Biosensors and Bioelectronics 74 (2015) 440–446

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Efficient one-pot synthesis of hydrophilic and fluorescent molecularly imprinted polymer nanoparticles for direct drug quantification in real biological samples

Hui Niu, Yaqiong Yang, Huiqi Zhang*

State Key Laboratory of Medicinal Chemical Biology, Key Laboratory of Functional Polymer Materials (Ministry of Education), Collaborative Innovation Center of Chemical Science And Engineering (Tianjin), and College of Chemistry, Nankai University, Tianjin 300071, PR China

ARTICLE INFO

Article history:
Received 8 June 2015
Accepted 28 June 2015
Available online 4 July 2015

Keywords:
Molecularly imprinted polymers
Chemosensor
Nanoparticles
Fluorescence quenching
Biological samples

ABSTRACT

Efficient one-pot synthesis of hydrophilic and fluorescent molecularly imprinted polymer (MIP) nanoparticles and their application as optical chemosensor for direct drug quantification in real, undiluted biological samples are described. The general principle was demonstrated by preparing tetracycline (Tc, a broad-spectrum antibiotic)-imprinted fluorescent polymer nanoparticles bearing hydrophilic polymer brushes via poly(2-hydroxyethyl methacrylate) (PHEMA) macromolecular chain transfer agent-mediated reversible addition-fragmentation chain transfer (RAFT) precipitation polymerization in the presence of a fluorescent monomer. The introduction of hydrophilic PHEMA brushes and fluorescence labeling onto/into the MIP nanoparticles proved to not only significantly improve their surface hydrophilicity and lead to their obvious specific binding and high selectivity toward Tc in the undiluted bovine serum, but also impart them with strong fluorescent properties. In particular, significant fluorescence quenching was observed upon their binding with Tc in such complex biological milieu, which makes these Tc-MIP nanoparticles useful optical chemosensor with a detection limit of 0.26 μM. Furthermore, such advanced functional MIP nanoparticles-based chemosensor was also successfully utilized for the direct, sensitive, and accurate determination of Tc in another biological medium (i.e., the undiluted pig serum) with average recoveries ranging from 98% to 102%, even in the presence of several interfering drugs.

1. Introduction

Molecularly imprinted polymers (MIPs) are synthetic receptors with tailor-made molecular recognition sites (Alexander et al., 2006; Haupt, 2003; Haupt and Mosbach, 2000; Hilt and Byrne, 2004; Hoshino and Shea, 2011; Sellergren, 2000; Wulff and Liu, 2012; Ye and Mosbach, 2008; Zhang et al., 2006). Their high affinity and specificity toward the target analytes as well as their good stability, ease of preparation, and low cost make them highly promising in many applications such as separation and purification, antibody mimics (immunoassay or biomedicine), chemical sensors, biomimetic catalysis, drug development, and drug delivery. In particular, MIPs-based sensors (or chemosensors) have attracted enormous attention because of their great potential in many important areas such as analyses of foodstuffs, environmental monitoring, and clinical diagnostics (Haupt and Mosbach, 2000; Volkert and Haes, 2014; Wackerlig and Lieberzeit, 2015; Whitcombe et al., 2011).

MIPs-based sensors (or chemosensors) can be readily fabricated by combining a MIP recognition element with various transducers based on optical (fluorescence and refractive index), electrochemical, and acoustic signals. Among them, fluorescent MIPs-based chemosensors are particularly interesting because fluorescence has proven to be a highly powerful transduction mechanism to report the chemical recognition event (due to the easy availability of many fluorescence techniques, their high sensitivity, broad linear range, little sampling, and high simplicity of operation) (Basabe-Desmonts et al., 2007; Canfarotta et al., 2013; Henry et al., 2005; Takeuchi et al., 2005). So far, many fluorescent MIPs-based chemosensors have been designed by incorporating fluorescent components (e.g., organic fluorescent moieties or inorganic quantum dots) into MIPs for the sensitive and label-free detection of a wide range of analytes (Awino and Zhao, 2014; Banerjee and König, 2013; Chao et al., 2014; Huy et al., 2014; Ivanova-Mitseva et al., 2012; Li et al., 2010; Tan et al., 2014; Ton et al., 2013; Turkewitsch et al., 1998; Wan et al., 2013; Wei et al., 2014; Wu et al., 2015; Yang et al., 2012; Zhang et al., 2014; Zhao et al., 2012). One of the main focuses in this field is to develop fluorescent MIPs that are able to directly detect small organic molecules in aqueous media because food, environmental, and

* Corresponding author.
E-mail address: Zhanghuiqi@nankai.edu.cn (H. Zhang).

http://dx.doi.org/10.1016/j.bios.2015.06.071
0956-5663/

clinical analyses are typically based on aqueous samples. Despite some progress made in the development of fluorescent MIPs-based chemosensors capable of directly sensing small organic analytes in relatively simple aqueous media such as distilled water (Turkewitsch et al., 1998), tap water (Wu et al., 2015; Yang et al., 2012; Zhao et al., 2012), river water (Li et al., 2010; Wei et al., 2014; Wu et al., 2015), or aqueous buffer solutions (Awino and Zhao, 2014), the design of fluorescent MIPs applicable for direct quantification of small organic analytes in real biological samples remains a formidable challenge due to the complex nature of such systems. Recently, some attempts to use fluorescent MIPs for direct optosensing of target analytes in biological solutions have been disclosed, however, either highly diluted biological samples were used (Chao et al., 2014; Huy et al., 2014) or the target analyte was highly water-soluble protein (Tan et al., 2014). Since high dilution of biological samples will largely decrease the analyte concentrations, which might make their direct trace analyses in such complex media more difficult or even become impossible, the development of fluorescent MIPs capable of direct quantification of small organic analytes in the undiluted biological samples is of significant importance.

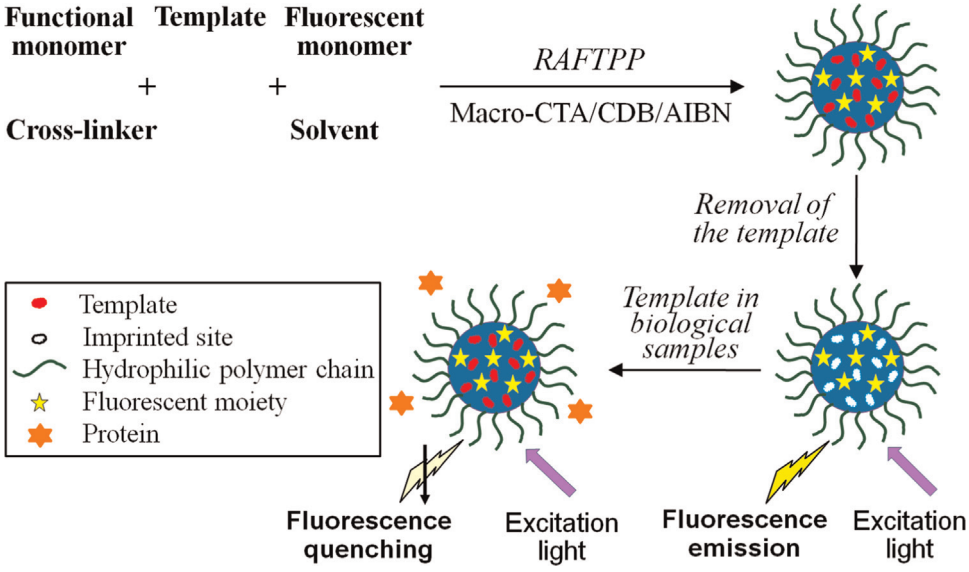
Over the past few years, our group has successfully prepared a series of hydrophilic MIP particles (i.e, MIP particles bearing hydrophilic polymer brushes) with efficient specific recognition ability toward small organic molecules in real, undiluted biological samples (e.g., bovine serum) by using our recently developed controlled/"living" radical precipitation polymerization techniques (Ma et al., 2013; Zhang, 2013, 2014; Zhao et al., 2014a, 2014b), which proved to be promising synthetic substitutes for biological receptors in bioanalytical applications. However, both centrifugation of the mixed solutions of such hydrophilic MIP particles and biological media and subsequent precipitation of proteins in the resulting supernatants have to be performed prior to the quantification of the analytes bound onto the MIPs by high performance liquid chromatography (HPLC), which is rather time-consuming and thus largely limits the practical applications of these hydrophilic MIP particles.

Herein, we report the facile and highly efficient one-pot synthesis of hydrophilic and fluorescent MIP nanoparticles and their application as the optical chemosensor for rapid, sensitive, and accurate drug (e.g., tetracycline (Tc), a broad-spectrum antibiotic, Scheme S1) quantification in real, undiluted biological samples (including bovine serum and pig serum). Such fluorescent MIP nanoparticles with surface-grafted hydrophilic polymer brushes were readily prepared via hydrophilic macromolecular chain-transfer agent (macro-CTA)-mediated reversible addition-fragmentation chain transfer (RAFT) precipitation polymerization (RAFTPP) (Zhang, 2013) with Tc as the template in the presence of a fluorescent monomer (Scheme 1). The introduction of hydrophilic polymer brushes and fluorescence labeling onto/into the MIP nanoparticles not only significantly improved their surface hydrophilicity and led to their compatibility with biological samples (Ma et al., 2013; Zhang, 2014; Zhao et al., 2014a, 2014b), but also imparted them with analyte binding-induced fluorescence quenching properties, thus making them useful optical chemosensor for direct drug quantification in complex biological milieu without the need of any sample pretreatment and expensive instrument. The versatility of hydrophilic macro-CTA-mediated RAFTPP for the one-pot synthesis of hydrophilic MIP nanoparticles and its good compatibility with noncovalent molecular imprinting system as well as the easy availability of hydrophilic macro-CTAs and polymerizable fluorescent monomers makes this strategy highly applicable. The finding we report here represents, to our knowledge, the first successful example of the one-pot generation of highly cross-linked fluorescent polymer or MIP nanoparticles with surface-grafted hydrophilic polymer brushes. Moreover, it also represents an important progress for molecular imprinting technology, since direct and rapid quantification of small organic analytes in complex biological media has long been a challenging dream in this field (Bowen et al., 2013; Pichon and Chapuis-Hugon, 2008; Vlatakis et al., 1993).

2. Experimental

2.1. Materials

Methacrylic acid (MAA, Tianjin Jiangtian Chemicals, 99%) and ethylene glycol dimethacrylate (EGDMA, Alfa Aesar, 98%) were purified by distillation under vacuum. Acetonitrile (Tianjin Kangkede Chemicals, Analytical grade (AR)) was refluxed over calcium hydride and then distilled. *N,N*-Dimethylformamide (DMF, Tianjin Jiangtian Chemicals, 99.5%) was dried with anhydrous magnesium sulfate and then distilled under vacuum. Methanol (Tianjin Jiangtian Chemicals, AR) was distilled prior to use. Azobisisobutyronitrile (AIBN, Chemical Plant of Nankai University, AR) was



Scheme 1. Schematic protocol for the synthesis of hydrophilic and fluorescent MIP nanoparticles for direct and rapid drug optosensing in real, undiluted biological samples.