Identification of peptide inhibitors of penicillinase using a phage display library

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A B S T R A C T

There is a constant need to identify novel inhibitors to combat β-lactamase-mediated antibiotic resistance. In this study, we identify three penicillinase-binding peptides, P1 (DHIHSYRGED), P2 (NYTTTPWGSNWS), and P3 (SHSLPASADLRR), using a phage display library. Surface plasmon resonance (SPR) is utilized for quantitative determination and comparison of the binding specificity of selected peptides to penicillinase. An SPR biosensor functionalized with P3-GGGC (SHSLPASADLRRGGGC) is developed for detection of penicillinase with excellent sensitivity (15.8 RU nM⁻¹) and binding affinity (Kₒ = 0.56 nM). To determine if peptides can be good inhibitors for penicillinase, these peptides are mixed with penicillinase and their inhibition efficiency is determined by measuring the hydrolysis of substrate penicillin G using UV–vis spectrophotometry. Peptide P2 (NYTTTPWGSNWS) is found to be a promising penicillinase inhibitor with a Kᵢ of 9.22 μM and a Kᵢ' of 33.12 μM, suggesting that the inhibition mechanism is a mixed pattern. This peptide inhibitor (P2) can be used as a lead compound to identify more potent small molecule inhibitors for penicillinase. This study offers a potential approach to both detection of β-lactamases and development of novel inhibitors of β-lactamases.

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Introduction

The synthesis of large numbers of antibiotics over the past few decades has caused complacency about the threat of bacterial resistance [1]. In recent years, increasing resistance of bacterial pathogens to clinically useful antibiotics has become a serious public health threat [2,3]. Among all antibiotics available nowadays, β-lactam antibiotics are still the most widely used and account for approximately 50–70% of total antibiotic use [4]. β-Lactam antibiotics include all antibiotic agents that contain β-lactam rings in their molecular structure. Typical β-lactam antibiotics include penicillins, cephalosporins, monobactams, and carbapenems [5]. Most β-lactam antibiotics work by irreversibly binding to penicillin-binding proteins (PBPs) to inhibit cross-linking of the peptidoglycan layer of bacterial cell walls, disrupting cell wall synthesis [6]. The primary cause of bacterial resistance to β-lactam antibiotics is the expression of β-lactamases, enzymes that attack and hydrolyze the β-lactam ring [7,8]. To combat β-lactamase-mediated antibiotic resistance, extended-spectrum β-lactam antibiotics have been introduced in an effort to circumvent the action of β-lactamases. However, the use of these agents has resulted in the emergence of mutants capable of hydrolyzing extended spectrum antibiotics [9]. An alternative method of combating β-lactamase-mediated resistance is the use of mechanism-based small molecule inhibitors. These inhibitors protect the β-lactam drug from hydrolysis by β-lactamases and restore the antibiotic effect. However, variants have now evolved that resist these inhibitors while maintaining the ability to hydrolyze β-lactam antibiotics [10,11]. Therefore, there is a constant need to find new β-lactamase inhibitors.

At present, small molecules such as clavulanic acid or clavulanate, sulbactam, and tazobactam are the mechanism-based β-lactamase inhibitors most commonly used to combat β-lactamase-mediated antibiotic resistance [12]. These molecules are often co-formulated with β-lactam antibiotics to combat microbial infec-

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protein (BLIP) produced by *Streptomyces clavuligerus* was proved to be more effective than clavulanic acid against some class A β-lactamases [15]. Bouagna et al. described several cysteinyl peptides as competitive inhibitors of *Bacillus cereus* zinc β-lactamase, with inhibition dissociation constants in the 10−1−10−3 M range [16]. Mandal et al. recently reported two novel five-amino-acids-long β-lactamase peptide inhibitors, which were tested in vivo in mice to neutralize bacterial resistance [17].

Phage display is a powerful selection technique for screening of novel peptides against a target molecule, in which a library of peptide variants is expressed on the outside of a phage virion, while the genetic material encoding each variant resides on the inside [18–20]. In the past, this technique has been used to identify peptide inhibitors for a broad range of enzymes [21]. Novel inhibitors have been isolated by screening phage display random peptide libraries on the immobilized enzymes, such as serine protease [22], trypsin [23], urease [24], β-glucosidase [25], transferase [26], ligase [27,28], and β-lactamase [29,30]. Huang et al. identified a peptide inhibitor of class A β-lactamase TEM-1 with a Ki of 136 µM. This peptide can also inhibit the class A Bla1 β-lactamase with a Ki of 42 µM and the class C P99 β-lactamase with a Ki of 140 µM, even though it was not optimized to bind these enzymes [29]. Sanschagrin et al. identified a peptide inhibitor of class B metallo-β-lactamase L-1 showing mixed inhibition with a Ki of 16 µM and K′ of 9 µM [30]. Typically, enzyme-linked immunosorbent assays (ELISA) were used for qualitative determination of whether selected peptide binds to the target, without knowing the actual binding affinity between the selected peptides and the target [29,31,32]. Surface plasmon resonance (SPR) is a surface-sensitive optical technique that allows real-time and label-free measurements of biomolecular interactions in small volumes and high sensitivity [33–36]. Those advantages of SPR make it a better choice for studying the binding between peptides and the target than ELISA, and for detection of β-lactamases than conventional phenotypic and genotypic methods [37,38]. By using selected peptides from phage display as molecular receptors and SPR as the detection method, the binding specificity of selected peptides to the target can be quantitatively determined and compared.

Here, we aimed to identify peptide inhibitors of penicillinase from *B. cereus* for development of novel antimicrobials. Using a phage display library, we performed an unbiased search to identify peptides from a random sequence library that would bind to the penicillinase. SPR was utilized for determination and comparison of the specificity of binding selected peptides by phage display experiments to the penicillinase. However, not all peptides that bind to penicillinase are good inhibitors. Therefore, inhibitory activity of potential peptide inhibitors to the penicillinase were assessed in a functional assay in which hydrolysis of penicillin G by the penicillinase was monitored using UV−vis spectrophotometry. This study provides a tool for development of novel peptide inhibitors of β-lactamases. These peptide inhibitors may be a useful starting point for the design of novel small molecule inhibitors for β-lactamase after their binding mechanisms and structures are fully understood.

**Materials and methods**

**Materials**

Penicillinase from *B. cereus* lyophilized powder, α-cysteine (97%), penicillin G (≥98.0%), and potassium clavulanate were purchased from Sigma–Aldrich (Singapore). A Ph.D.-12 phage display peptide library kit was purchased from New England Biolabs (United States). Biacore gold sensor chips (untreated), borate buffer (10 mM disodium tetraborate pH 8.5 and 1 M NaCl), glycine hydrochloride (10 mM glycine hydrochloride, pH 2.0), and 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). Peptides were synthesized by GenicBio Ltd. (Shanghai, China) with purity ≥95%. Deionized water (18 MΩ cm) was obtained from a Millipore filtration system.

**Phage display**

A Ph.D.-12 phage display peptide library (containing 1.0 × 1013 plaque-forming units (pfu) mL−1, ~2.7 × 109 12-mer random peptide sequences) was used to identify peptides that bind to immobilized penicillinase from *B. cereus*. The phage display library screening was employed according to a standard protocol with some modifications [39]. (See the Supplementary Material for more details.) Briefly, the screening was carried out by incubating a library of random 12-mer peptides (displayed on phage) on a plate coated with penicillinase (target). After rigorous washing steps, only those phage-expressing peptides that bind to penicillinase were eluted. The eluted phage were then amplified in *Escherichia coli* and used in the next round of selection to enrich the binding peptide sequences (Fig. 1). After three rounds, individual clones were characterized by DNA sequencing. Peptide sequences binding to the target molecule were deduced from the phage DNA sequences.

**Binding assays by SPR**

To immobilize consensus peptides selected from phage display, a peptide linker, GGGC, was added to the C-terminus of these peptides. The peptides were synthesized and immobilized on gold surfaces through cysteine [39,40]. All SPR measurements were done using a Biacore T200 SPR system. First, peptide was immobilized on a bare gold sensor chip. Borate buffer (pH 8.5) containing 1.0 mg mL−1 of peptide flowed over the chip surface at 1 μL min−1 for 350 min. The surface density of immobilized peptide was determined using net increase of SPR response (for Biacore T200, a response of 1 RU is equivalent to 1 pg mm−2) [41]. The sensor chip was flushed with HBS-EP buffer for more than 30 min to remove unreacted peptide. Next, the sensor chip surface was blocked with borate buffer containing 10 mM of cysteine, which is known to reduce nonspecific adsorption on the gold surface [36,42,43]. To test binding of penicillinase to the immobilized peptide, HBS-EP buffer containing penicillinase was injected at 2 μL min−1 over a period of 3 min. After the injection, the flow cell was flushed with fresh HBS-EP buffer for 3.5 min to remove unbound penicillinase. The binding level of penicillinase was determined by measuring the
SPR response shift before and after exposure to the penicillinase solution. Finally, the flow cell was regenerated with glycine hydrochloride solution (pH 2.0) for 1 min. The background signal (0 μM penicillinase) was subtracted from the binding signal to minimize signal variations.

Inhibition assays

The consensus peptides selected from the phage display library and a peptide inhibitor (RRGHYY) of β-lactamase reported by Huang et al. [29] were synthesized and used as penicillinase inhibitors. To study inhibition of penicillinase activity, peptides or a small-molecule inhibitor (potassium clavulanate) were incubated with 20 nM of penicillinase for 1 h in PBS (10 mM, pH 7.4). Concentrations of peptides tested were 0, 50, 100, and 200 μM. Following the incubation, different concentrations (10, 25, 50, 100, 500, and 1000 μM) of a penicillinase substrate, penicillin G, were added. Hydrolysis of penicillin G was determined using a UV–vis spectrophotometer (Cary 50, Varian) by measuring the decrease in absorbance at 240 nm. All experiments were carried out in triplicate.

Results and discussion

Selection of penicillinase-binding peptides

The goal of phage display experiments was to perform an unbiased search to identify peptides from a random sequence library that might bind to penicillinase. For this purpose, a phage display library displaying randomized 12-mer peptides was chosen as a biopanning tool, and the target was immobilized penicillinase. After three rounds of screening, 16 blue phage colonies were randomly selected for DNA sequencing to determine if the library was converging on a particular sequence. Table 1 showed that phages displaying 12 different peptide sequences were discovered in this experiment. It was noticed that hydrophobic aromatic amino acids (F, Y, W; 16.7%), hydrophilic amino acids (L, I, V, M; 15.3%), and basic amino acids (R, H, K; 15.3%) were abundant. This is probably because penicillinase from B. cereus (pl ~ 5.5) [44] is negatively charged in TBST buffer (pH 7.5) during the screening process. Moreover, three consensus sequences, P1 (DHHRSYRGFGDGGCC), P2 (NYITTPWSNWSGGGC), and P3 (SHSLPASDLRGGGC), were synthesized. These peptides were immobilized on SPR sensor chips through gold–thiol bonds. Surface densities of the immobilized peptides were at a level of 10−1 molecule nm−2, which is consistent with our previous study [39], Fig. 2 showed responses of SPR to different concentrations of penicillinase (0, 0.08, 0.2, 0.4, 1.0, 1.5, and 2.0 μM) on the P3-GGCC-modified sensor surface (for P1-GGCC- and P2-GGCC-modified sensor surfaces see Fig. S-1 in the Supplementary Material). Our results showed that with increasing concentrations of penicillinase, the SPR shift increased accordingly. However, when the penicillinase concentration was 2.0 μM, the SPR shift did not increase further, probably because the surface was saturated with penicillinase. These results were modeled using a Langmuir isotherm to obtain equilibrium binding responses at different penicillinase concentrations on different peptide-modified surfaces (Fig. 3) [46]. The dissociation constant of binding (K_D) was determined from the Langmuir isotherm calibration curve of 1/(SPR shift) versus 1/(concentration of penicillinase) [47], and the sensitivity was defined as the slope of SPR shift response versus concentration of penicillinase at low analyte concentrations [39]. Among these three peptides, P3-GGCC displayed the best sensitivity (15.8 RU nM−1) and the smallest K_D (0.56 nM), whereas P1-GGCC showed the second best sensitivity (5.05 RU nM−1) and a slightly larger K_D at 1.81 nM (Table 2). These results were not surprising, because the arginine residues within the peptides played an important role in β-lactamase binding [29]. For P2-GGCC, K_D was 294 nM, which was higher than that of the other two peptides. However, the binding affinity of P2-GGCC to the penicillinase was still quite good, since typical dissociation constants of protein–ligand interactions are in the micromolar to nanomolar range [48]. These results confirmed that our phage display experiments were successful. By using SPR as binding assays, we not only quantitatively determined the binding specificity of selected peptides (P1, P2, and P3) to the penicillinase target, but also developed three peptides were selected and synthesized for subsequent tests.

Table 1

<table>
<thead>
<tr>
<th>Peptide no.</th>
<th>Sequence</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DHHRSYRGFGDGGCC</td>
<td>3/16</td>
</tr>
<tr>
<td>2</td>
<td>NYITTPWSNWSGGGC</td>
<td>3/16</td>
</tr>
<tr>
<td>3</td>
<td>SHSLPASDLRGGGC</td>
<td>3/16</td>
</tr>
</tbody>
</table>

Note. Frequency indicates the number of times a particular sequence appeared in the 16 sequenced clones.

Binding of selected peptides to penicillinase

Surface plasmon resonance (SPR) was used to compare binding of selected peptides to penicillinase. Three peptide sequences, P1-GGCC (DHHRSYRGFGDGGCC), P2-GGCC (NYITTPWSNWSGGGC), and P3-GGCC (SHSLPASDLRGGGC), were synthesized. These peptides were immobilized on SPR sensor chips through gold–thiol bonds. Surface densities of the immobilized peptides were at a level of 10−1 molecule nm−2, which is consistent with our previous study [39], Fig. 2 showed responses of SPR to different concentrations of penicillinase (0, 0.08, 0.2, 0.4, 1.0, 1.5, and 2.0 μM) on the P3-GGCC-modified sensor surface (for P1-GGCC- and P2-GGCC-modified sensor surfaces see Fig. S-1 in the Supplementary Material). Our results showed that with increasing concentrations of penicillinase, the SPR shift increased accordingly. However, when the penicillinase concentration was 2.0 μM, the SPR shift did not increase further, probably because the surface was saturated with penicillinase. These results were modeled using a Langmuir isotherm to obtain equilibrium binding responses at different penicillinase concentrations on different peptide-modified surfaces (Fig. 3) [46]. The dissociation constant of binding (K_D) was determined from the Langmuir isotherm calibration curve of 1/(SPR shift) versus 1/(concentration of penicillinase) [47], and the sensitivity was defined as the slope of SPR shift response versus concentration of penicillinase at low analyte concentrations [39]. Among these three peptides, P3-GGCC displayed the best sensitivity (15.8 RU nM−1) and the smallest K_D (0.56 nM), whereas P1-GGCC showed the second best sensitivity (5.05 RU nM−1) and a slightly larger K_D at 1.81 nM (Table 2). These results were not surprising, because the arginine residues within the peptides played an important role in β-lactamase binding [29]. For P2-GGCC, K_D was 294 nM, which was higher than that of the other two peptides. However, the binding affinity of P2-GGCC to the penicillinase was still quite good, since typical dissociation constants of protein–ligand interactions are in the micromolar to nanomolar range [48]. These results confirmed that our phage display experiments were successful. By using SPR as binding assays, we not only quantitatively determined the binding specificity of selected peptides (P1, P2, and P3) to the penicillinase target, but also developed
an antibody-free SPR biosensor with excellent sensitivity for detection of the target penicillinase.

**Screening of peptide inhibitors**

Not all peptides that bind to penicillinase are good inhibitors. After comparing binding affinity of selected peptides to penicillinase, we tested inhibition efficiency of each peptide. A broad-spectrum peptide inhibitor (RRGHYY) for β-lactamase reported by Huang et al. [29] was used as a benchmark for comparison. A commonly used small molecule inhibitor, potassium clavulanate, was also used for comparison.

To evaluate inhibition efficiency quantitatively, a modified Michaelis–Menten model was employed,

\[
V = \frac{V_{\text{max}}[S]}{\alpha K_m + \alpha'[S]},
\]

where \(V\) is reaction rate, \(V_{\text{max}}\) is maximum reaction rate, \([S]\) is substrate concentration, \(K_m\) is the Michaelis–Menten constant, and the modifying factors \(\alpha\) and \(\alpha'\) are defined by inhibitor concentration \([I]\) and its two dissociation constants \(K_i\) and \(K'_i\):

\[
\alpha = 1 + \frac{[I]}{K_i},
\]

\[
\alpha' = 1 + \frac{[I]}{K'_i}.
\]

The dissociation constant of the enzyme–inhibitor complex \(K_i\) describes the binding ability of a competitive inhibitor to the enzyme, whereas the dissociation constant of the enzyme–substrate–inhibitor complex \(K'_i\) describes the binding ability of an uncompetitive inhibitor to the enzyme–substrate complex. Mixed inhibition can be described by using \(K_i\) and \(K'_i\) together.

Equation (1) can be simplified to Eq. (4) as a Langmuir equation by introducing \(V_{\text{app}}\) and \(K_{\text{app}}\) as follows,

\[
V = \frac{V_{\text{max}}[S]}{K_{\text{app}}^\text{app} + [S]},
\]

where

\[
V_{\text{max}}^\text{app} = \frac{V_{\text{max}}}{\alpha},
\]

\[
K_{\text{app}}^\text{app} = \frac{\alpha}{\alpha' K_m}.
\]

By using UV–vis spectrophotometry and the Beer–Lambert law, \(V\) was determined experimentally using initial absorbance decrease at 240 nm [49]. The difference of extinction coefficients between the product and the substrate (penicillin G) was \(\Delta_{240} = -560 \text{ M}^{-1} \text{ cm}^{-1}\) and the path length was 1 cm [50]. \(V_{\text{max}}\) and \(K_{\text{app}}\) were determined from the Michaelis–Menten kinetics curve by Eq. (4). Then \(K_i\) was calculated by combining Eqs. (2), (5) and (6) to express \(K_{\text{app}}^\text{app}/V_{\text{max}}\) as a function of \([I]\) with an \(x\)-intercept of 0. Similarly, \(K'_i\) was calculated by combining Eqs. (3) and (5), which gave 1/\(V_{\text{max}}\) as a function of \([I]\) with an \(x\)-intercept of \(-K'_i\).

First, 100 \(\mu\text{M}\) of potential inhibitors was mixed with penicillinase and substrate penicillin G to determine their inhibition efficiency. The Michaelis–Menten kinetics curves in Fig. 4 demonstrated that P2 was a good inhibitor for penicillinase, even though it was not as good as potassium clavulanate. P1 showed no inhibition on penicillinase at all, whereas both P3 and RRGHYY acted as weak inhibitors for penicillinase. Kinetic parameters of these potential inhibitors are shown in Table 3. In the absence of inhibitors, \(K_m\) for penicillinase was 60 \(\mu\text{M}\), which was consistent with the \(K_m\) value provided by Sigma–Aldrich. When 100 \(\mu\text{M}\) of P2 was added, the apparent maximum reaction rate (\(V_{\text{app}}\)) was 1.17 \(\mu\text{M s}^{-1}\), the turnover number (\(k_{\text{cat}}\)) was 1.17 \(\mu\text{M s}^{-1}\), the number of substrate molecules converted to product per enzyme molecule per second) was 58.5 \(\mu\text{M s}^{-1}\), which is less than that when other 100 \(\mu\text{M}\) peptides were used and 48% lower than when no inhibitor was used, and the constant (\(k_{\text{cat}}/K_m\), a measure of how efficiently an enzyme converts a substrate into product) decreased to

![Fig.3. Comparison of equilibrium SPR responses to penicillinase using SPR sensor chips modified with different peptides (P1-GGGC, P2-GGGC, or P3-GGGC).](Image)

<table>
<thead>
<tr>
<th>Peptides</th>
<th>MW</th>
<th>Sensitivity (RU nM⁻¹)</th>
<th>(K_0) (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1-GGGC</td>
<td>1805.91</td>
<td>5.05</td>
<td>1.81</td>
</tr>
<tr>
<td>P2-GGGC</td>
<td>1699.82</td>
<td>0.12</td>
<td>294</td>
</tr>
<tr>
<td>P3-GGGC</td>
<td>1583.75</td>
<td>15.8</td>
<td>0.56</td>
</tr>
</tbody>
</table>

![Fig.4. Michaelis–Menten kinetics curves of penicillinase with substrate penicillin G in the presence of no inhibitor, 100 \(\mu\text{M}\) peptides (P1, P2, P3, or RRGHYY), or 100 \(\mu\text{M}\) potassium clavulanate.](Image)
0.73 μM⁻¹ s⁻¹, which is also lower than that when other 100 μM peptides were used and 61% lower than without inhibitor.

To further confirm that P2 is an inhibitor of penicillinase and determine its inhibition efficiency, the concentration-dependent inhibition of peptides on penicillinase activity was studied (Fig. 5). Michaelis–Menten kinetics curves showed that P1 exhibited little inhibition of penicillinase activity even at a concentration as high as 200 μM (Fig. 5A). P3 and RRGHYY presented some weak inhibition of penicillinase activity, but the inhibition efficiency was not concentration-dependent (Fig. 5C and D). Only P2 showed concentration-dependent inhibition of penicillinase activity (Fig. 5B). As the concentrations of P2 increased from 0 to 200 μM, the V_max decreased from 2.24 to 0.49 μM s⁻¹, and both k_cat and k_cat/K_m decreased accordingly (Table 3). P2 was found to inhibit penicillinase with a K_i of 9.22 μM and a K_i' of 33.12 μM, showing a mixed inhibition fashion. This meant P2 can bind to both penicillinase and penicillinase–penicillin G complex. The control peptide RRGHYY, claimed to be a broad-spectrum peptide inhibitor of β-lactamase [29], did not perform as well as P2 in terms of inhibition efficiency. Since different β-lactamases share low sequence identity and have major differences in their active site [30], new peptide inhibitors need to be identified for each specific type of β-lactamase to achieve maximum inhibition efficiency. Among three selected peptides, although P2 did not show the best binding affinity to the penicillinase, it showed the highest inhibition efficiency on penicillinase activity. These results were not surprising because good binding alone, as defined by ensemble measurements of affinity, was not sufficient to make a good enzyme inhibitor [51]. Therefore, after initial screening of inhibitors by affinity binding, it is crucial to test the inhibition efficiency of selected peptides to ensure the identification of good inhibitor candidates.

Table 3
Michaelis–Menten constants and kinetic parameters of penicillinase with substrate penicillin G in the presence of no inhibitor, 100 μM peptides (P1, P2, P3, or RRGHYY), 100 μM potassium clavulanate, or other concentrations of P2 (50 and 200 μM).

<table>
<thead>
<tr>
<th>Potential inhibitors</th>
<th>V_max (μM s⁻¹)</th>
<th>K_max (μM)</th>
<th>k_cat (s⁻¹)</th>
<th>k_cat/K_m (μM⁻¹ s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No inhibitor</td>
<td>2.24 ± 0.12</td>
<td>60</td>
<td>112.0 ± 6.2</td>
<td>1.87 ± 0.10</td>
</tr>
<tr>
<td>100 μM P1</td>
<td>2.28 ± 0.22</td>
<td>50</td>
<td>114.0 ± 10.9</td>
<td>2.28 ± 0.22</td>
</tr>
<tr>
<td>100 μM P2</td>
<td>1.17 ± 0.06</td>
<td>80</td>
<td>58.5 ± 3.1</td>
<td>0.73 ± 0.04</td>
</tr>
<tr>
<td>100 μM P3</td>
<td>1.64 ± 0.08</td>
<td>55</td>
<td>82.0 ± 3.9</td>
<td>1.49 ± 0.07</td>
</tr>
<tr>
<td>100 μM RRGHYY</td>
<td>1.89 ± 0.03</td>
<td>70</td>
<td>94.5 ± 1.4</td>
<td>1.35 ± 0.02</td>
</tr>
<tr>
<td>100 μM Potassium clavulanate</td>
<td>0.63 ± 0.06</td>
<td>110</td>
<td>31.5 ± 3.1</td>
<td>0.29 ± 0.03</td>
</tr>
<tr>
<td>50 μM P2</td>
<td>1.66 ± 0.05</td>
<td>40</td>
<td>83.0 ± 2.7</td>
<td>2.08 ± 0.07</td>
</tr>
<tr>
<td>200 μM P2</td>
<td>0.49 ± 0.06</td>
<td>80</td>
<td>24.5 ± 2.8</td>
<td>0.31 ± 0.04</td>
</tr>
</tbody>
</table>

Fig.5. Michaelis–Menten kinetics curves of penicillinase with substrate penicillin G in the presence of 0 (black squares), 50 (red squares), 100 (green squares), or 200 (blue squares) μM peptides: (A) P1, (B) P2, (C) P3, (D) RRGHYY. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
Conclusions

In this paper, we have successfully identified peptide P2 (NIYTPWGNSWS) as a potent penicillinase inhibitor. Its inhibition behavior is considered to be a mixed pattern (K_i = 9.22 μM and K_D = 33.12 μM), which means that P2 can bind to both penicillinase and penicillinase–penicillin G complex. Its inhibition efficiency is better than a reported peptide inhibitor, RGCHYY, but not as good as the small-molecule inhibitor potassium clavulanate. This peptide inhibitor (P2) may be a useful starting point for the design of novel small-molecule inhibitors for penicillinase after the structure of P2 and its binding mechanisms is fully understood. The other two peptides, P1 (DHHRSYREGED) and P3 (SHSLPASADLLR), although they bind to penicillinase strongly, as shown in SPR, show weak or no inhibition efficiency on penicillinase.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jab.2015.10.009.

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