Biosensors and Bioelectronics 35 (2012) 271–276

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

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

Oligopeptides functionalized surface plasmon resonance biosensors for detecting thiacloprid and imidacloprid

Xiaokang Ding[a], Wei Zhang[a], Dan Cheng[b], Jianzhong He[b], Kun-Lin Yang[a,*]

[a] Department of Chemical and Biomolecular Engineering, National University of Singapore, 4 Engineering Drive 4, Singapore 117576, Singapore
[b] Department of Civil and Environmental Engineering, National University of Singapore, 1 Engineering Drive 3, Singapore 117576, Singapore

ARTICLE INFO

Article history:
Received 17 December 2011
Received in revised form 25 February 2012
Accepted 28 February 2012
Available online 7 March 2012

Keywords:
SPR
Biosensor
Phage display
Peptide receptor
Neonicotinoid

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).

* Corresponding author. Tel.: +65 6516 6614.
E-mail address: cheyk@nus.edu.sg (K.-L. Yang).

0956-5663/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2012.02.060

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

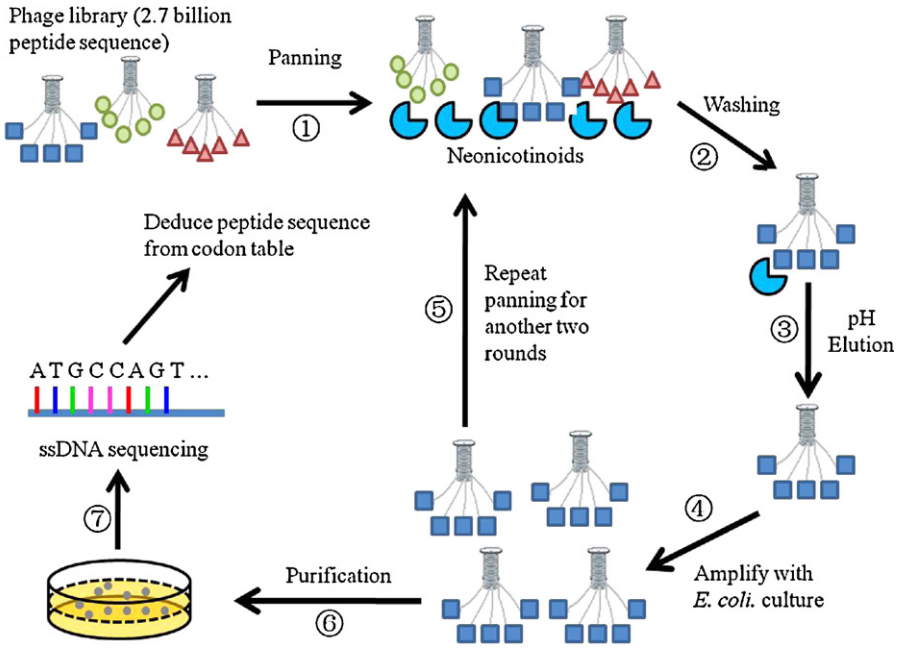

Fig. 1. Schematic illustration of the phage display screening protocol for identifying peptides binding to thiacloprid or imidacloprid molecules. (1) A phage library is incubated with molecular forms of neonicotinoids (thiacloprid or imidacloprid), (2) washing with TBST buffer to remove non-specific 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.

of blue plaques was counted to determine phage titer. This panning procedure was repeated for two more rounds by using the amplified phage eluate from the previous round as input phage to enrich phages. For the second and third round of screening, the concentration of Tween-20 was increased to 0.3% (v/v) and 0.5% (v/v), respectively, to increase stringency of binding to thiacloprid or imidacloprid targets.

2.3. DNA sequencing

The phages after three rounds of panning against thiacloprid or imidacloprid were purified with plate tittering. First, we cultured uninfected *E. coli* overnight. Then, the culture was diluted (1:100) with LB medium. Then, the diluted culture was dispensed into 12 culture tubes (1.5 mL for each tube). Subsequently, a blue plaque was picked from the LB/IPTG/Xgal plate, and transferred to each culture tube. These tubes were incubated at 35 °C under vigorous shaking, allowing the phage to infect *E coli.* After 5 h, the phage-containing cultures in these tubes were centrifuged twice at 10,000 rpm at 4 °C (each time for 10 min) to remove *E coli* residues. Subsequently, 1 mL of the supernatant was transferred to a microcentrifuge tube, and 400 μL of PEG/NaCl solution (20% (w/v) PEG-8000, 2.5 M NaCl) was added and incubated for 2 h at 4 °C. After that, the solution was centrifuged at 10,000 rpm for 10 min and the phage was precipitated as a pellet. To extract phage DNA, the pellet was dissolved in 100 μL of iodide buffer (10 mM Tris–HCl, pH 8.0, 1 mM EDTA, 4 M NaI) and 250 μL of ethanol. After incubation for 10 min, the DNA of phages was precipitated by centrifugation at 12,000 rpm for 15 min. Then, the DNA was resuspended in 100 μL of TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8.0). The purified DNA from 12 single-clone phages was sequenced by AIT Biotech (Singapore) using the -96gIII sequencing primer provided in the Ph.D.-12 phage display kit. Finally, the oligopeptide sequence can be deduced according to codon table.

2.4. Fluorescence test for binding specificity

Two custom synthesized oligopeptides, RKRIRRMMPRPS (**P_1**) and RNRHTHLRTRPR (**P_2**), were labeled with FITC. First, 40 μM of oligopeptide (**P_1** or **P_2**) was incubated in PBS buffer (pH 7.4) containing 10 μg/mL of FITC for 2 h at room temperature. Subsequently, the FITC-labeled oligopeptide was dialyzed against fresh PBS buffer to remove unreacted FITC by using a dialysis membrane (MWCO: 1000 Da). Next, we added 5 mg of either thiacloprid or imidacloprid crystal into 200 μL of solution containing FITC-labeled **P_1** or **P_2**. The solution was then shaken vigorously on a rocking platform for 30 min. After that, the thiacloprid and imidacloprid crystals were washed with 1% SDS solution for 5 times to remove unbounded oligopeptides. Finally, fluorescence images of these crystals were taken by using a fluorescent microscope (Eclipse E200, Nikon) equipped with mercury lamp (Intensilight, Nikon). The exposure time was 1 s for all fluorescence images.

2.5. Immobilization of oligopeptides on SPR sensor chip

Oligopeptides CGGGRKRIRRMMPRPS (Cys-**P_1**) and CGGGRNRHTHLRTRPR (Cys-**P_2**) were also synthesized for SPR experiments. CGGG was added to the NH_2-terminus of the original oligopeptide to provide an anchoring point (through cysteine) and a spacer. To immobilize the oligopeptides, borate buffer (10 mM disodium tetraborate and 1 M NaCl, pH 8.5), containing 0.1 mg/mL of either Cys-**P_1** or Cys-**P_2**, was injected slowly (1 μL/min) into a flow cell (FC-1 or FC-2) on a gold sensor chip (BIAcore) for 60 min. Then, the flow cell was flushed with fresh HBS-EP buffer (2 μL/min) for 60 min to remove unreacted oligopeptides. To increase the surface density of immobilized oligopeptides, the concentrations of Cys-**P_1** and Cys-**P_2** were increased to 1.0 mg/mL, and the injection time was increased to 325 min for FC-3 and FC-4 flow cells.

2.6. SPR experiments

HBS-EP buffer containing 10 μM of thiacloprid was injected into a flow cell functionalized with Cys-**P_1** (FC-1) at 2 μL/min for 5 min. Subsequently, the flow cell was flushed with fresh HBS-EP buffer for 15 min to remove unbounded thiacloprid. After that, the Cys-**P_1** functionalized surface was regenerated by flowing glycine-HCl (pH 2.0) buffer for 1 min (5 μL/min) to remove the bounded thiacloprid. We refer to the three steps above (injection, flushing, and regeneration) as one "binding cycle". In control experiments, HBS-EP buffer containing 10 μM of imidacloprid was injected into FC-1 with the same parameters as described above. Similarly, we repeated the same experiment by using another flow cell (FC-2) functionalized with Cys-**P_2**.

Next, HBS-EP buffer with increasing thiacloprid concentrations (2, 10, 20, 40, 60, 80, and 100 μM) were injected sequentially into FC-1 (or FC-3) at a flow rate of 2 μL/min. Each injection step took 5 min. Similarly, after each injection, the flow cell was flushed with fresh HBS-EP buffer for 15 min to remove unbounded thiacloprid, followed by glycine-HCl (pH 2.0) for 1 min (5 μL/min) to regenerate the sensor chip. All experiments were repeated three times and the average binding responses were calculated and plotted. The same experiments were repeated for imidacloprid by using FC-2 (or FC-4).

3. Results and discussion

Table 1 shows the result of phage display library when two neonicotinoids, thiacloprid and imidacloprid, were used as target molecules after 3 rounds of panning. When thiacloprid was used, 5 (out of 12) colonies displayed oligopeptide RKRIRRMMPRPS (**P_1**), and 2 colonies displayed TNLKSIRRPQIP. Interestingly, 7 out of 12 of the oligopeptides have Ile-Arg-Arg (IRR) and one oligopeptide has Ile-Arg-Gln (IRQ) in their sequences. We point out that arginine (R) and glutamine (Q) have similar side chains and similar hydrophobicity at pH 7.0 (Monera et al., 1995). Therefore, IRR or IRQ is most likely to be responsible to the binding of thiacloprid. In contrast, when imidacloprid was used, 6 (out of 12) colonies displayed oligopeptide RNRHTHLRTRPR (**P_2**), and 2 displayed RRPKRKHRMHLS. Among all imidacloprid binding oligopeptides, 7 out of 12 of the oligopeptides show Arg-Pro-Arg (RPR) in their sequences, and 3 other oligopeptides have Arg-Pro-Lys (RPK). Because arginine (R) and lysine (K) exhibit similar side chains and similar hydrophobicity (Monera et al., 1995) at pH 7.0, RPR or RPK is the potential sequence to bind imidacloprid. Although the nature of the peptide–neonicotinoid binding is still not completely understood, we hypothesize that the abundant amino acid residues, IRR or RPR, are responsible for the binding. However, they do not always appear at the N-terminus of the oligopeptides, sometimes they also appear in the middle, or the C-terminus of oligopeptide sequence. This is different from previous reported results (Jaworski et al., 2008). Moreover, we notice that the two most abundant oligopeptides (RKRIRRMMPRPS (**P_1**) and RNRHTHLRTRPR (**P_2**)) show certain similarities in their sequences. For example, the positively charged residues, such as R, K, and H, are abundant. On the other hand, residues that heavily negatively charged, such as E and D, are absent. Table 1 also shows that the p*I* values and average hydrophilicity of **P_1** and **P_2** are similar. Because of the high abundance of these two oligopeptides **P_1** and **P_2**, they are chosen as the best molecular receptors for binding thiacloprid and imidacloprid, respectively.

Table 1
Oligopeptide sequences and physical properties[a] identified through phage display library against thiacloprid and imidacloprid after 3 panning rounds.

Targets	Oligopeptide sequence [N] → [C]	p*I*	Average hydrophilicity	Hydrophobic ratio	Freq.
Thiacloprid	RKRIRRMMPRPS	13	1.2	42%	5/12
	TNLKSIRRPQIP	12.4	0.3	50%	2/12
	LRKRNNPIRTKM	12.7	0.8	42%	1/12
	SSTRMISIRQST	12.4	0.1	42%	1/12
	KMPRQSNRRLMM	12.7	0.6	42%	1/12
	RHLQTSPIMLQI	11	−0.5	67%	1/12
	RHTNSTRLNRPT	12.7	0.5	50%	1/12
Imidacloprid	RNRHTHLRTRPR	13	1	50%	6/12
	RRPKRKHRMHLS	12.9	1.2	42%	2/12
	ISKHTPLRTRPR	12.7	0.6	58%	1/12
	RRPKRQHTMQLS	12.7	0.7	42%	1/12
	SMRMRIHIPSSH	12.4	0	58%	1/12
	RQIIQPSPKQRR	12.7	0.8	33%	1/12

[a] The physical properties of oligopeptides, including p*I*, net charge, average hydrophilicity, and hydrophilic ratio are calculated from peptide property calculator: http://www.innovagen.se/custom-peptide-synthesis/peptide-property-calculator/peptide-property-calculator.asp (accessed on June 28, 2011).

To test their specificity for binding their neonicotinoid targets, thiacloprid powder was incubated in PBS buffer (pH 7.4) containing 40 μM of either FITC-labeled **P1** or **P2** with vigorous shaking. After 2 h, the solution was decanted, and the neonicotinoid solid was washed with 1.0% SDS for 5 times to remove unbounded oligopeptides. Fig. 2 shows that only **P1** can bind to thiacloprid and gives green fluorescence, whereas **P2** cannot bind to thiacloprid and no fluorescence can be observed. In contrast, when solid imidacloprid powder was used in the binding experiment, only **P2** can bind to its surface and gives green fluorescence. This experiment suggests that oligopeptides **P1** and **P2** can bind to their respective targets specifically, and the cross-binding is minimal despite the similarity of **P1** and **P2**. As mentioned earlier, this excellent binding specificity may come from IRR or RPR residues in **P1** and **P2**, and some other factors, including charge distribution and polarity of these two oligopeptides. For example, both thiacloprid and imidacloprid have a negatively charged tip, such as cyano group or nitro groups, which can interact favorably with the positively charged amino acid residue (R and K) distributed on **P1** or **P2**. However, the exact mechanism has not been confirmed yet. More experiments are needed to shed lights on the specificity.

To design a biosensor for detecting thiacloprid by using SPR, a slightly different oligopeptide with a sequence of CGGGRKRIRRMMPRPS (Cys-**P1**) was synthesized. After immobilization of Cys-**P1** on a sensor chip, SPR shows an increase of 50.2 resonance units (RU), corresponding to a surface density of 0.016 molecule/nm^2 on the surface (see Supporting Information). In contrast, when RKRIRRMMPRPS (**P1**) is used, the SPR signal only

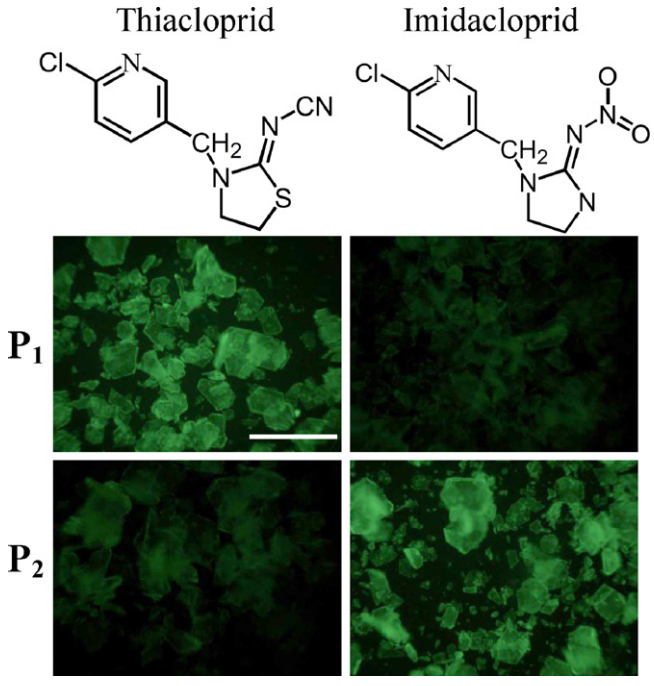

Fig. 2. Fluorescent images of thiacloprid and imidacloprid powders bind to oligopeptide **P1** and **P2**, respectively. Scale bar: 500 μm.

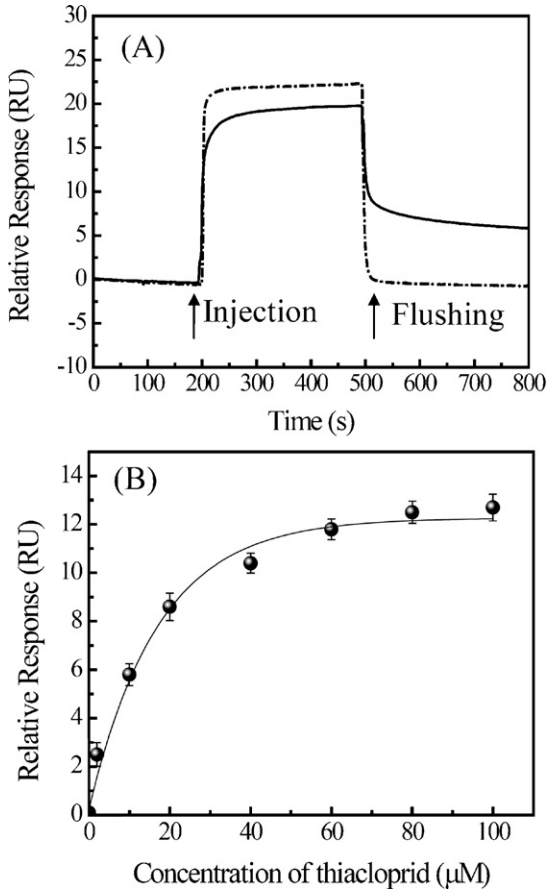

Fig. 3. (A) Response of an Cys-**P1** modified SPR gold chip to 10 μM of thiacloprid (solid) and 10 μM of imidacloprid (dashed) and (B) binding curve of Cys-**P1** modified SPR gold chip to HBS-EP buffer containing thiacloprid with concentration of 2, 10, 20, 40, 60, 80, and 100 μM. The error bars represent the standard deviation of three measurements.

increases by 5 RU. These results suggest that Cys-**P_1** is immobilized on the gold sensor chip through the formation of an Au—S bond on the surface. To detect thiacloprid, 10 μM of thiacloprid solution was injected into the same flow cell at 2 μL/min. Sensorgram in Fig. 3A shows that after one binding cycle, a net increase of 5.8 RU caused by the formation of oligopeptide (Cys-**P_1**)-thiacloprid complex on the surface can be observed. In contrast, when 10 μM of imidacloprid is used during the binding cycle, the net increase in the final response is negligible. This phenomenon indicates that imidacloprid cannot bind to Cys-**P_1** despite the similar molecular structures between thiacloprid and imidacloprid. Furthermore, nonspecific adsorption of thiacloprid or imidacloprid on a bare-gold SPR sensor chip, was also tested. Our result shows that the nonspecific binding is negligible, probably because both thiacloprid and imidacloprid are small molecules, they do not adsorb on solid surfaces like proteins (see Supporting Information).

Fig. 3B shows the binding curve for thiacloprid from 2 to 100 μM. We notice that when the bulk concentration of thiacloprid increased to 60 μM, the binding curve reaches plateau, indicating that the binding sites of immobilized Cys-**P_1** for thiacloprid are saturated. In the BIAcore SPR system, a response of 1000 RU corresponds to a change in surface density of about 1 ng/mm^2 (Yang et al., 2005). In this case, we can calculate the surface mass density ($\Delta m/A$) of thiacloprid molecules that bind to surface immobilized oligopeptide. Then, dissociation constant K_D for thiacloprid can be deduced from Langmuir adsorption isotherm

$$\frac{\Delta m/A}{\Delta m_{max}/A} = \frac{C}{C + K_D} \quad (1)$$

where $\Delta m_{max}/A$ is the maximum mass density and C is the thiacloprid concentration in the bulk. We obtained a dissociation constant (K_D) of 9.3 μM for thiacloprid by using least-squares fitting method (Bi and Yang, 2009).

To detect imidacloprid, we synthesized oligopeptide Cys-**P_2**, with a sequence of CGGGRNRHTHLRTRPR, and immobilized it on a sensor chip with a surface density of 0.022 molecule/nm^2 (see Supporting Information). Then, we repeated the same binding experiment with 10 μM of imidacloprid as binding targets. Sensorgrams in Fig. 4A show that imidacloprid molecules can bind to Cys-**P_2** immobilized surface, and causes a net increase of 6.2 RU. In contrast, when 10 μM of thiacloprid was used, the net increase in the binding signal is negligible. Therefore, we can conclude that thiacloprid cannot bind to Cys-**P_2**. Fig. 4B also shows the sigmoidal correlation between the SPR binding signals and the imidacloprid concentration. Similarly, we obtained a dissociation constant of 11.5 μM for imidacloprid from Langmuir adsorption isotherm by using least-squares fitting method.

However, we noticed that when the bulk concentration of thiacloprid or imidacloprid is 60 μM, the binding signal has reached a plateau, indicating that the surface binding sites have been

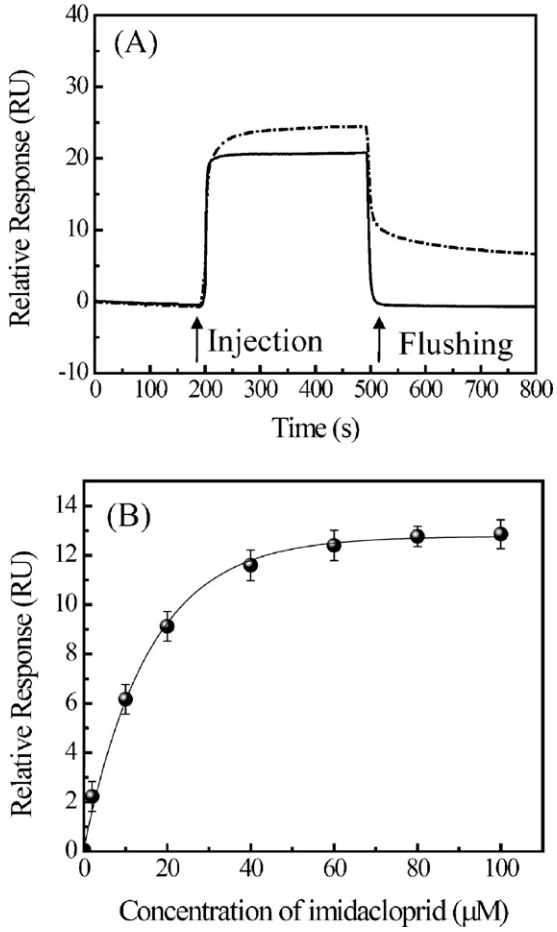

Fig. 4. (A) Response of an Cys-**P_2** modified SPR gold chip to 10 μM of thiacloprid (solid) and 10 μM of imidacloprid (dashed) and (B) binding curve of Cys-**P_2** modified SPR gold chip to HBS-EP buffer containing imidacloprid with concentration of 2, 10, 20, 40, 60, 80, and 100 μM. The error bars represent the standard deviation of three measurements.

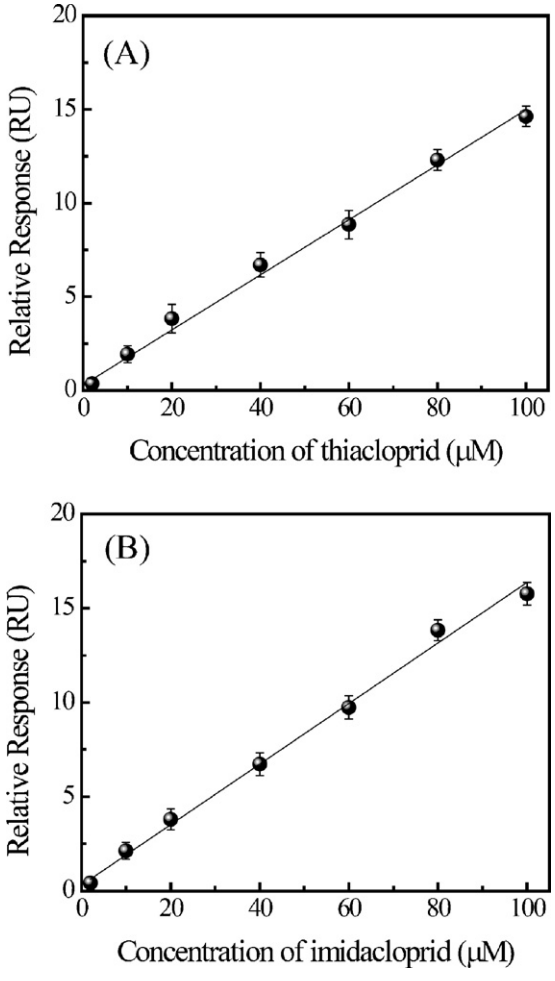

Fig. 5. (A) The relationship of SPR final responses when HBS-EP buffer containing thiacloprid with concentration of 2, 10, 20, 40, 60, 80, and 100 μM flows over Cys-**P_1** modified surface. (B) The relationship of SPR final responses when HBS-EP buffer containing imidacloprid with concentration of 2, 10, 20, 40, 60, 80, and 100 μM flows over Cys-**P_2** modified surface. The error bars represent the standard deviation of three measurements.

saturated. In this case, the linear response range to thiacloprid and imidacloprid is small. To address this issue, we increased the surface densities of immobilized Cys-**P$_1$** and Cys-**P$_2$** to 0.47 molecule/nm^2 and 0.60 molecule/nm^2, respectively (see Supporting Information). Fig. 5 shows the SPR response to thiacloprid and imidacloprid from 2 to 100 μM. In this case, the surface is not saturated even when the concentration is as high as 100 μM, which is close to the solubility of both compounds in the buffer solution. Since the response is linear in this range, we can estimate the sensitivity for thiacloprid and imidacloprid are 0.14 RU/μM and 0.16 RU/μM, respectively. From Fig. 5, we can also determine the limit of detection (LOD) for thiacloprid and imidacloprid are 1.2 μM and 0.9 μM, respectively.

4. Conclusions

In conclusion, two oligopeptides RKRIRRMMPRPS (**P$_1$**) and RNRHTHLRTRPR (**P$_2$**) were identified by using phage display library and their binding capabilities for thiacloprid and imidacloprid were studied. Surprisingly, despite the similar molecular structures between thiacloprid and imidacloprid, cross-binding of **P$_1$** and **P$_2$** to these two organic compounds is minimal. We believe that the abundant amino acid residues, IRR and RPR, are responsible for the binding. By using **P$_1$** and **P$_2$**, we successfully developed a biosensor for detecting thiacloprid and imidacloprid. The sensitivities for thiacloprid and imidacloprid are 0.14 RU/μM and 0.16 RU/μM, respectively, whereas the LODs are 1.2 μM and 0.9 μM, respectively. Oligopeptides identified in this study may also shed lights on the binding mechanism of neonicotinoids to nicotinic acetylcholine receptors.

Acknowledgment

The work was supported by a research scholarship from the National University of Singapore.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2012.02.060.

References

Bi, X.Y., Hartono, D., Yang, K.L., 2008. Langmuir 24, 5238–5240.
Bi, X.Y., Yang, K.L., 2009. Anal. Chem. 81, 527–532.
Brighamburke, M., Edwards, J.R., Oshannessy, D.J., 1992. Anal. Biochem. 205, 125–131.
Catalina, M.I., de Mol, N.J., Fischer, M.J.E., Heck, A.J.R., 2004. Phys. Chem. Chem. Phys. 6, 2572–2579.
Cerruti, M., Jaworski, J., Raorane, D., Zueger, C., Varadarajan, J., Carraro, C., Lee, S.W., Maboudian, R., Majumdar, A., 2009. Anal. Chem. 81, 4192–4199.
Devlin, J.J., Panganiban, L.C., Devlin, P.E., 1990. Science 249, 404–406.
Dover, J.E., Hwang, G.M., Mullen, E.H., Prorok, B.C., Suh, S.J., 2009. J. Microbiol. Methods 78, 10–19.
Ferrer, I., Thurman, E.M., Fernandez-Alba, A.R., 2005. Anal. Chem. 77, 2818–2825.
Hennion, M.C., Barcelo, D., 1998. Anal. Chim. Acta 362, 3–34.
Hwang, K.S., Lee, M.H., Lee, J., Yeo, W.-S., Lee, J.H., Kim, K.-M., Kang, J.Y., Kim, T.S., 2011. Biosens. Bioelectron. 30, 249–254.
Iqbal, S.S., Mayo, M.W., Bruno, J.G., Bronk, B.V., Batt, C.A., Chambers, J.P., 2000. Biosens. Bioelectron. 15, 549–578.
Jaworski, J.W., Raorane, D., Huh, J.H., Majumdar, A., Lee, S.W., 2008. Langmuir 24, 4938–4943.
Jones, R.B., Gordus, A., Krall, J.A., MacBeath, G., 2006. Nature 439, 168–174.
Kim, H.J., Liu, S.Z., Keum, Y.S., Li, Q.X., 2003. J. Agric. Food Chem. 51, 1823–1830.
Lee, J.K., Ahn, K.C., Park, O.S., Kang, S.Y., Hammock, B.D., 2001. J. Agric. Food Chem. 49, 2159–2167.
Li, K., Li, Q.X., 2000. J. Agric. Food Chem. 48, 3378–3382.
Li, X., Husson, S.M., 2006. Langmuir 22, 9658–9663.
Monera, O.D., Sereda, T.J., Zhou, N.E., Kay, C.M., Hodges, D.R.S., 1995. J. Pept. Sci. 1, 319–329.
Nauen, R., Ebbinghaus-Kintscher, U., Schmuck, R., 2001. Pest Manag. Sci. 57, 577–586.
Navalon, A., GonzalezCasado, A., ElKhattabi, R., Vilchez, J.L., FernandezAlba, A.R., 1997. Analyst 122, 579–581.
Nunes, G.S., Toscano, I.A., Barcelo, D., 1998. Trac—Trends Anal. Chem. 17, 79–87.
Obana, H., Okihashi, M., Akutsu, K., Kitagawa, Y., Hori, S., 2003. J. Agric. Food Chem. 51, 2501–2505.
Pang, G.-F., Liu, Y.-M., Fan, C.-L., Zhang, J.-J., Cao, Y.-Z., Li, X.-M., Li, Z.-Y., Wu, Y.-P., Guo, T.-T., 2006. Anal. Bioanal. Chem. 384, 1366–1408.
Scott, J.K., Smith, G.P., 1990. Science 249, 386–390.
Smith, G.P., Petrenko, V.A., 1997. Chem. Rev. 97, 391–410.
Tomizawa, M., Casida, J.E., 2003. Annu. Rev. Entomol. 48, 339–364.
Tomizawa, M., Casida, J.E., 2005. Annu. Rev. Pharmacol. Toxicol. 45, 247–268.
Tomizawa, M., Lee, D.L., Casida, J.E., 2000. J. Agric. Food Chem. 48, 6016–6024.
Vilchez, J.L., ElKhattabi, R., Fernandez, J., GonzalezCasado, A., Navalon, A., 1996. J. Chromatogr. A 746, 289–294.
Watanabe, E., Baba, K., Eun, H., Miyake, S., 2007. Food Chem. 102, 745–750.
Yang, C.Y., Brooks, E., Li, Y., Denny, P., Ho, C.M., Qi, F.X., Shi, W.Y., Wolinsky, L., Wu, B., Wong, D.T.W., Montemagno, C.D., 2005. Lab Chip 5, 1017–1023.