Integrated optical biosensor for detection of multivalent proteins

Dan Kelly and Karen M. Grace

Nonproliferation and International Security Division, Los Alamos National Laboratory, Los Alamos, New Mexico 87545

Xuedong Song and Basil I. Swanson

Chemical Science and Technology Division, Los Alamos National Laboratory, Los Alamos, New Mexico 87545

Daniel Frayer, Sergio B. Mendes, and Nasser Peyghambarian

Optical Sciences Center, University of Arizona, Tucson, Arizona 85721

Received June 29, 1999

We have developed a simple, highly sensitive and specific optical waveguide sensor for the detection of multivalent proteins. The optical biosensor is based on optically tagged glycolipid receptors embedded within a fluid phospholipid bilayer membrane formed upon the surface of a planar optical waveguide. Binding of multivalent cholera toxin triggers a fluorescence resonance energy transfer that results in a two-color optical change that is monitored by measurement of emitted luminescence above the waveguide surface. The sensor approach is highly sensitive and specific and requires no additional reagents and washing steps. Demonstration of protein-receptor recognition by use of planar optical waveguides provides a path forward for the development of fieldable miniaturized biosensor arrays.

OCIS codes: 130.0130, 130.6010, 170.0170.

Environmental detection of biological agents and rapid diagnosis of infectious diseases represent formidable challenges for fieldable detection technologies. Recent research[1–12] into biological detection has been driven by this need for reliable diagnostics of infectious disease, biological toxins, and pathogens. What are needed are simple, reagentless, and fieldable sensor systems that are capable of simultaneous detection of multiple species with sensitivities and specificities comparable with those of the best current laboratory-based technologies. The two mature detection technologies, gene probe methods based on nucleotide sequencing[1–3] and immunoassays based on antibody–antigen recognition,[4–7] are not amenable to simple, low-cost hand-held sensor arrays that are needed for field or point-of-care analysis. These approaches require excessive front-end sample preparation, multiple unstable reagents, and several steps, and they are costly as well as personnel intensive.

We recently used flow cytometry to demonstrate a detection platform that is based on cell-membrane mimics and optical signal transduction triggered by protein-receptor binding.[8] This detection platform relies on proximity-based fluorescence resonance energy transfer triggered by protein-receptor binding for multivalent proteins. Multivalent proteins, such as cholera and avidin, bind multiple receptors, thereby bringing them into close proximity and inducing the fluorescence resonance energy-transfer signal. Cholera binds Gm_1, a glycolipid receptor that decorates the upper leaf of the target cell membrane. We have functionalized lyso-Gm_1 to attach donor and acceptor fluorophores and then embedded the tagged Gm_1 into the fluid upper leaf of a phospholipid supported bilayer that is coated onto glass beads for flow cytometry measurements. This approach exhibits high sensitivities and specificities that are comparable with those of the best laboratory-based immunoassay approaches (low tens of picomolar in sensitivity).

Detection efforts based on the structure-function dependence of cell surface receptors show promise in developing highly specific, highly sensitive, and reagent-free biological detection methods that address some of the limitations of immuno-based assays and DNA sequencing schemes.[8–12] Some researchers have exploited such methods by attaching recognition elements of planar optical transduction platforms and measuring luminescence or fluorescence signals or by measuring effective refractive-index changes through interferometry. In particular, phospholipid-based membranes are suitable for developing surfaces that can be tailored to recognize and adsorb biological species.[8,10] Phospholipid bilayers also provide ideal surfaces to minimize the effect of nonspecific binding. Nonspecific binding of proteins to the optically tagged receptor molecules was studied with albumin and peanut lectin and found to give no response even at concentrations 2–3 orders of magnitude higher than that of cholera toxin.[8]

Although flow cytometry offers an established and sensitive detection method, there is an increasing demand for sensors that are less expensive and extremely compact and that have low power requirements. To address these needs we recently adapted the biosensor approach outlined in Ref. 8 to evanescent fluorescent techniques that use planar optical waveguides. We report here preliminary experimental results for an integrated optical biological toxin sensor. The sensor

0146-9592/99/231723-03$15.00/0

employs a planar optical waveguide as a transduction platform. The sensing element comprises a phospholipid membrane that incorporates optically tagged toxin receptors that is formed upon the surface of the waveguide. Toxin detection is achieved by measurement of the ratio of emission intensity from a donor–acceptor pair of fluorophores that are tagged onto the receptors. The ratio of fluorescent emission intensity depends on the concentration of toxin.

The experimental setup is shown schematically in Fig. 1 and explained below. A waveguide structure is fabricated by sputter deposition of a 400-nm-thick film of Corning 7059 glass ($n = 1.56$) onto one surface of a standard microscope slide ($n = 1.51$). A surface relief grating for incoupling of light to the waveguide structure is fabricated onto the slide by holographic exposure in photoresist and ion milling. The waveguide is housed within a fluid cell into which a 3×10^{-4} M solution of palmitoyl, 9-octadecenoyl phosphatidylcholine (POPC) vesicles is flowed, resulting in formation of a hybrid POPC bilayer through vesicle fusion on the waveguide surface. Before bilayer formation, the POPC vesicles are incubated with a 1.5×10^{-6} M solution of optically tagged cell-membrane ganglioside receptors (Gm_1). Gm_1 is the receptor targeted by cholera toxin protein. The donor–acceptor optical tags are a pair of fluorophores chosen such that the emission curve of the donor significantly overlaps the adsorption curve of the acceptor. For the research reported here, half of Gm_1 is tagged with Bodipy TMR (BTMR, donor fluorophore) and half is tagged with Bodipy TR (BTR, acceptor fluorophore). We couple incident laser light from a frequency-doubled YAG laser ($\lambda = 532$ nm) into the waveguiding layer by aiming the circular laser spot onto a diffraction grating (period $\Lambda = 400$ nm) at the appropriate angle of impingement. This coupling produces a streak of light visible to the eye, approximately 3 mm wide, that propagates along the Corning glass layer. Experimental parameters are such that the streak propagates approximately 13 mm along the waveguide surface. Along the path of the streak the evanescent tail of the waveguided light penetrates from the Corning glass waveguiding layer into the POPC bilayer. Fluorescence emission is collected from outside the fluid cell by a fiber-optic spectrophotometer. A 5-mm-diameter lens ($f = 10$ mm) is positioned approximately 20 cm from the light streak and collimates emitted fluorescence into a 600-μm-diameter fiber. A long-pass filter with a cut-on wavelength of 550 nm is used to filter out the laser excitation source.

Shown in Fig. 2 are the spectral curves for fluorescence emission from the POPC bilayer for exposure to varying concentrations of cholera toxin. Cholera toxin was introduced into the fluid cell through a syringe port. Successively increasing cholera toxin concentrations were achieved by addition of sufficient quantities of a stock cholera toxin solution to the flow cell to produce the effective concentrations shown in Fig. 2. In all cases spectral curves were obtained within minutes of toxin addition to the fluid cell. The intensities of the spectral curves have been normalized at the isobestic point (610 nm) for BTR-Gm_1 and BTMR-Gm_1 emission from a POPC bilayer.[13] The wavelengths of peak fluorescence emission for BTMR and BTR tagged to Gm_1 and incorporated within the POPC bilayer are 575 and 625 nm, respectively. The inset of Fig. 2 shows the ratio of BTR to BTMR peak intensity taken from the spectral curves and denoted R_i, i.e., $R_i = I_{\lambda=625\,\mathrm{nm}}/I_{\lambda=575\,\mathrm{nm}}$. As can be seen from Fig. 2, a concentration ratio of 200:1 for (POPC):(Gm_1) results in an initial (before cholera toxin exposure) R_i of approximately unity. This is not surprising considering the relatively high concentration of fluorophore-tagged Gm_1 receptors distributed on the waveguide surface. Assuming a site area of 5 nm/POPC molecule and a random distribution of Gm_1 receptors within the bilayer on the surface, the average distance between fluorophores before the addition of cholera toxin is approximately 10 nm. Energy from the 532-nm laser source is four times more efficient at stimulating fluorescence from BTMR-Gm_1 than from BTR-Gm_1

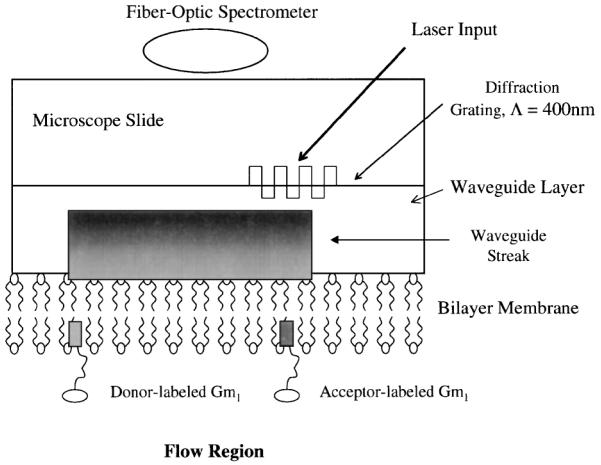

Fig. 1. Overhead schematic view of integrated optical biosensor (see text for explanation).

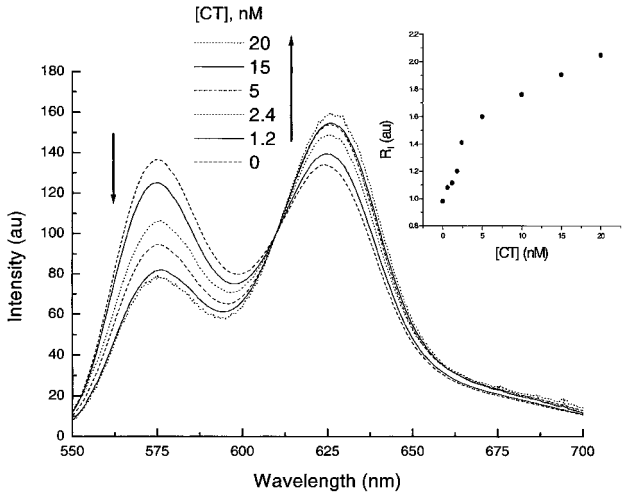

Fig. 2. Fluorescence spectra of BTMR-Gm_1 and BTR-Gm_1 within a POPC bilayer attached to the waveguide surface parametric in concentration of cholera toxin (CT). Inset, ratio of fluorescence emission at peak intensity for BTR-Gm_1–BTMR-Gm_1 as a function of exposure to CT.

incorporated in a POPC bilayer. Hence the emission from BTMR-Gm_1 is due primarily to laser excitation, whereas the emission from BTR-Gm_1 is due to a combination of laser excitation and resonance energy transfer from BTMR-Gm_1. These observations are further borne out by the fact that decreasing tagged Gm_1 while keeping POPC constant results in R_i of less than unity. The inset of Fig. 2 indicates that R_i increases nearly linearly from 1 to 1.55 with increasing CT concentration for CT from 0.6 to 5 nM. Above CT concentrations of 5 nM the increase in R_i slows with increasing CT, reaching 2.1 at CT = 20 nM.

The linear increase of R_i with CT at lower concentrations mimics results obtained previously[8] for cholera toxin detection by use of a POPC bilayer incorporating BTMR-Gm_1–BTR-Gm_1 receptors deposited upon glass microbeads and analyzed with flow cytometry. Our results support the explanation that addition of pentavalent CT induces an aggregation of tagged Gm_1 receptors. The aggregation of BTMR and BTR within the upper leaf of the POPC bilayer brings these fluorophores within close proximity to one another, allowing for efficient fluorescence resonance energy transfer. This proximity-induced energy transfer accounts for the increase in R_i with increasing CT exposure. We note that detection over the CT range shown in Fig. 2 is comparable with that of previous studies that involved the detection of cholera[8] and streptavidin[10] by use of receptors bound within a POPC bilayer. In our present research we assume that measurements of fluorescence emission collected from the waveguided streak of light are representative of the entire microscope slide surface; i.e., we have a uniform POPC bilayer with randomly distributed Gm_1 receptors on the waveguide surface. Further assuming irreversible CT adsorption within this uniform POPC bilayer allows us to convert liquid-phase molar concentrations to an effective site-area based mass loading of toxin. In doing so we find that adsorption of all toxin molecules from a fluid cell with CT = 1 nM corresponds to a mass loading of 300 pg/mm^2 on the waveguide surface.

The kinetics and reversibility of binding are complicated owing to the multivalent nature of the receptor–protein interaction. The binding is reversible but only on an extremely long time scale,[14] and the rate-determining steps are the diffusion of the protein to the membrane surface and the first Gm_1–cholera binding step. Subsequent binding of additional Gm_1 receptors is fast owing to the short diffusion times of receptors on the two-dimensional membrane surface. The change of the normalized ratio of the acceptor to donor emission varies linearly with cholera loading up to a cholera/receptor ratio of 1/5, at which point the emission change saturates. The use of this sensor to quantify cholera concentration is therefore related to the rate of change of the ratio of emission intensities under relatively high cholera concentrations for which equilibrium is not attained. However, at extremely low (picomolar) cholera concentrations the system is in equilibrium, the normalized ratio of emission intensities does not saturate, and the concentration of cholera is directly related to the absolute change in the ratio of emission intensity.

The benefits of the detection method described here are numerous. Fluorophores incorporated within the upper leaf of a biomimetic membrane are relatively stable and free from interaction with adsorbing interferents. Also, measuring the fluorescence emission ratio from an energy-transfer pair of fluorophores provides a detection signal that is independent of nonspecific adsorption of the analyte or binding of interferent proteins. This energy-transfer detection signal is not susceptible to temperature fluctuations or fluorescence quenching and alleviates the need for measuring absolute fluorescence intensities. Finally, this sensor system responds in a time of the order of minutes and does not require the addition of reagents to facilitate detection.

This work was supported by Los Alamos National Laboratories discretionary funding under the auspices of the U.S. Department of Energy. K. M. Grace's e-mail address is kgrace@lanl.gov.

References

1. R. Elghanian, J. J. Storhoff, R. C. Mucic, R. L. Letsinger, and C. A. Mirkin, Science **277,** 1078 (1997).
2. B. D. Hames and S. J. Higgins, eds., *Gene Probes* (IRL Press, New York, 1995).
3. J. Wang, E. Palecek, P. E. Nielsen, G. Rivas, X. H. Cai, H. Shiraishi, N. Dontha, D. B. Luo, and P. A. M. Farias, J. Am. Chem. Soc. **118,** 7667 (1996).
4. P. Wood, in *Principles and Practice of Immunoassay*, C. P. Price and D. J. Newman, eds. (Stockton, New York, 1991), p. 365.
5. J. R. Astles and W. G. Miller, Anal. Chem. **66,** 167 (1994).
6. S. P. A. Fodor, J. L. Read, M. C. Pirrung, L. Stryer, A. T. Lu, and D. Solas, Science **251,** 767 (1991).
7. B. A. Cornell, V. L. B. Braach-Maksvytis, L. G. King, P. D. Osman, B. Raguse, L. Wieczorek, and R. J. Pace, Nature **387,** 580 (1997).
8. X. Song, J. Nolan, and B. I. Swanson, J. Am. Chem. Soc. **120,** 11514 (1998).
9. J. Dubendorfer, R. E. Kunz, E. Schurmann, G. L. Duveneck, and M. Ehrat, J. Biomed. Opt. **2,** 391 (1997).
10. B. Klee, G. L. Duveneck, P. Oroszlan, M. Ehrat, and H. M. Widmer, Sensors Actuators B **29,** 307 (1995).
11. G. L. Duveneck, M. Pawlak, D. Neuschafer, E. Bar, W. Budach, U. Pieles, and M. Ehrat, Sensors Actuators B **38–39,** 88 (1997).
12. S. J. Choquette, L. Locascio-Brown, and R. A. Durst, Proc. SPIE **1368,** 258 (1990).
13. The isobestic point for BTMR-GM_1 and BTR-GM_1 incorporated within a POPC bilayer was determined in Ref. 8.
14. X. Song and B. I. Swanson, Anal. Chem. **71,** 2097 (1999).