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Direct and fast detection of *Alexandrium minutum* algae by using high frequency microbalance

Célia Sousa [a,b,c], Chantal Compère [d], Catherine Dreanno [d], Marie-Pierre Crassous [d], Fabienne Gas [e], Beatrice Baus [e], Hubert Perrot [a,b,*]

[a] Sorbonne Universités, UPMC Univ Paris 06, UMR 8235, Laboratoire Interfaces et Systèmes Electrochimiques, F-75252 Paris, France
[b] CNRS, UMR 8235, LISE, F-75005 Paris, France
[c] Universidade Federal do Rio de Janeiro, Instituto de Química, Av. Athos da Silveira Ramos, 149, CT, Bl. A, sala 408, Ilha da Cidade Universitária, CEP: 21.941-909 Rio de Janeiro, RJ, Brazil
[d] IFREMER, Centre de Brest, Laboratoire Détection, Capteurs et Mesures, F-29280 Plouzané, France
[e] CEA Marcoule, Direction des Sciences du Vivant, iBEB/SBTN/LDCAE, F-30200 Bagnols sur Cèze, France

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ABSTRACT

In this paper, a simple detection of a toxic algae, *Alexandrium minutum*, was developed using highly sensitive quartz crystal microbalance. In terms of performance, compared with other conventional analytical tools, the main interest of our immunosensor is based on a fast and direct detection of these living cells. This system requires the use of one monoclonal antibody directed against the surface antigen of *A. minutum*. We demonstrate that the whole living and motile algae are caught and detected. The high specificity of the biosensor is also demonstrated by testing several other dinoflagellate species. The frequency shift is correlated to the *A. minutum* cell concentration. This simple system is potentially promising for environmental monitoring purposes.

1. Introduction

Although the red tides, called harmful algal blooms (HABs), are a natural occurring phenomenon, in the last decades, they have become a serious environmental and economic problem due to their adverse impact on the ecosystem and on human health (Hallegraeff, 1993; Van Dolah, 2000). Their frequency, intensity and geographic ranges have seriously increased since the 1970's. The increasing occurrence of algal blooms reflects the alteration in ecological systems as a consequence of human activities. HABS are often attributed to eutrophication, ballast water introduction and climate change (Hallegraeff, 2010). They are, nowadays, considered as one of the major problems that coastal countries must face, and it seems that this will be even greater in the near future (Masó and Garcès, 2006). In Europe, the genus *Alexandrium* is often involved in paralytic shellfish poisoning (PSP) events. The current method for the accurate identification of this dinoflagellate is based on the microscopic examination of its specific morphological features. This procedure requires considerable broad taxonomic expertise; it is time consuming and costly. As a result, the rapid high throughput of samples is beyond the detection capabilities of most monitoring programs and does not effectively prevent or mitigate the adverse effects of these toxic events. Consequently, improved, reliable and cost effective monitoring methods are urgently needed. The application of biosensor technology is ideal for marine environmental monitoring and detection as the sensors are portable and provide selective and rapid responses in real time in the field (Kröger and Law, 2005; Zielinski et al., 2009).

Various types of sensors are currently being developed to detect microorganisms in biological and environmental samples. They are based on different transducers such as optical, electrochemical, gravimetric systems and several sensitive layers like immunomolecules or nucleic acid molecules. Quartz crystal microbalance (QCM) technology is particularly interesting for environmental monitoring and diagnosis due to its rapid analysis, satisfactory sensitivity, simple low cost instrumentation (Hao et al., 2009) and has become widely used for pathogen detection (Olsen et al., 2003; Buchatip et al., 2010; Hong et al., 2009; Shen et al., 2011; Guntupalli et al., 2012). The immunosensors are advantageous since they can be based on the direct detection of the whole cell through antibody selective layers immobilized on the transducer surface. The key feature of this approach is to obtain suitable antibodies particularly in terms of affinity and specificity. Nakanishi et al. (1996) were pioneers in using a conventional QCM and different methods of antibody immobilization to detect *Chatonella marina*.

* Corresponding author at: University Paris 06, LISE, F-75252 Paris 05, France. Tel.: +33 144277216; fax: +33 144274074.
E-mail address: hubert.perrot@upmc.fr (H. Perrot).

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Electrical sensing combined with carbon nanotube structures was recently set up to detect *Aureococcus anophagefferens* (Ishikawa et al., 2009). Nucleic sensors are currently developed since their specificity appears to be excellent (Nayak et al., 2009). However, these systems do not allow the direct detection of whole targeted microorganisms. Preliminary steps of preparation such as cell lysis and targeted DNA amplification are needed before performing the detection step. Nevertheless, sophisticated sensors have already been designed thanks to conventional DNA immobilizations, through electrochemical detection (Diercks et al., 2008), optical detection (Lagier et al., 2007) or gravimetric detection (Gamby et al., 2008; Lazerges et al., 2006). An electrochemical genosensor for the detection of *Alexandrium minutum* was developed by Diercks et al. (2008). This system uses oligonucleotide probes hybridizing rRNA genes in the ribosomes of target cells. It depends on the rRNA content of the cells, but the rRNA level varies according to the cell growth cycle leading to error estimations in cell concentrations.

A sophisticated Environmental Sample Processor (ESP) based on nucleic hybridization was successfully designed for detecting plankton and microorganisms in the field in real time (Scholin et al., 2001; Scholin, 2010). This autonomous device is state of the art technology and nowadays it is too costly for routine environmental monitoring in any national program. In France, recurrent blooms of *A. minutum* mainly occurred in Brittany (f.i Bay of Morlaix, Penzé estuary, Bay of Brest, Rance estuary). These yearly blooms developed in the period June–July and often reached cell concentrations $>1 \times 10^5$ cells L^{-1}. For sanitary monitoring purposes, when the alert threshold of 10 000 cell L^{-1} is exceeded, biotoxin analysis is then performed in shellfishes. Therefore, the sensitivity of the biosensor or monitoring system needed to meet this alert threshold. It could be achieved by the immunosensor itself or by combining a phytoplankton concentration step prior the detection analysis.

In this paper, we explore for the first time, the possibility of using QCM as a simple, rapid and label free method to detect and quantify directly *A. minutum* algae after a simple and single step of concentration. The monoclonal antibody is prepared against these microalgae and is immobilized on the gold electrodes of quartz resonators. Fast and in situ measurements are obtained using high frequency microbalance based on a stable and thermo-regulated oscillator. The limits of detection, the dose responses and the selectivity of this QCM sensor are carefully evaluated.

2. Materials and methods

2.1. Quartz microbalance apparatus

9 MHz AT-cut planar quartz crystals (14 mm diameter) were purchased from RAKON Company (RAKON FRANCE, Mougins, France). Two identical gold electrodes were deposited on both sides of the crystal with a standard keyhole shape and they were connected to a BNC connector. The crystal was mounted between two O-Ring seals inserted in a Plexiglas cell whose chamber volume was about 50 μL. Only one side of the quartz resonator was in contact with the solution. To produce a flow system, a P-1 peristaltic pump (Pharmacia) was connected to the chamber; a constant flux (70 μL min^{-1}) of solutions was maintained during the experiments. A homemade oscillator was designed to drive the quartz crystal at 27 MHz (3rd overtone). A frequency counter (Philips PM 6685) was coupled to the QCM to monitor the microbalance frequency, f_m (Fig. 1a). Under the gravimetric regime, a linear relationship was obtained between the mass change, Δm, due to the different interactions on the resonator surface and the frequency change, Δf_m. At a 27 MHz resonant frequency, a theoretical value of 0.36 ng Hz^{-1} was deduced and an experimental value of 0.35 ng Hz^{-1} was used according to Bizet et al. (2000) and to Rodriguez-Pardo et al. (2004).

To validate the gravimetric regime of such a device, electroacoustic measurements were performed on this system under the same experimental conditions. The experimental set up was based on a standard network analyzer (HP 4194A) and was computer-controlled through a lab made software written in HP-VEE® language. Electrical admittance measurements were automatically carried out with a 10 mV perturbation signal at 201 frequencies around the resonance frequency. They were monitored in real time during the different reactions and the equivalent circuit could be estimated (Garcia-Jareno et al., 2000). The series resonant frequencies, f_s, and the motional resistance, R, were extracted during the experiments every 1.5 min.

2.2. Chemical reagents

Phosphate buffered saline solution (PBS; 137 mM NaCl, 2.7 mM KCl, 10 mM $Na_2HPO_4 \cdot 2H_2O$, 2 mM KH_2PO_4, pH 7.4), H_2SO_4 95%, H_2O_2 30%, HCl, NaCl, and bovine serum albumin (BSA) were purchased from Sigma Aldrich. All the other chemicals used in this study were analytical grade.

2.3. Immobilization of the monoclonal antibody against A. minutum cells

The AMI6 monoclonal antibody against *A. minutum* was selected because of its high specificity for this study. The production, specificity and characteristics have been previously reported (Gas et al., 2009, 2010). This IgG was purified on a protein G HiTrap1® affinity column (GE Healthcare Life Sciences) prior to immobilization and diluted in PBS at the final concentration of 100 μg mL^{-1}.

The gold-coated QCM electrodes were cleaned by soaking in a Piranha solution (30% H_2O_2 and concentrated H_2SO_4, 1:3 v/v) for 20 min, rinsed thoroughly with distilled water and then, dried under argon prior to assembling them in the QCM cell. The cleaned electrodes were flushed with 0.1 M HCl, distilled water and then PBS. The antibody solution flowed through the QCM cell for 90 min at ambient temperature. The excess and unbound antibodies were removed by rinsing the chamber with PBS for 30 min. Then, a PBS solution containing 1% BSA solution was injected for 10 min to block any non-specific interactions that might occur between the gold substrate and antigens (Brewer etal., 2005). After

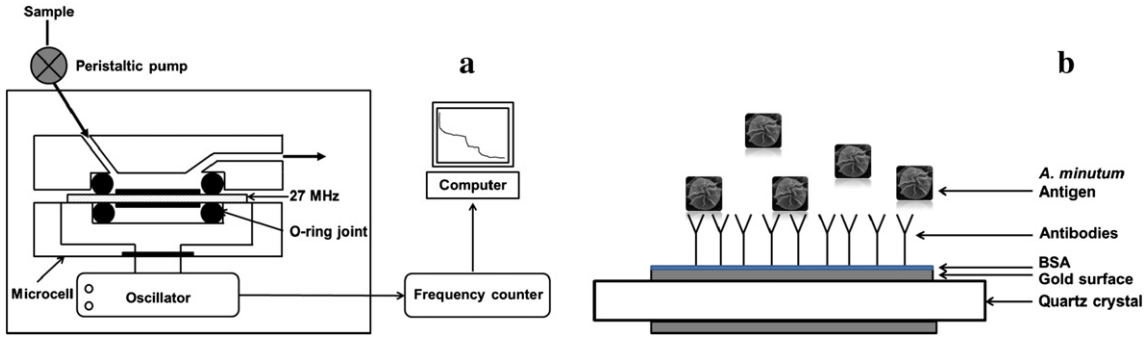

Fig. 1. Schematic illustration of the experimental QCM detection system (a) and functionalized QCM sensor by monoclonal AMI6 antibodies (b).