Hydrolysis by Cathepsin B of Fluorescent Peptides Derived From Human Prorenin

Paulo Cezar Almeida, Vitor Oliveira, Jair Ribeiro Chagas, Morten Meldal, Maria Aparecida Juliano, Luiz Juliano

Abstract—Cathepsin B is a lysosomal thiolprotease that, because of its colocalization with renin and its ability to activate prorenin, has been proposed as a prorenin processing enzyme. To characterize the biochemical aspect of this potential cathepsin B activity in more detail, we synthesized and assayed with human cathepsin B the internally quenched fluorescent peptide Abz-FSQPMKRTLGLGNTTQ-EDDnp (Abz, ortho-aminobenzoic acid fluorescent group and EDDnp, N-[2,4-dinitrophenyl]-ethylenediamine quencher group) that contains 7 amino acids for each side of the R-L bond that is the processing site of human prorenin. Human cathepsin B hydrolyzed this peptide at the correct site (R-L bond), with $k_{cat}/K_m = 75 \text{ mmol/L}^{-1} \text{s}^{-1}$. Analogues of this peptide obtained by Ala scanning at positions P$_5$ to P$_5'$ were also synthesized and assayed as substrates for human cathepsin B. The obtained specificity constant ($k_{cat}/K_m$) values have a significant parallel with the previous data of prorenin activation by AtT-20 cells and in vitro by cathepsin B. In addition, we demonstrated the presence of cathepsin B–like activity in rat mesangial cells and the ability of its whole soluble fraction lysates, as well as that of purified cloned rat cathepsin B, to hydrolyze Abz-IKKSSF-EDDnp at the K-S bond, which contains 6 amino acids of rat prorenin processing site. The specificity data of cathepsin B toward peptides derived from prorenin processing site support the view that human or rodent cathepsin B could be involved in the intracellular processing of prorenin that is locally synthesized or taken up from the extracellular compartment. (Hypertension. 2000;35:1278-1283.)

Key Words: renin ♦ prorenin ♦ cathepsin B ♦ mesangium ♦ substrate, fluorogenic

Renin, a very specific aspartyl protease, catalyzes the rate-limiting step that generates angiotensin I from plasma angiotensinogen. Renal juxtaglomerular cells are the main source of active plasma renin. In those cells, active plasma renin is synthesized as preprorenin and is then converted to prorenin on its insertion into the endoplasmic reticulum (for a more comprehensive review and references, see Hsueh and Baxter). The sorting to regulated pathway depends on the presence of protease processing site, which is constituted in human prorenin of the basic amino acid pair Lys-Arg. The protease that activates prorenin to renin in the regulated pathway is still unknown, although PCs and cathepsin B have been indicated as candidates. Prorenin released constitutively from renal and extrarenal tissues also circulates in the blood, and its plasma concentration is 10 times higher than that of active renin. There is substantial evidence of local production of angiotensin that is independent of the circulating renin-angiotensin system. The presence of all components of renin-angiotensin system has been demonstrated in several tissues. The origin of this local prorenin, renin, and angiotensinogen is still not clear, because particularly in heart, endothelium, and vascular smooth muscle cells there is evidence that these components are taken up from circulating plasma. It has also been reported that the internalization and activation of prorenin by cardiac cells are mediated by mannose 6-phosphate receptors. Immunoactive renin was detected in proximal tubular cells, (possibly in lysosomes) by immunoelectron microscopy. Because these cells synthesize very little renin mRNA, the renin found there could be taken up from the duct lumen. On the basis of these arguments, it is reasonable to admit that cathepsin B and renin could occupy the same subcellular compartments, particularly the late endosomes and lysosomes. As a consequence, cathepsin B could activate the prorenin that was taken up from the milieu. Although in the renin prosegment there are several potential sites that could be cleaved by cathepsin B, that enzyme processed in vitro the human prorenin secreted by transfected AtT-20 cells, to produce active renin with the expected molecular weight range in SDS-PAGE electrophoresis. Mutated human prorenins resulted from Ala scanning of amino acids P$_3$ to P$_5'$ around the cleavage site (Schechter and Berger nomenclature) and were processed by cathepsin B with different efficiency. AtT-20 cells were unable to secrete active renin
from an R/A prorenin mutated at P, position, except when cotransfected with cathepsin B.21 Radiographic structures available for cathepsin B indicate that its occluding loop is located toward the prime region of the active site of this enzyme. The occluding loop is also responsible for the low kcat values for cathepsin B endopeptidase activity previously reported.23 To evaluate the human cathepsin B efficiency and to determine whether the cleavage occurs in the correct prorenin processing peptide bond, we synthesized and assayed with human cathepsin B the internally quenched fluorescent peptide Abz-FSQPMKRLTGLNTTQ-EDDnp. Abz, ortho-aminobenzoic acid and EDDnp, N-[2,4-dinitrophenyl]-ethylene diamine were the fluorescent and quencher groups, respectively, and glutamine (Q) was introduced as a requirement for the peptide solid-phase synthesis.24 This peptide contains the amino acid sequence from P7 to P7 of the human prorenin cleavage site. An Ala scanning was also performed from P7 to P7, and the resulting peptides were assayed as substrates for human cathepsin B. Kinetic parameters for the hydrolysis of these peptides were determined with bovine trypsin, a reference enzyme that is commonly used to activate prorenin. In conjunction with this investigation of prorenin processing by cathepsin B and as a result of a previous report25 stating that mesangial cells secrete prorenin and renin and also take up renin from the medium by a specific receptor binding,26 we investigated cathepsin B activity in rat mesangial cells and its activity on the internally quenched fluorescent peptide Abz-IKKSSF-EDDnp, which is derived from the rat prorenin cleavage site.

Methods

Peptides
Z-Phe-Arg-MCA and Z-Arg-Arg-MCA were purchased from Nova Biochem. All the internally quenched fluorescent peptides of the general structure Abz-peptidyl-Q-EDDnp (qf-peptides) containing Gin (Q) at the C-terminus were synthesized by the solid-phase method24 and were subjected to purification by preparative reverse phase high-pressure (performance) liquid chromatography. MALDI-TOF mass spectrometry (TofSpec-E, Micromass) and a review of amino acid compositions were used to check the molecular weight and purity of synthesized peptides. In addition, qf-peptide 1 was examined by nuclear magnetic resonance, which revealed the random structure of that peptide. The concentrations of the peptides were determined by colorimetric determination of the 2,4-dinitrophenyl group (extinction coefficient at 365 nm, 17300 mol/L·cm−1).

Enzymes
Human recombinant cathepsin B and rat recombinant cathepsin B were obtained as previously described.23,27 The molar concentrations of the enzyme solutions were determined by active site titration with E-64.28 β-Trypsin was purified as described elsewhere28 from a twice-crystallized bovine trypsin (Biobras Co) previously treated with tosyl phenylalanyl chloromethyl ketone (TPCK), and the operational molarities were determined by active site titration.30

Fluorometric Enzyme Assay
Hydrolysis of the qf-peptides by cathepsin B was performed at 37°C in 50.0 mmol/L phosphate buffer, 200.0 mmol/L NaCl, 2.5 mmol/L DTE, and 5.0 mmol/L EDTA (pH 6.0), after which the fluorescence was measured at λex=420 nm and λem=320 nm according to a Hitachi F-2000 spectrophotometer. A 1-cm path-length cuvette containing 2 mL of the substrate solution was placed in a thermostatically controlled cell compartment for 5 minutes before the enzyme solution was added, after which the increase in fluorescence with time was recorded continuously for 10 minutes. The slope was converted into moles of substrate hydrolyzed per minute on the basis of the fluorescence curves of standard peptide solutions before and after total enzymatic hydrolysis.31 The kinetic parameters were calculated by nonlinear regression according Wilkinson.32 The β-trypsin activity was measured in 100 mmol/L Tris-HCl buffer (pH 8.0) containing 10 mmol/L CaCl2 at 37°C. The cleavage sites of peptides were identified by mass spectroscopy as indicated above and by peptide sequencing that used a protein sequencer PPSQ-23 (Shimadzu).

Mesangial Cell Culture
The glomeruli were isolated from Wistar rats as previously described.33 The rats were anaesthetized with ethyl ether and underwent bilateral nephrectomy. The kidneys were decapsulated, and cortical macrodisssection was performed. The cortex was separated from the outer medulla and was sliced and forced through a graded series of stainless-steel meshes (60, 100, and 200 mesh, respectively). The glomeruli were then collected from the surface of the third sieve and were fully disrupted by being forced through a 2×7-gauge needle. The decapsulated glomeruli were plated at a density of ∼300 glomeruli/cm2 onto RPMI 1640 supplemented with 20% fetal calf serum, 50.0 U/mL penicillin, 2.6 g acid HEPES, and 2.0 mmol/L glutamine. Culture flasks were kept in a humidified environment (95% air and 5% CO2) at 37°C. The medium was replaced every 36 hours. After 3 weeks in the primary culture, mesangial cells were harvested with trypsin. Subcultures were grown in the same medium. This procedure was repeated up to the fifth subculture, when the cells were prepared for the experiment. Mesangial cells (fifth passage) were incubated for 20 hours with RPMI and without fetal bovine serum, and mesangial cells and medium were collected for the experiments. The mesangial cells were characterized as previously described.34

Cell Disruption
Whole-cell homogenates were obtained by suspending the washed cells in PBS, cooling them on ice, and disrupting them by sonication (3×30 s-pulses at a power setting of 60%) by means of a Sonic Dismembranator with a microprobe (Heat Systems). The resulting suspensions were centrifuged at 1000g for 5 minutes at 4°C. The supernatants were removed, divided into aliquots, and stored at −20°C. The total protein concentrations were determined according to Bradford.35 The fluorometric assays using qf-peptides were performed as described above, and with respect to Z-Phe-Arg-MCA and Z-Arg-Arg-MCA, the initial velocities were also recorded spectrofluorometrically but λex=380 nm and λem=460 nm were used. Before the reaction was initiated by addition of 10 μL of substrate stock solution prepared in 30% DMSO, the lysate (10 to 100 μL) was preincubated for 10 minutes in a thermostated cuvette at 37°C with 2.0 mL of buffer containing the inhibitors of hydrolytic activity that we wanted to suppress (5 μmol/L pepstatin, 1 mmol/L PMSF, 1 mmol/L EDTA) and thiol activator was added (5 mmol/L DTT). Initial velocities were obtained by continuously recording the fluorescence for 5 to 10 minutes. The following buffers were used: 0.1 mol/L potassium phosphate (pH 3.0 to 3.5), 0.1 mol/L sodium acetate (pH 3.5 to 5.5), 0.1 mol/L sodium phosphate (pH 5.5 to 8.0), and 0.1 mol/L Tris-HCl (pH 8.0 to 9.0).

Results

Hydrolysis of qf-Peptides Derived From the Human Prorenin Processing Site
Table 1 shows the sequences of all synthesized qf-peptides related to human prorenin as well as their molecular masses determined by MALDI-TOF mass spectrometry. The qf-peptide 1 has the sequence of the human prorenin processing site from P7 to P7. The qf-peptides 2 to 11 have the same size

---

Almeida et al  Fluorescent Peptides Derived from Human Prorenin 1279
and sequence of the qf-peptide 1, except that Ala was put in the position of each amino acid from P5 to P1 sites. All peptides were susceptible to hydrolysis by human cathepsin B. The peptide 1 and those with Ala at nonprime positions (P3, P4, P5) (peptides 2 to 4, Table 2) were hydrolyzed at the K-R and R-L bonds, except peptide 8, which was cleaved at the R-L bond as the peptide 1. The kinetic parameters for the hydrolysis of all these substrates, which are presented in Table 2, were determined. The kinetic constant values for those substrates with 2 cleavages are also shown to give a comparative order of magnitude of hydrolysis because these are apparent constants. All the modifications on the natural sequence (peptide 1) resulted in substrates hydrolyzed by human cathepsin B with higher specificity constants (kcat/Km), except peptides 3 and 6, in which Ala substituted Pro and Arg at positions P4 and P5, respectively. Peptide 1 was hydrolyzed with the higher kcat and Km values, which indicates that cathepsin B hydrolyzes peptide 1 with higher velocity but with lower affinity than any other analogue. Curiously, peptides 10 and 11 were hydrolyzed by cathepsin B, which has higher affinity; this indicates that positions P9 and P9 have significant influence on substrate binding. Peptides 12 and 13 were synthesized and assayed to verify the effects of Phe and Gly at the P4 position on the hydrolysis of qf-peptides by cathepsin B, because these modifications were performed on site-mutated human prorenin to force or release conformation restrictions at that position. Pro was described to be critical for prorenin processing, and mutants with Ala, Phe, or Gly impaired the capacity of AtT-20 cells to process prorenin. The observed low kcat/Km values for hydrolysis of peptides 12 and 13 are in parallel with the resistance to be processed of P/F-mutated, P/G-mutated, and P/A-mutated prorenin mentioned above. Table 2 also shows the kinetics parameters for hydrolysis by trypsin of those previously mentioned peptides with human prorenin sequence. All peptides were susceptible to hydrolysis by trypsin, and the R-L bond was essentially the cleavage bond except in peptide 6, in which Ala was substituted for Arg and the cleavage shifted to the K-A bond. The kinetic parameters presented in Table 2 indicate that trypsin, when compared with human cathepsin B, has lower

### Table 1. Internally Quenched Fluorogenic Peptides Related to the Human Prorenin Processing Site and Their Molecular Masses Obtained by MALDI-TOF Mass Spectroscopy

<table>
<thead>
<tr>
<th>No.</th>
<th>Peptides</th>
<th>Molecular Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Calculated</td>
</tr>
<tr>
<td>1</td>
<td>Abz-FSQPKRLTLGNTTQ-EDDnp</td>
<td>2049</td>
</tr>
<tr>
<td>2</td>
<td>Abz-FSQPKRLTLGNTTQ-EDDnp</td>
<td>1992</td>
</tr>
<tr>
<td>3</td>
<td>Abz-FSQPKRLTLGNTTQ-EDDnp</td>
<td>2023</td>
</tr>
<tr>
<td>4</td>
<td>Abz-FSQPKRLTLGNTTQ-EDDnp</td>
<td>1989</td>
</tr>
<tr>
<td>5</td>
<td>Abz-FSQPKRLTLGNTTQ-EDDnp</td>
<td>1992</td>
</tr>
<tr>
<td>6</td>
<td>Abz-FSQPKRLTLGNTTQ-EDDnp</td>
<td>1964</td>
</tr>
<tr>
<td>7</td>
<td>Abz-FSQPKRLTLGNTTQ-EDDnp</td>
<td>2007</td>
</tr>
<tr>
<td>8</td>
<td>Abz-FSQPKRLTLGNTTQ-EDDnp</td>
<td>2019</td>
</tr>
<tr>
<td>9</td>
<td>Abz-FSQPKRLTLGNTTQ-EDDnp</td>
<td>2063</td>
</tr>
<tr>
<td>10</td>
<td>Abz-FSQPKRLTLGNTTQ-EDDnp</td>
<td>2006</td>
</tr>
<tr>
<td>11</td>
<td>Abz-FSQPKRLTLGNTTQ-EDDnp</td>
<td>2006</td>
</tr>
<tr>
<td>12</td>
<td>Abz-FSQPKRLTLGNTTQ-EDDnp</td>
<td>2009</td>
</tr>
<tr>
<td>13</td>
<td>Abz-FSQPKRLTLGNTTQ-EDDnp</td>
<td>2010</td>
</tr>
</tbody>
</table>

The replaced residues are in bold and italic.

### Table 2. Kinetic Parameters and Peptide Bond Cleaved for the Hydrolysis of Internally Quenched Fluorogenic Substrates Related to Human Prorenin Processing Site by Human Cathepsin B and Trypsin

<table>
<thead>
<tr>
<th>Human Cathepsin B</th>
<th>Trypsin</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td></td>
</tr>
<tr>
<td>Km, µmol/L</td>
<td>kcat, s⁻¹</td>
</tr>
<tr>
<td>kcat/Km, mmol/L⁻¹ s⁻¹</td>
<td>Cleavage Sites</td>
</tr>
<tr>
<td>1 6.00 0.45 75.00 1 R-L</td>
<td>28.00 24.00 857.00 R-L</td>
</tr>
<tr>
<td>2 2.00 0.25 125.00 1 R-L</td>
<td>4.00 9.00 2250.00 R-L</td>
</tr>
<tr>
<td>3 6.00 0.10 16.00 1 R-L</td>
<td>7.00 14.00 2000.00 R-L</td>
</tr>
<tr>
<td>4 1.40 0.20 142.00 1 R-L</td>
<td>9.00 7.30 811.00 R-L</td>
</tr>
<tr>
<td>5 1.00 0.11 110.00 *R-L/L-T (1/5) 1 R-L</td>
<td>4.00 11.00 2750.00 R-L</td>
</tr>
<tr>
<td>6 2.30 0.06 26.00 1 K-A</td>
<td>29.00 6.00 207.00 K-A</td>
</tr>
<tr>
<td>7 3.00 0.25 83.00 *R-A/K-R (1/1) 1 R-L</td>
<td>11.00 22.20 2018.00 R-L</td>
</tr>
<tr>
<td>8 1.00 0.11 110.00 1 R-L</td>
<td>2.20 9.70 4850.00 R-L</td>
</tr>
<tr>
<td>9 1.00 0.18 180.00 *R-L/K-R (9/1) 1 R-L</td>
<td>7.80 10.80 1384.00 R-L</td>
</tr>
<tr>
<td>10 0.36 0.20 555.00 *R-L/K-R (1/1) 1 R-L</td>
<td>7.50 8.00 1066.00 R-L</td>
</tr>
<tr>
<td>11 0.47 0.10 213.00 *R-L/K-R (1/1) 1 R-L</td>
<td>15.00 19.00 1267.00 R-L</td>
</tr>
<tr>
<td>12 38.00 0.13 3.50 1 R-L</td>
<td>4.00 16.00 4500.00 R-L</td>
</tr>
<tr>
<td>13 2.90 0.05 17.20 1 R-L</td>
<td>7.00 12.00 1714.00 R-L</td>
</tr>
</tbody>
</table>

*Two cleavages were detected, and the numbers in parentheses indicate the hydrolysis ratio. The standard errors of Km and kcat were <7%.
affinity but higher catalytic efficiency toward the same peptides, which results in $k_{\text{cat}}/K_m$ values for trypsin 1 order of magnitude higher than human cathepsin B. As observed with cathepsin B, peptides 1 and 6 were hydrolyzed with the lower $k_{\text{cat}}/K_m$ values, and peptide 1 also presented the higher $k_{\text{cat}}$ and $V_m$ and $L$. We synthesized the peptide Abz-IKKSSF-EDDnp, which contains the sequence of the rat prorenin processing site, and (by analogy to the prorenin of other species) the cleavage site would be the K-S bond. Rat cathepsin B hydrolyzed this peptide at this bond. Abz-IKKSSF-EDDnp was assayed as substrate for the soluble fraction of whole rat mesangial cell lysates and was also hydrolyzed at the K-S bond (pH 3.5 to 8) in the presence of 5 mmol/L pepstatin, 1 mmol/L PMSF, and 1 mmol/L EDTA. This hydrolytic activity was also fully activated by 5 mmol/L DTT and disappeared in the presence of 1 mmol/L E-64. Figure 2 shows the pH profiles activities of rat mesangial cell lysate and rat cathepsin B on Abz-IKKSSF-EDDnp, and both profiles of activities have an almost complete overlapping. The following kinetic parameters were obtained for hydrolysis of Abz-IKKSSF-EDDnp by rat cathepsin B: $k_{\text{cat}}=0.021 \pm 0.01 \text{ s}^{-1}$, $K_m=0.22 \pm 0.04 \text{ mmol/L}$, and $k_{\text{cat}}/K_m=95 \text{ mmol/L}^{-1} \text{ s}^{-1}$, and by whole rat mesangial cell lysates: $K_m=0.40 \pm 0.04 \text{ mmol/L}$ and $V_m=8.5 \text{ nmol/min}$. These data indicate that isolated rat cathepsin B and the cathepsin B activity obtained from the mesangial cells lysate are hydrolytic activities of the same enzyme.

**Discussion**

Human cathepsin B hydrolyzed the qf-peptide 1 with reasonable efficiency ($k_{\text{cat}}/K_m=75 \text{ mmol/L}^{-1} \text{ s}^{-1}$) for its endopeptidase activity when compared with the hydrolysis of Abz-AFRSAAQ-EDDnp ($k_{\text{cat}}/K_m=1.9 \text{ mmol/L}^{-1} \text{ s}^{-1}$) and of Abz-ARRSAAQ-EDDnp ($k_{\text{cat}}/K_m=14 \text{ mmol/L}^{-1} \text{ s}^{-1}$) (data not published). The relative low $k_{\text{cat}}/K_m$ values of endopeptidase activity of cathepsin B compared with those of mutated cathepsin B in which its occluding loop contacts were removed ($k_{\text{cat}}/K_m=928 \text{ mmol/L}^{-1} \text{ s}^{-1}$) demonstrates that the occluding loop limits the access of substrates to the enzyme active-site cleft. This restriction could be the origin of cathepsin B specificity to the human prorenin processing site, although the renin prosegment has 5 sites potentially susceptible to cathepsin B. A turn-like conformation on the prorenin (analog to the prorenin of other species) the cleavage site would be the K-S bond. Rat cathepsin B hydrolyzed this peptide at this bond. Abz-IKKSSF-EDDnp was assayed as substrate for the soluble fraction of whole rat mesangial cell lysates and was also hydrolyzed at the K-S bond (pH 3.5 to 8) in the presence of 5 mmol/L pepstatin, 1 mmol/L PMSF, and 1 mmol/L EDTA. This hydrolytic activity was also fully activated by 5 mmol/L DTT and disappeared in the presence of 1 mmol/L E-64. Figure 2 shows the pH profiles activities of rat mesangial cell lysate and rat cathepsin B on Abz-IKKSSF-EDDnp, and both profiles of activities have an almost complete overlapping. The following kinetic parameters were obtained for hydrolysis of Abz-IKKSSF-EDDnp by rat cathepsin B: $k_{\text{cat}}=0.021 \pm 0.01 \text{ s}^{-1}$, $K_m=0.22 \pm 0.04 \text{ mmol/L}$, and $k_{\text{cat}}/K_m=95 \text{ mmol/L}^{-1} \text{ s}^{-1}$, and by whole rat mesangial cell lysates: $K_m=0.40 \pm 0.04 \text{ mmol/L}$ and $V_m=8.5 \text{ nmol/min}$. These data indicate that isolated rat cathepsin B and the cathepsin B activity obtained from the mesangial cells lysate are hydrolytic activities of the same enzyme.

**Hydrolysis of qf-Peptide Derived From the Rat Prorenin Processing Site by Rat Cathepsin B and Whole Rat Mesangial Cell Lysates**

The thiol-protease activities in the soluble fraction of whole rat mesangial cell lysates were examined by means of susceptible substrates, specific inhibitors for this class of protease, and pH-profile of hydrolytic activities. Figures 1 A and 1 B show the pH profile of hydrolytic activity of mesangial cell lysate on Z-Phe-Arg-MCA and Z-Arg-Arg-MCA, respectively, in the presence of 5 mmol/L pepstatin, 1 mmol/L PMSF, and 1 mmol/L EDTA. These hydrolyses were fully activated by 5 mmol/L DTT and disappeared in the presence of 1 mmol/L E-64. Two peaks of activity were detected with Z-Phe-Arg-MCA. The pH profile of Z-Arg-Arg-MCA hydrolysis presented only 1 peak that overlapped to the same pH interval of the more alkaline peak detected with Z-Phe-Arg-MCA. This substrate is hydrolyzed by both cathepsin L and B; however, Z-Arg-Arg-MCA is hydrolyzed only by cathepsin B.28,36 Therefore, mesangial cells have at least 2 thiol-proteases with a substrate, inhibitor specificity, and pH range of activity similar to those of cathepsin B and L.
cathepsin B\textsuperscript{24} and by AtT-20 cells\textsuperscript{6} of wild-type human prorenin compared with that of mutatedzymogens at position P\textsubscript{s} by Ala, Phe, and Gly. The K\textsubscript{m} value we obtained with peptide 1 is 3 orders of magnitude higher than that reported for the hydrolysis of prorenin by cathepsin B.\textsuperscript{3} Although prorenin and peptide 1 were hydrolyzed at same site, other strong interactions, possibly from the cathepsin B catalytic site, occurred between the 2 proteins.

The kinetic parameters presented in Table 2 for hydrolysis by human cathepsin B of peptides derived from the human prorenin processing site in which an Ala scan was performed from P\textsubscript{5} to P\textsubscript{s} have a good overall similarity to the same modification by mutation of human prorenin processed by AtT-20 cells\textsuperscript{6} or in vitro by human cathepsin B.\textsuperscript{31} Namely, the substitutions by Ala at nonprime sites resulted in a decrease of hydrolysis of the resulting substrates, and the inverse was observed with the Ala substitution on the prime site. The substitution of Arg at P\textsubscript{1} by Ala decreased the k\textsubscript{cat}/K\textsubscript{m} value for hydrolysis of peptide 6 to one third of that of peptide 1. The most striking difference between our data with prorenin-qf-peptides hydrolysis and that obtained with activation of prorenin by AtT-20 cells\textsuperscript{6} or in vitro by cathepsin B was observed with the Lys-to-Ala modification at P\textsubscript{2} site. Whereas peptide 5 (Table 2) was hydrolyzed with a k\textsubscript{cat}/K\textsubscript{m} higher than that of peptide 1, the equivalent mutated prorenin was barely processed by AtT-20 cells\textsuperscript{6} or, in vitro, by cathepsin B. The origin of this difference might be the lower affinity of the peptide to cathepsin B as opposed to prorenin, as mentioned above. The low K\textsubscript{m} values obtained with the hydrolysis of qfptides 10 and 11, where Ala was substituted for Gly and Asn at position P\textsubscript{4} and P\textsubscript{5}, respectively, is surprising. These peptides were hydrolyzed at 2 sites, with almost the same enzyme preference for each one, in a similar manner to that of peptide 7, which has Ala at P\textsubscript{1} position. Because the occluding loop of cathepsin B is located at the prime side of its catalytic cleft, it is possible that Ala at P\textsubscript{1} and P\textsubscript{5} induces a set of conformations on the substrate that alters its interaction with this segment of the protease.

The larger difference observed on the hydrolysis by trypsin of the prorenin processing site-related qf-peptides was that of the low k\textsubscript{cat}/K\textsubscript{m} value obtained with the hydrolysis of peptide 6 and modified at P\textsubscript{1} position (Table 2). A similar resistance to maturation by trypsin of R/A mutated prorenin secreted by transfected CHO cells was also observed.\textsuperscript{6} Because the cleavage in this peptide occurs at the K-A bond, the lower susceptibility of peptide 6 to trypsin may be due to the well-known lower activity of this enzyme on substrates with Lys in comparison to that of Arg at P\textsubscript{1} site.\textsuperscript{37} In humans, plasma and tissue kallikreins activate prorenin;\textsuperscript{38,39} however, peptide 1 was resistant to hydrolysis by human urinary kallikrein, and plasma kallikrein hydrolyzed this peptide at the R-L bond with kinetic parameters similar to those obtained with cathepsin B (K\textsubscript{m}=1.17 μmol/L, k\textsubscript{cat}=1 s\textsuperscript{-1}, k\textsubscript{cat}/K\textsubscript{m}=85.0 mmol/L\textsuperscript{-1} s\textsuperscript{-1}).

Rat mesangial cells, which secrete prorenin and renin\textsuperscript{25} and also take up renin from the medium by a specific receptor binding,\textsuperscript{26} have a cathepsin-B–like activity, as demonstrated by the pH-profile activity of the soluble fraction of whole rat mesangial cell lysates on Z-Phe-Arg-MCA and Z-Arg-Arg-MCA (Figure 1) as a result of activation by DTT and specific inhibition by E-64. Purified cloned rat cathepsin B hydrolyses Abz-IKKSSF-EDDnp at the K-S bond with a reasonable k\textsubscript{cat}/K\textsubscript{m} value (95 mmol/L\textsuperscript{-1} s\textsuperscript{-1}) when compared with those for human cathepsin B (Table 2). The same cleavage point with a similar pH-profile was observed with the hydrolysis of Abz-IKKSSF-EDDnp by a soluble fraction of whole rat mesangial cell lysates.

In conclusion, these biochemical results, particularly that of the specificity of cathepsin B toward peptides derived from the human prorenin processing site, support the view that human or rodent cathepsin B could be involved in the processing of prorenin that is taken up from the extracellular compartment or is synthesized by the cells (as, for example, by mesangial cells).

Acknowledgments

This work was supported by the following Brazilian research foundations: FAPESP, CNPq, and PADCT; the INCO-DC program (EU Contract Number ERBIC18CT970225); and the Danish National Research Foundation.

References


Hydrolysis by Cathepsin B of Fluorescent Peptides Derived From Human Prorenin
Paulo Cezar Almeida, Vitor Oliveira, Jair Ribeiro Chagas, Morten Meldal, Maria Aparecida Juliano and Luiz Juliano

Hypertension. 2000;35:1278-1283
doi: 10.1161/01.HYP.35.6.1278
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2000 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://hyper.ahajournals.org/content/35/6/1278

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published
in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial
Office. Once the online version of the published article for which permission is being requested is located,
click Request Permissions in the middle column of the Web page under Services. Further information about
this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org/subscriptions/