 g), the resultant mixture was degassed by bubbling with N_2 for at least 20 min and then sealed. The mixture was placed in an oil bath at 65 °C for 24 h with stirring in the dark. The guanine imprinted MIP particles were collected by centrifugation and washed with deionized water and methanol, and then dried under vacuum until they reached a constant weight. The bulk MIP was crushed and milled. Guanine in the polymer was removed by Soxhlet extraction with 200 mL of a methanol/acetic acid mixture (9:1 v/v) for 24 h, followed by 200 mL of methanol for 24 h in the dark. A lengthy Soxhlet extraction was used to remove all the template molecules embedded in the MIP either through specific or non-specific binding. The resultant MIP was dried at 40 °C under vacuum until it reached a constant weight. A control non-imprinted polymer (NIP) was prepared and treated in exactly the same way as the guanine-imprinted polymer, except that guanine was not used in the polymerisation procedure. Both MIP and NIP materials were stored at room temperature in the dark.

2.4. Spectroscopic characterisation and photoisomerization studies

Spectroscopic characterisation of the MAPDIA monomer and MIP was performed in 0.10 mol L^{-1} phosphate buffer solution using a 1.0 cm path length quartz cuvette at room temperature. Suspension of the MIP was maintained with the help of a magnetic stirrer, and the suspension was irradiated at 365 nm and then at 440 nm. The kinetics of the photoisomerization (*trans*/*cis*) were analyzed using Eq. (1) (Tang et al., 2010).

$$\ln\frac{A_0 - A_\infty}{A_t - A_\infty} = kt, \quad (1)$$

where A_0, A_t, and A_∞ are the absorbances of the azobenzene chromophores at their corresponding wavelengths at times 0, t and at the photo-stationary stage, respectively, and k is the rate constant of the photoisomerization process.

2.5. Binding characteristics for guanine

To investigate the binding kinetics of the MIP and NIP, 0.25 g of MIP or NIP was incubated in 50.0 mL of a 5.0×10^{-5} mol L^{-1} guanine solution in methanol: phosphate buffer solution (5:95, v/v) in the dark at 25 °C under stirring; methanol was used to increase