Biosensors and Bioelectronics 25 (2009) 568–571

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Direct detection of analyte binding to single molecularly imprinted polymer particles by confocal Raman spectroscopy

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ARTICLE INFO

Article history:
Received 6 October 2008
Received in revised form 17 December 2008
Accepted 13 January 2009
Available online 23 February 2009

Keywords:
Molecular imprinting
Confocal Raman spectroscopy
Microsphere
Molecular recognition
Synthetic receptor

ABSTRACT

We describe the use of Raman spectroscopy to detect and quantify, for the first time, the presence of the imprinting template in single molecularly imprinted polymer microspheres. The polymers were imprinted with the β-blocking drugs propranolol and atenolol, and precipitation polymerization was used to obtain spherical particles of diameters of 200 nm and 1.5 μm. The size of the Raman laser spot being between 1 μm and a few μm, the nanoparticles were used for bulk detection whereas with micrometer-sized particles, quantitative measurements on single particles were possible. The laser power, and consequently the acquisition times, needed to be adapted as a function of the polymer and template used in order to avoid burning. Analyte quantification from Raman spectra is straightforward by determining the peak height of a typical Raman band of the analyte, and by using a typical polymer peak for normalization. Relatively low detection limits down to 1 μM have been reached for the detection of S-propranolol through bulk measurements on MIP nanoparticles.

1. Introduction

Micro-biochips are an area drawing considerable recent interest for their possible use in clinical testing, environmental and food monitoring, security, etc. The target analyte is bound by recognition elements present in the form of a pattern at the chip surface. The recognition elements are in most cases biomacromolecules such as enzymes, antibodies or DNA. The specific molecular recognition phenomenon generates a change in one or more physicochemical parameters of the system, which is then translated by a transducer into an easily quantifiable output signal. For some application areas, biomacromolecules are not perfectly suited since they tend to be unstable out of their native environment. For certain target analytes a natural receptor may not exist or may be difficult to obtain in pure form. Thus, a main trend in research has long been the creation of synthetic receptors for a desired molecular target.

A simple way of generating synthetic macromolecular receptors is through the molecular imprinting of polymers (Arshady and Mosbach, 1981; Wulff and Sarhan, 1972; Zimmerman and Lemcoff, 2004). Here, the target analyte or a derivative thereof acts as a template around which functional and cross-linking monomers are co-polymerized to form a molecular mould. When the template is subsequently removed, binding sites are revealed that are complementary to the template in size, shape, and chemical functionality. Their conformation is preserved by the polymer matrix that is highly cross-linked. Thus, the polymer is now capable of selectively recognizing and binding the target.

For use in biochips, these molecularly imprinted polymers (MIPs) have to be patterned on surfaces and interfaced with a transducer. Patterning methods that are commonly used are standard microspotting techniques such as ink-jetting (Blanchard et al., 1996), mechanical microspotting (Schena et al., 1995), or microcontact printing (Quist et al., 2005). For example, arrays of silicon microcantilevers have been used to deposit biomolecules onto glass slides (Belaubre et al., 2003). The diameter of the dots can vary but is normally between a few micrometers and a few hundreds of micrometers. For smaller dots, it is possible to use scanning probe microscopy techniques like dip-pen nanolithography (DPN), or the nanofountain pen (NFP) (Lewis et al., 1999; Taha et al., 2003). Some of these techniques have already been used with MIPs (Belmont et al., 2007; Lin et al., 2006; Vandevelde et al., 2007).

However, working with very small single structures of MIPs presents critical challenges as far as detection of binding and releasing of target molecules are concerned. Commonly employed transduction methods, such as surface plasmon resonance, quartz crystal microbalance, interferometric or reflectometric techniques are often not sensitive enough to detect such minute amounts of analyte. In addition, these label-free techniques are non-specific and report merely on accumulation of any material on the surface of the sensor. Sometimes, the analyte might be fluorescent

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doi:10.1016/j.bios.2009.01.020

and can be detected directly by fluorescence microscopy techniques (Vandevelde et al., 2007), or a competitive assay or sensor format with a fluorescent molecular probe can be used. The latter may even be incorporated into the polymer if the form of a "signaling monomer" (Matsui et al., 1998; Turkewitsch et al., 1998). Anyhow, at least during the development phase of such microarrays there is a strong need of a general technique that can detect, identify and if possible quantify the imprinting template in a microdot or other microstructure. Possible general analytical techniques that could be used are infrared microscopy or micro-Raman spectroscopy. Both FTIR and Raman measurements have already been demonstrated to be useful for the analysis of MIPs (Jakusch et al., 1999; Kostrewa et al., 2003; Uibel and Harris, 2005), and sometimes quantitative measurements were reported (Jakusch et al., 1999; McStay et al., 2005). An additional advantage of these techniques is the higher information content of the spectra that in some cases allows not only to quantify but also to identify an analyte.

In the present work we describe the use of micro-Raman spectroscopy to detect the presence of the imprinting template in nanobeads and in single microbeads of MIP. As model templates, we chose the β-blocking drugs S-propranolol and S-atenolol.

2. Experimental

2.1. Materials

Ethylene glycol dimethacrylate (EGDMA), divinylbenzene (DVB), methacrylic acid (MAA), (S)-propranolol hydrochloride, S-atenolol and ^{3}H-RS-propranolol were from Sigma–Aldrich. 2,2′-Azobis-(2,4-dimethylvaleronitrile) (Vazo-52) was from DuPont Chemicals. All solvents were of HPLC grade. The polymerization inhibitor was removed from DVB by treatment with basic alumina. (S)-propranolol hydrochloride was converted into the free base by extraction from a sodium carbonate solution at pH 9 into chloroform.

2.2. Synthesis of MIP particles

MIP particles were synthesized by precipitation polymerization. The template molecule S-propranolol or S-atenolol (1 mmol), the polymerization initiator Vazo-52 (0.89 mmol), the cross-linking monomer EGDMA or DVB (40 mmol), and the functional monomer MAA (8 mmol) were dissolved in the appropriate solvent (toluene or acetonitrile, 5% monomer concentration in the case of DVB and 2% in the case of EGDMA) in a round bottom flask equipped with a magnetic stirring bar and sealed with a Suba-Seal^{TM} silicon rubber septa. Solutions were placed on ice and purged with nitrogen. Polymerization was carried out in an oil bath at 60 °C for 12 h. The particles were collected by centrifugation at 18,000 rpm for 20 min, and the template was extracted by three incubations in methanol:acetic acid 9/1 for 1 h, followed by three incubations in methanol. The particles were then dried under vacuum and stored at ambient temperature until use. Non-imprinted polymers (NIP) were synthesized under identical conditions in the absence of the template molecule.

2.3. Radioligand binding experiments

The MIP particles were suspended in acetonitrile containing 0.5% (v/v) acetic acid and kept under agitation. From this stock suspension, a series of suspensions ranging in concentration from 0.01 to 1.8 mg/mL were prepared in Eppendorf tubes, and 0.4 pmol of ^{3}H-labeled propranolol was added to each tube, which contained a final volume of 1 mL. The tubes were incubated overnight on a rocking table. The tubes were then centrifuged, and a 500 mL aliquot of the supernatant was pipetted from each tube into scintillation vials that contained 4 mL of scintillation fluid (Beckman Coulter). The concentration of free radioligand was measured with a scintillation counter (Beckman LS-6000 IC).

2.4. Binding assays and Raman spectroscopy

Polymer particles were dispersed in acetonitrile containing 0.5% (v/v) acetic acid and kept under agitation, and from this stock suspension, equal volumes containing 2 mg/mL of polymer were distributed to Eppendorf test tubes. (S)-propranolol or S-atenolol were added at final concentrations from 1 μM to 2 mM. The total volume was adjusted to 1 mL. The samples were placed on a rocking table for 1 h. After incubation, an appropriate volume of the particle suspension was deposited on a porous alumina membrane coated with 50 nm of gold, and the incubation solvent was removed using a home-made vacuum system.

Raman experiments were performed with a Horiba Jobin Yvon LabRAM HR High Resolution Raman Microscope equipped with Labspec 4.02 software, in a backscattering configuration. An argon-ion laser operated at 514 nm illuminating the sample through the microscope objective under normal incidence was used for excitation. Raman spectra were obtained with an appropriate power output at the sample using a 100× objective (NA = 0.8) with an acquisition time of 10 s to 20 min. The laser spot was about 1 μm in diameter. All spectra were baseline corrected, averaged and smoothed.

3. Results and discussions

3.1. Polymer synthesis

We have synthesized molecularly imprinted polymer beads of two different sizes, around 200 nm for bulk measurements, and above 1 μm for measurements on single beads. MIPs in the form of bulk monoliths, selective for S-propranolol were originally reported by Andersson (1996). For the present work we have adapted the initial recipe to the precipitation polymerization technique, which allows to obtain spherical particles (Ye et al., 2002). With EGDMA as the cross-linker we obtained homodisperse spherical particles with a diameter of 200 nm (Fig. 1A). In order to verify that the particles were indeed molecularly imprinted, we first performed radioligand binding experiments with tritium-labeled propranolol. Fig. 1B shows the binding of ^{3}H-propranolol by the MIPs and the corresponding control polymers to an increasing amount of polymer. As can be seen, the MIPs adsorbed the radioligand and shows saturation-type behavior, whereas the non-imprinted control polymer shows nearly no binding even at higher polymer concentrations. This confirms that molecular imprinting has indeed taken place in the MIP.

3.2. Raman spectroscopy of MIPs

Raman measurements were first done on layers of 200 nm-sized MIP particles imprinted with propranolol. Since the laser spot is approximately 1 μm in diameter, the spectra represent an average of a number of particles. We were interested in quantitative measurements and in recording binding isotherms. Therefore, MIPs were incubated with a series of analyte concentrations and the measurements of bound propranolol were done at equilibrium. In order to remove the solvent containing the free fraction of the analyte, an appropriate volume of the particle suspension was pipetted on a nanoporous alumina membrane covered with 50 nm of gold. The solvent was removed by applying vacuum through the membrane.

The particle layer was then measured in the Raman microscope with a 514 nm Ar-ion laser. Fig. 2 shows the spectrum obtained, and for comparison the spectrum of pure propranolol. As can be seen,