Biosensors and Bioelectronics 77 (2016) 131–136

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Virus-based assay for antigen detection using infective growth as signal transduction mechanism

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ARTICLE INFO

Article history:
Received 1 July 2015
Received in revised form
10 September 2015
Accepted 11 September 2015
Available online 12 September 2015

Keywords:
Phage
Antibody
Infection
Z Domain
Visual detection

ABSTRACT

Viruses have the ability to infect and thereby confer new phenotypes on host cells. *E. coli*, for example, if infected by viruses containing antibiotic resistance genes, can benefit by surviving in the presence of the corresponding antibiotics to grow into colonies observable by the naked eye. Using this concept as a signal transduction mechanism for our immunoassay, we have engineered ampicillin resistant virions to display a dimer of the z domain from Protein A. This zz-domain selectively binds to the conserved heavy domain of IgG across various species. As commercially available antibodies are in no short supply, this engineered virion can be used modularly with existing antibodies for converting the presence of target antigen into a visually detectable colony forming unit. Here we demonstrate that this scheme for zz-phage transfection and selective growth of infected *E. coli* can facilitate sub-nanomolar detection limits for target antigen. Moreover, this phage infectivity assay works over a range of concentrations competitive with existing ELISA techniques. Because this system is derived from self-regenerating components (*i.e.*, virus and bacteria) and furthermore obviates the need for chromogenic substrates or spectroscopic equipment, we find it particularly suitable for use in regions where cost effective detection is a necessity.
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1. Introduction

Viruses such as filamentous bacteriophage have been widely used for their ability to be engineered to control the display of functional peptides and proteins for library screening and drug lead discovery among other applications (Cwirla et al. 1990; Griffiths and Duncan 1998; Merzlyak and Lee 2006). Within the realm of engineered virus systems, phagemids have been commonly used as they are able to provide small vectors for simple genetic modification of the minor phage coat proteins such as p3 which is present in 3–5 copies displayed on the end of the phage virion (Barbas et al. 1991). In addition, because the phagemid can be easily designed to possess genes for antibiotic resistance, engineered virions carrying a beta lactamase gene, for instance, can confer ampicillin resistance (Amp^r) to a host cell after infection (de la Cruz and Davies 2000). As a result, infected host *E. coli* can be rapidly selected and enriched by applying the ampicillin antibiotic to the media to hinder growth of any non-infected cells. In doing so, the number of colony forming units (C.F.U.) corresponding to the amount of viral infections is easily determined. By further engineering of this phagemid system to display an IgG binding domain (derived from protein A) on the p3 coat, we produced a modular phage capable of selectively attaching to the conserved domain (Fc region) of antibodies derived from several organisms. A wealth of previous reports have shown that this three helix domain, called z-domain, derived from staphylococcal protein A can bind with high affinity to the Fc region of IgG (Nilsson et al. 1987). Because the z-domain (and Protein A in general) can bind a broad range of IgG classes, they have been particularly attractive components of immunosensor technologies (Jose et al. 2009; Ko et al. 2009; Lee et al. 2003). The z-domain has even been integrated into the p3 coat of filamentous phage for a range of immunological applications including receptor discovery (Braisted and Wells 1996; Nord et al. 1996). Here we incorporate a dimer of this z-domain, specifically the zz sequence designed as part of the tandem affinity purification tag (Rigaut et al. 1999), into the amino terminus of the p3 gene on an ampicillin resistant phagemid system. The resulting virions using this phagemid system, referred to herein as zz-phage, prove to be an effective probe for transducing an antibody–antigen binding event into a visually detectable colony *via* infection and selective growth of host *E. coli*. The benefit of this approach compared to standard phage ELISA is an enhancement in the target detection limit. We find that this approach can be utilized with antibodies derived from several mammalian hosts which are widely commercially available. While

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http://dx.doi.org/10.1016/j.bios.2015.09.028
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this virus-based transduction approach requires a longer time for completion, it compensates by utilizing inexpensive self-replicating components thereby mitigating the need for costly chromogenic substrates or spectroscopic equipment.

The use of viruses as components of detection systems for targets of interest is relatively rare but certainly gaining in popularity. Perhaps the most intuitive use of phage in sensing is for detecting the presence of bacteria using phage as the selective recognition element which has been demonstrated to be highly selective (Fernandes et al. 2014; Tawil et al. 2012). Recent virus-based sensing systems have also shown detection of small chemicals, including the explosive trinitrotoluene, by using the ability of liquid crystalline filamentous virus films to change color in response to target analyte binding (Oh et al. 2014). Other effective virus-based sensors have used modification of a phage ELISA technique in which displayed peptide fragments (Wang et al. 2015) or protein A coupled p3 systems (Brasino et al. 2015) provide selectivity through antibody binding along with reporter enzymes coupled to the virus main coat to achieve high quality colorimetric detection. Similarly, coupling of the virus major coat with DNA-gold nanoparticles has shown the possibility of transduction of the binding event into a colorimetric signal (Lee et al. 2012) or alternatively detection through Raman scattering (Lee et al. 2014). In contrast to these approaches, we provide a detection strategy for quantifying the presence of target antigen without the need for extensive virus processing or additional coupling reagents. In this proof of concept, we show that engineered phage containing the zz domain can directly serve as an effective transducer of antibody binding information into visually observable colony forming units allowing detection of small amounts of protein target (Figs. 1 and S1). In doing so, we make use of the natural infectivity of a single virus to generate a new phenotype within a host *E. coli* cell which amplifies the signal into a quantized single colony of bacteria for each individual virus after incubation.

Here we demonstrate this unique phage infectivity based assay for the proof of concept case of detecting target glucose oxidase in order to provide a model system for assessing the range and detection limits of our system. Glucose oxidase was chosen as the target for this proof of concept, since it can be quantified by kinetic assays to ensure the amount of target present. We provide a direct evaluation of our work relative to existing ELISA/phage-ELISA approaches to allow assessment of our zz-phage infection system relative to traditional enzyme-driven colorimetric reporter based approaches. Furthermore, we confirm that our zz-phage will work effectively with IgG derived from mouse, human, and rabbit due to the inherent specificity of the z-domain. We reveal significant benefits of our approach in target sensitivity for our form of virus-based detection over phage-based ELISA. Furthermore, because direct quantification is achievable without the need for spectroscopic tools, we find our approach to offer a lower cost than traditional ELISA Table S1.

2. Materials and methods

2.1. Genetic engineering of zz-phage

Primers were purchased from Cosmogenetech Co. Ltd. (South Korea) and restriction enzymes as well as ligase were purchased from New England Biolabs (USA). The zz-phage were constructed based on a phagemid/helper phage system utilizing a modified pCantab6 amp^r phagemid (McCafferty et al. 1996) and were rescued using M13KO7 helper phage. The pCantab6 phagemid p3 gene was genetically modified to contain the zz domain near the amino terminus after the leader signal sequence for SecYEG mediated periplasmic translocation (Scott et al. 2008). The insert containing the zz domain was obtained by PCR of a TAP tag insertion cassette derived from pFA6a-TAP-His3MX (Ghaemmaghami et al. 2003) using the following primers (zz for: ACAACTGCAGGTGGACAACAAATTCAACAAAGAACAAC; zz rev: TATTGCGGCCGCCTGATGATTCGCGTCTACTTTCG) to generate PstI and NotI restriction sites, respectively. The vector in which the insert was to be ligated was produced by vector PCR of pCantab6 DNA with the following primers (pcanta for: AAGCGGCCGCGACTGTTGAAAGTTGTTTAGCAAAACCTC; pcanta rev: CGAGCTCCTGCAGTTGGACC) to yield corresponding PstI and NotI restriction sites for subsequent cloning. After PCR, the insert and vector DNA were digested with DpnI for 1 h at 37 °C followed by column purification and then underwent double restriction digest with PstI and NotI for 1 h at 37 °C. The DNA was again column purified and eluted with 20 uL of sterile water. The combined insert and vector (8.5 uL total) was mixed with 1 uL of T4 ligase buffer and 0.5 uL T4 DNA ligase and incubated for 2 h at 22 °C. The ligation was directly added to 100 uL of chemically competent TG1 *E. coli* for transformation by heat shock and subsequent growth on LB-ampicillin plates overnight at 37 °C for selection of successful clones confirmed by sequencing. A schematic of the zz phage virion and phagemid vector

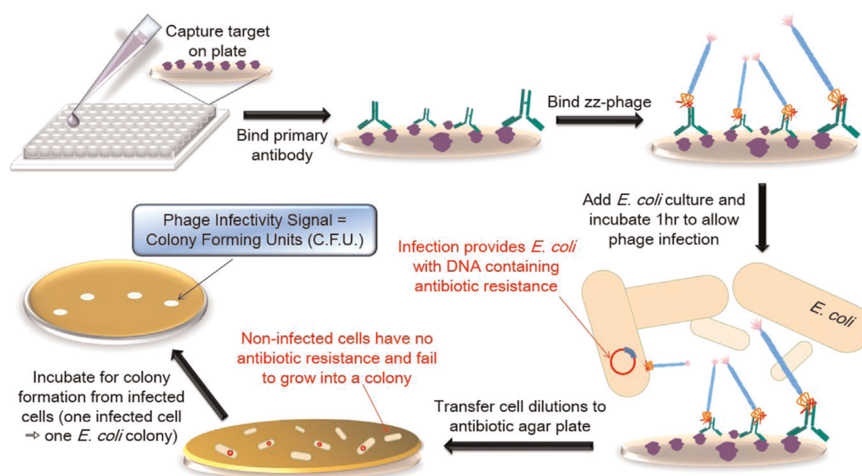

Fig. 1. A workflow schematic of the phage infection based method for detecting bound target antigens. Immobilized antigen is first bound by primary antibody, followed by addition of zz-phage virions able to selectivity by with the Fc region of IgG. Addition of E. coli to the wells results in phage infection of the *E. coli* to confer resistance to ampicillin antibiotic. The number of phage, determined as colony forming units, provides an effective measure of the amount of bound antigen with low detection limits.

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