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Molecularly imprinted polyurethane grafted calcium alginate hydrogel with specific recognition for proteins

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ABSTRACT

Polyurethane grafted calcium alginate hydrogel microspheres intended for bovine serum albumin imprinting were prepared and characterized. The grafting modification was performed prior to the assembling of protein template with hydrogel, which was advantageous to maintain protein conformation and construct more accessible imprinted sites. It was found that the imprinted hydrogel had higher affinity to template molecules after modification. Investigations of grafting ratio influence on specific rebinding and competitive properties indicated that grafted side chains were beneficial to the selective recognition of protein.

1. Introduction

Molecularly imprinted polymers (MIPs) are cross-linked polymeric networks designed to specifically recognize a target molecule and to be capable of mimicking natural systems [1]. With the development of MIPs, it has been successfully applied to recognizing small molecules such as sugars, metal ions and amino acids [2–5]. However, the properties of protein such as large size and fragile conformation bring challenges to MIPs designed for protein imprinting and recognizing [6,7]. These drawbacks could be overcome to some extent by adopting hydrogel as imprinting matrix. Hydrogel makes it possible to form imprinted polymers in aqueous media, which is advantageous to maintain protein conformation and bioactivity. Furthermore, its swelling characteristic allows for easy diffusion of macromolecules through the material [8].

Hydrogel-based protein imprinted polymers, either synthetic or natural, have been documented over the past decade [9,10]. Alginates can be ionically cross-linked (usually by Ca^{2+}) to form hydrogel materials which have good biocompability and biodegradability [11,12]. One of the representative attempts at protein imprinting using calcium alginate-based microcapsules was reported by Zhang et al. [13] using an inverse suspension method which involved the use of organic chemicals. Recently, Herrero et al. presented a new biocompatible technology of protein imprinting without toxic chemicals [7,14]. Although alginate is an ideal candidate for protein imprinting, it is still challenged by limitations in material stability and recognizing sites' precision. Considering these difficulties, some modifications of alginate have been developed, for example, alginate interpenetrating networks [13], alginate blending [15] and alginate-grafted polymer [16]. However, most of the studies involved radical reaction in the presence of protein template, which lead to the denaturation of protein and deteriorate the specific recognition of MIPs.

In this research, it is presented the preparation of protein imprinted hydrogel based on polyurethane grafted calcium alginate (PU-g-CaA). The adsorption behaviour, imprinting efficiency and selectivity of the hydrogel are characterized. The grafted PU side chains have been previously confirmed to construct physical cross-linking points and improve the mechanical and chemical stability of hydrogel [17], which is therefore expected to be benefit for protein recognition. Moreover, protein templates are protected from initiator radical denaturation because the grafting modification is performed prior to adding protein templates.

2. Experiment and methods

Materials: Sodium alginate (SA) was purchased from Shanghai Chemical Reagents Corporation. Isophorone diisocyanate (IPDI) was provided by Three Trees Co. Ltd, China. 2-Hydroxyethyl methacrylate (HEMA) and dimethylolpropionic acid (DMPA) were obtained from Shanghai crystal pure reagent Co. Ltd, China. Tris-(hydroxymethyl) aminomethane (tris) was purchased from Aladdin Industrial Corporation. Bovine serum albumin (BSA, pI=4.7, M_w=66 kDa), Ovalbumin (OVA, pI=4.7, M_w=46 kDa), bovine hemoglobin (BHb, pI=6.9, M_w=64.5 kDa) were supplied by Sigma-Aldrich.

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Synthesis of polyurethane grafted sodium alginate (PU-g-SA): Synthesis of PU-*g*-SA has been introduced elsewhere [17]. The differences are that the grafting reaction is carried out in aqueous media before gelating with Ca^{2+} and PU is synthesized with IPDI, HEMA and DMPA instead of diethylene glycol (DEG). The modified samples were extracted using acetone to remove the remained reactants and homopolymers and then dried under vacuum at 50 °C for 12 h. The grafting ratio was calculated as follows:

$$\text{grafting ratio}(\%) = \frac{(m_1 - m_0)}{m_0} \times 100 \quad (1)$$

wherein m_0 and m_1 were the weight of alginate and grafted product, respectively.

Preparation of protein imprinted microspheres: BSA solution (2 mg/mL, 10 mL) was mixed with PU-*g*-SA and allowed assembling for 4 h. Then microspheres were prepared by dropping the mixture from a injector into $CaCl_2$ aqueous solution (2% w/w) and gelating for 1 h. The generated beads were eluted by a mixture of 1% $CaCl_2$ solution and 0.05 M Tris–HCl buffer (pH=7.58). The concentration of BSA was determined by UV/vis spectrophotometer at 280 nm. This process was conducted at room temperature until no protein was detected in the eluant. The template removal was quantified via the ratio of BSA in eluant to the total template amount, which was up to 70%. Non-imprinted polymers (NIPs) were synthesized under the same condition as above, except the absence of BSA template.

Protein rebinding tests: The rebinding experiments were all carried out at room temperature. Accurately weighed imprinted and non-imprinted microspheres were placed in Erlenmeyer flasks containing protein solution. The equilibrium rebinding quantity (Q_e) was calculated using Eq. (1).

$$Q_e = \frac{(C_0 - C_e)V}{m} \quad (2)$$

wherein C_0 and C_e were initial and equilibrium concentrations of protein, respectively; V was the volume of protein solution, and m was the mass of microspheres.

The recognition ability was evaluated by imprinting efficiency (IE), which was calculated by Eq. (2).

$$\text{IE} = \frac{Q_M}{Q_N} \quad (3)$$

wherein Q_M and Q_N were protein rebinding capacities of MIPs and NIPs, respectively.

The selectivity of imprinted microspheres was evaluated by static distribution coefficient K_D and separation factor α, which were calculated using Eqs. (3) and (4).

$$K_D = \frac{Q_e}{C_e} \quad (4)$$

$$\alpha = \frac{K_{D1}}{K_{D2}} \quad (5)$$

wherein K_{D1} and K_{D2} were static distribution coefficients of template and competing molecules, respectively. The concentration of competitive proteins in the supernatant was measured by UV/vis spectrometer at 280 nm for OVA and 405 nm for BHb.

3. Results and discussion

In the previous study, PU-*g*-CaA was successfully synthesized by radical grafting polymerization. The grafted PU segments formed crystallizing area and acted as physical crosslinking points, improving thermodynamic and anti-swelling stability [17]. It is therefore expected that PU-*g*-CaA hydrogel be applicable for protein imprinting and the grafted PU segments help to maintain the molecular imprints and facilitate the recognition specificity.

Adsorption capacity and IE of MIPs with different grafting ratio: The adsorption capacity and IE of MIPs with different grafting ratios are measured and the results are shown in Fig. 1. With the increase of grafting ratio, the adsorption capacity of MIPs and IE decreased at first and then increased (up to 1.29 mg/g and 6.41, respectively). The imprinted polymers with lower grafting ratios are difficult to maintain the specific steric structure and the specific binding capacity is reduced. When the grafting degree increased, more accessible and stable imprinting sites are constructed in MIPs, which provide a higher specific recognition. However, the grafted side chains make protein transport in hydrogel beads difficult. As a result the non-specific adsorption is decreased, which can be confirmed by the reduction in adsorption capacity of NIPs. Based on this result, the PU-*g*-CaA with grafting ratio of 20% is chosen as the imprinted material for investigating the adsorption isotherms and rebinding specificity.

Adsorption isotherms of PU-g-CaA beads: The equilibrium rebinding experiments are carried out at different initial concentrations of BSA over the range of 0.05–6 mg/mL and the results are shown in Fig. 2a. MIPs display significantly higher rebinding amounts than NIPs and both of them reach an adsorption plateau after the concentration of 4 mg/mL. The maximum adsorption capacity of MIPs and NIPs are 4.5 mg/g and 2.5 mg/g, respectively. This observation indicates an obvious protein imprinting effect on adsorption capacity. It is also suggested that the imprinted sites will be saturated and the adsorption will be limited to a maximum value even when the materials are incubated in protein solution with a higher concentration. This provides an evident that protein is binding with the functional sites (e.g. –OH etc.) rather than general adsorption or diffusion into the hydrogel.

The Freundlich model is employed to describe the adsorption isotherms. The linear form of Freundlich model is expressed as follows [18]:

$$\lg Q_e = \lg K_F + n \lg C_e \quad (6)$$

wherein K_F is the Freundlich isotherm constant and n is the Freundlich isotherm exponent constant.

Fitting of the data on Freundlich equation yields a good fit for the MIPs (R=0.9606) and NIPs (R=0.9640) (Fig. 2b). This result demonstrates that the adsorption of BSA on PU-*g*-CaA microspheres can be characterized by multi-site adsorption isotherm.

Rebinding specificity: The specific recognition property is one of the important considerations for MIPs in practical applications. In addition to the template BSA, two other protein (OVA, BHb) are chosen as references to run a single protein batch rebinding test.

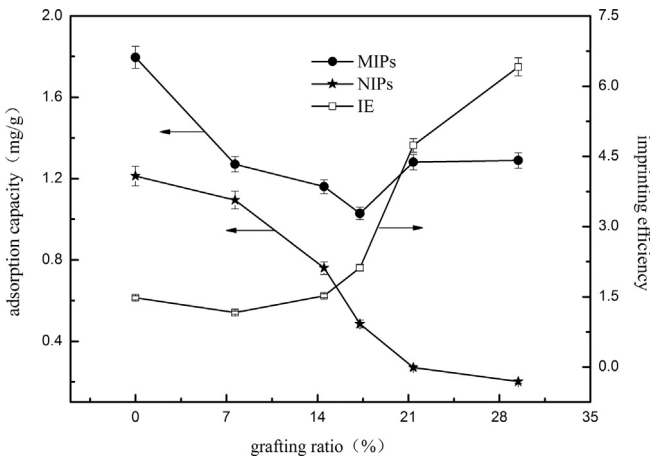

Fig. 1. The adsorption capacity and IE of PU-*g*-CaA beads at different grafting ratios.