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Probing the interaction of a membrane receptor with a surface-attached ligand using whole cells on acoustic biosensors

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ABSTRACT

Two different types of acoustic sensors, a surface acoustic wave device supporting a Love-wave (Love-SAW) and a quartz crystal microbalance system with dissipation (QCM-D), were used to demonstrate the potential of acoustic devices to probe the binding of a cell membrane receptor to an immobilized ligand. The class I Major Histocompatibility Complex molecule HLA-A2 on the surface of whole cells and anti-HLA monoclonal antibodies immobilized on the sensor were used as an interaction pair. Acoustic measurements consisted of recording the energy and velocity or frequency of the acoustic wave. Results showed that both devices could detect the number of cells in solution as well as the cells bound to the surface. In addition, the Love-wave sensor, which can sense binding events within the relatively short distance of ~50 nm from the device surface, was sensitive to the number of bonds formed between the cell membrane and the device surface while the QCM-D, which can sense deeper within the liquid, was found to respond well to stimuli that affected the cell membrane rigidity (cytochalasin D treatment). The above results suggest that acoustic biosensors can be a powerful tool in the study of cell/substrate interactions and acoustic devices of different type can be used in a complementary way.

1. Introduction

Molecular interactions that govern cell–cell and cell–substrate communication are of great importance in biology. Assays that employ whole cells and focus on the interactions that drive a particular biological process in a non-invasive way are particularly useful (Cooper, 2004; Fang et al., 2006a). Biosensors offer certain undoubted advantages: speed of analysis, versatility, no use of labels and non-invasiveness. In particular, acoustic biosensors can be extremely advantageous for studying events such as cell adhesion and motility, since they respond not only to net mass changes, but also to differences in the physiological conditions of cells or cell layers (Heitmann et al., 2007). Quartz crystal microbalance (QCM) sensors have been successfully used in several studies of dynamic cellular processes (Ergezen et al., 2007; Galli Marxer et al., 2003; Hong et al., 2006; Li et al., 2007, 2008b; Marx et al., 2001; Wegener et al., 2000). In these works, the QCM signal (frequency change) was shown to depend on the degree of attachment of the cell body via interactions between proteins on the cell membrane and the sensor surface, as well as the distance between the cell membrane and device surface and the condition of the cytoskeleton. Cell attachment was also shown to cause increased energy dissipation of the acoustic wave (Janshoff et al., 1996; Li et al., 2005). However, despite these efforts, it is still unclear how much and in what way each variable, i.e., cell type, size and physiology, membrane molecules and surface modification, contributes to the acoustic signal (Heitmann et al., 2007). Another type of biosensor, the surface acoustic wave (SAW) sensor, has also been employed in such studies. Due to the fact that SAW sensors generally operate at higher frequencies than QCM, they are potentially more sensitive (Ballantine et al., 1997). In particular, the Love-wave configuration of SAW sensors (Gizeli, 1997) offers the means to probe cell surface receptor/immobilized ligand interactions since the acoustic wave is confined where the actual binding occurs, i.e., within ~50 nm from the surface, and does not extend further in the cell body. Signal change has been shown to depend on the number of bonds between cell membrane proteins and surface immobilized antibodies (Saitakis et al., 2008).

In this work, we compare the response of the two aforementioned acoustic sensors, QCM-D and Love-SAW, during the study of specific cellular binding events using whole cells. Specifically, the acoustic signals, i.e., energy loss expressed as amplitude or dissipation and phase or frequency change, were compared during the binding of the class I MHC molecule HLA-A2 (used as a cell surface receptor) to the immobilized anti-HLA-A2 monoclonal

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antibody. The probed HLA/anti-HLA binding pair was chosen as a model system to mimic the function of this cell membrane molecule that mediates cell–cell intercommunication (Bjorkman and Parham, 1990). A Surface Plasmon Resonance (SPR) sensor was also employed for additional support information. The advantages of the two acoustic sensors are clearly demonstrated and are also compared to each other.

2. Materials and methods

2.1. Preparation of biosensor surfaces

Gold-coated device surfaces were plasma-etched in oxygen plasma with a Harrick Plasma Etcher (Harrick). The surfaces were then incubated in a protein G (Calbiochem) solution (1 mg mL^{-1}) for 1 h at room temperature. Following protein adsorption, each device was inserted in the device holder, washed and left to equilibrate with PBS phosphate buffer (Sigma) at a flow rate of 50 $\mu L\,min^{-1}$. A solution of 10 $\mu g\,mL^{-1}$ of the anti-HLA-A2 monoclonal antibody BB7.2 (Becton Dickinson) was pumped over each biosensor surface under a 25 $\mu L\,min^{-1}$ flow rate and the interaction with the gold-adsorbed protein G was monitored in real time. The surface density of the immobilized proteins was measured using SPR (SR7000, Reichert, USA) and found to be 2.19 ng mm^{-2} for protein G and 1.46 ng mm^{-2} for anti-HLA. All experiments were performed at 25 °C. Experiments with cells involved addition of cell suspensions with cell number ranging from 6.0×10^4 to 3.0×10^6 cells mL^{-1} over the anti-HLA immobilized antibody at a flow rate of 10 $\mu L\,min^{-1}$.

2.2. Cell cultures and treatments

The EBV-transformed human B-lymphoblastoid cell line LG2 (HLA-A*$0201^{+/+}$) (kindly provided by Dr. H. Reyburn, Dept. of Immunology and Oncology, National Centre of Biotechnology, Spain) was used in this work. RPMI 1640 (GIBCO Inc.) supplemented with 1 mg L^{-1} gentamycin and 10% of fetal bovine serum was used as culture medium. Culture flasks were kept in humidified 5% CO_2 atmosphere at 37 °C. The medium was exchanged every 2–3 days. The cell density was 3–8 $\times 10^5$ cells mL^{-1} in normal cultures and 2–3 $\times 10^6$ cells mL^{-1} in high density cultures. Viability was checked prior to experiments by the trypan blue exclusion technique and cell numbers were counted on a Neubauer slide. Dead cells were never over 5% of the total. Cells were washed with PBS buffer, centrifuged at 250 g and resuspended in PBS prior to addition to the sensor surface.

To prepare mild acid-treated cells, in order to remove cell surface HLA-A2 associated peptides, LG2 cells were briefly (90 s) treated with ice-cold pH 3.2 citric acid-Na_2HPO_4 buffer (van der Burg et al., 1995) (mixture of an equal volume of 0.263 M citric acid and 0.123 M Na_2HPO_4) and then washed with PBS. Using an indirect quantitative immunofluorescence assay (QIFIKIT, Dako) and flow cytometry (FACSculiber, Becton Dickinson) as previously described (Saitakis et al., 2008), the number of HLA-A2 molecules on the cell surface was calculated.

2.3. Love-wave sensor setup

The Love-SAW acoustic device, operating at 110 MHz, has been described elsewhere (Gizeli, 1997, 2000). Devices were cleaned and mounted on a special holder and liquid was pumped over the sensing area using a peristaltic pump (Gilson) and a flow-through cell. The flow cell exposed a sensing area of 12 mm^2. A Hewlett-Packard 4195A network analyzer was used to monitor the amplitude and phase of the wave and LabVIEW (National Instruments) software for collecting the data.

2.4. QCM-D sensor setup

The Q-sense D300 system (Q-sense, Sweden) was used in this study. The system offers simultaneous measurements at operating frequencies of 5, 15, 25 and 35 MHz. Following preparation, the device was inserted in the special holder, providing temperature control. The accompanying software QSoft 3.1 was used to monitor and collect acoustic data, i.e., frequency and energy dissipation (Rodahl et al., 1997). The flow was controlled with a peristaltic pump.

2.5. Microscopic observation of sensor surfaces

Following the end of a Love-wave experiment, the biosensor surface was observed under the microscope. Since the sensor chip is not opaque, no fluorescent staining was needed for the observation of cells. Similarly, at the end of each experiment performed on a SPR refractometer (SR7000, Reichert Analytical Instruments), the SPR sensor surfaces (bare gold chips, obtained from XanTec) were observed under the microscope with no need for staining. Cells on the sensor surfaces were counted from at least three areas.

For the QCM-D experiments, the chip was removed from the system and prepared for staining with propidium iodide (Sigma) in order to measure the number of cells attached on the sensor surface. Briefly, the surface was incubated at room temperature for 20 min with paraformaldehyde 4%. After washing three times with PBS, triton-X 0.1% in PBS was added for 5 min. Following that treatment, the surface was washed three times with PBS and incubated for 5 min at dark with propidium iodide (1/1000, v/v). The sensor surface was then observed under a Nikon Eclipse E800 microscope and pictures were taken with an attached ProgRes CF camera (Jenoptik).

2.6. Treatment with cytochalasin D

Cytochalasin D (Sigma), at a concentration of 5 μM in PBS containing 0.05% dimethylsulfoxide (DMSO) (Sigma), was pumped over the sensor surface for ~100 min. Negative controls were performed using PBS containing 0.05% DMSO. No significant cellular toxicity was observed in the time frame of the acoustic experiments. For the QCM experiments, the sensor surfaces were stained with rhodamine-conjugated phalloidin (Fluka) which binds with higher affinity to actin filaments than to actin monomers allowing direct observation of the state of actin polymerization inside the cell. Briefly, the sensor chip was removed from the system and incubated at room temperature with paraformaldehyde 4% for 40 min. After washing three times with PBS, triton-X 0.5% in PBS was added for 10 min. Following that treatment, the surface was washed with PBS and 1% bovine serum albumin in PBS and was incubated for 40 min (dark) with rhodamine-conjugated phalloidin (1/100, v/v). The surfaces were then observed under the microscope.

3. Results

For the study of HLA/anti-HLA interaction, the LG2 B-lymphoblastoid cell line was used expressing the human class I MHC molecule HLA-A2 at the cell membrane. The LG2 cells were attached to the sensor after treatment of the gold surface with protein G followed by an anti-HLA monoclonal antibody (Fig. 1). Protein G which adsorbs irreversibly on gold was used to specifically bind antibodies via their Fc fragment resulting in an oriented immobilization (Fahnestock et al., 1986; Saha et al., 2003). The anti-HLA molecule applied in this work recognizes the α-chain of HLA when the latter exists in a heterotrimer form; this form consists of α chain, β_2-microglobulin and a bound peptide (Hogan and Brown, 1992; Parham and Brodsky, 1981). It has been shown that anti-HLA adsorbed on the sensor surface under these conditions forms