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Detection of refractive index changes in individual living cells by means of surface plasmon resonance imaging

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ABSTRACT

Real time imaging of living cell activation is an increasing demand in disciplines of life science and medicine. We previously reported that surface plasmon resonance (SPR) sensors detect large changes of refractive index with living cells, such as mast cells, keratinocyte, human basophils and B-cells activated by biological stimuli. However, conventional SPR sensors detect only an average change of refractive index with thousands of cells at detectable area on a sensor chip. In this study, we developed an SPR imaging (SPRI) sensor with a CMOS camera and an objective lens in order to analyze refractive index of individual living cells and their changes upon stimuli. The SPRI sensor could detect reactions of individual rat basophilic leukemia (RBL-2H3) cells, mouse keratinocyte (PAM212) cells, and human epidermal carcinoma (A431) cells in response to either specific or non-specific stimuli, such as antigen, phorbol ester or epidermal growth factor, with or without their inhibitors, resembling signals obtained by a conventional SPR sensor. Moreover, we distinguished reactions of different type cells, co-cultured on a sensor chip, and revealed that the increase of refractive index around nuclei is rapid and potent as compared to that in peripheries in the reaction of RBL-2H3 cells against antigen. This system may be a useful tool to investigate the mechanism of refractive index-changes evoked in near-membrane fields of living cells, and to develop a system of high-throughput screening for clinical diagnosis.

1. Introduction

There is a continuously increasing demand for biosensors to detect living cell activation (Ziegler and Copel, 1998). We previously reported that surface plasmon resonance (SPR) sensors could detect real-time large changes of refractive index (RI) in response to the activation of living cells, such as mast cells, keratinocytes, basophils and B lymphocytes on a sensor chip without labeling, suggesting the potential of SPR as a new diagnostic method for allergy and immunology (Hide et al., 2002; Yanase et al., 2007a,b; Suzuki et al., 2008). Moreover, similar RI changes upon epidermal cell activation were also observed by resonant waveguide grating (Fang et al., 2006). The RI change may reflect interactions of a variety of biomolecules, including proteins, oligonucleotides, lipids, and even small structures (<500 nm) such as cells, in the field of evanescence (Rich and Myszka, 2000; Karlsson, 2004; Cooper, 2003; Szabo et al., 1995). However, we have demonstrated that reactions detected by SPR sensors are not limited to changes of the area of cell adhesion, the binding of ligands to cell surface and subcellular structure of living cells which are observed using an ordinary light microscope (Yanase et al., 2007a). In mast cells, the activation of the protein kinases Syk, Lat, Gads and protein kinase C β (PKCβ) are indispensable for the antigen-induced RI increase of mast cells detected by SPR biosensors (Tanaka et al., 2008). Thus, SPR sensor possesses great potential to reveal nano-scale living-cell actions in the field of evanescence. However, conventional SPR sensors detect only an average RI change in the presence of thousands of cells in an area of the sensor chip, and could offer only a small number of sensing channels (<10). Therefore we could not detect RI changes in individual cells, so that reactions of target cells in a mixture of different cell types could easily be overlooked. In consequence, construction of array systems for cell activation was difficult. Finally, conventional

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SPR sensors could not reveal the intracellular distribution of RI changes, thus impairing progress in understanding the mechanism of RI changes upon cell activation. A recently developed SPR imaging (SPRI) system determines a spatial RI distribution at the SPR-active surface by measuring the distribution of light intensity which proportionally reflects RI on the surface of sensor chip in an image captured by a camera (Berger et al., 1998; Homola et al., 2005). It can be used as multichannel SPR sensor if the sensing area is divided into multiple spots. Here, we developed an SPRI system for living cell analysis in order to detect RI changes in individual cells induced by stimuli, analyze subcellular distribution of RI changes and confirm the relationship between RI changes and morphological changes predicted by a conventional SPR, and investigated the utility of the sensor for multiple spots cell analysis for clinical diagnosis.

2. Materials and methods

2.1. Reagents

The chemicals used were obtained from the following sources: bovine serum albumin (BSA), diniro-phenol-conjugated human serum albumin (DNP-HSA) and DNP-specific rat monoclonal IgE form Sigma–Aldrich Japan (Tokyo, Japan). Hydrocell from Cell Seed Inc. (Tokyo, Japan). Phorbol 12-myristate 13-acetate (PMA), ionomycin, Gö6976, genistein, wortmannin and cytochalasin D from Calbiochem (SanDiego, CA). Anti-human IgE antibody from BETYL (Montgomery, TX), Human IgE purified from CHEMICON International (Temecula, CA). Epidermal Growth Factor (EGF) from R&D systems (Minneapolis, MN). The cDNA of human FcεRIα-subunit (Kochan et al., 1988), rat γ-subunit (Blank et al., 1989) and pEF-BOS, were kindly provided by Dr. G Alber (University of Leipzig, Germany) and Dr. J.P. Kinet (Beth Israel Deaconess Medical Center, Boston, MA) and Dr. Nagata (Osaka Bioscience Institute, Osaka, Japan) respectively.

2.2. Instrument for SPR imaging (SPRI)

Developed SPRI sensor was composed of a light source (630 nm diode laser, Sigma Koki Co., Ltd., Tokyo, Japan), P-Polarizer (Sigma Koki Co., Ltd., Tokyo, Japan), prism (S-LAL-10, RI = 1.72), thermostat (Olympus Corporation, Tokyo, Japan), objective lens (×4, Edmund Optics Japan, Tokyo, Japan) andCMOS camera (Monochrome-digital CMOS camera, 1280 × 1024 pixel, 15 fps, Fortissimo Corporation, Tokyo, Japan) (Fig. 1a). The sensor chips (S-LAL-10, 20 mm × 20 mm × 1 mm, RI = 1.72) were coated with gold thin film (1 nm Cr layer and 49 nm gold layer) by means of vapor deposition (Osaka Vacuum Industrial Co., Ltd., Osaka, Japan). Flow chamber was composed of cover glass, PEEK tubes and silicone rubber (20 mm × 20 mm × 5 mm) of which the center was cut out as flow space. Obtained images and changes of light intensity of individual cells were analyzed with Image-Pro (Media Cybernetics, Bethesda, MD).

2.3. Cell culture

RBL-2H3 cells were cultured in Roswell Park Memorial Institute (RPMI) medium supplemented with 10% fetal calf serum (FCS), 100 U/ml penicillin, and 100 μg/ml streptomycin as described previously (Yanase et al., 2007a,b, 2010). The day before experiments, RBL-2H3 cells were harvested using trypsin. They were then cultured in the presence or absence of 50 ng/ml anti-DNP IgE on a sensor chip for SPRI analysis or in hydrocell for floating culture. RBL-2H3 cells cultured in a floating condition were placed onto a sensor chip 20 min before measurement. PAM212 cells and A431 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FCS, 100 U/ml penicillin, and 100 μg/ml streptomycin. On the day before experiments, cells were harvested using trypsin and then cultured on the sensor chip for SPRI analysis.

2.4. SPRI measurement

Sensor chip, on which living cells were cultured, was placed on prism with matching fluid (RI = 1.72). The flow cell was placed on a sensor chip and then washed with buffer (1,4-piperazinediethanesulfonic acid (PIPES) buffer for RBL-2H3 cells and RBL-3D4 cells, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer for PAM212 cells and A431 cells (Yanase et al., 2007a)). For pretreatments with inhibitors, cells were exposed to inhibitors for indicated time followed by stimulation. The stimulation was performed in the flow chamber by injection with a manual syringe at 37 °C. Images which show the distribution of reflected light intensity were taken every 10 s using CMOS camera. The change of light intensity (refractive index changes) in individual cells surrounded by AOI(area of interest), from which the intensity of the area with-

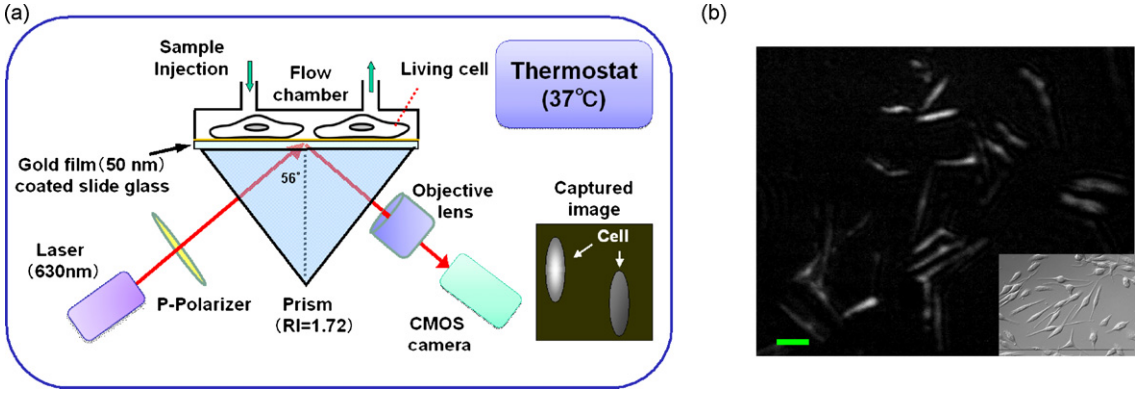

Fig. 1. Development of surface plasmon resonance imaging (SPRI) sensor for individual living cells. (a) The sensor is composed of a diode laser emitting at the wavelength of 630 nm, P-polarizer, S-LAL-10 glass prism, sensor chip (S-LAL-10) coated with thin gold film (50 nm), flow chamber for living cells, thermostat for regulating the temperature at 37 °C, objective lens (×4), CMOS camera. The light was directed to the prism surface at the incident angle of 56°. Two-dimensional images were created from the light reflected at the interface between gold film and glass using objective lens and CMOS camera. Living cells were visualized by the difference of RI between buffer solution and living cells. (b) Thin gold film-coated slide was observed under phase contrast microscopy (upper panel) and SPRI system (lower panel). Diameter of each gold dot is 30 μm. (c) Distribution of RI intensity on the surface of a sensor chip on which RBL-2H3 cells are cultured. Inlet shows RBL-2H3 cells observed under differential interference contrast microscopy (Carl Zeiss, Oberkochen, Germany). Bar shows ca. 20 μm.