Biosensors and Bioelectronics 99 (2018) 289–295

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

# Selection of affinity peptides for interference-free detection of cholera toxin

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ARTICLE INFO

*Keywords:*
*Vibrio cholerae*
Cholera toxin
Phage display
Affinity peptide
LSPR
SERS

ABSTRACT

Cholera toxin is a major virulent agent of *Vibrio cholerae,* and it can rapidly lead to severe dehydration, shock, causing death within hours without appropriate clinical treatments. In this study, we present a method wherein unique and short peptides that bind to cholera toxin subunit B (CTX-B) were selected through M13 phage display. Biopanning over recombinant CTX-B led to rapid screening of a unique peptide with an amino acid sequence of VQCRLGPPWCAK, and the phage-displayed peptides analyzed using ELISA, were found to show specific affinities towards CTX-B. To address the use of affinity peptides in development of the biosensor, sequences of newly selected peptides were modified and chemically synthesized to create a series of affinity peptides. Performance of the biosensor was studied using plasmonic-based optical techniques: localized surface plasmon resonance (LSPR) and surface-enhanced Raman scattering (SERS). The limit of detection (LOD) obtained by LSPR with 3σ-rule was 1.89 ng/mL, while SERS had a LOD of 3.51 pg/mL. In both cases, the sensitivity was much higher than the previously reported values, and our sensor system was specific towards actual CTX-B secreted from *V. cholera,* but not for CTX-$AB_5$.

## 1. Introduction

Cholera is an acute intestinal infectious disease caused by the bacterium *Vibrio cholerae* (Espineira et al., 2010; Garrido-Maestu et al., 2015; Haddour et al., 2006; Jin et al., 2013). This bacterium can produce an endotoxin, cholera toxin (CTX), which can lead to severe dehydration and shock (Jin et al., 2013; Seo et al., 2012; Yamazaki et al., 2008). Therefore, CTX is considered as not only one of the major virulent factors of cholera, but also a common causative agent for diarrhea in humans (Cheng et al., 2004; Shirai et al., 1991). CTX has two subunits (Bunyakul et al., 2009; Kuramitz et al., 2011; Seo et al., 2012), A-subunit and B-subunit, which can assemble together into a hexametric arrangement of $AB_5$ family toxins. A-subunit (encoded by *ctxA* gene) is composed of two polypeptides responsible for the toxic activity, while B-subunit (encoded by *ctxB* gene) is a homo-pentameric, non-toxic subunit. It is responsible for specific binding of the toxin to GM1 ganglioside receptors on the surfaces of intestinal cells, and therefore, it is recognized as an adjuvant in the development of cholera vaccines for monitoring and controlling cholera outbreaks.

Conventional methods for CTX detection can be performed only after *V. cholerae* are isolated from the patients, which is a time-consuming and laborious approach (Jin et al., 2013; Shirai et al., 1991; Yamazaki et al., 2008). Thus, there has been increasing demand for development of new diagnostic techniques that are capable of simple, rapid, and inexpensive CTX detection. In recent years, tremendous analytical methods such as fluoro-immunoassay (Bunyakul et al., 2009; Haddour et al., 2006), microarray-based detection (Kim et al., 2012; Seo et al., 2012), colorimetric assay with polymers (Schofield et al., 2007), and nanoparticle-based multiplexing detection (Cheng et al., 2004; Connelly et al., 2012) have been proposed. For example, lactose-tethered gold nanoparticles or ganglioside GM1-tethered polymers can achieve rapid and sensitive detection of CTX at concentrations as low as 0.2 pmol and 54 nmol, respectively (Kilian et al., 2007; Schofield et al., 2007). However, specific recognition of these methods is mainly based on either antibody, GM1, or both (Kuramitz et al., 2011). For point-of-care applications, development of new recognition agents over antibodies for early prediction and detection of cholera may be preferred.

Phage display is a powerful method that enables identification of new target-specific peptides in a short period of time (Hwang et al., 2015, 2016, 2017; Park et al., 2010, 2015; Sidhu, 2001; Wu et al., 2011). There are a number of successful examples of this approach: identification of GM1-

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http://dx.doi.org/10.1016/j.bios.2017.07.075
Received 12 April 2017; Received in revised form 28 July 2017; Accepted 30 July 2017
Available online 31 July 2017
0956-5663/ 

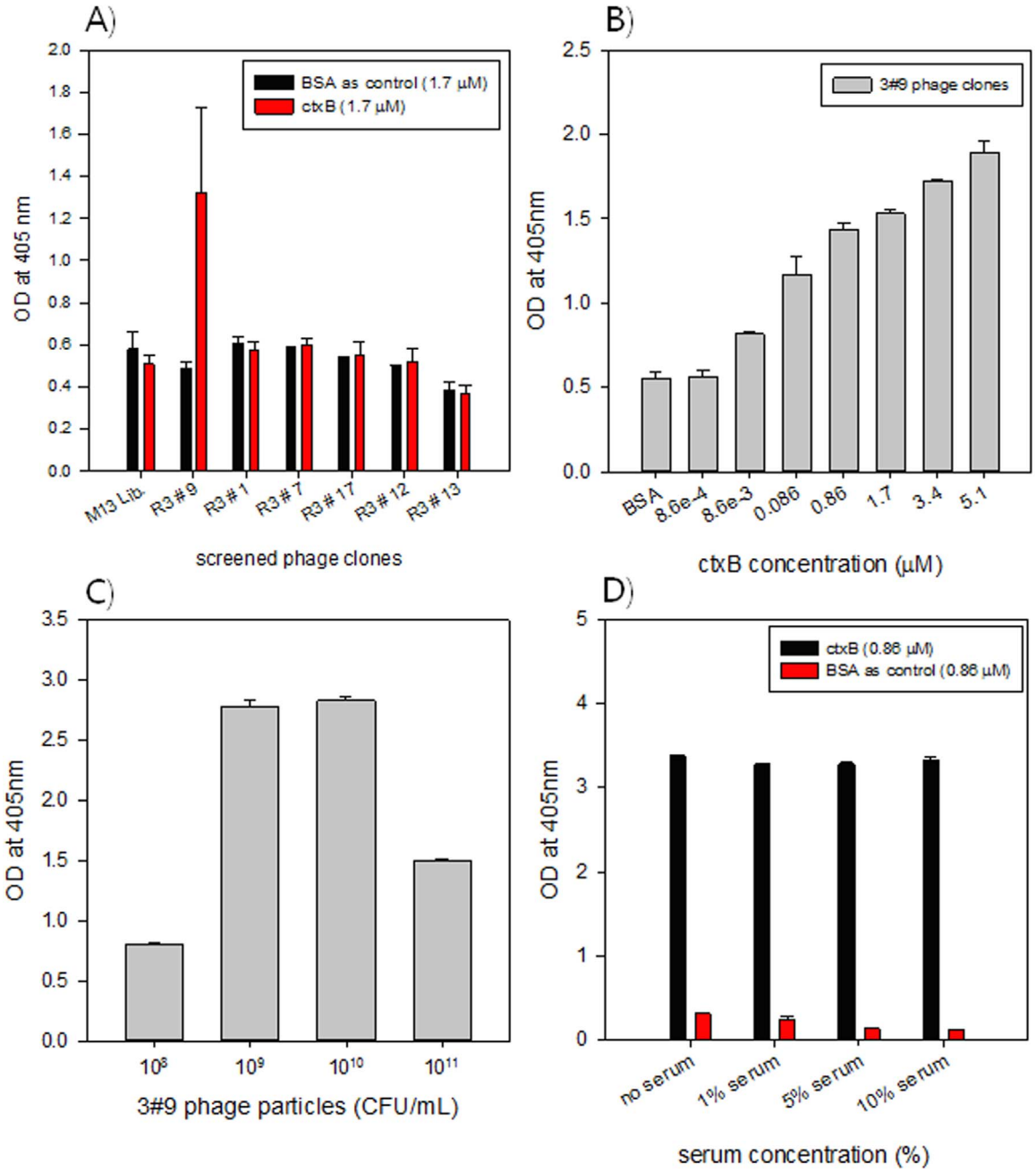


**Fig. 1.** Characterization of selected peptide-displayed phage clones by ELISA. A) Relative binding affinity of identified phage-displayed peptides. B) Relative binding affinity of selected phage-displayed peptides at different CTX-B concentrations. C) Relative binding affinity of selected phage-display peptides. D) Effects of serum on binding interactions. All measurements were performed in triplicate and error bars represent standard deviations.

specific binding peptides (Matsubara et al., 1999), identification of inorganic material-binding peptides (Gabryelczyk et al., 2013; Sarikaya et al., 2003; Sawada et al., 2013), and selection of unique peptides or proteins (Park et al., 2010; Sano et al., 2004; Wu et al., 2010). The advantages of using affinity peptides as recognition elements are their small sizes and cost-efficiencies in developing new bioassay systems (Hwang et al., 2015; Wu et al., 2011). More importantly, affinity peptides require less cost for manipulation, production, and show lower immunogenicity (Park et al., 2010).

Meanwhile, a number of bioanalytical detection platforms have been developed, which can be applied in clinical and biotechnological applications. Among various biosensing methods, localized surface plasmon resonance (LSPR) and surface enhanced Raman spectroscopy (SERS)-based bioanalytical techniques are frequently used for their rapid response, label-free detection, and reliability (Heo et al., 2016; Sepulveda et al., 2009). Thus, both methods can provide an ultra-sensitive level of detection of desired targets (Chon et al., 2010; Huh and Erickson, 2010).

In this study, we performed biopanning of M13 phage library to screen unique and short linear peptides that enable the recognition and detection of CTX-B. After several rounds of biopanning, CTX-B binding peptides were identified, chemically synthesized, and their binding affinity was characterized by using enzyme-linked immunosorbent assay (ELISA), LSPR, and SERS.

## 2. Materials and methods

### 2.1. Chemicals

Biotinylated cholera toxin subunit B, Tween 20, and 2,2′-azino-