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Interfacing Whispering Gallery Mode Optical Microresonator Biosensors with the Plant Defense Elicitor Chitin

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ABSTRACT

The biomaterial class of chitooligosaccharides (chitin), commonly found in insects and fungi, is one of the most abundant on earth. Substantial evidence implicates chitin in mediating a diverse array of plant cellular signaling events, including the induction of plant defense mechanisms against invading pests. However, these recognition and mediation mechanisms, including the binding kinetics between chitin and their plant recognition receptors, are not fully understood. Therefore, the creation of a platform capable of both interfacing with chitin and plant cell receptors, and monitoring their interactions, would significantly advance our understanding of this plant defense elicitor. Recently, a label-free, highly sensitive biosensor platform, based on Whispering Gallery Mode optical microresonators, has been developed to study such biomolecular interactions. Here, we demonstrate how this unique platform can be interfaced with chitin using simple carbohydrate chemistry. The surface chemistry is demonstrated using X-ray photoelectron spectroscopy, fluorescence microscopy, optical profilometry, ellipsometry, and contact angle measurements. The resulting surface is uniform, with an average surface roughness of 1.25 nm, and is active toward chitin recognition elements. Optical loss measurements using standard quantitative cavity analysis techniques demonstrate that the bioconjugated platforms maintain the high performance ($Q > 10^6$) required to track binding interactions in this system. The platform is able to detect lectin, which binds COs, at 10 μg/mL concentration. This biosensor platform's unique capabilities for label-free, high sensitivity biodetection, when properly interfaced with the biomaterials of interest, could provide the basis for a robust analytical technique to probe the binding dynamics of chitin–plant cell receptors.

1. Introduction

A great number of agriculturally important crops suffer devastating losses every year from plant diseases. Of these diseases, fungal pathogens lead to the largest economic loss [1]. Plants depend upon their innate immune response to defend against these pathogens [2–4]. This immune response is triggered by two levels of microbial recognition: (1) the detection of pathogen (microbe) associated molecular patterns [P(M)AMPs] by pattern recognition receptors (PRRs) and (2) effector triggered immunity (ETI) [3]. PAMPs are characterized both by being structurally conserved, or identical, across a wide array of microbes, and by being not produced by the host. Numerous studies have shown that PAMPs elicit an immune response in a number of agriculturally significant plants, such as soybean, rice, barley, and wheat [5–7]. PAMP signaling is also a key element in animal, including human, innate immunity systems [8,9].

The ability of plants to recognize various PAMPs enables plants to quickly defend against fungal, bacterial, and insect invaders. One of the most common PAMPs is chitin, a fungal cell wall constituent [10]. Chitin is comprised of polymers of N-acetylglucosamine (GlcNAc) residues linked together through a β1–4 glycosidic

Abbreviations: CO(s), chitooligosaccharides; P(M)AMPs, pathogen (microbe) associated molecular patterns; PRRs, pattern recognition receptors; ETI, effector triggered immunity; GlcNAc, N-acetylglucosamine; CEBiP, elicitor binding protein; Os, oryzasativa; CERK1, chitin elicitor receptor kinase 1; MAPK, mitogen-activated protein kinase; ELISA, enzyme-linked immunosorbent assay; WGM, Whispering Gallery Mode; SPR, surface plasmon resonance; APTMS, 3-aminopropyltrimethoxysilane; XPS, X-ray photoelectron spectroscopy; Q factor, quality factor; FITC, fluorescein isothiocyanate.

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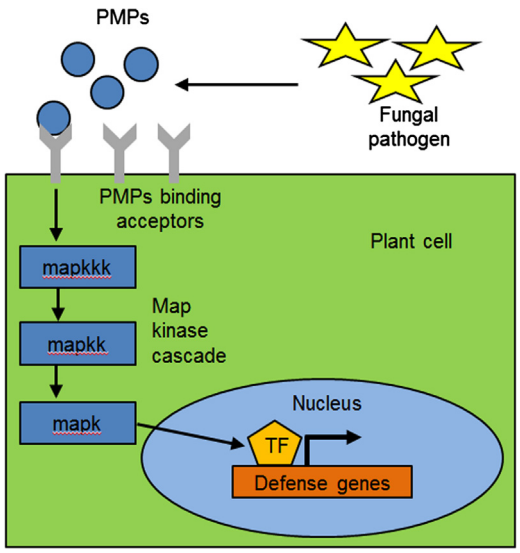

Fig. 1. Model of the PAMP signaling pathway. Fungal pathogens release pathogen-associated molecular patterns (PAMPs), which bind to plant membrane associated pattern recognition receptors. The binding of PAMP leads to activation of the MAPK cascade, ultimately activating transcription factors, which mediate the expression of defense related genes.

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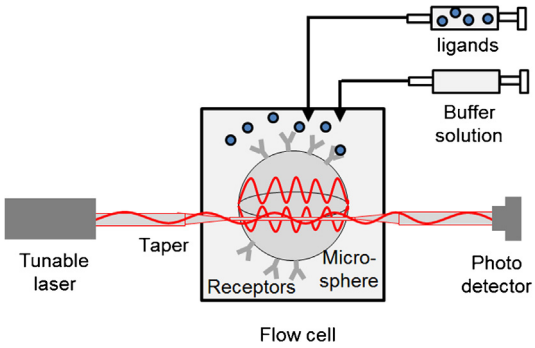

Fig. 2. Schematic of a WGM optical biosensor to detect ligands in buffered solutions. In this WGM optical biosensor platform, the bioconjugated silica microsphere WGM optical microresonator is immersed in a buffer solution within a "micro-aquarium" style housing. Incoming laser light from a tunable laser is coupled into the microsphere by a tapered optical fiber. When analytes are injected into the "micro-aquarium," they are able to bind to the immobilized receptors on the bioconjugated device. This changes the optical properties of the circulating light in the device, leading to detectable shifts in the device's resonant frequency.

linkage. Chitin is synthesized through the action of chitin synthases, which represent a very large gene family, and is found in fungi, insects, bacteria, protozoa, and vertebrates [11]. In order for chitin to induce an immune response in plants, plasma membrane proteins, known as pattern recognition receptors (PRRs), must recognize and bind the chitin (Fig. 1) [12–14].

Several different chitin elicitor binding proteins have been identified in several plant species. Each elicitor has at least one lysine (LysM) domain. For instance, in *Arabidopsis thaliana*, a chitin elicitor receptor kinase (AtCERK1) with three LysM domains and an intracellular serine/threonine kinase domain, has been identified (AtCERK1/LYK1) [15,16]. It is well known that only chitooligosaccharides (COs) of dp > 7 have significant elicitor activity, at least on rice and arabidopsis. However, a key issue with the AtCERK1 structure is its relatively low K_d values, in the μM range, while a variety of biological assays have shown that COs can act at concentrations below 10 nM. Once COs bind to the specific receptors, downstream signaling will occur. Chitin binding to AtCERK1 results in activation of a mitogen-activated protein kinase (MAPK) cascade through phosphorylation (Fig. 1). This signaling cascade is responsible for activating the transcription of genes directly involved in pathogen defense, as well as transcription factors that will activate other defense-related genes (in Fig. 1) [17–19].

Although some aspects of the chitin–receptor systems are reasonably understood, their fundamental interaction processes need to be studied further to elucidate how they trigger immune responses, using a broad range of biological, chemical, and physical approaches [20]. Previous work with label-based affinity binding assays, isothermal titration calorimetry, and molecular methods have established techniques for determining if a plant cell receptor will interact with chitin, but these methods lack either or both the sensitivity or specificity required to further elucidate the kinetic parameters of their actual interactions [20]. An alternative approach, based on a robust, sensitive and specific biosensor, would allow more detailed studies of the kinetic parameters of these interactions, as well as structural data to explain the chitin receptors affinity for various COs.

Optical biosensors are non-destructive sample interrogation methods with an ability to perform extremely sensitive detection in liquid environments due to their high signal-to-noise ratios. Common, high sensitivity optical methods for biosensing include labeled immunoassays, such as the ELISA assays and fluorescent immunoassays. Label-free optical biosensors, such as optical waveguides [21–23], surface plasmon waveguides and resonators [24–29], and WGM microresonators [30], have demonstrated high sensitivity detection in multi-component systems when paired with an appropriate recognition element. These types of highly sensitive optical sensors have easily demonstrated μM to nM thresholds of sensitivity, and in the case of WGM microresonators, shown in Fig. 2, are capable of detection down to the single nanoparticle or single virus level (aM to fM thresholds) [31–34].

The functionality of WGM microresonators as biosensor platforms derives from their high sensitivity to changes in the environment [35]. WGM microresonators can efficiently confine light at specific resonant frequencies, which is then circulated within the microresonator's periphery. Due to their unique geometric optics and inherent signal amplification, WGM microresonators can achieve ultra-low detection limits [33,36–39]. This is because the WGM microresonators' optical field extends slightly into the surrounding environment through evanescence [40,41]. The primary gauge of WGM microresonator performance is the device's quality factor, or Q factor, which describes the photon lifetime (and is dependent on the microresonator's optical loss) in the microresonator and is directly related to its ultimate or ideal sensitivity. For example, a high-Q device ($Q > 1.0E+08$) has a high photon lifetime (>100 ns); a single photon in such a device will circulate, and potentially interact, over 100,000 times [40]. In comparison, planar evanescent sensors, like waveguide sensors or SPR platforms, typically allow input photons to interact only once with the environment. Using high-Q WGM microresonators, the interaction of molecules with the optical field can be detected via shifts in the resonance frequency of the microresonator. This shift is a result of a change in the effective refractive index of the optical field, which itself can be due to small changes in diameter, optical loss, etc. In these devices, the high photon lifetime within the resonator imparts a high degree of sensitivity to the device, enabling the label-free detection of molecules, such as antigens, antibodies, and bacteria [40].

Recently, WGM microresonators have also been used to study the binding kinetics of biomolecular interactions. For example, Soteropulos et al. [42] determined the kinetic parameters of biotin–streptavidin binding using silica microsphere WGM microresonators. For these reasons, this highly sensitive biosensor platform has the potential to be very useful in defining kinetic