Esterolytic Activity of Acid Carboxypeptidase from *Aspergillus saitoi*

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Received November 11, 1972

A new type of acid carboxypeptidase from *Aspergillus saitoi* catalyzes the hydrolytic liberation of amino acids from the carboxy-terminal of the substrates in acidic pH range.^{1~4)} However, unlike pancreatic carboxypeptidase A (EC 3.4.2.1) or carboxypeptidase B (EC 3.4.2.2) but similar to *Citrus* carboxypeptidase,⁵⁾ it is unstable at pH above 6, and is not inactivated by EDTA. The highly purified enzyme was found to be essentially homogeneous by such criteria as sedimentation in the ultracentrifuge and disc electrophoresis on polyacrylamide at pH values of 9.5, 8.0 and 2.3. It was concluded in the previous paper that with small synthetic substrates of the type R-X-Y, where the X-Y bond is broken, the acid carboxypeptidase exhibits a preference for aromatic or carboxyl group in the X position.

The present paper describes the preliminary account of a sensitive esterolytic activity for Bz-Arg-OEt hydrolysis at acidic pH range of the acid carboxypeptidase from *A. saitoi*.

The basis for the rapid spectrophotometric assay is the release of benzylarginine from 1.58×10^{-3} M Bz-Arg-OEt by the esterase action of the acid carboxypeptidase, which is followed by measurement of the increase in absorbances at 258 m μ , according to the modified method of Schwert and Takenaka.⁶⁾ The standard assay conditions were as follows: To an exact volume (2 ml) of a stock solution

of Bz-Arg-OEt in quartz reference and sample cuvettes (1-cm pathlength) at 30°C was added an 1.5 ml of McIlvain's citrate buffer, pH 5.2. The absorbances were balanced at 258 m μ , preferably in a double-beam recording spectrophotometer of Hitachi model 124. An appropriate amount of the acid carboxypeptidase in a volume of 200 μ l was added to the sample cuvette to a final volume of 3.7 ml. The increase in absorbance was followed for about 10 min. It was found that about 14 Z-Glu-Tyr hydrolase munits of the enzyme in the reaction mixture could be satisfactorily assayed. The sensitivity of the Bz-Arg-OEt method thus compared favourably with the ninhydrin assay for Z-Tyr-Leu and Z-Glu-Tyr hydrolysis, in which the $V_{\max}$ values of 1.64×10^3 μ moles leucine/min for 1 unit OD at 280 m μ and 1.9×10 μ moles tyrosine/min for 1 unit OD at 280 m μ had been obtained.¹⁾

The relation between pH and relative rate of activity shown in Fig. 1 for the hydrolysis of Bz-Arg-OEt by the acid carboxypeptidase is bell in shape. The optimum pH with Bz-Arg-OEt as substrate was 5.2. From the slopes of a pH-relative rate plot in the same figure, it appears that two ionizing groups participate in the catalysis. These groups had pK_a' values of about 4 and 6, suggesting

FIG. 1. Effect of pH for the Hydrolysis of Bz-Arg-OEt by Acid Carboxypeptidase from *Aspergillus saitoi* at 30°C.

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Abbreviation: Ac-, Acetyl; Bz-, benzoyl; R-, acyl; To-, tosyl; Z-, benzyloxycarbonyl; -OEt, ethyl ester; -OMe, methyl ester; -TyrI₂, 3,5-diiodotyrosine.

that they could represent participation of two ionizing carboxyl groups or one ionizing carboxyl group and one ionizing imidazole group. In the previous paper,^{3,4)} the two ionizing groups of $pK_{es1}=2.3$ and $pK_{es2}=5.1$ had also been implicated in the action mechanism of the acid carboxypeptidase for Z-Tyr-Leu hydrolysis. The K_m value of 8×10^{-4} M and the V_{max} value of $1.7 \times 10^4 \mu\text{moles benzoylarginine/min}$ for 1 unit O.D. at 280 m μ were calculated from Lineweaver-Burk plots obtained with $0.3\text{--}3.5 \times 10^{-3}$ M substrate concentration at 30°C.

The rate of esterolytic activity for Bz-Arg-OEt hydrolysis was found to be inhibited by the addition of the competitive inhibitor, hydrocinnamic acid,³⁾ of the carboxypeptidase activity towards Z-Glu-Tyr. It may be concluded that the esterolytic activity on Bz-Arg-OEt is its intrinsic property.

The acid carboxypeptidase also possessed some esterolytic activity on Ac-Tyr-OEt according to the spectrophotometric method at 234 m μ of Schwert and Takenaka.⁶⁾ The optimum pH was 5.5. In the previous paper,^{1,4)} slow liberation of the carboxyterminal amino acid from an acetyl substrate, Ac-Phe-TyrI₂, had also been observed.

The acid carboxypeptidase had no action on To-Arg-OMe. The esterolytic properties of the acid carboxypeptidase from *A. saitoi*

indicate that this fungal enzyme is similar to pancreatic trypsin (EC 3.4.4.4) and baker's yeast proteinase C,⁷⁾ but nevertheless shows important differences in the optimum pH for digestion of Bz-Arg-OEt. Furthermore, the acid carboxypeptidase has no action on To-Arg-OMe and Bz-Arg-*p*-nitroanilide in acidic pH range, unlike trypsin.

A part of this study was supported by the research grant of the Ministry of Education. We thank Professors T. Takahashi, R. Hamada, T. Kobayashi and A. Kawamura for their encouragement.

REFERENCES

- 1) E. Ichishima, *Biochim. Biophys. Acta*, **258**, 274 (1972).
- 2) E. Ichishima, S. Sonoki, K. Hirai, Y. Torii and S. Yokoyama, *J. Biochem.*, **72**, 1045 (1972).
- 3) E. Ichishima and T. Arai, Proc. Symposium on Biochemistry of the 45th Japanese Biochemical Soc., p. 326 (1972); *Biochim. Biophys. Acta*, **293**, 444 (1973).
- 4) E. Ichishima, "Fermentation Technology Today," ed. by G. Terui, The Soc. of Fermentation Technol., Osaka, p. 259 (1972).
- 5) H. Zuber, *Hoppe-Seyler's Z-Physiol. Chem.*, **349**, 1339 (1968).
- 6) G. W. Schwert and Y. Takenaka, *Biochim. Biophys. Acta*, **16**, 570 (1955).
- 7) E. Doi, R. Hayashi and T. Hata, *Agr. Biol. Chem.*, **31**, 160 (1967).