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Short communication

Porous silicon-based optical microsensor for the detection of L-glutamine

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Abstract

The molecular binding between the glutamine-binding protein (GlnBP) from *Escherichia coli* and L-glutamine (Gln) is optically transduced by means of a biosensor based on porous silicon nano-technology. The sensor operates by the measurement of the interferometric fringes in the reflectivity spectrum of a porous silicon Fabry–Perot layer. The binding event is revealed as a shift in wavelength of the fringes. Due to the hydrophobic interaction with the Si–H terminated surface of the porous silicon, the GlnBP protein, which acts as a molecular probe for Gln, penetrates and links into the pores of the porous silicon matrix. We can thus avoid any preliminary functionalization process of the porous layer surface, which is also prevented from oxidation, at least for few cycles of wet measurements. The binding of Gln to GlnBP has also been investigated at different concentration of GlnBP.

Keywords: Porous silicon; Optical biosensor; L-Glutamine; Proteins

1. Introduction

In a biosensor, the interaction between the analyte and the biological recognition system is normally detected by the transducer element, which converts the molecular event into an electrical or optical signal. A great number of experimental works, concerning the worth noting properties of porous silicon (PSi) in chemical and biological sensing, has been recently reported in literature (Dancil et al., 1999; Arrand et al., 1999; Gao et al., 2000; De Stefano et al., 2004a,b).

Due to its sponge-like structure, PSi is an almost ideal material as a transducer: its surface has a specific area of the order of 200–500 $m^2\,cm^{-3}$, so that a very effective interaction with several adsorbates, liquid or gaseous, is assured. Moreover, PSi is an available and low cost material, completely compatible with standard IC processes. Therefore, it could usefully be employed in the so-called smart sensors (De Stefano et al., 2004a,b).

The PSi is produced by the electrochemical etching of a silicon (Si) wafer in a hydrofluoridic acid solution. It is well known that the porous silicon "as-etched" has a Si–H terminated surface due to the Si dissolution process (Canham, 1997). From the chemical point of view, the Si–H bonds make the PSi surface strongly hydrophobic and very reactive. The hydrophobic interaction between biological molecules and several templates has been extensively studied (Yu et al., 1997; Yu and Mosbach, 2000).

PSi optical sensors are based on changes of photoluminescence or reflectivity when exposed to the target analytes (Mulloni and Pavesi, 2000), which substitute the air into the PSi pores. The effect depends on the chemical and physical properties of each analyte, so that the sensor can be used to recognize the pure substances. Due to the sensing mechanism, these kind of devices are not able to identify the components of a complex mixture. In order to enhance the sensor selectivity through specific interactions, some researchers have proposed to chem-

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ically or physically modify the PSi surface (Ligler and Rowe Taitt, 2004). The common approach is to create a covalent bond between the porous silicon surface and the biomolecules, which specifically recognize the unknown analytes (Dancil et al., 1999; Hart et al., 2003; Yin et al., 2004).

On the other hand, ligand-binding proteins are good candidates in designing highly specific biosensors for small analytes: in particular, the glutamine-binding protein (GlnBP) from *Escherichia coli* is a monomeric protein composed of 224 amino acid residues (26 kDa) responsible for the first step in the active transport of L-glutamine (Gln) across the cytoplasmic membrane. Gln is a major source of nitrogen and carbon in cell culture media: its monitoring is thus important in bioprocesses control. The GlnBP consists of two similar globular domains, the large domain (residues 1–84 and 186–224) and the small domain (residues 90–180), linked by two peptides. The deep cleft formed between the two domains contains the ligand-binding site. Of the naturally occurring amino acids, only Gln is bound by GlnBP with a dissociation constant K_d of 5×10^{-9} M (D'Auria et al., 2005).

In this communication, we report some preliminary data on the development of a new reagentless microsensor for the optical interferometric detection of Gln based on the interaction between an unmodified porous silicon nano-structured monolayer and GlnBP. The obtained results show that when adsorbed into the PSi pores, the outer amino acids of GlnBP, which are hydrophobic in nature, link to the Si–H bonds and, as a consequence, block the protein to the PSi surface. GlnBP can thus sufficiently strongly interact with the porous silicon surface and also work as a molecular probe for the detection of Gln.

2. Materials and methods

Apart from the previously cited advantages of PSi in the sensing field, it is also a very attractive optical material for the possibility of fabricating single layer, like Fabry–Perot interferometers, and multilayer structures, such as Bragg or rugate filters, with high quality optical response (Theiss, 1997). In our experiments we used a porous silicon Fabry–Perot interferometer fabricated by electrochemical etching of p^+-type (1 0 0) crystalline silicon (resistivity 8–12 mΩ cm) in HF/EtOH (50:50) solution. The etching current had a value of 550 mA cm^{-2} and has been applied for 0.9 s. The layer thickness was about 5 μm and the porosity about 70% (values estimated by fitting the optical reflectivity data). The reflectivity spectrum in white light exhibited lot of fringes in the wavelength range between 500 nm and 1100 nm. The optical set-up required for our sensing experiments was very simple (see Fig. 1): a tungsten lamp (400 nm < λ < 1800 nm) inquired the sensor, through an optical fibre and a collimator. The reflected beam was collected by an objective, coupled into a multimode fibre, and then directed in an optical spectrum analyser (Ando, AQ6315A). The reflectivity spectra have been measured with a resolution of 0.2 nm. The solutions containing the molecular probe and the analyte were directly spotted on the sensor surface.

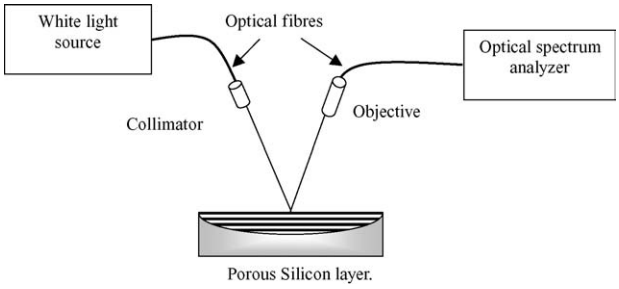

Fig. 1. The experimental set-up.

Previous ellipsometric characterization has shown that the surface of porous silicon layer as prepared could be covered by a 100–200 nm thin parasitic film of very low porosity (<20%) due to hydrogen contamination of the silicon wafer (Gaillet et al., 2004). Such film prevents not only the pores from filling but also any biochemical interaction with the hydrogenated porous silicon surface after the etching process. For sensing purposes it is therefore mandatory to avoid the formation of the parasitic film by thermal treating the wafer at 300 °C in nitrogen atmosphere before the electrochemical etch (Chamard et al., 1998).

The presence of Si–H bonds on the porous silicon surface has been monitored by means of infrared spectroscopy with a Fourier transform spectrometer (FT-IR Nicolet Nexus) (Socrates, 2001).

2.1. Preparation and purification of GlnBP

GlnBP from *E. coli* was prepared and purified according to a standard procedure (Dattelbaum and Lakowicz, 2001). The protein concentration was determined by the method of Bradford (1976) on a double beam Cary 1E spectrophotometer (Varian, Mulgrade, Vic., Australia) with bovine serum albumin as standard.

2.2. Labeling of GlnBP

A solution of homogenous GlnBP 2.0 mg ml^{-1} in 1.0 ml of 0.1 M bicarbonate buffer, pH 9.0 was mixed with 10 μl of fluorescein isothiocyanate (FITC) (Molecular Probes) solution in *N,N*-dimethylformamide (DMF) (1.0 μg FITC/100 μl DMF). The reaction mixture was incubated for 1 h at 30 °C and the labeled protein was separated from unreacted probe by passing over a Sephadex G-25 column equilibrated in 50 mM phosphate buffer, 100 mM NaCl, pH 7.0.

To assess the protein penetration into the pores, we spotted on the porous silicon chip 20 μl of 1.0 mM sodium bicarbonate buffer containing the dye labeled protein. For ligand–protein interaction measurement, we prepared a 20 mM L-glutamine in the same buffer solution.

3. Experimental results

Since the hydrophobic interaction is the only binding mechanism between the PSi and the GlnBP, it is crucial to assess the presence of Si–H bonds in the samples before spotting the pro-