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Fluorescent molecularly imprinted polymers based on 1,8-naphthalimide derivatives for efficiently recognition of cholic acid

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ABSTRACT

Fluorescent molecularly imprinted polymers (MIPs) have attracted increasing attentions in recent years due to their high selectivity and sensitivity for target molecules. In this study, two cholic acid imprinted fluorescent polymers, i.e., MIP_1 and MIP_2, were prepared using 4-dimethylamino-N-allylnaphthalimide (F_1) and 4-piperazinyl-N-allylnaphthalimide (F_2) as the fluorescent functional monomers, respectively. The fluorescence intensity of MIP_1 decreased linearly with the increase of the template concentration in the range of 1.50–120.0 μM, while the fluorescence intensity of MIP_2 increased linearly with the increase of the template concentration in the range of 0.40–110.0 μM. The detection limits of MIP_1 and MIP_2 for cholic acid were 0.42 and 0.083 μM, respectively. The mechanisms of the fluorescence responsive of the imprinted polymers were discussed. The results of fluorescence measurement and binding experiments demonstrated that both imprinted polymers have high recognition abilities and binding affinities for the template. The imprinted polymers have been successfully applied to the determination of cholic acid in human serums. The present study indicated that 1,8-naphthalimide can be used as a modular building block for design and construction of various fluorogenic molecularly imprinted materials for practical sensing and separation.

1. Introduction

Cholic acid is one of the major bile acids produced by the human liver. Determination of cholic acid is very important and necessary in body fluids due to its both pharmaceutical and clinical significances. Today, gas chromatography–mass spectrometry (GC/MS) [1], high performance liquid chromatography–mass spectrometry (HPLC/MS) [2–9] and capillary electrochromatography [10] are the most commonly used methods for cholic acid analysis. These traditional detection methods are mostly dependent on analytical equipment, and are involved in complicated and time-consuming sample pre-treatment steps or requiring a derivatization process. Moreover, these methods always suffer from the interference from the coexisting substances which are structurally and chemically similar to cholic acid. Up to now, many efforts have been devoted to improve the selectivity and sensitivity of cholic acid detection [11–13].

Molecular imprinting is a promising method for preparing artificial receptors which can bind target analytes with high selectivity and sensitivity. Synthesizing molecularly imprinted polymer (MIP) involves the copolymerization of functional monomers and cross-linkers in the presence of the template, and the special tailor-made binding sites are exposed after removal of the template. Molecularly imprinted materials have received growing attention because of their high selectivities toward target molecules, chemical and mechanical stabilities, low costs and ease of fabrications for possible applications [14–26]. Molecular imprinting technology (MIT) has also been used to detect cholic acid. Wu et al. [27] prepared molecularly imprinted photonic hydrogels which displayed optical changes in responsive to cholic acid concentration. Gültekin et al. developed MIP based-quartz crystal microbalance (QCM) nanosensor [28] and gold–silver-nanoclusters [29] for specific recognition of cholic acid.

Fluorescent molecularly imprinted materials have also attracted considerable attention in recent years because they can not only bind target analytes selectively, but also generate detectable optical signals as a result of the molecular binding [30–45]. Two main approaches are being explored for introducing fluorescent species into molecularly imprinted materials: (1) encapsulating gold nanoclusters [29], quantum dots (QDs) [31–33], carbon nanotube-quantum dots [34], carbon dots [35], etc. in molecularly imprinted materials; (2) Using fluorescent functional monomers for preparing molecularly imprinted materials [36–44]. In the second approach, the fluorescent functional monomers must have intrinsic fluorescence variation when exposed to the target analytes. Therefore, in most cases fluorescent functional monomers need to be specially designed and synthesized. Compounds such as 1,8-naphthalimide derivatives [37], pyrenedimethacrylamide [38], dansyl [39,40], 8-hydroxyquinoline [41], zinc(II)–protoporphyrin [42], etc. have already been utilized as fluorescent functional monomers for preparation of fluorescent MIPs.

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Recently, Rouhani et al. [37] reported the synthesis of fluorescent imprinted polymer for caffeine using N-allyl-4-ethylenediamine-1,8-naphthalimide as the fluorescent functional monomer because of their excellent properties such as high fluorescence quantum yield, good stability, being easily modified, etc. [45–47]. 1,8-naphthalimide derivatives may be used as modular building blocks for construction of various fluorogenic molecularly imprinted materials for target molecules. In this study, the utilization of 1,8-naphthalimide derivatives as fluorescent functional monomers in molecular imprinting is further investigated. In order to gain more insight into the mechanism of fluorescence responsive of fluorescent MIPs, two polymerizable 4-substituted naphthalimide, i.e., 4-dimethylamino-N-allylnaphthalimide (F_1) and 4-piperazinyl-N-allylnaphthalimide (F_2) were synthesized and were used as fluorescent functional monomers for prepared cholic acid-imprinted polymers. The imprinted polymers displayed fluorescence response to cholic acid concentration. The mechanisms of fluorescence responsive of the imprinted polymers were discussed. The imprinting effect and the selectivity of the imprinted polymers were confirmed by the change of fluorescence intensity and binding experiments. In addition, cholic acid level in human plasmas was determined by using the prepared fluorescent MIPs.

2. Experimental section

2.1. Materials and instruments

2.1.1. Materials

4-Bromo-1,8-naphthalic anhydride (98%) was purchased from Adamas Reagent Co., Ltd. (Shanghai, China). 2-Methoxymethanol (99%) was obtained from Macklin Biochemical Co., Ltd. (Shanghai, China). Dimethylamine aqueous solution (40%), 2,4-dichlorophenoxyacetic acid (2,4-D), allyamine and piperazine hexahydrate were obtained from Aladdin (Shanghai, China). Cholesterol, testosterone and bisphenol A (BPA) were purchased from Sigma-Aldrich (Shanghai, China). Cholic acid was obtained from Fuchen Chemical Plant (Tianjin, China). Ethylene glycol dimethacrylate (EGDMA) was obtained from Jiangsu Anli Chemical Plant (Jiangsu, China). Before use, EGDMA was distilled under vacuum after being extracted with 10% sodium hydroxide and dried over anhydrous magnesium sulfate. 2,2-azobis(isobutyronitrile) (AIBN) was obtained from Shanghai Chemical Plant (Shanghai, China) and recrystallized from methanol. Dimethyl sulfoxide (DMSO) was dried with molecular sieves 3A and then distilled under reduced pressure. Ultrapure water (18.2 MU cm) obtained from an ELGA LabWater System (Vivendi Water Systems Ltd. High Wycombe, Buckinghamshire, UK) was used throughout the experiments. All the solvents for HPLC analysis were of HPLC grade. All the other chemicals used were of analytical grade. The structures of cholic acid and some other compounds used in this study are shown in Fig. 1.

2.1.2. Characterization

The FT-IR spectrum was recorded on an IRPrestige21 instrument (Shimadzu, Japan). The ^{1}H NMR spectrum was measured on a Mercury-plus 300 MHz spectrometer (Varian, USA). The elemental analysis was measured on a Vario EL CHNS Elemental Analyzer (Elementar, Germany). UV–vis absorption spectra were recorded with a UV-2501PC spectrophotometer (Shimadzu, Japan). Fluorescence emission was measured using a RF-5301PC spectrofluorometer (Shimadzu, Japan). Scanning electron microscopy (SEM) imaging was carried out on a JEOL JSM-6700F Field Emission Scanning Electron Microscope (Tokyo, Japan).

A Shimadzu CTO-10AVP HPLC system (Shimadzu, Japan) equipped with a LC-10 AD pump, a SPD-10AVP UV–vis detector and a C_{18} analytic column (250 mm × 4.6 mm, 5 μm) was used for detection of the concentrations of cholesterol, cholic acid and testosterone. The mobile phase was prepared with acetonitrile and isopropanol (80:20, *v*/*v*). The UV detection wavelength was 210 nm and the flow rate of the mobile phase was 0.5 ml min^{-1}. The column temperature was set at 30 °C and the sample injection volume was 10 μl.

2.2. Synthesis of fluorescent functional monomers (F_1 and F_2)

The fluorescent monomers (F_1 or F_2) were synthesized via two steps from 4-bromo-1,8-naphthalic anhydride. Scheme 1 describes the processes of synthesis of the fluorescent monomers.

2.2.1. Synthesis of 4-bromo-N-allylnaphthalimide

4-bromo-1,8-naphthalic anhydride (12.0 mmol, 3.32 g), allylamine (2.0 ml) and hydroquinone (10 mg) (as a polymerization inhibitor) were added into 70 ml of ethanol. The mixture was heated to 82 °C under nitrogen atmosphere, and magnetically stirred for 3.0 h. The intermediate, 4-bromo-N-allylnaphthalimide, was precipitated when the mixture was cooled to room temperature. The solid product was collected by filtration, washed with 10 ml of ethanol and dried under vacuum at room temperature. Yield: 91.4%. m.p.: 139–140 °C. Elemental

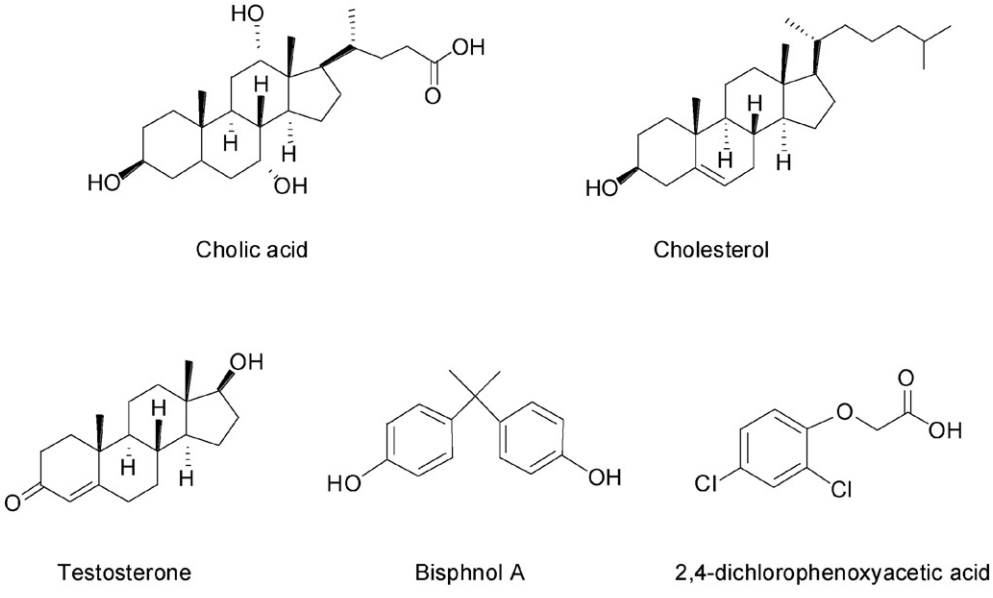

Fig. 1. Structures of cholic acid and related substrates used in this study.