Biosensors and Bioelectronics 79 (2016) 187–192

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Thermo-sensitive imprinted polymer embedded carbon dots using epitope approach

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ARTICLE INFO

Article history:
Received 16 September 2015
Received in revised form
30 November 2015
Accepted 10 December 2015
Available online 11 December 2015

Keywords:
Molecularly imprinted polymer
Carbon dots
Epitope approach
Fluorescent recognition

ABSTRACT

A new type of thermo-sensitive receptor carbon dots/SiO_2/molecularly imprinted polymer (CDs/SiO_2/MIP) was prepared by surface imprinting procedure and the epitope approach. The synthetic CDs/SiO_2/MIP was able to selectively capture target protein with fluorescence quenching via the special interaction between them and the recognition cavities. The receptor exhibited the linear fluorescence quenching to cytochrome c (cyt c) in the range of 0.1–40 μM, and the detection limit was 89 nM. The precision for five replicate detection of cyt c at 20 μM was 3.11%. Moreover, the receptor owned the temperature-sensitive element that allowed for swelling and shrinking in response to temperature changes to realize recognition of the target cytochrome c. The proposed strategy revealed the feasibility of fabrication of a thermo-sensitive imprinted polymer based on CDs and surface imprinting procedure and the epitope approach.

1. Introduction

Molecular imprinting technique (MIT) is an attractive method to recognize target by tailor-made binding sites with memory of the shape, size, and functional groups of the template (Chen et al., 2011). So, the molecularly imprinted polymer (MIP) can be regarded as synthetic analogues of antibodies with advantages of low cost, good stability, resistance to harsh environments, and selective recognition (Çakir et al., 2013; Kryscio and Peppas, 2012; Shi et al., 1999). For now, the synthesis of MIP toward small molecules is straightforward, however, the imprinting of biological macromolecules like protein continues to be a significant challenge due to their high molecular weight, as well as multitude of functional group, which together with low stability in non-physiological conditions hinder the imprinting process (Gao et al., 2007; Guerreiro et al., 2014; Verheyen et al., 2011).

The epitope imprinting is one of the proposed methods toward macromolecules, which is inspired by the recognition between antigen and antibody (Rachkov and Minoura, 2000). Following this concept, only a small peptide fragment called epitope rather than the whole protein plays the roles of template in the epitope imprinting process. Compared with traditional protein imprinting, the epitope imprinting possesses several advantages. First, the choices of short peptides that serve as epitopes are abundant. Second, epitope-cavities are flexible enough to incorporate a protein and more specific and stronger interactions with the epitope can lower the non-specific binding and improve the affinity (Berglin et al., 2009; Tai et al., 2005b). Third, epitope-cavities not only owns rigid configuration, but are also easy to acquire and beneficial to the preparation of imprinted polymers to recognize the protein with defined orientation (Sellergren, 2010).

In recent years, luminescent nanometer materials combining with MIT to generate fluorescent MIP were proposed due to the high sensitivity of luminescent nanometer materials and the high selectivity of molecular imprinting technique (Feng et al., 2015; Li et al., 2015a, 2013; Ma et al., 2013; Stringer et al., 2010; Tan et al., 2014; Wang et al., 2009; Xu et al., 2013; Yang et al., 2014b; Zhang et al., 2011, 2012a, 2012b, 2014; Zhao et al., 2012). Carbon dots (CDs) as the emerging carbon nanocrystals exhibit many fascinating optical properties, such as tunable photoluminescence properties, low cytotoxicity, eco-friendliness, and excellent biocompatibility (Baker and Baker, 2010; Mao et al., 2012; Wang et al., 2015; Zhou et al., 2014). However, to the best knowledge, the thermo-sensitive fluorescence imprinting receptor based on CDs and the epitope approach for the recognition of proteins has not been reported to date. Herein, we fabricated a novel thermo-

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http://dx.doi.org/10.1016/j.bios.2015.12.016
0956-5663/

sensitive receptor based on surface imprinting and the epitope approach with CDs as the signal-transduction element and N- and C-terminal nonapeptides of target protein as the common templates.

In this work, CDs were first functionalized with silanization, which played the role of fluorescent source and the carrier for the further imprinting procedure. Next, the polymer was fabricated with nonapepides AYLKATNE and GDEKKKI as the common templates, *N*-isopropylacrylamide (NIPAM) and methacrylic acid (MAA) as the functional monomers, N, N-methylenebis acrylamide (MBA) as the cross-linker, and ammonium persulfate (APS) as the initiator. The NIPAM was introduced as temperature-sensitive element that allowed for swelling and shrinking in response to temperature changes to realize recognition of the target cytochrome c (cyt c). The synthetic CDs/SiO_2/MIP was able to selectively capture the target cyt c with fluorescence quenching via the special interaction between them and the recognition cavities. The CDs/SiO_2/MIP exhibited specific recognition behavior to the corresponding target protein, revealing the feasibility of fabrication of a thermo-sensitive polymer based on CDs and surface imprinting procedure and the epitope approach.

2. Experimental section

2.1. Materials and chemicals

N-isopropylacrylamide (NIPAM), citric acid, ethylenediamine, ammonium hydroxide ($NH_3 \cdot H_2O$), 3-aminopropyltriethoxysilane (APTES), tetraethoxysilicane (TEOS), trifluoroacetic acid (TFA) and methacylic acid (MAA) were purchased from J&K Chemical Co. *N*, *N*-Metylenebisacryamide (MBA), ammonium persulfate (APS) were purchased from Sangon Biotech. The standard peptides AYLKKATNE and GDVEKGKKI were synthesized by GL Biochem Co., Ltd (Shanghai, China). Cytochrome c (cyt c, MW 12.4 kDa, *pI* 10.2), lysozyme (Lyz, MW 14.4 kDa, *pI* 11), trypsin (Try, MW 23.3 kDa, *pI* 10.8) and acetonitrile (MeCN) were obtained from Sigma–Aldrich Co. (St. Louis, MO).

2.2. Instrumentation

High-resolution transmission electron microscopy (HRTEM) imaging was performed on a Tecnai G^2 F20 transmission electron microscope with the voltage of 200 kV (FEI, Holland). The X-ray photo-electron spectroscopy (XPS) measurements were performed on a Kratos Axis Ultra DLD spectrometer employing a monochromated Al-Kα X-ray source ($h\nu$=1486.6 eV) (Krato, UK). The source X-rays were not filtered and the instrument was calibrated against the C1s band at 285.0 eV. The identication of the crystalline phase of carbon dots was performed on a Rigaku D/max/2500 v/pc (Japan) X-ray diffractometer (XRD) with a Cu K a source. The 2θ angles probed were from 3° to 80° at a rate of 4° min^{-1}. Fourier-transform infrared (FT-IR) spectra (4000–400 cm^{-1}) in KBr were recorded on a FTS6000 spectrophotometer (Bio-rad, USA). UV–vis spectra (200–800 nm) were recorded on a UV-2450 spectrophotometer (Shimadzu, Japan). Fluorescence (FL) measurements were performed on an F-4500 fluorospectrophotometer (Hitachi, Japan). Removal of template was confirmed by the LC-20AD HPLC (Shimadzu, Japan) equipped with a vacuum degasser, ternary pump (G1311B), and variable wavelength detector system (G1314F) connected to a reversed-phase column (Kromasil C18, 5 μm, 4.6 mm × 250 mm, Tianjin, China). The column temperature was 30 °C, and the mobile phase for analysis were solvent A (0.1% TFA/MeCN) and solvent B (0.1% TFA/water) with a flow rate of 1 mL/min.

2.3. Synthesis of CDs

The preparation of CDs was performed according to previous reference (Zhu et al., 2013). In brief, 1.0507 g of citric acid and 335 μL of ethylenediamine were dissolved in 10 mL of ultrapure water; the mixture was then transferred to a 30 mL of poly (tetrafluoroethylene) (Teflon)-lined autoclave and heated at 150 °C for 5 h. After the reaction, the reactors were cooled to room, and the mixture was filtrated by 0.22 μm membrane.

2.4. Synthesis of CDs/SiO_2

CDs were doped in silica spheres based on the stober method with some modifications (Stöber et al., 1968). In brief, 40 μL of APTES and 40 μL of TEOS were added into the CDs solution, then 40 mL of ethanol and 40 μL of $NH_3 \cdot H_2O$ were added in the mixture and the mixture was kept stirring for 6 h. The resultant CDs/SiO_2 was centrifuged and washed with ultrapure water and ethanol respectively for three times to remove the excess reactants.

2.5. Preparation of CDs/SiO_2/MIP

The CDs/SiO_2 composites were dispersed in 20 mL of PBS buffer solution (pH=7.3), in which 84.8 mg of NIPAM, 24 μL of MAA, and 10 mg of AYLKATNE and 10 mg of GDEKKKI, and 20.0 mg of MBA were added, which was incubated 6 h under stirring for prepolymerization. Then, the solution was deaerated by N_2 for 30 min, and the polymerization was initiated by addition of 10 mg of ammonium persulfate and 50 μL of TEMED, and the polymerization was continued at 37 °C for 24 h. The resultant particles were centrifuged and washed with MeOH/HAc (9:1, v/v) until no template was detected by HPLC. The non-imprinted polymer (CDs/SiO_2/NIP) was prepared under the same conditions in the absence of the template. Meanwhile, in order to compare the selectivity of the CDs/SiO_2/MIP, another molecularly imprinted polymers CDs/SiO_2/MIP-1 and CDs/SiO_2/MIP-2 were prepared by the same method, except that the template was only C-terminal AYLKATNE or N-terminal GDEKKKI, respectively (Table 1).

2.6. Fluorescence measurement

The fluorescence measurements were carried out with the excitation wavelength of 340 nm when the spectrofluorometer was set in the fluorescence mode. The slit widths of the excitation and emission were both 10 nm, and the photomultiplier tube voltage was set at 950 V. The appropriate CDs/SiO_2/MIP or CDs/SiO_2/NIP was added to a 5 mL standard colorimetric tube, and the given concentration of cyt c was added successively, and the mixture was diluted to 2.5 mL with PBS buffer solution (pH=7.3) and mixed thoroughly.

3. Results and discussion

3.1. Preparation and characterization of CDs/SiO_2/MIP

The general protocol for the synthesis of CDs/SiO_2/MIP is illustrated in Fig. 1. In the first step, CDs were doped into silica to form the uniform CDs/SiO_2 composites. Silica is optically transparent, which could protect the luminescent CDs and provide the resultant core-shell particles with water dispersibility, biocompatibility and surface functionality. Then, the C- and N-terminals of cyt c and NIPAM and MAA were pre-assembled through double hydrogen bonds. The NIPAM was introduced as the temperature-sensitive element that allowed for swelling and shrinking in response to temperature changes to realize recognition and release of target protein cyt c.