Comparative Stability of Newly Introduced β-Lactam Antibiotics to Renal Dipeptidase

HIDETADA MIKAMI, MIEKO OGASHIWA, YUSHI SAINO, MATSUHISA INOUE,* AND SUSUMU MITSUHASHI

Department of Microbiology and Laboratory of Drug Resistance in Bacteria, School of Medicine, Gunma University, Maebashi, Japan

Received 18 March 1982/Accepted 15 July 1982

Renal dipeptidase purified from swine kidney hydrolyzed N-formimidoyl thienamycin, carpetimycins A and B, and Sch29482, but not aztreonam, penicillin G, or cephaloridine.

During the last several years a number of new β-lactam antibiotics have appeared which differ markedly in both their side chains and their ring structures from the classical penicillins and cephalosporins. Some of these antibiotics include carbapenem antibiotics such as thienamycin (5), epithienamycins (11), olivamic acids (1), PS-5 (10), carpetimycins (9), and aspapenemycins (6). Recently, it was found that thienamycin, N-formimidoyl thienamycin (7, 14), and related carbapenem compounds were considerably inactivated in vivo by renal dipeptidase (dehydropeptidase-I, EC3.4.14.11) (H. Kropp, J. G. Sundelof, R. Hajdu, and F. M. Kahan, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 20th, New Orleans, La., abstr. no. 272, 1980). Renal dipeptidase is known to be distributed widely in various mammalian species. The enzyme has been purified from swine (3) and human kidney (12). This paper deals with the stability of newly introduced β-lactam antibiotics to renal dipeptidase isolated from swine kidney.

The carbapenem antibiotics N-formimidoyl thienamycin (MK0787) and carpetimycins A and B were gifts from Merck & Co., Inc., Rahway, N.J., and Tokyo Research Laboratory, Kowa Co., Ltd., Tokyo, Japan, respectively. A new penem antibiotic, Sch29482 (V. M. Girijavallabhan, A. K. Ganguly, S. W. McCombie, P. Pinto, and R. Rizvi, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 21st, Chicago, Ill., abstr. no. 829, 1981), was supplied by Schering Corp., Bloomfield, N.J. Azthreonam (SQ26,776) (13), a synthetic monocyclic β-lactam (monobactam), was obtained from The Squibb Institute for Medical Research, Princeton, N.J. Penicillin G and cephaloridine were commercially available materials.

Renal dipeptidase was purified from swine kidney cortex by a modification of the procedures reported by Campbell et al. (2, 3) with glycyldihydrophenylalanine as an assay substrate.

Fresh swine kidney tissue (about 1.5 kg) was homogenized in a Bio-mixer (BM-2; Nihon Seiki Co., Ltd., Tokyo, Japan) with 2 volumes of ice water. From the resulting supernatant, dipeptidase was solubilized by treatment with n-butanol and then subjected to ammonium sulfate fractionation as described (2). The enzyme, which precipitated between 50 and 75% saturation of ammonium sulfate, was further purified by carboxymethyl-cellulose column chromatography, followed by isoelectric focusing and gel filtration through Sephadex G-200. The isoelectric focusing was carried out with a carrier ampholyte of pH 3.5 to 10.0 and a sucrose gradient in a 110-ml column supplied with a constant potential of 300 V at 4°C.

As shown in Table 1, the renal dipeptidase was purified about 700-fold with an overall recovery of 0.75%. The final preparation had a specific activity of 9.48 U/mg of protein and gave a single protein band on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The molecular weight of the purified enzyme, estimated from electrophoretic mobility in the sodium dodecyl sulfate-polyacrylamide gel, was about 47,000, which was very similar to the published value of 47,200 (3). The isoelectric point (pI) of the enzyme, determined from isoelectric focusing chromatography, was 5.5.

Hydrolysis of the compounds was assayed spectrophotometrically (2, 4) by measuring the decrease in absorbance at the substrate specific wavelength in a temperature-controlled spectrophotometer (Beckman model 24). The absorbance maxima of substrates were as follows: glycyldihydrophenylalanine, 279 nm; N-formimidoyl thienamycin, 303 nm; Sch29482, 328 nm; carpetimycin A, 288 nm; carpetimycin B, 285 nm; aztreonam, 213 nm; penicillin G, 233 nm; and cephaloridine, 260 nm. One unit of the
TABLE 1. Summary of the purification of renal dipeptidase from swine kidney

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Total activity (U)*</th>
<th>Sp act (U/mg of protein)</th>
<th>Purification factor</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Homogenization</td>
<td>2,668</td>
<td>0.0137</td>
<td>1.00</td>
<td>100</td>
</tr>
<tr>
<td>2. Precipitation at pH 5.0</td>
<td>1,288</td>
<td>0.0113</td>
<td>0.82</td>
<td>48.3</td>
</tr>
<tr>
<td>3. Washing with phosphate buffer</td>
<td>686</td>
<td>0.0089</td>
<td>0.65</td>
<td>25.7</td>
</tr>
<tr>
<td>4. Solubilization with n-butanol</td>
<td>130</td>
<td>0.182</td>
<td>13.3</td>
<td>4.87</td>
</tr>
<tr>
<td>5. Fractionation with ammonium sulfate</td>
<td>101</td>
<td>1.03</td>
<td>75.2</td>
<td>3.79</td>
</tr>
<tr>
<td>6. Chromatography on carboxymethyl cellulose</td>
<td>32.9</td>
<td>1.38</td>
<td>101</td>
<td>1.23</td>
</tr>
<tr>
<td>7. Isoelectric focusing</td>
<td>21.7</td>
<td>6.46</td>
<td>472</td>
<td>0.81</td>
</tr>
<tr>
<td>8. Sephadex G-200 gel filtration</td>
<td>20.0</td>
<td>9.48</td>
<td>692</td>
<td>0.75</td>
</tr>
</tbody>
</table>

* Enzyme units were determined spectrophotometrically with 50 μM glycyldidehydrophenylalanine as the substrate (see text).

TABLE 2. Kinetics of hydrolysis of various β-lactam antibiotics by the renal dipeptidase purified from swine kidney

<table>
<thead>
<tr>
<th>Substrate</th>
<th>$K_m$ (mM)</th>
<th>$V_{max}$ (U/mg of protein)</th>
<th>Relative hydrolysis rate*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycyldehydrophenylalanine</td>
<td>1.10</td>
<td>200</td>
<td>5,330</td>
</tr>
<tr>
<td>N-Formimidoyl thienamycin</td>
<td>1.38</td>
<td>3.75</td>
<td>100</td>
</tr>
<tr>
<td>Sch29482</td>
<td>0.998</td>
<td>1.21</td>
<td>32</td>
</tr>
<tr>
<td>Carpetimycin A</td>
<td>0.229</td>
<td>13.9</td>
<td>371</td>
</tr>
<tr>
<td>Carpetimycin B</td>
<td>0.323</td>
<td>8.67</td>
<td>231</td>
</tr>
</tbody>
</table>

* Aztreonam, penicillin G, and cephaloridine were not detected.

Values are expressed as percent hydrolysis of N-formimidoyl thienamycin.

The enzyme was defined as the amount of enzyme which hydrolyzed 1 μmol of substrate per min per mg of protein at 35°C in 50 mM MOPS [3-(N-morpholino)propanesulfonic acid; Wako Pure Chemical Industries Ltd., Osaka, Japan] (pH 7.2). The concentration of protein was estimated by the method of Lowry et al. (8), using bovine serum albumin as the standard. The Michaelis constant ($K_m$) and maximum rate of hydrolysis ($V_{max}$) were determined from Lineweaver-Burk plots with substrate concentration between 200 and 25 μM.

The kinetic parameters of enzyme activity for various compounds are shown in Table 2. N-Formimidoyl thienamycin, carpetimycins A and B, and Sch29482 were appreciably hydrolyzed by the enzyme, although the $V_{max}$ values of these substrates were one to two orders of magnitude lower than that of glycyldehydrophenylalanine. The enzyme hydrolyzed carpetimycins A and B two to four times more rapidly than N-formimidoyl thienamycin. The $K_m$ values of N-formimidoyl thienamycin and Sch29482 were almost equal to that of glycyldehydrophenylalanine. Carpetimycins A and B had a somewhat higher affinity for the enzyme, as indicated by their relatively low $K_m$ values.

On the other hand, no detectable hydrolysis was observed with aztreonam, penicillin G, or cephaloridine. These results were also confirmed by microbiological assay (data not shown).

Renal dipeptidase has been known to be more active against carbapenem antibiotics with an N-acetyl group on the cysteamine side chain at the 3-position than against the unacetylated forms (11). A similar situation may be involved in the case of carpetimycins A and B and Sch29482.

Our results and those of earlier workers (6, 11) suggest that carbapenem antibiotics and, in addition, penem antibiotics have a common property: they are susceptible to hydrolysis by renal dipeptidase. This property presumably causes a discrepancy between in vitro and in vivo activities and reduces the urinary recovery of these drugs.

Although the physicochemical properties and substrate specificities of renal peptidase may vary from organism to organism, the in vitro susceptibility test described here will be a useful screening method to search for newer β-lactam antibiotics possessing improved stability in vivo.

We thank K. Saida for supplying swine kidney tissues and Y. Hattori for his technical assistance.

This work was supported in part by scientific research grant 56030023 from the Ministry of Education, Science and Culture, Japan.

LITERATURE CITED

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