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Oligopeptides functionalized surface plasmon resonance biosensors for detecting thiacloprid and imidacloprid

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ABSTRACT

By using phage display library, we identified two highly specific oligopeptide sequences RKRIRRMMPRPS and RNRHTHLRTRPR for binding neonicotinoids such as thiacloprid and imidacloprid. The former shows high affinity for thiacloprid whereas the latter shows high affinity for imidacloprid. Surprisingly, cross binding is minimal despite the similarity of the two molecules. To develop a neonicotinoid biosensor, these two oligopeptides are synthesized and immobilized on the surface of a surface plasmon resonance (SPR) chip with a bare-gold surface. This oligopeptide functionalized SPR biosensor can rapidly detect thiacloprid and imidacloprid in buffer solutions in a real-time manner. The limit of detection (LOD) for thiacloprid and imidacloprid is 1.2 μM and 0.9 μM, respectively.

1. Introduction

Neonicotinoids such as thiacloprid and imidacloprid are a class of small molecules which have molecular structures similar to nicotine. Therefore, they are able to block nicotinic acetylcholine receptors in the central nervous system of insects and act as pesticides (Nauen et al., 2001; Tomizawa and Casida, 2005; Tomizawa et al., 2000). While the use of neonicotinoids has greatly improved crops production, abuse of these pesticides may cause potential risk to consumers and the environment. As a result, the maximum allowable thiacloprid or imidacloprid residues have been regulated by European Union (EU), ranging from 0.01 to 3 mg/kg for many fruits and vegetables (Ferrer et al., 2005). Detection of neonicotinoids can be accomplished by using standard analytical methods such as GC/MS (Navalon et al., 1997; Pang et al., 2006; Vilchez et al., 1996) or LC/MS (Ferrer et al., 2005; Obana et al., 2003). Recently, using ELISA for the detection of neonicotinoids, such as imidacloprid (Lee et al., 2001; Li and Li, 2000; Watanabe et al., 2007) and thiamethoxam (Kim et al., 2003) was also reported. These ELISA-based methods show decent selectivity and sensitivity (μg/L); however, the production of antibodies is time-consuming and expensive, and the batch-to-batch variation is a concern. Furthermore, small molecules such as neonicotinoids do not trigger immune reaction in animal's body easily (Hennion and Barcelo, 1998; Nunes et al., 1998). These issues hinder the widespread use of ELISA for routine analysis of neonicotinoids.

To develop a biosensor which can be used to detect and differentiate different types of neonicotinoids without using antibodies, the greatest challenge is how to design a neonicotinoid receptor which can bind to a particular neonicotinoid molecule with superior specificity. In the past, several groups have attempted to design sensitive layers by using molecular imprinting techniques (Bi and Yang, 2009; Li and Husson, 2006). For example, Bi et al. (Bi and Yang, 2009) reported a two-dimensional molecular imprinted monolayer of alkanethiol which can recognize imidacloprid and thiacloprid. However, the mechanism of recognition sites of this molecular imprinting method is based only on differences in the size and shape of template molecules. As a result, some cross-binding still occurs because of the close similarity of neonicotinoid molecules. In contrast, synthetic oligopeptides can be used to mimic the binding domain of the antibody, and their sequence can be tailored to bind to a wide range of target molecules such as biomolecules (Brighamburke et al., 1992; Catalina et al., 2004; Jones et al., 2006), TNT (Cerruti et al., 2009; Jaworski et al., 2008) or DNT (Hwang et al., 2011). Compared to more complex antibody molecules, short oligopeptides have several potential advantages for the development of biosensors. For example, oligopeptides are more stable and resistant to harsh environments, and they can be synthesized with desired sequences (Dover et al., 2009; Iqbal et al., 2000). Furthermore, additional amino acids (such as cysteine) can be incorporated into oligopeptides sequences to control its immobilization on solid surfaces (Cerruti et al., 2009), or obtain a desired orientation upon immobilization (Bi et al., 2008).

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Herein, inspired by the high specificity of neonicotinoid receptors to neonicotinoid in the central nervous system of insects (Tomizawa and Casida, 2003; Tomizawa et al., 2000), we identified two 12-mer oligopeptides which bind specifically to thiacloprid and imidacloprid, respectively, by using modified phage display library (Devlin et al., 1990; Jaworski et al., 2008; Scott and Smith, 1990; Smith and Petrenko, 1997). In this phage display library, we used solid crystals of thiacloprid and imidacloprid (instead of coated petri dish) as targets because both thiacloprid and imidacloprid have low solubility in water. Thus, unbound phage can be washed out easily without losing thiacloprid and imidacloprid during screening. To the best of our knowledge, this is the first study showing that oligopeptide fragments can be used as a bio-recognition element for neonicotinoids.

2. Materials and methods

2.1. Materials

Thiacloprid and imidacloprid were purchased from Fluka (Singapore). Ph.D.-12 phage display library kit was purchased from New England Biolabs (U.S.A.). Luria broth base was purchased from Invitrogen (Singapore). Agar, isopropyl β-D-thiogalactoside (IPTG), ethylenediaminetetraacetic acid (EDTA), polyethylene glycol (PEG, MW = 8000 g/mol), glycine, bovine serum albumin (BSA), Tween-20, fluorescein isothiocyanate isomer I (FITC), sodium hydroxide, sodium azide and sodium iodide were purchased from Sigma (Singapore). Sodium chloride (99.8%) was purchased from GCE Laboratory Chemicals (Singapore). 5-Bromo-4-chloro-3-indolyl-β-D-galactoside (Xgal) was purchased from Duchefa (Netherlands). Tris buffer (1 M, pH 8.0), phosphate buffer saline (PBS buffer, 10×) and sodium dodecyl sulfate (SDS) were purchased from 1st Base. Ethanol (AR grade) and hydrochloric acid (37%, fuming) were purchased from Merck (Singapore). Spectra/Por dialysis membrane (MWCO: 1000 Da) was purchased from Fisher Scientific (Singapore). Oligopeptides were synthesized by Sigma–Aldrich with a purity ≥ 95%. BIAcore sensor chip Au (untreated gold surface), borate buffer (10 mM disodium tetraborate, pH 8.5, and 1 M NaCl), glycine-HCl (10 mM glycine-HCl, pH 2.0), HBS-EP buffer (0.01 M HEPES, pH 7.4, 0.15 M sodium chloride, 3 mM EDTA, and 0.005%, v/v surfactant P20) were purchased from GE Healthcare (Singapore). Deionized water (18 MΩ-cm) was obtained from a Millipore filtration system.

2.2. Phage display

Fig. 1 is a schematic showing a standard protocol of phage display library employed in this study. Briefly, 10 μL of phage library (1.5×10^{13} pfu/mL, complexity 2.7×10^{9}) was added to 1.5 mL of 0.1% TBST buffer which contains 50 mM of Tris–HCl (pH 7.4), 150 mM NaCl, and 0.1% (v/v) of Tween-20. Then, this solution was mixed with 5 mg of crystalline neonicotinoids (either thiacloprid or imidacloprid) and incubated under vigorous shaking. Because the solubility of neonicotinoids is very low (0.185 mg/mL and 0.51 mg/mL for thiacloprid and imidacloprid, respectively), we used neonicotinoid crystals in the phage display instead of plate-coating methods, which are commonly used for biomolecular targets. After 30 min, the buffer solution was decanted, and the neonicotinoid crystals were washed with 2 mL of fresh 0.1% TBST buffer 10 times to remove non-specific adsorbed phages. Bounded phages were eluted with 1 mL of 0.2 M glycine-HCl (pH 2.2), 1 mg/mL of BSA, and the solution was neutralized with 150 μL of 1 M Tris–HCl (pH 9.1). Next, the eluted phages were amplified with 20 mL of early-log phase *Escherichia coli* culture (ER2738) for 5 h at 35 °C under vigorous shaking. The phage-infected *E. coli* culture was centrifuged twice at 10,000 rpm for 10 min to remove cell residues of *E. coli*, and the supernatant, which contains the phage released from *E. coli* was collected. After that, 4 mL of PEG/NaCl solution (20%, w/v PEG-8000, 2.5 M NaCl) was added to the supernatant, and this solution was incubated at 4 °C. After 2 h, the solution was centrifuged for 15 min at 10,000 rpm at 4 °C to precipitate phage. The pellet containing phage was dissolved in 1 mL of TBS buffer containing 0.02% of NaN_3. This amplified phage was titered on LB/IPTG/Xgal plates as recommend by the supplier (New England Biolabs), and the number

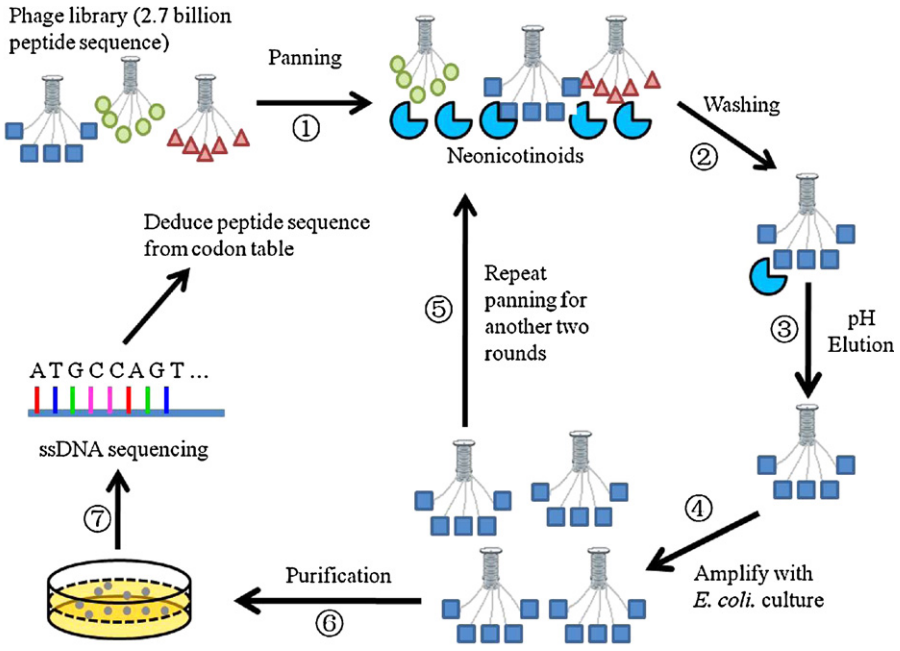

Fig. 1. Schematic illustration of the phage display screening protocol for identifying peptides binding to thiacloprid or imidacloprid molecules. (1) A phage library is incubated with molecular forms of neonicotinoids (thiacloprid or imidacloprid), (2) washing with TBST buffer to remove non-specific bind phages, (3) elute the binding phages by lowering the pH, (4) amplify the eluted phage with *E. coli* culture, (5) repeat the screening with amplified phage another two times for the strongest binding phages, (6) purification of phage colony with plate tittering, and (7) DNA sequencing.