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Short communication

A proteomic biosensor for enteropathogenic *E. coli*

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Abstract

The study of proteins and the molecules with which they interact on an organismwide scale is critical to understanding basic biology, and understanding and improving human health. New platform technologies allowing label-free, quantitative array-based analysis of proteins are particularly desirable. We have developed an analytical technology, reflective interferometry (RI), which provides specific, rapid, and label-free optical detection of biomolecules in complex mixtures. In order to evaluate the suitability of RI for proteomics, we have prepared a series of arrays bearing the extracellular domain of the secreted enteropathogenic *Escherichia coli* (EPEC) protein Translocated Intimin Receptor (Tir). These arrays are able to selectively detect the extracellular domain of the protein Intimin, Tir's natural binding partner. Furthermore, we demonstrate the use of RI and Tir-functionalized arrays for the selective detection of EPEC directly from culture.

Keywords: Interferometry; Biosensor; Proteomics; EPEC

1. Introduction

The burgeoning field of proteomics promises to yield vital new information about fundamental cellular processes, and is also likely to lead to new diagnostic tests for a wide variety of human diseases. Developing new technologies for the rapid and sensitive detection, identification, and quantitation of large numbers of proteins in parallel is crucial to furthering both these fundamental and applied goals. To date, the primary technologies employed in the proteomics field for protein detection include 2-D gel electrophoresis (Hakansson et al., 2003), mass spectrometry (Nedelkov et al., 2002), and protein arrays in which a signal is generated via a fluorescently tagged antibody or sandwich assay (Huang et al., 2001; Belov et al., 2001). While each of these technologies has led to notable advances, it is generally recognized that each has drawbacks as well (Mitchell, 2001; Howbrook et al., 2003). Clearly, the development of new quantitative analytical techniques for use in protein detection is important. In particular, methods that are label-free, allowing for direct detection of the target protein without need for additional labeling or secondary antibody steps, are most desirable. Of these, surface plasmon resonance (SPR) (Daly et al., 2000) is the most broadly used and available. Commercial SPR instruments for single analytes have been produced for some time, and an array-based technique termed SPR imaging has been described by several groups (Smith et al., 2003; Shumaker-Parry and Campbell, 2004; Nelson et al., 2001). We recently introduced a new label-free analytical technique, termed reflective interferometry (RI), as a potential alternative to SPR (Lu et al., 2004) and other interferometric methods (Tünnemann et al., 2001; Arwin, 2000). While our initial efforts focused on DNA detection, several aspects of RI, particularly its label-free nature and compatibility

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with standard immobilization chemistry, made it highly attractive as an array-based protein detection method. We present herein our first studies on the application of RI to proteomics.

Detection strategies and instrumentation developed in the context of proteomics efforts also have broad applicability in medical diagnostics. Bacterial identification, traditionally dependent on culture, staining, and visual inspection by trained microbiologists, is one area where such methods can have a particular impact. Enteropathogenic *Escherichia coli* is the primary cause of diarrhea and mortality in children worldwide (Clarke, 2001; Nataro and Kaper, 1998), and employs a complex, but well studied, network of proteins in its mechanism of infection, making it an ideal target on both fundamental and applied grounds. Using arrays functionalized with the Intimin-binding domain of Translocated Intimin Receptor (Tir), a key component of EPECs virulence mechanism, we have been able to selectively detect EPEC directly from culture.

EPEC and other pathogenic *E. coli* strains secrete Tir via the Type III secretory pathway (Zaharik et al., 2002). Following secretion, Tir inserts itself into the intestinal epithelium, acting much like a "molecular harpoon". Tir is then able to bind Intimin, a protein expressed on the EPEC cell surface, providing a docking site for the EPEC cell (Goosney et al., 2000). Extensive biochemical studies by the Finlay and Strynadka labs led to the observation that the extracellular domains of Intimin and Tir (herein Intimin-ECD and Tir-IBD, respectively) from enteropathogenic *E. coli* retain significant affinity for one another, and culminated in a determination of the Tir-IBD/Intimin-ECD structure by X-ray crystallography (Fig. 1) (Luo et al., 2000). In our hands, the affinity of Tir-IBD for Intimin-ECD, as measured by isothermal titration calorimetry (350 nM), was on par with the reported literature value (312 nM). Secreted proteins such as Tir are particularly attractive as probe molecules, since they (or, more precisely, their extracellular domains) are typically simple to produce in large quantity. Furthermore, the corresponding cell surface protein Intimin is present in high abundance on the surface of EPEC, effectively "amplifying" the amount of signal (in terms of numbers of binding events) that can be produced by a single bacterium.

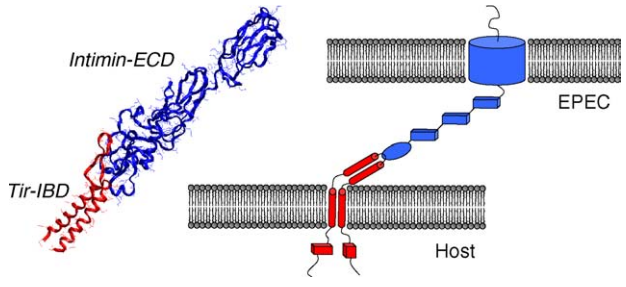

Fig. 1. Left: Extracellular fragments of the EPEC proteins Intimin (Intimin–ECD) and Tir (Tir–IBD). Right: Schematic of EPEC anchoring mediated by Intimin–Tir binding.

2. Materials and methods

2.1. Preparation, purification, and quantification of proteins

Vectors for both 6xHis–Tir-IBD and 6xHis–Intimin-ECD were obtained from the Strynadka and Finlay groups, and transformed into expression strain BL21(DE3) and BL21(DE3)pLysS, repsectively. Proteins were expressed as described in Luo et al. Because the proteins were expressed as 6xHis-tagged fusions, purifications were completed using HiTrap Chelating HP columns (Pharmacia). Samples were added to the column by a peristaltic pump at 0.5 mL/min to facilitate coordination to the resin. Pure fractions were then subjected to dialysis for 24 h to purge the remaining imidazole introduced during the eluting process. Tir-IBD was dialyzed into a buffer containing 20 mM HEPES and 150 mM NaCl at pH 7.5 (HBS), and Intimin-ECD was dialyzed into HBS with 3 mM EDTA and 0.005% Tween-20 at pH 7.3 (HBS-ET). Concentrations of dialyzed proteins were measured by UV absorbance at 280 nm and theoretical molar extinction coefficients.

2.2. Chip preparation and functionalization

12 mm × 25 mm chips were diced from 6 in. silicon wafers bearing a 1400 Å coating of SiO_2, supplied by Silicon Valley Machines. Chips were then cleaned with Piranha solution (1:2, H_2O_2:H_2SO_4) by immersion for 30 min. With a sufficiently clean surface, derivitization chemistry could begin with the addition of a solution of (3-aminopropyl) triethoxysilane (APTES) (1:20, 5% APTES in ddH_2O:acetone) for 15 min. After thorough rinsing with ddH_2O and drying under a stream of N_2, chips were baked at 100 °C for 30 min. A glutaraldehyde solution (1:39, 50% aqueous glutaraldehyde:50 mM phosphate buffer, 150 mM NaCl, pH 7.5) was then introduced to the chips for 20 min. After again rinsing with ddH_2O and drying under a stream of N_2, this provided aldehyde-functionalized chips ready for covalent attachment of amines. For the specificity experiment, 2 μL of 500 μM Tir-IBD and 2 μL of 500 μM Ubiquitin were manually spotted on each chip and allowed to stand in a humidified chamber for 60 min. Remaining aldehydes were deactivated by immersion of each chip in a 500 μM solution of glycine methyl ester (GME) in water (pH 1.0) for 45 min. Prior to exposure, chips were rinsed with ddH_2O and dried under a stream of N_2.

2.3. EPEC and controls

EPEC serotype RDEC-1 (O15:H^-) was purchased from the American Type Culture Collection (ATCC) as a freeze-dried pellet. A small amount was used for a 25 mL overnight growth in Luria–Bertani (LB) media. A 2 mL aliquot was then harvested by centrifugation and resuspended in Dubecco's Modified Eagle Medium (DMEM). The aliquot then was used to inoculate 50 mL of DMEM, in order to upregulate Intimin