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Detection of odorant molecules via surface acoustic wave biosensor array based on odorant-binding proteins

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ABSTRACT

In this paper, we present an array of biosensors for vapour phase detection of odorant molecules based on surface acoustic wave (SAW) resonators coated with odorant-binding proteins (OBPs). For the first time, the sensing capabilities of three different OBPs, as sensitive layers for SAW devices, are studied and compared. The SAW biosensor array is composed of three SAW devices coated by the droplet method with the wild-type OBP from cow (wtbOBP), a double mutant of the OBP from cow (dmbOBP) and the wild-type OBP from pig (wtpOBP). An uncoated device is used to compensate the variations of the environmental parameters. The SAW devices consist of two-port resonators fabricated on quartz (ST-cut, x propagation) with electrodes made of aluminium covered with a thin gold film (2 nm thick). The obtained surface densities of OBP layers are between 1.18×10^{-6} kg/m^2 and 2.31×10^{-6} kg/m^2 and were calculated measuring the resonant frequency shift of the SAW devices after the coating. The SAW biosensor array was tested in nitrogen upon exposure to vapours of R-(−)-1-octen-3-ol (octenol), in the range of concentration between 13 and 61 ppm, and R-(−)-carvone (carvone), in the range between 9 and 80 ppm. The highest sensitivity for detection of octenol (25.9 Hz/ppm) was obtained using the wtpOBP-based SAW biosensor, while the highest sensitivity for detection of carvone (9.2 Hz/ppm) was obtained using the dmbOBP-based SAW biosensor.

1. Introduction

The demand for versatile technologies that can be used for developing chemical and biological sensors for the detection of volatile species has rapidly increased in the last decade (Fanget et al., 2011). Multiple areas of application include homeland security (Demirev et al., 2005), environmental pollution control, health and wellness (Varriale et al., 2012), to mention some examples. Specifically, the mandatory need for detection of volatile organic compounds (VOCs), which have a potential impact on climate and long-term health effect (Cheol Gil et al., 2000), and of chemical warfare agents for counter-terrorism actions (Joo et al., 2007), has stimulated an extensive research activity for the development of several types of sensitive devices. These sensors have to provide precision, high sensitivity, reversibility, selectivity, low cost, fast response time and compactness (Fernández et al., 2007; Pellejero et al., 2012). Finally, the methodologies devoted to the sensory assessment of foods and based on the global evaluation of the odour intensity require sensor arrays to develop artificial olfactory systems (Garcia-Gonzalez and Aparicio, 2002).

The capabilities of SAW devices to measure physical parameters, such as force, acceleration, pressure, electric and magnetic fields, potentials, etc., or chemical and biochemical values, such as gas, vapours or ion concentrations, are widely exploited since many years (Ballantine et al., 1997; Benetti et al., 2004). In particular, in the last two decades, SAW devices have attracted the attention of the biochemical scientific community for bio/sensing applications. Some examples of SAW based biosensor systems suitable to detect bacteria, proteins, DNA, sugars, viruses and cells in liquids are reported in the literature (Berkenpas et al., 2006; Branch and Edwards 2007; Länge et al., 2007; Rupp et al., 2008).

For vapour phase applications, SAW biosensors seem to be a powerful tool to measure small concentrations of volatile compounds (Sang-Hun et al., 2005). In fact, they can overcome the intrinsic low-selectivity of polymer coated SAW chemical sensors (Alizadeh and Zeynali 2008), and, at the same time, ensure the high sensitivity and fast response time typical of these sensors. However, the development of SAW biosensors for in-air applications has been delayed because of the knowledge that biomolecules maintain their three-dimensional structure and, hence, their prescribed functionality, only in an aqueous environment (Stubbs et al., 2002). On the contrary, some works on acoustic wave biosensors for vapour phase detection based on commercially available quartz crystal microbalance (QCM), which operate on thickness shear mode resonation, were reported in the literature several years ago (Guilbault 1983; Ngeh-Ngwainbi et al., 1986). QCM sensors for mass detection of formaldehyde and organophosphorous pesticides based on films of enzymes and antibodies are

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described previously (Guilbault 1983; Ngeh-Ngwainbi et al., 1986).

To date, only a limited number of works on SAW devices utilizing biological molecules as sensing material and able to detect small molecules in air have been proposed (Hunt et al., 2003). A SAW resonator immunosensor array is described in (Sang-Hun et al., 2005) demonstrating the detection of low vapour pressure plastic explosives containing nitro groups such as RDX and TNT. The monoclonal anti-RDX and anti-TNT have been immobilized on the metal electrodes of the SAW resonator via protein-A cross linker. Stubbs et al., (2005) detected cocaine vapours by anti-benzoylecgonine antibodies using a SAW platform.

Vertebrate OBPs are small extracellular proteins belonging to the lipocalin superfamily (Briand et al., 2002; Dal Monte et al., 1993; Lobel et al., 2002; Spinelli et al., 1998). It has been hypothesized that OBPs play a role in receptorial events of odour detection by carrying, deactivating, and/or selecting odorant molecules (Blanchet et al., 1996; Herent et al., 1995; Tegoni et al., 1996). The OBPs share a conserved folding pattern, an 8-stranded β-barrel flanked by an α-helix at the C-terminal end of the polypeptide chain. The β-barrel creates a central apolar cavity whose role is to bind and transport hydrophobic odorant molecules. These proteins reversibly bind odorants with dissociation constants in the micromolar range. Although their functions are still not fully understood, OBPs are also believed to participate in the deactivation of odorants (Briand et al., 2002; Dal Monte et al., 1993). Since the discovery of the first vertebrate OBP in the bovine nasal mucus, OBPs have been identified in a variety of species, and different OBP subtypes have been reported to simultaneously occur in the same animal species (Briand et al., 2002). The binding properties investigated in the three OBPs from rat demonstrated that they were specially tuned towards distinct chemical classes of odorants (Garibotti et al., 1997; Pes et al., 1992; Vincent et al., 2000).

In this work, we present a comparison of the sensing capabilities of three different OBPs as probes for odorant molecules by using a SAW biosensor system.

The proposed solution exploits the flexibility and the well-known features of SAW based sensors in conjunction with the adaptable selectivity of the OBPs. In particular, the use of this class of proteins was suggested by the fact that they can be easily modified by genetic engineering techniques to modulate their binding specificity (Ramoni et al., 2007) and preserve their full functionality when exposed to air environment. Moreover, the possibility to use wtbOBPs to implement SAW biosensors, able to detect odorant molecules, has been demonstrated (Di Pietrantonio et al., 2009).

The proposed sensor system is based on an array configuration composed of three SAW resonators coated with three different OBPs, characterized by different binding specificity, plus an uncoated SAW device used as reference. The chosen proteins are the wtbOBP, a dmbOBP and the wtpOBP. Tests were performed exposing the SAW biosensor array to concentrations of octenol and carvone vapours in nitrogen atmosphere. These odorants are largely used in food industry and their detection is a first approach towards the recognition of food flavours.

Experimental results showed different sensitivities of the three OBP-based SAW biosensors for detection of the investigated compounds.

2. Material and methods

2.1. OBPs purification and functionality test with 1-amino-antharacene

A 6xHis affinity tag was placed at the N-terminal of the wtbOBP and mutant the double protein dmbOBP by Polymerase Chain Reaction (PCR) with specific primers (Ramoni et al., 2007). The fused cDNAs were sub-cloned in the expression vector pT7-7 and the expressions of the two proteins were realized in BL21-DE 3 *Escherichia coli*. The purification of the two proteins was obtained by affinity chromatography with a Ni-NTA Agarose (Quiagen, Germany) according to the manufacturer's instructions, followed by a second chromatography step on the anion exchange column Resource Q (Amersham Biosciences, Italy), on an Fast Performance Liquid Chromatography (FPLC) chromatographic system. The purity of the two proteins was determined by SDS-PAGE (Poly-Acrylamide Gel Electrophoresis) and the concentration of the two purified proteins was calculated by the absorbance value at 280 nm (48,000 M^{-1} cm^{-1}) (Ramoni et al., 2007).

Pig (*Sus scrofa*, breed Large White) nasal mucosa was obtained from the local slaughterhouse. The tissue was collected within 20 min after death and quickly utilized for the extraction of pOBP. Protein was purified from fresh porcine nasal tissue according to the procedure described by (Dal Monte et al., 1991). This procedure yielded on average 15 mg of purified pOBP from a single animal.

The functionalities of the recombinant wtbOBP, dmbOBP and pOBP were determined by direct titrations using the fluorescent ligand 1-amino-anthracene (AMA). Briefly, 1.0 ml of 1.0 μM OBPs, in 20 mM Tris–HCl buffer pH 7.8, was incubated overnight at 4 °C in the presence of increasing concentrations of AMA (0.156–10 μM).

Fluorescence emission spectra were recorded between 450 nm and 550 nm by fluorometer (ISS K2 model) (excitation and emission slits were set at 2.0 nm) at a fixed excitation wavelength of 380 nm. The formation of the AMA-OBPs complex was followed as the increase of the fluorescence emission intensity at 480 nm. In particular, the functional assay is based on the resonance energy transfer phenomenon between the indolic residues of the protein and the AMA localized in protein binding site. In fact, the indolic residues of OBP are excited upon irradiation at 295 nm. Since in the very proximity of them are located AMA molecules (Forster distance), there is no emission from the indolic residues of OBP, but there is energy transfer to AMA molecules that emit at about 480 nm. The presence of the odorant molecules competes with AMA molecules for the protein active site, and since odorant molecules have a higher affinity than AMA for the protein active centre, they displace AMA molecules and interrupt the energy transfer phenomenon. This means that in the presence of the odorant molecules we cannot observe emission from AMA molecules.

2.2. SAW biosensor system

SAW biosensors consist of two-port SAW resonators obtained by two inter-digital transducers (IDTs) synchronously arranged between reflecting gratings and operating at approximately 392 MHz. The devices were implemented on α-quartz substrates (ST-cut, x propagation) and the metallic electrodes are made of Al film (100 nm thick), which is conventionally used in SAW devices for the low density, good conductivity (Zhu et al., 1996). A thin Au film (2 nm thick) is deposited on the Al electrodes to increase the adhesion of protein. In fact, Au forms strong bonds with the thiol groups of the proteins (Lee et al., 2003; Wilson et al., 2001). Both Au and Al films were grown by radio frequency (RF) magnetron sputtering technique, using a commercial MRC 8620 system, from a 99.9% and 99.999% pure Au and Al target (6.5 in.), respectively. The metals were deposited in an Ar atmosphere at a pressure of 3 mTorr and a flow rate of 90 sccm. The deposition rate was 0.167 nm/s for Al and 0.087 nm/s for Au films. The thickness of the films was estimated measuring the deposition time. The patterns were transferred by a photolithography process, using poly(methyl methacrylate) (PMMA) resist exposed by deep UV radiation, and lift-off procedure. Each IDT