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Comparison of surface plasmon resonance spectroscopy and quartz crystal microbalance techniques for studying DNA assembly and hybridization

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Abstract

In this study we evaluate the strengths and weaknesses of surface plasmon resonance (SPR) spectroscopy and quartz crystal microbalance (QCM) technique for studying DNA assembly and hybridization reactions. Specifically, we apply in parallel an SPR instrument and a 5 MHz QCM device with dissipation monitoring (QCM-D) to monitor the assembly of biotinylated DNA (biotin-DNA) on a streptavidin-modified surface and the subsequent target DNA hybridization. Through the parallel measurements, we demonstrate that SPR is more suitable for quantitative analysis of DNA binding amount, which is essential for interfacial DNA probe density control and for the analysis of its effect on hybridization efficiency and kinetics. Although the QCM is not quantitative to the same extent as SPR (QCM measures the total mass of the bound DNA molecules together with the associated water), the dissipation factor of the QCM provides a qualitative measure of the viscoelastic properties of DNA films and the conformation of the bound DNA molecules. The complexity in mass measurement does not impair QCM's potential for a kinetic evaluation of the hybridization processes. For quantification of target DNA, the biotin-DNA modified SPR and QCM sensors are exposed to target DNA with increasing concentration. The plots of SPR/QCM signals versus target DNA concentration show that water entrapment between DNA strands make the QCM sensitivity for the hybridization assay well comparable with that of the SPR, although the intrinsic mass sensitivity of the 5 MHz QCM is ∼20 times lower.

Keywords: Surface plasmon resonance spectroscopy; Quartz crystal microbalance; DNA assembly; DNA hybridization

1. Introduction

Surface plasmon resonance (SPR) spectroscopy and the quartz crystal microbalance (QCM) technique have been known independently as surface analytical techniques capable of in situ monitoring of interfacial processes. One of the current trends in SPR and QCM research is to use a combined SPR and QCM data collection mode and analysis [via either dual probed devices that have both the QCM and SPR functions (Baily et al., 2002; Laschitsch et al., 2002; Bund et al., 2003; Wang et al., 2003; Zhou et al., 2004) or parallel measurements using separate instruments (Graneli et al., 2004; Laricchia-Robbio and Revoltella, 2004; Larsson et al., 2003; Höök et al., 2001a)] in order to obtain complementary details of a particular binding event. This becomes possible as SPR spectroscopy and QCM are based on different physical principles; each method being sensitive to different properties of the materials studied. SPR spectroscopy, for example, is an optical technique that detects changes in the refractive index of thin films assembled on a noble-metal surface. The measured signals are proportional to the molecular weight of the adsorbed materials, and can be used to quantify the number density of different types of adsorption. On the other hand, QCM is an acoustic wave device. It measures thin films mechanically coupled to a metal electrode on a quartz disk. The QCM oscillation frequency and quality are

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related to the mass loading and the viscoelastic properties of the adsorbed materials, respectively. For macromolecular films, QCM is sensitive to both the bound molecules and the associated solvent, e.g. water.

Since the earliest SPR- and QCM-based biosensors were reported in 1983 (Liedberg et al., 1983) and 1972 (Shons et al., 1972), respectively, these two devices have been widely used for biological analysis and clinical diagnosis (Cavic et al., 1999; Englebienne et al., 2003). The merits of the SPR- and QCM-based bioassays lie in the fact that the biomolecular binding reactions can be monitored in a label-free manner, which shortens the assay time and eliminates the use of hazardous materials and expensive lab equipments. In addition, the liquid cell configuration of the two devices makes them suitable for real-time studies of bioaffinity reactions at relevant solution conditions of temperature, flow rate, pH, ionic strength, etc.

Recently, increasing concern has been raised about the strengths and weaknesses of SPR and QCM devices if used as sensing platforms for various biological analyses. Comparisons of SPR and QCM performances for immunoassays (Kösslinger et al., 1995; Sellborn et al., 2003; Su and Zhang, 2004), blood plasma coagulation determination (Vikinge et al., 2000), enterotoxin detection (Spangler et al., 2001), enzymatic analysis (Su and O'Shea, 2001), structural analysis of proteins (Laricchia-Robbio and Revoltella, 2004; Stevens et al., 2004), DNA hybridization analysis (Larsson et al., 2003; Cho et al., 2004), and DNA–protein interactions (Su et al., 2005) have been reported. In these studies, correlations between results obtained using the two techniques are reported and SPR and QCM devices are evaluated to determine whether the sensitivity, reliability, and ease of operation are suitable for the specific bioassays.

In our study here, we compare the strengths and weaknesses of the SPR and QCM techniques for the study of DNA assembly and hybridization reactions. Specifically, we apply in parallel an SPR instrument (AutoLab ESPR) and a 5 MHz QCM device with dissipation monitoring (QCM-D) to follow the assembly of a biotinylated 30-mer oligonucleotide on a streptavidin-modified gold electrode employed for the hybridization analysis. By a combined data collection and analysis (SPR angle shift, QCM frequency shift, and QCM energy dissipation factor), we demonstrate how the different sensing principles of the SPR and QCM benefit the study of DNA film structure and how the DNA probe density affects hybridization efficiency/kinetics, as well as the viscoelastic properties of the DNA films. Also, we compare the QCM and SPR sensitivity for target DNA quantification.

2. Experimental

2.1. Materials and surface preparation

Streptavidin (SA) was purchased from Sigma. 30-Mer oligonucleotides were obtained from MWG (Germany). The probe DNA was prepared with a biotin label at the 5′-end (5′-biotin-GCACCTGACTCCTGTGGAGAAGTCT-GCCGT-3′) and the target DNA contains fully complementary sequences to the probe DNA (3′-CGTGGACTGA-GGACACCTCTTCAGACGGCA-5′). Phosphate buffered saline (PBS), composed of 10 mM phosphate buffer, 137 mM NaCl, 2.7 mM KCl, pH 7.4, was used as a carrier buffer for SA immobilization, DNA assembly and target DNA hybridization.

The gold electrodes of the SPR and QCM disks were first cleaned with hot piranha solution (a 3:1 mixture of H_2SO_4 and H_2O_2. Cautions!). The freshly cleaned disks were then immersed in a binary biotin-containing thiol mixture (10% biotin–thiol, 90% ethylene glycol–thiol at a net concentration of 1 mM in ethanol) overnight (these thiol compounds were synthesized in our laboratory in Mainz). The formula can be seen in previous papers (Su et al., 2004; Spinke et al., 1993). After rinsing with ethanol followed by a drying step using nitrogen, the disks were ready to use.

2.2. SPR measurement and data treatment

SPR measurements were conducted using a double channel, AutoLab ESPR (Eco Chemie, The Netherlands). The configuration of this equipment is described elsewhere (Su and O'Shea, 2001). The instrument is equipped with a cuvette. Gold sensor disks (diameter 17 mm) mounted to the optical lens through index-matching oil form the base of the cuvette. An autosampler (Eco Chemie, The Netherlands) is used to inject or remove the tested solutions, but the measurement of the SPR angle shift ($\Delta\theta$) was done at non-flow liquid condition, i.e. with the circulating pump paused, and at room temperature. The noise level of the SPR angle is ∼1 mdegree.

The measured SPR angle shifts were converted into mass uptakes using a sensitivity factor of 122 mdegrees = 100 ng/cm^2. In the data conversion we assumed the same equivalent SPR response per unit coverage for protein, single-stranded DNA and double-stranded DNA, respectively. This assumption is reasonable (Larsson et al., 2003; Peterson et al., 2002), as the dn/dC values (the incremental change in refractive index with concentration) for protein and DNA are very similar.

2.3. QCM-D measurement and data modeling

The QCM-D measurements were conducted using a Q-sense instrument (Q-Sense, Göteborg, Sweden). This instrument allows for a simultaneous measurement of frequency change (Δf) and energy dissipation (ΔD) change by periodically switching off the driving power of the oscillation of the sensor crystal and by recording the decay of the damped oscillation. The time constant of the decay is inversely proportional to D, and the period of the decaying signal gives f. Five megahertz AT-cut quartz crystals (Q-Sense AB. Göteborg,