Biochemical Characterization of SHV-55, an Extended-Spectrum Class A β-Lactamase from *Klebsiella pneumoniae*

We biochemically characterized the *Klebsiella pneumoniae* extended-spectrum SHV-55 enzyme carrying the amino acid substitutions Tyr7→Phe (as in SHV-28) and Gly238→Ser and Glu240→Lys (both found in SHV-5) identified in a previous study (5). The SHV-55 extended-spectrum β-lactamase differed from SHV-5 only in the signal peptide region (1). The *bla*<sup>SHV-55</sup> gene was obtained as described by Mendonça et al. (6), and transformants were selected on Luria broth agar supplemented with 30 μg/L kanamycin/ml and 16 μg/ml amoxicillin/ml. SHV-55 was extracted and purified according to the previously described protocol (6). The Michaelis constant (K<sub>m</sub>) and catalytic activity (k<sub>cat</sub>) of purified extracts of SHV-55 were obtained by using a computerized microacidimetric method and a 702 SM Tititino pH-stat apparatus (Metrohm, Herisau, Switzerland) (3). The complete hydrolysis time courses were analyzed, and the kinetic progress curves were fitted by nonlinear least-squares regression. These kinetic parameters were determined and compared to those of the SHV-1 enzyme for 10 β-lactams (Table 1).

SHV-55 has a high affinity (K<sub>m</sub>, 5 to 10 μM) for penicillins, similar to that of SHV-5 (1) and higher than that of SHV-1 (K<sub>m</sub>, 11 to 31 μM). SHV-55 presented higher affinity values (K<sub>m</sub>, 9 to 58 μM) than SHV-1 (K<sub>m</sub>, 40 to 257 μM) for narrow-, extended-, and broad-spectrum cephalosporins and monobactams. This finding may be a consequence of the Gly238→Ser substitution present in the active sites of both SHV-55 and SHV-5, which pushes the β-strand out and away from the reactive Ser70 (2). This effect results in a slightly expanded active site that may improve binding and accommodate cephalosporins with bulky side chains (4). SHV-55 presented a higher affinity for cefotaxime than for cefazidime (K<sub>m</sub>s, 21 and 58 μM, respectively), as did SHV-5 (1, 7). This finding is surprising because both enzymes possess the Glu240→Lys substitution, which increases hydrolytic activity against cefazidime (8) due to the change in the electrostatic charge of the exposed group at position 240 (2). The enzymatic activities (k<sub>cat</sub>) of SHV-55 for penicillin G and amoxicillin were 84- and 45-fold lower, respectively, than those of SHV-1, and the catalytic efficiency (k<sub>cat</sub>/K<sub>m</sub>) ratio against penicillins was more than 10-fold higher for SHV-1 (k<sub>cat</sub>/K<sub>m</sub> ratio, 20 to 84 μM<sup>−1</sup>·s<sup>−1</sup>) than for SHV-55 (k<sub>cat</sub>/K<sub>m</sub> ratio, 2 to 5 μM<sup>−1</sup>·s<sup>−1</sup>). However, the enzyme activity and catalytic efficiency against extended- and broad-spectrum cephalosporins were higher for SHV-55 (k<sub>cat</sub>, 7 to 24 s<sup>−1</sup>, and k<sub>cat</sub>/K<sub>m</sub> ratio, 0.2 to 1 μM<sup>−1</sup>·s<sup>−1</sup>) than for SHV-1 (note, however, that the values for monobactam were undeterminable), although the catalytic efficiencies of the two enzymes against cefalothin were similar (k<sub>cat</sub>/K<sub>m</sub> ratios, 3.2 and 4.4 μM<sup>−1</sup>·s<sup>−1</sup>). This result may be due to the amino acid substitutions in SHV-55 causing conformational modifications in the active site. Fifty percent inhibitory concentrations (IC<sub>50</sub>) indicated that SHV-55 was nine-fold more susceptible to the inhibitor activity of clavulanate than SHV-1 (IC<sub>50</sub> of clavulanate, 0.02 versus 0.17 μM).

In conclusion, these results confirmed the extended-spectrum activity of the SHV-55 enzyme, which is important due to the magnitude of extended- and broad-spectrum SHV β-lactamases described to date and not biochemically characterized, in spite of the ease of sequencing genes (http://www.lahey.org/studies).

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<tr>
<th>Antibiotic</th>
<th>SHV-1&lt;sup&gt;b&lt;/sup&gt;</th>
<th>SHV-55&lt;sup&gt;b&lt;/sup&gt;</th>
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<tr>
<td></td>
<td>K&lt;sub&gt;m&lt;/sub&gt; (μM)</td>
<td>k&lt;sub&gt;cat&lt;/sub&gt;/K&lt;sub&gt;m&lt;/sub&gt; ratio (μM&lt;sup&gt;−1&lt;/sup&gt;·s&lt;sup&gt;−1&lt;/sup&gt;)</td>
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<tr>
<td>Clavulanic acid</td>
<td>23 ± 0.42</td>
<td>1,937 ± 82</td>
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<tr>
<td>Penicillin G</td>
<td>31 ± 1.29</td>
<td>1,044 ± 10</td>
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<tr>
<td>Amoxicillin</td>
<td>11 ± 3.40</td>
<td>220 ± 49</td>
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<tr>
<td>Ticarcillin</td>
<td>24 ± 0.53</td>
<td>1,490 ± 96</td>
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<td>Piperacillin</td>
<td>40 ± 1.46</td>
<td>128 ± 33</td>
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<td>Cephalothin</td>
<td>80 ± 0.59</td>
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<tr>
<td>Cefuroxime</td>
<td>142 ± 3.18</td>
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<td>Cefotaxime</td>
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<tr>
<td>Aztreonam</td>
<td>ND</td>
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<tr>
<td>Cefepime</td>
<td>149 ± 7.30</td>
<td>&lt;0.1</td>
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<sup>a</sup> Values (except IC<sub>50</sub>) are means ± standard deviations.

<sup>b</sup> Data are from reference 6.

<sup>c</sup> ND, not determinable.

TABLE 1. Kinetic constants of SHV-55 and SHV-1 β-lactamases^a^
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REFERENCES


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