Amplified detection of avidin-biotin binding using terahertz wave technology

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Abstract

A new affinity biosensor based on a novel terahertz (THz) wave technology, differential time domain terahertz spectroscopy (DTDS), has been used to monitor the binding between biotin and avidin molecules. Amplified detection of ligand-receptor binding on supported membranes composed of a biotin bilayer on quartz modified octadecanol surface is a useful model lipid membrane for studying avidin-biotin complexes. To this end, and in order to increase the sensitivity of terahertz optical biosensor, we have developed a new amplification methodology based on agarose beads. Conjugating agarose particles with avidin and applying the conjugate to already bound-biotin on the quartz surface, the biotin binds the conjugate rapidly and causes an enhancement of THz difference signal, between biotin and biotin-avidin complexes, by more than six fold when compared to the same sample without agarose beads. Using this method we have been able to detect less than 10.3 ng/cm^2 avidin, thus giving THz system a powerful detection capability of sub-thin solid films better than ellimsometry and reflectometry techniques. This amplification procedure may trigger a cascade of applications in THz sensing and imaging, for example by encapsulating highly scattering beads, such as gold and carbon, with residues that might localize tumors, the technique should be able to help increase the image special resolution, and consequently detect cancer in its earliest stages.

Key words: differential THz time-domain spectroscopy, biosensor, biotin-avidin
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1 INTRODUCTION

In the panoply of biosensor technology a plethora of transducers has been developed and used successfully for detection purposes. In the UV- visible optical region, detection of ligand-analyte binding is mainly accomplished by the application of either surface plasmon resonance spectroscopy (SPR) or fluorescence spectroscopy [1,2]. These methods have registered enormous success and now being used routinely in analytical laboratories; however, to the best of our knowledge, the number of biosensors operating in the far infrared region is either scarce or inexistent. The only techniques that have been recognized as a potentially useful tool for monitoring the on-off binding in the far infrared region are attenuated total reflection Fourier transform infrared (ATR-FTIR) and Raman spectroscopy [3,4], but the complications associated with cryogenic detectors (FTIR), and Raleigh lines (Raman) are among the anomalies that hampered FTIR and Raman spectroscopy from being widely used. Recently, a new optical method has emerged as an alternative option. Time domain terahertz (TH-TDS) with a non-ionizing source has emerged as a new optical sensing technique to monitor the on off binding of label free antigen-antibody, lipid-protein interactions, DNA hybridization, and drug discovery without system perturbation [5–7].

In the ever-advancing field of biosensing systems there is always a need for methods to increase the system sensitivity to signal resulting from binding of one biological molecule to another. There is also a desire for flexibility in the types of ligands that mimic phenomena occurring in nature. To satisfy these criteria, we have developed a novel biochemical technique, based on small agarose beads, which can offer one such solution owing to some of their unique features: (a) they provide a large outer surface area where target molecules can be attached; (b) their spherical shapes provide a very flexible, cell membrane-like environment. Therefore, by employing a secondary interaction with a small agarose beads, conjugated with a ligand or receptor, which binds the surface-bound compound, the THz optical difference signal between ligand and ligand-analyte affinity bound component can be amplified.

Avidin is a protein, which is comprised of four identical subunits, each binding one biotin molecule. The affinity binding between avidin and biotin is so high (Ka 1015 mol^{-1}) in a way that the formation of this complex can be regarded as nearly irreversible, on a scale nearly comparable to a covalent bond [8]. The high affinity binding of this system has found many applications, e.g., in affinity chromatography and in attaching antibodies to solid surfaces, precipitating liposomes, or targeting cells with liposomes [9,10]. Importantly, it has been shown that biotin can be adsorbed to hydrophobic surface without loosing its specificity towards avidin, leading to the possibility of studying ligand-analyte interactions on supported lipid membrane.

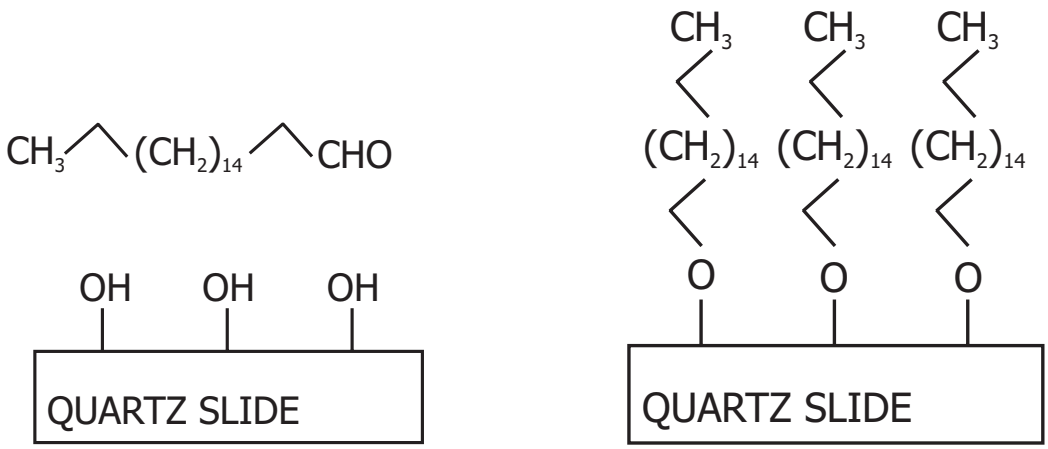
The aim of this paper is to demonstrated the capability of biochemical means to amplify the optical terahertz signal from a previously affinity bound compound in a lipid membrane like environment. This amplification methodology is simple, non-invasive, inexpensive, and does not require hardware modification as in conventional methods.

2 MATERIALS AND METHODS

2.1 Chemicals

The following chemicals were used as obtained without further purification: Octadecanal, bovine serum albumin, biotin, agarose beads conjugate avidin in 0.01 M sodium phosphate pH 7.0, containing 0.02% sodium azide from Sigma (St. Louis, MO). Quartz microscope slides (25×25 mm) were obtained from Electron Microscopy Sciences (Washington, PA), and tested for thickness homogeneity before use. All reagents were HPLC grade and used as received. Aqueous solutions were prepared in doubly distilled de-ionized water.

2.2 Sample Preparation



(a) Biotin unattached (b) Biotin attached

Fig. 1. Modification of quartz crystal surface by self-assembly octadecanol molecule. the hydrophobic moiety of octadecanol allows interaction with biotin through hydrophobic interaction.

Prior to deposition, the quartz slides were cleaned in 50% hot nitric acid for an hour, and then rinsed thoroughly in double distilled water. The quartz

slides were further subjected to 1 mg/ml octadecanal solution for half an hour, after drying and washing with double distilled water, the sample was dipped in 0.5 mg/ml biotin dissolved in chloroform/methanol (5:1) and allowed to incubated for 45 minutes. The spontaneous organization of biotin lipid into the octadecanol self-assembled bilayers guarantees certain level of stability to the octadecanol-biotin complexes. To eliminate the effect of non specific interactions, the quartz slides were further incubated in 1% bovine serum albumin, dried and washed several time with phosphate buffer solution (PBS). Either 10.3 ng of avidin in 200 ml double distilled water or avidin conjugated to agarose beads was spread on 1 cm^{-2} quartz surface modified biotin and allowed to incubate for half an hour. After drying the sample was washed with water to remove unbound molecules.

2.3 THz Spectrometer

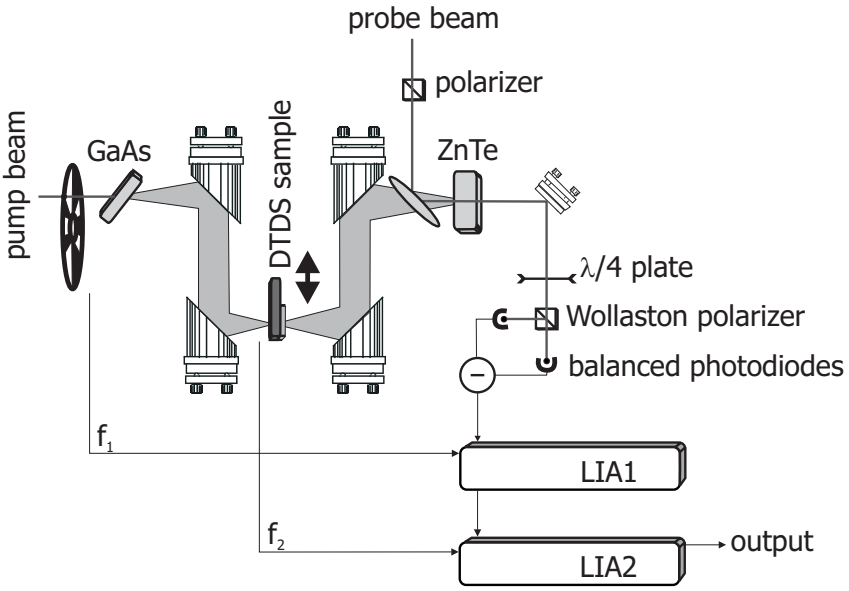


Fig. 2. Terahertz biosensor set up showing an unbiased GaAs crystal THz emitter, and ⟨110⟩ ZnTe crystal detector. The sample is dithered in and out of the THz beam by a galvanometer.

The double modulated terahertz differential spectroscopy DTDS used in this work was described in more details in our previous paper [7], and schematically represented in figure 2. Briefly, the femtosecond laser source generates 150-fs pulses at 86 MHz and 1.5 W of average power. A beam splitter separates the laser beam into pump and probe pulses. The probe pulse illuminates an unbiased GaAs semiconductor emitter wafer to generate a THz beam, which is collimated and focused onto an electro-optic sampling crystal with paraboloidal mirrors. A pellicle after the second parabolic mirror allows the reference beam to travel collinearly with the THz wave across the electro-optic crystal ⟨110⟩

ZnTe. A quarter wave plate (QWP), a Wollaston prism (P) and a pair of photodiodes are assembled for the balanced detection of the THz radiation. The reference and sample on quartz slides was mounted in a galvanometer and dithered in the THz beam at 10 Hz over a peak-to-peak distance of about 10 mm.

3 RESULTS & DISCUSSION

The need for a new non-invasive biosensor technology in health care, food monitoring and weapon detection is becoming increasingly important in our daily life. Optical detection techniques have been used for more than a decade in the analytical laboratory since they provide a possible means of meeting such needs. Up to date the most successful optical biosensor in the market place is surface plasmon resonance (SPR); however, more recently a new detection method based on terahertz wave operating on the same principal as SPR has been proposed, and used successfully to detect minute amount of label-free analysis of DNA molecules [5]. An integrated wave-guide approach, incorporating resonant Hz structures, has been presented by Nagel's team (at the Rheinisch-Westflalish Technisch Hochschule, Aachen, Germany). Using the newly developed THz system the authors achieved a detection limit of a femtomole sensitivity label free of DNA hybridization [5]. Although, this approach is still in progress and may open up new avenues for label-free detection, it is far from being prone of drawbacks. Hardware modification, difficulty of interconnecting a large number of resonator for the simultaneous detection of thousands of genomes in a single experiment, and delicate sample handling are just few of the problems facing the aforementioned work. To overcome these difficulties, we have developed a new biochemical methodology, based on agarose beads that can achieve higher sensitivity without hardware modification. Clearly, by conjugating agarose particles to avidin and applying the conjugate to half of the quartz surface modified biotin, the THz difference signal between biotin and biotin- avidin complex increased sharply. We have been able to detect less than 10.3 ng/cm^2 avidin on quartz surface, indicating the potential capability of the newly developed method for future application of DNA hybridization and antigen-antibody interactions. Compared to similar avidin-biotin experiment, but without agarose beads, an enhancement factor of six folds has been observed (Figure 3). Although the origin of signal enhancement is still under investigation, we believe that this fact is most probably due to the enhanced refractive index of agarose particles. If it is the case, further enhancement can be easily achieved by conjugating target molecules to gold or carbon micro spheres.

In order to test for the non-specific interaction of biotin towards avidin, and avoid false positive results, a control sample has been prepared. In this re-

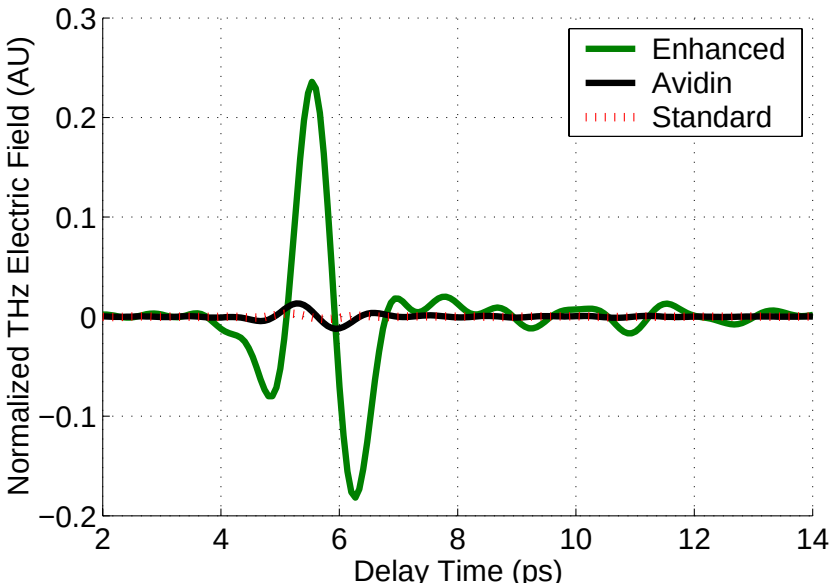


Fig. 3. Time domain THz pulses obtained by measuring the difference signal between a) biotin and biotin-avidin conjugated agarose, (b) biotin and biotin-avidin complexes without beads, (c) biotin and biotin-DGDG (control sample).

gard, when a quartz crystal bearing biotin was incubated with a suspension of galactosyldiacyl glycerol lipid (DGDG) instead of avidin tagged agarose, we found that the THz differential signal drops dramatically, indicating that the previously observed THz signal is solely due to biotin-agarose conjugated avidin. The THz signal resulting from biotin-DGDG interaction is presumably due to the surface of biotin inhomogeneity or the non-specific adsorption of DGDG on biotin surface. Selectivity was not quantified in this report, but we demonstrated that the specificity of biotin towards DGDG is practically negligible. This is a significant result that exemplifies the ability to use molecular recognition as the primary source of biotin selectivity towards avidin. The inherently high affinity of avidin-biotin complementarity makes this immunosensor highly attractive as an analytical device for a wide variety of biochemical study. Owing to the technical difficulties encountered in the preparation of protein markers, the biotin-avidin system has proved of particularly broad application; almost any biologically active molecule can be tagged with biotin or avidin with only minimal effect on biological activity. Likewise antibody-antigen technology would also benefit from this new amplified THz detection method. Since antibodies can be produced to almost all classes of substances, e.g., proteins, polysaccharides, nucleic acids, and to more complex particles such as pollens, infectious agents, viruses, and tissue cells, we expect that the enhanced DTHz system should be able to detect minute amount of a wide range of biomolecules.

4 CONCLUSIONS & FUTURE WORK

Much attention has long been devoted to the surface modification of metal and carbon electrodes with other biological materials for the purpose of obtaining high performance biosensor.

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