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Synthesis of L-phenylalanine imprinted hydrogels with anti-biofouling capability by using a novel zwitterionic functional monomer

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ABSTRACT

A novel zwitterion was synthesized and used as a functional monomer in the synthesis of L-phenylalanine (L-phe) imprinted hydrogel (MIH). Molecular simulation demonstrated that the binding energy of the zwitterion with L-phe was −23.50 kcal mol^{-1} in the aqueous phase, which was higher than the binding energies of L-phe with other traditional functional monomers. Binding experiments also showed that MIH made using the zwitterion had a higher adsorption capacity and imprinting factor (IF) compared to MIHs made using either acrylic acid or 4-vinylpyridine. Optimization studies showed that the best recognition ability of MIH was obtained when the molar ratio of zwitterion to L-phe was 2:1 in the synthesis of MIH. Adsorption kinetics experiments showed that MIH could equilibrate within 120 min, and adsorption isotherms obtained experimentally demonstrated that MIH had a good adsorption capacity of 9.8 mg g^{-1} with a high imprinting factor of 2.0 in 0.3 mg mL^{-1} concentrated L-phe solution. Circular dichroism spectroscopy confirmed the excellent chiral resolution ability of MIH for racemic phenylalanine. Furthermore, MIH prepared using the zwitterion exhibited strong anti-fouling capability and could be utilized to extract L-phe from the protein solution.

1. Introduction

Molecular imprinting is a promising technique for artificial synthesis of affinity recognition sites in polymeric matrices. Molecularly imprinted polymers (MIPs) offer several advantages in terms of good mechanical and chemical stability, high specific recognition property, and relative ease of mass production. Therefore, they are useful in a wide range of applications, such as in biosensors, bio-separation, medical diagnostics and drug delivery [1–4]. Till date, molecular imprinting technology has been undergoing rapid developments, especially in the case of biomolecular imprinting [5–7]. A large number of researches focusing on the preparation of amino acids, polypeptides, proteins and even whole cell imprinted polymers have been carried out and reported [8–10]. However, these ingenious recognition materials are now facing a big challenge of preventing the adhesion and non-specific adsorption of proteins, cells, and bacteria [11]. These undesirable biofouling could induce to the deposition of blood proteins on MIPs [12], the blockage of imprinted membranes in bio-separation process [13,14], and the reduction of MIPs sensors' detection sensitivity [15]. Hence, research focusing on the preparation of MIPs, having good abilities to prevent the adsorption of proteins, is a matter of great urgency to enable its practical applications in biomolecular imprinting technology.

Poly(ethylene glycol) (PEG) and its derivatives are the most commonly used antifouling materials [16,17]. Modification of the surface of PEG, which has repeating units of ethylene glycol, decreases its interaction with proteins. This in turn, improves the circulation and residence time of nanoparticles in a biological environment. However, the antifouling abilities of the shorter oligo (ethylene glycol) chains are limited when exposed to blood [18]. Furthermore, reports suggest that degradation can occur in the presence of oxygen and transition metal ions, or in the presence of an enzyme, in vivo [19]. Particularly, the enzymatic degradation of low molecular weight PEG results in toxic metabolites [20]. As a result, search for alternatives to PEG has received much attention.

The use of zwitterions against fouling was inspired by the fact that the external surface of the mammalian cell membrane are rich in phospholipids bearing zwitterionic head groups [21]. As a new generation of antifouling materials, zwitterions including phosphobetaine, carboxybetaine and sulfobetaine can strongly resist nonspecific protein adsorption by means of a hydration layer bound through solvation of the charged terminal groups [22,23]. Therefore, combining the antifouling capabilities of zwitterions with MIPs is a promising strategy for obtaining recognition materials with good resistance to non-specific protein adsorption.

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Very recently, a novel kind of zwitterion, 3-(bis(2-acrylamidoethyl) (methyl) ammonio) propane-1-sulfonate (AMAS) was synthesized and utilized as both, an antifouling monomer and a crosslinker, in the preparation of diclofenac MIP [24]. The obtained MIP exhibited high imprinting efficiency in aqueous phase due to the multi-functionality of AMAS. Moreover, MIPs based on AMAS demonstrated strong resistance to non-specific protein adsorption, and hence could extract diclofenac from the solution that also contained protein. Li et al. also used 2-methacryloyloxyethyl phosphorylcholine (MPC) as an assistant monomer in the preparation of bovine serum albumin (BSA) imprinted magnetic microspheres [25]. In addition to selectively recognizing the template protein via imprinted sites, the obtained MIPs could also reduce the non-specific adsorption of proteins on their surfaces. In spite of the excellent work done in above mentioned researches, studies on the use of zwitterions in MIPs for resistance of non-specific protein adsorption are still inadequate.

In this work, a sulfobetaines, namely, 1-vinyl-3-sulfopropylimidazolium (VSPIM) was synthesized and utilized as a functional monomer to prepare L-phe imprinted hydrogels, as shown in Fig. 1. The thermo-sensitive monomer, N-isopropylacrylamide (NIPAm), used in the fabrication of hydrogels, facilitated the rapid removal and rebinding of L-phe. The anti-biofouling capability and specific recognition ability of the resultant MIHs were investigated by resistance non-specific protein adsorption experiments and chiral resolution experiments.

2. Materials and methods

2.1. Materials

N-isopropylacrylamide (NIPAm) was purchased from Acros Organics (Morris Plains, NJ, USA). 1-Vinylimidazole (VIM), *N*,*N*-methylenebisacrylamide (MBA), ammonium persulfate (APS), *N*,*N*,*N*,*N*-tetramethylenebis(acrylamide) (TEMED), acrylic acid (AA), 4-vinylpyridine (4-VP) and bovine serum albumin (BSA; MW 66.4 kDa, pI 4.8), were procured from Sigma–Aldrich (Tokyo, Japan). 1,3-Propane sultone was purchased from Beijing J&K Co., Ltd. (Beijing, China). L-Phenylalanine (L-phe, ≥99.5%), D-Phenylalanine (D-phe, ≥99.5%) and racemic phenylalanine were purchased from Xi'an Wolsen Biological Co., Ltd. (Xi'an, China). Deionized water was obtained using a Millipore water system. All other chemicals were of analytical grade and were used as received.

2.2. Characterization

Fourier transform infrared (FTIR) spectra were recorded on a Tensor 27 FT-IR spectrometer (Bruker). The specimens were prepared by mixing each sample with KBr powder and pressing into a compact pellet. The absorption spectra were obtained using a UV spectrophotometer (Varian, Cary-1E). Circular dichroism (CD) experiments were carried out using an Applied Photophysics Chirascan instrument. The ^{1}H nuclear magnetic resonance (^{1}H NMR) spectra were recorded using an Avance 300 MHz spectrometer. Scanning electron microscopy (SEM) was carried out using an Inca Oxford instrument to investigate the hydrogel morphology. Elemental analysis using Elementar Vario EL (Germany) was carried out to determine chemical composition. Protein and amino acid identification was carried out using a Shimadzu LC-2010A series HPLC (Japan) instrument, equipped with a C18 column. Pore volume and surface area analysis was characterized according to Brunauer–Emmett–Teller (BET) method by using nitrogen adsorption–desorption isotherms with a Trestar 3020 surface area analyzer (Micromeritics, USA). Thermal gravimetric analysis (TGA) was performed under a nitrogen atmosphere with a 10 °C per minute heating rate (TA Instruments, Hi-Res TGA Q50).

2.3. Synthesis and characterization of 1-vinyl-3-sulfopropylimidazolium

VIM (1.88 g, 0.02 mol) was dissolved in 10 mL acetonitrile at 50 °C. Under vigorous magnetic stirring 1,3-propanesultone

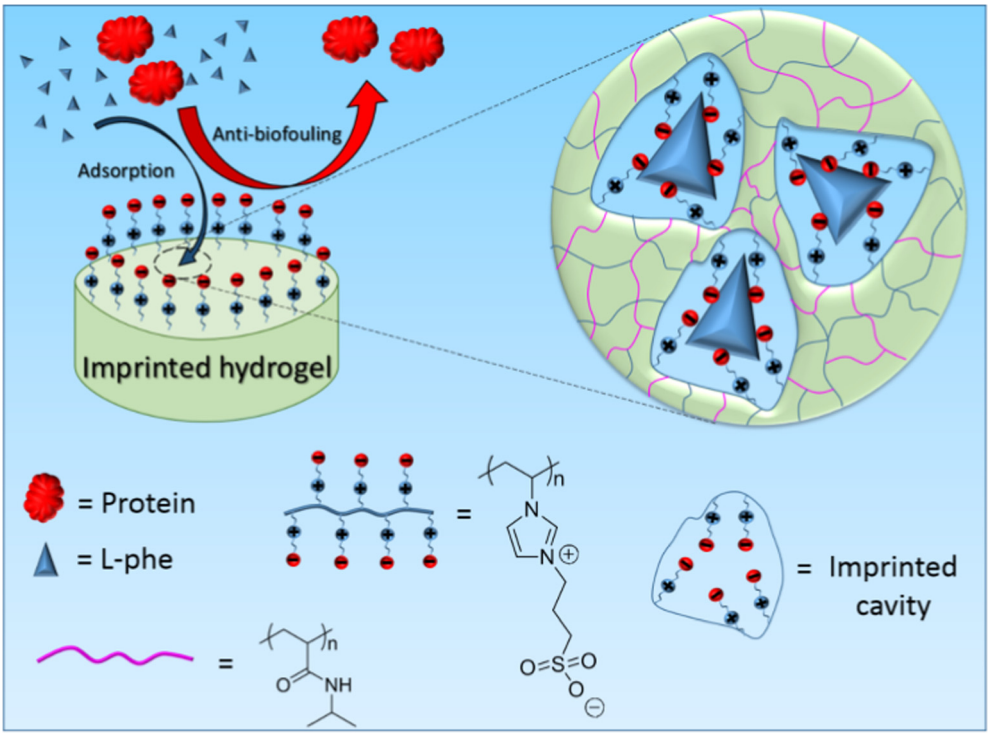

Fig. 1. Schematic representation of the recognition ability of MIH for L-phe and its anti-biofouling capability.