Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biomaterials 26 (2005) 5737–5745
Biomaterials
www.elsevier.com/locate/biomaterials

Chitosan beads as molecularly imprinted polymer matrix for selective separation of proteins

Tian-Ying Guo*, Yong-Qing Xia, Jin Wang, Mou-Dao Song, Bang-Hua Zhang

State Key Laboratory of Functional Polymer Materials for Adsorption and Separation, Institute of Polymer Chemistry, College of Chemistry, Nankai University, Tianjin 300071, China

Received 30 September 2004; accepted 23 February 2005
Available online 9 April 2005

Abstract

Two kinds of molecularly imprinted polymers were prepared using hemoglobin as the imprinting molecule, acrylamide as the functional monomer, chitosan beads and maleic anhydride-modified chitosan beads as matrixes, respectively. Static adsorbing experimental results showed that an equal class of adsorption was formed in the imprinted polymers and the adsorption equilibrium constant and the maximum adsorption capacity were evaluated. Chromatographic characteristics showed that the column bedded with the hemoglobin imprinted beads could separate hemoglobin and bovine serum albumin effectively from their mixture, which indicates that the imprinted beads have very higher selectivity for hemoglobin than the non-imprinted with the same chemical composition.

Keywords: Chitosan beads; Polyacrylamide; Protein separation; Molecular imprinting

1. Introduction

Molecular imprinting is a technique for creating recognition sites for a specific analyte in a synthetic polymer [1–3]. The technique involves the formation of complexes between imprinting molecules and functional monomers based on three different interactions: covalent [4–5], non-covalent [6–10] and metal ion coordination [11–13]. These complexes are fixed by polymerization with a degree of cross-linking. Removal of the imprinting molecules from the obtained polymer network affords complementary binding sites that can selectively rebinding the same imprinting molecules. Chemically and mechanically stable molecularly imprinted polymers (MIPs) have been used as the stationary phase in chromatography [14], as artificial antibodies in immunoassays [15] and as recognition elements in sensors [16]. This technique is a conceptually simple and straightforward method to apply to a wide variety of target molecules. However usually, only relatively low molecular weight compounds such as amino acid derivatives, certain drugs and pesticides [17–20] are used as imprinted molecules, and biomacromolecule such as protein is seldom [21–24].

Polyacrylamide gel has been proven useful for the separation of proteins in HPLC systems [25,26]. But the polyacrylamide gel was soft and its mechanical strength was not well as the stationary phase in HPLC systems, it could only allow relatively low flow rate.

In our previous work [27], we have fabricated a hemoglobin imprinted polymer material with acrylamide as the functional monomer and cross-linked chitosan beads as the supporting matrix. The MIP was achieved by entrapment of the selective soft polyacrylamide gel in the pores of the cross-linked chitosan beads by letting acrylamide monomer and the protein diffuse into the pores of chitosan beads before starting the polymerization. And we have characterized the

*Corresponding author. Tel.: +86 22 23501597; fax: +86 22 23501597.
E-mail address: tyguo@nankai.edu.cn (T.-Y. Guo).

0142-9612/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2005.02.017

imprinted polymer preliminary with the static adsorbing methods. The results indicated that the MIP was a very good adsorbent to Hb. Considering there is no chemical bonds between the chitosan matrix and the polyacrylamide gel, the polyacrylamide gel could be washed out of the pores of the chitosan beads after multiple using cycles. In this work we have further prepared a novel chitosan matrix by introducing double bonds to the chitosan beads first, then the polyacrylamide gel was grafted to the modified chitosan beads. Thus, the interaction forces of the selective polyacrylamide gel and chitosan matrix could be strengthened by chemical bonds. The work in this article was chiefly focused on the comparisons between the grafting MIP and the MIP prepared by simply entrapment method in the material morphology and adsorptive properties. At the same time, the dynamic characteristics for adsorption and desorption of the materials were characterized by chromatographic method, which confirmed that the materials have a very promising prospect in highly selective separation of proteins.

2. Experimental

2.1. Materials

Chitosan was purchased from Boao Bio-Technology Company, Shanghai (China). Deacetylation degree is 90%; the viscosity average molecular weight of the chitosan was determined to be 503,495 by viscometric method. Epichlorhydrin (ECH) and maleic anhydride (MAH) were purchased from Tianjin no.1 Chemical Reagent Factory (China). Acrylamide (Am) was purchased from Miou Chemical Factory (Tianjin, China). *N*, *N*′-methylenebisacrylamide (MBA) was purchased from Tianjin Special Reagent Factory (China). Potassium persulfate (KPS) was obtained from Tianjin no.3 Chemical Reagent Factory. Hemoglobin (Hb) and bovine serum albumin (BSA) were purchased from Sino-America Biotechnology Company and the protein solutions were prepared using 0.01 M sodium dihydrogen phosphate buffer (pH 6.8). Am and KPS were recrystallized before used. Other chemicals were analytical grade and used as received.

2.2. Preparation of MIP

2.2.1. Preparation of cross-linked chitosan beads

Chitosan beads were prepared as previous [24]. Simply, 3.0 g chitosan dissolved in 100 mL, 2%(v/v) acetic acid. The solution was dropped through a 7-gauge needle into 2 M sodium hydroxide solutions, and the gelled spheres formed instantaneously. This process was accomplished by using a model 100 push–pull syringe pump. The formed chitosan beads remained in the sodium hydroxide for 24 h and was washed with distilled water and stored in distilled water for use.

The wet non-cross-linked beads were put into a flask with 100 mL sodium hydroxide solution (pH = 10), some amount of cross-linking agent, epichlorohydrin was added and stirred at 60 °C for 6 h. The cross-linked chitosan beads were then washed extensively with distilled water to remove any unreacted epichlorohydrin.

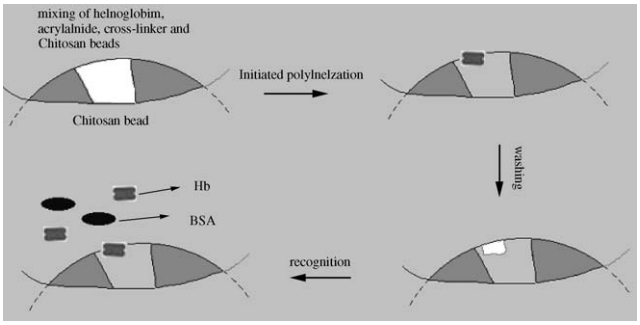
2.2.2. Preparation of MIP

For the preparation of MIP, 16.0 g wet cross-linked chitosan beads (use filter to absorb the surface water), 1.9 g Am, 0.1 g MBA, 20.4 mg KPS, 600 mg Hb, 28 mL 0.01 M sodium dihydrogen phosphate buffer (pH 6.8) were put into a 100 mL, four-necked flask equipped with a nitrogen inlet and a mechanical stirrer. The mixture was stirred continuously under a nitrogen atmosphere for 45 min, then 5 mL of sodium dihydrogen phosphate buffer containing 0.16% (w/v) $NaHS0_3$ was added. The mixture was stirred under the nitrogen atmosphere for 2 h at 4 °C. The formed beads were put into a nylon stocking to press out the surrounding polyacrylamide gel, and the freed chitosan beads were washed with 10% (v/v) acetic acid containing 10% (w/v) SDS solution to desorb the hemoglobin till the color was pale (but was still somewhat colored). Then the beads were equilibrated with 0.01 M sodium dihydrogen phosphate buffer (pH 6.8) for 24 h. The general preparing procedure can be depicted as Scheme 1.

The non-imprinted polymer (NIP) was prepared by the same procedure without addition of hemoglobin.

2.2.3. Modification of the porous chitosan beads with maleic anhydride (MAH–Cs)

The cross-linked chitosan beads were modified chemically with MAH to introduce double bonds. 21.0 g wet chitosan beads, 9.8 g MAH and 50 mL 95% ethanol were put into a 100 mL, four-necked flask equipped with mechanical stirrer at 70 °C, then 1.02 g triethylamine (Et_3N) was dropped into the mixture and heated to 80 °C for 8 h, the obtained MAH–chitosan



Scheme 1. Procedure for preparing protein imprinted polymer.