Mode of Action on Fluorogenic Substrates of Acid Carboxypeptidases from Aspergillus

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Acid carboxypeptidases (CPases) from Aspergillus can release most amino acid residues, including proline, from the carboxyterminus of peptides and proteins at acidic pHs. The enzymes are not inhibited by ethylenediaminetetraacetate or o-phenanthroline; thus they differ from the pancreatic CPase A and B. The metallo-CPases only catalyze the hydrolysis of peptide bonds that are alpha to the terminal free carboxyl group. On the other hand, the acid CPases from plants, molds, and yeast act as amidases and/or esterases for TV-substituted amino acid derivatives. Recently, Kunugi et al. reported that the CPases Y, P, and W hydrolyzed peptide-MCA at pH 7 by releasing 7-amino-4-methyl coumarin (AMC). There is little information about the action of the acid CPases from Aspergillus for peptide-MCAs. In this paper, the mode of action of the acid CPases from Aspergillus on fluorogenic substrates is identified.

The peptide-MCAs were purchased from the Peptide Institute, Ina, Mino-shi, Osaka, Japan. The enzymes used in this experiment were A. oryzae acid CPase O (MW, 155 K), O-1 (MW, 63 K), and A. saitoi and CPase (MW, 125 K). The CPase activities were assayed as in our previous papers. The enzymes were purified as in our previous papers. The enzyme preparations used were homogeneous on disc gel electrophoresis at pH 9.4 and had no endopeptidase activity. Protein concentrations were usually estimated from the absorbance at 280 nm by a Hitachi model 101 spectrophotometer. Amidase activities for peptide-MCA hydrolysis were measured under conditions similar to those in an earlier paper, except for the buffer. An acetate buffer (pH 4.9) at 0.05 M was used for A. saitoi acid CPase and McIlvaine buffer (pH 5.8) at 0.05 M was used for A. oryzae acid CPase O.

The acid CPase O and A. saitoi acid CPase can hydrolyze the amide bond between the amino acid and AMC in the peptide-MCAs, PFR-MCA, t-butoxycarbonyl(Boc)VPR-MCA, and Boc-VLK-MCA. The optimum pH of the acid CPase O for hydrolysis of Boc-VPR-MCA was estimated to be pH 5.8. The optimum pH of the A. saitoi acid CPase for hydrolysis of PFR-MCA was estimated to be pH 4.9. The enzymes did not hydrolyze Boc-EKK-MCA, succinyl(Suc)-LLVY-MCA, benzoyl(Bz)-R-MCA, benzylxoycarbonyl(Z)-RR-MCA, Boc-IEGR-MCA, Suc-AAPF-MCA, Boc-(O-benzyl)-GR-MCA, Boc-LGR-MCA, Boc-LTR-MCA, Boc-LSTR-MCA, Boc-FSR-MCA, Suc-GP-MCA, GP-MCA, glutaryl-GR-MCA, or KA-MCA. The acid CPase O and A. saitoi acid CPase displayed identical specificities for the peptide-MCAs. When the P2 position was occupied by hydrophobic amino acid residues, the enzymes released the AMC at a rather high rate. In spite of the presence of hydrophobic amino acid residues at the P2 position, Suc-LLVY-MCA and Suc-AAPF-MCA were not hydrolyzed by the enzymes because of the presence of a succinyl group in the P5 position. All of the peptide-MCAs used in this experiment were not hydrolyzed by the acid CPase O-1, which is a low-molecular-weight acid CPase from A. oryzae. We concluded that the specificity of the acid CPase O-1 was different from the other ones. Kinetic parameters of the enzymes for hydrolysis of peptide-MCAs are shown in the table.

Table I. Kinetic Parameters of Aspergillus Acid Carboxypeptidases for the Hydrolysis of Peptide-MCAs

<table>
<thead>
<tr>
<th>Substrates</th>
<th>A. saitoi acid CPase</th>
<th>A. oryzae acid CPase O</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_m$ (mM)</td>
<td>$k_{cat}$ (s$^{-1}$)</td>
</tr>
<tr>
<td>P-F-R-MCA</td>
<td>0.01</td>
<td>0.14</td>
</tr>
<tr>
<td>Boc-V-P-R-MCA</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>Boc-V-L-K-MCA</td>
<td>0.07</td>
<td>0.01</td>
</tr>
</tbody>
</table>

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Table I. PFR-MCA was the best of the substrates tested for the enzymes.

Kunugi et al.\(^8\) reported that CPase Y from yeast hydrolyzed Suc-LLVY-MCA and Suc-AAPF-MCA rapidly. But the acid CPases from Aspergillus did not hydrolyze these peptide-MCAs. These enzymes were distinguished by their substrate specificities for the peptide-MCAs. A previous study\(^12\) has demonstrated that an aspartic proteinase from A. saitoi relases R-MCA from Boc-LSTR-MCA and Boc-IEGR-MCA. In this report, we demonstrated that the acid CPase from A. saitoi did not hydrolyze Boc-LSTR-MCA and Boc-IEGR-MCA. These results suggest that Boc-LSTR-MCA and Boc-IEGR-MCA may be useful substrates for the detection of contaminating aspartic proteinase activity in the preparation of the purified A. saitoi acid CPase.

The \(K_m\) values for peptide-MCAs are similar to the values\(^3-4\) for bradykinin hydrolysis. But the \(k_{cat}/K_m\) values of the enzymes for the bradykinin are about 100-fold higher than those for the hydrolysis of Boc-VPR-MCA. Although the \(k_{cat}/K_m\) values of the enzymes for hydrolysis of peptide-MCAs are low, the fluorometric assay is useful for the detection of acid CPase activities from Aspergillus because of the high sensitivity.

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References