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ScienceDirect
Biosensors and Bioelectronics 22 (2007) 2322–2328
BIOSENSORS & BIOELECTRONICS
www.elsevier.com/locate/bios

Surface imprinted beads for the recognition of human serum albumin

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Received 3 May 2006; received in revised form 21 December 2006; accepted 21 December 2006
Available online 17 January 2007

Abstract

The synthesis of poly-aminophenylboronic acid (ABPA) imprinted beads for the recognition of the protein human serum albumin (HSA) is reported. In order to create homogeneous recognition sites, covalent immobilisation of the template HSA was exploited. The resulting imprinted beads were selective for HSA. The indirect imprinting factor (IF) calculated from supernatant was 1.6 and the direct IF, evaluated from the protein recovered from the beads, was 1.9. The binding capacity was 1.4 mg/g, which is comparable to commercially available affinity materials. The specificity of the HSA recognition was evaluated with competitive experiments, indicating a molar ratio 4.5/1 of competitor was necessary to displace half of the bound HSA. The recognition and binding of the imprinted beads was also tested with a complex sample, human serum and targeted removal of HSA without a loss of the other protein components was demonstrated. The easy preparation protocol of derivatised beads and a good protein recognition properties make the approach an attractive solution to analytical and bio-analytical problems in the field of biotechnology.
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Keywords: Molecularly imprinted polymers; Human serum albumin; 3-Aminophenylboronic acid; Serum depletion; Proteome

1. Introduction

Molecularly imprinted polymers (MIPs) are polymers prepared in presence of a template that serves as a mould for the formation of template-complementary binding sites (Mosbach, 1994; Sellergren, 2001a,b). Thus, MIPs can be created to recognise a large variety of target molecules often with affinity and selectivity comparable to those exhibited by poly- or monoclonal antibodies. MIPs are less expensive and quicker to prepare than biological receptors. Additionally, they are capable of withstanding much harsher conditions than antibodies, such as high temperature, pressure, extreme pH, and organic solvents. These properties have made them extremely attractive for solving problems in the fields of preparative chemical separations (Sellergren, 2001a,b), solid phase extraction (Lanza and Sellergren, 2001; Andersson and Schweitz, 2003) and sensing (Chianella et al., 2003; Haupt and Mosbach, 2000), or for the removal of specific molecular targets from food (Whitcombe et al., 1997; Ramstrom et al., 2001).

MIPs have been prepared using polypeptides (Kempe et al., 1995; Andersson et al., 1995; Minoura and Rachkov, 2000), bacteria (Dickert and Hayden, 2002), low molecular mass compounds (Katz and Davis, 2000; Chianella et al., 2003) and proteins (Burow and Minoura, 1996; Bossi et al., 2001; Guo et al., 2004) as templates. In the case of proteins, however, only modest success has been obtained due to the specific properties of these templates. First, all proteins are water-soluble compounds that are not always compatible with mainstream MIP technologies, which relies on the use of organic solvents for the polymer preparation. Second, proteins have a large amount of functional groups available for the interaction with functional monomers. Third, proteins have a flexible structure and conformation, which can be easily affected by changes in temperature or the nature of the solvent. Thus, from a thermodynamic and practical standpoint, it is difficult to develop successful imprints for such molecules.

In the last 10 years, some new approaches have been proposed, which use both covalent and non-covalent methods (Wulff, 1993; Mosbach and Ramstrom, 1996). The immobilisation of the protein template on a supporting surface (Shi et al., 1999; Yilmaz et al., 2000; Shiomi et al., 2005) provides a number of advantages, i.e. it allows the imprinting of templates

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doi:10.1016/j.bios.2006.12.034

independently of their solubility in the polymerisation mix, it minimises protein aggregation and it creates more homogeneous binding sites.

In the present contribution, we report the preparation of imprinted beads for the recognition of human serum albumin (HSA). The protein template is immobilised at the surface of the beads, with an approach modified from Shiomi et al. (2005) in which covalent immobilisation was achieved by derivatising silica beads with aldehyde groups, these were then exploited to form imine bonds with the amino groups of lysine in the HSA. The polymer was made of 3-aminophenyl boronic acid (APBA) (Piletsky et al., 2001; Bossi et al., 2001; Pribyl and Skladal, 2006) using a polymerisation process described earlier by our group (Bossi et al., 2001). The beads thus obtained were characterised in order to evaluate the percentage of polymer derivatised, the binding capacity for HSA, the binding kinetics, the specificity of the HSA binding and the recovery of bound protein.

Finally, the imprinted beads were applied to a real biological fluid, human serum, in which HSA is the most abundant protein (ca. 60% of the total proteins) (Andersson and Anderson, 2002; Tirumalai et al., 2003). The high quantity of HSA in serum is considered as a drawback, since it seriously hampers the detection of low abundant proteins, which are often marker of diseases. It has been demonstrated that if serum is selectively depleted of albumin, it would facilitate the analysis of such low-abundant proteins. Albumin removal has been achieved through adsorption to immobilized dyes (Ahmed et al., 2003), immuno-affinity extraction (Wang et al., 2003) or affinity capture by immobilized phage-derived peptides (Sato et al., 2002), but to date there have been no reports concerning the use of molecularly imprinted polymers. In the present case, we attempt the use of a synthetic receptor, the imprinted beads, for the specific removal of HSA from serum.

2. Materials and methods

2.1. Materials and reagents

Acetic acid, aminopropyl silica (particle size 15–40 μm, mean pore size 60 Å), 3-amino propyl trimethoxysilane (APTMS), ammonium persulfate (APS), Bradford assay, [(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), ethanol, glycine, glutaraldehyde, human serum albumin (HSA) (Mr 66 kDa), methilic acid, 2N-morpholine ethane sulfonic acid (MES), oxalic acid, propyl triethoxy silane, sodium chloride (NaCl), SDS, Sypro Ruby, thiourea, trizma, urea, were obtained from Sigma–Aldrich Chemie GmbH (Steinheim, Germany). Acrylamide, *N*,*N*-methylenebisacrylamide, *N*,*N*,*N*,*N*-tetramethyl-ethylenediamine (TEMED), dithiothreitol (DTT), linear Immobiline dry strips pH gradient 3–10 (7 cm) were obtained from Bio-Rad (Hercules, CA, USA).

2.2. Preparation of human serum albumin (HSA)-imprinted silica using immobilized templates

Silica beads (1 g) were derivatised with 10% (w/v) APTMS in methanol in order to introduce NH_2 functional groups. The beads were washed three times with ethanol and three times with deionised water and then dried at 50 °C for 24 h. They were then incubated with 10 mM 2N-morpholine ethane sulfonic acid (MES) buffer, pH 5.5 containing 1% glutaraldehyde for 12 h at room temperature, in order to introduce aldehyde groups. The product was washed repeatedly with deionised water. Finally, 1 ml of a 10 mM 3N-morpholino propane sulfonic acid (MOPS) solution (pH 7.0) containing 2.0 mg/ml human serum albumin (HSA) and 0.1 M NaCl was admixed as the template for 3 h at 4 °C, in order to covalently bind the protein on the aldehyde groups. The beads were then incubated with 1 M Tris for 30 min in order to block the un-reacted aldehyde groups. Washes with deionised water followed and finally 1 ml of 50 mM ABPA water solution was added to the beads. After 45 min incubation, 1 ml of 25 mM ammonium persulfate (APS) water solution was added in order to start the polymerisation reaction. The polymerisation was carried out at room temperature for 1 h, after which the beads were washed again with deionised water for five times. Finally, 1 ml of 1 M oxalic acid was added in order to remove the template. This step was carried out at room temperature for 12 h. The derivatisation protocol was checked at each step by FT-IR spectroscopy with a Magna FT-IR 760 Spectrophotometer (Nicolet, Offenbach Germany), samples were prepared mixing 10 mg of beads with 200 mg KBr, sample reading was 32×. The beads were finally conditioned with 10 mM phosphate buffer, pH 8.0, in order to increase the pH and remove the free HSA in solution produced by the treatment with 1 M oxalic acid.

2.3. Evaluation of the percentage of derivatisation

In order to evaluate the percentage of silica beads derivatised, 0.500 g of beads were derivatised (with APTMS, glutaraldehyde, APBA, as previously described). After each step, the beads were dried overnight at 87 °C and they were weighed. The degree of derivatisation was calculated as follows:

$$\mathrm{DG}\ (\%) = \frac{(\text{mg of ``polymer'' bound})}{(\text{g beads})} \times 100.$$

2.4. Calculation of the binding capacity

In order to evaluate the binding capacity, 0.500 g of beads were conditioned with 10 mM phosphate buffer, pH 8.0 and incubated with 300 μl of HSA solution at different concentrations (0.036, 0.058, 0.073, 0.18, 0.3, 1.0, 2.6, 2.74 and 4.08 μg/μl) for different times (3, 5, 20, 60 min). The beads were centrifuged at 4000 × *g* for 3 min at room temperature, then the supernatant was transferred in new tubes and it was quantified by Bradford assay at 595 nm. The protein bound was expressed as the difference between the total micrograms of HSA loaded and micrograms of HSA in solution after the binding.

2.5. Binding kinetics

In order to evaluate the binding kinetics, a 0.500 g of control or MIP beads, conditioned with 10 mM phosphate buffer pH 8.0, were incubated with 300 μl of HSA solution at different

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