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ACTION ON PEPTIDES BY WHEAT CARBOXYPEPTIDASE

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Key Word Index—*Triticum aestivum*; Gramineae; wheat; carboxypeptidase; peptides; kinetic parameters.

Abstract—A kinetic analysis has been performed with purified wheat carboxypeptidase by the use of *N*-acyl dipeptides, Z–Gly–Pro–Leu–Gly (Z = benzyloxycarbonyl), angiotensin II and bradykinin. The values of k_{cat} were dramatically influenced by amino acid residues occupying the penultimate position from the carboxyl terminus of substrates. The structure of the substrate did not appreciably affect the K_m values.

INTRODUCTION

Wheat carboxypeptidase, isolated and crystallized from wheat bran, is a new enzyme with a MW of 118 000 and an isoelectric point of 6.0, possessing active serine [1]. The enzyme was apparently different from germinated wheat carboxypeptidase [2, 3] in isoelectric point, MW and optimum pH. Wheat carboxypeptidase acted on bitter peptides from the peptic hydrolysates of milk casein and released hydrophobic amino acids from their carboxyl termini, followed by a decrease in bitter taste [4]. Serine carboxypeptidase (EC 3.4.16.1) releases most carboxyl-terminal amino acids, including proline, from peptides and proteins. The rate of hydrolysis of serine carboxypeptidase from *Phaseolus vulgaris* [5], *Citrus natsudaidai* [6], *Aspergillus saitoi* [7], *Penicillium janthinellum* [8] and yeast [9] is influenced by the amino acid residue occupying the penultimate position of the carboxyl terminus.

This paper describes a kinetic study of wheat carboxypeptidase with *N*-acyl dipeptides and longer substrates as Z–Gly–Pro–Leu–Gly, angiotensin II and bradykinin.

RESULTS AND DISCUSSION

Table 1 shows the kinetic parameters of wheat carboxypeptidase toward *N*-acyl dipeptides, Z–Gly–Pro–Leu–Gly, angiotensin II and bradykinin.

The value of k_{cat} was most influenced by amino acid residues occupying the penultimate position from the carboxyl terminus of substrates. When the penultimate residues had an aromatic or aliphatic side chain, the k_{cat} value was high. When the penultimate position was glutamic acid, the k_{cat} value was relatively high. When glycine or proline was in the penultimate position from the carboxyl terminus, the k_{cat} value was low. In particular, the k_{cat} value in the case of proline was *ca* 1/300 of that in the case of aromatic or aliphatic residues. The k_{cat} value seems to become greater with an increase in the hydrophobicity [10] of the side chain on the penultimate amino acid from the carboxyl terminus, except for proline.

The K_m values for substrates of different structures showed no variation compared to those of k_{cat}. The largest K_m value for Z–Gly–Pro–Leu–Gly was *ca* 10-times the smallest one for Z–Glu–Tyr. The nature of the amino acid in the penultimate position from the carboxyl terminus does not appear to have a strong effect on binding between the enzyme and the substrate. In phaseolain [5] and carboxypeptidase Y [9], alternation of the amino-blocking group had a decisive effect on the reaction rate of *N*-acyl dipeptide. In this study, there was a little difference in K_m value between *N*-acyl dipeptides and longer peptides such as Z–Gly–Pro–Leu–Gly, angiotensin II and bradykinin. The active site, corresponding to the third amino acid residue from the carboxyl terminus, may be rather flexible, though amino terminal substituents in the dipeptide substrate play a similar role to amino acid residues of longer substrates.

The presence of proline in the third or penultimate position from the carboxyl terminus of angiotensin II (–$\overset{6}{\text{His}}$–$\overset{7}{\text{Pro}}$–$\overset{8}{\text{Phe}}$) or bradykinin (–$\overset{7}{\text{Pro}}$–$\overset{8}{\text{Phe}}$–$\overset{9}{\text{Arg}}$) did not affect the K_m value; on the other hand the k_{cat} value for angiotensin II showed a marked decrease compared to that for bradykinin. Nakagawa and Kaiser[11] reported that yeast carboxypeptidase might form an acyl enzyme, using *p*-nitrophenyl trimethylacetate as substrate. A simi-

Table 1. Kinetic parameters of wheat carboxypeptidase toward *N*-acyl dipeptides, Z Gly–Pro–Leu–Gly, angiotensin II and bradykinin at 30°

Substrate $P_3 - P_2 - \overset{\downarrow}{P_1} - P'_1$	pH	K_m (mM)	k_{cat} (S^{-1})	k_{cat}/K_m ($S^{-1} \cdot mM^{-1}$)
Synthetic peptides				
Z–Tyr–Glu	4.0	0.15	132	880
Z–Glu–Tyr	4.0	0.19	20	105
Z–Gly–Pro–Leu–Gly	4.0	1.5	145	97
Z–Gly–Leu	3.7	0.82	10	12
Bz–Gly–Lys	4.0	0.20	1.8	9.0
Angiotensin II*				
–$\overset{5}{\text{Ile}}$–$\overset{6}{\text{His}}$–$\overset{7}{\text{Pro}}$–$\overset{8}{\text{Phe}}$	4.0	0.20	0.46	2.4
Bradykinin†				
–$\overset{6}{\text{Ser}}$–$\overset{7}{\text{Pro}}$–$\overset{8}{\text{Phe}}$–$\overset{9}{\text{Arg}}$	4.0	0.17	156	930

*The amino acid sequence of angiotensin II is Asp–Arg–Val–Tyr–Ile–His–Pro–Phe.

†The amino acid sequence of bradykinin is Arg–Pro–Pro–Gly–Phe–Ser–Pro–Phe–Arg.

lar reaction mechanism may occur for wheat carboxypeptidase. The rate constant for deacylation may be made small by the presence of proline in the penultimate position from the carboxyl terminus of substrates. In wheat carboxypeptidase, the nature of the amino acid in the penultimate position from the carboxyl terminus of the substrate generally appears to be subjected to the influence of the rate constant for deacylation.

In conclusion, the K_m value of wheat carboxypeptidase was not appreciably influenced by the structure of the substrate. However, the nature of the penultimate amino acid from the carboxyl terminus of the substrate dramatically changed the k_{cat} value.

EXPERIMENTAL

Materials. *N*-Acyl dipeptide, Z–Gly–Pro–Leu–Gly, angiotensin II and bradykinin were purchased from the Protein Research Foundation, Osaka.

Carboxypeptidase from wheat. Wheat bran from bread and common wheat (*Triticum aestivum* L.) was purchased from Nissin Seifun Co. Ltd., Tokyo. The crystalline wheat carboxypeptidase from wheat bran was prepared acccrding to ref. [1]. The enzyme used here was homogeneous on disc electrophoresis at pH 4 and on analytical ultracentrifugation.

Kinetic studies. The values of K_m and k_{cat} were determined graphically from Lineweaver–Burk plots. When *N*-acyl dipeptides were used as substrates, the released amino acids were determined by the ninhydrin method. The initial rates of hydrolysis of Z–Gly–Pro–Leu–Gly, angiotensin II and bradykinin were determined with an automatic amino acid analyser, Hitachi model 835-30. No amino acids other than the carboxyl-terminal amino acid from the substrate used in the kinetic study were observed.

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AN ACETYLENIC TRIOL FROM *HYOSERIS LUCIDA**

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Key Word Index—*Hyoseris lucida*; Compositae; acetylenic compound; C_{14}-enediyne.

Abstract—The aerial parts of *Hyoseris lucida* afforded a new acetylenic triol. The structure was elucidated by spectroscopic methods and a few chemical transformations.

So far little is known on the chemistry of the small genus *Hyoseris* (tribe Cichorieae). A preliminary investigation of the roots of *H. lucida* L. gave no characteristic compounds [1]. However, from the aerial parts, we have now isolated a crystalline compound, molecular formula $C_{14}H_{20}O_3$, which was covered rapidly with a deep blue coloured polymer when exposed to light. This was an indication that an acetylenic compound may be present. The characteristic UV maxima of an enediyne [2] established this proposal. The ^{1}H NMR spectrum (Table 1) showed the typical signals of a *trans*-configurated propenyl end group and four lowfield signals (δ 3.69 and 3.75 *dt*, 3.59 and 3.47 *dddd*) indicated the presence of a primary and two secondary hydroxyl groups. A broadened triplet at δ 2.50 was obviously due to a methylene group adjacent to the triple bonds. Spin decoupling showed that the secondary hydroxyl groups were at vicinal carbons while the signals of the primary hydroxyl group were coupled with a multiplet at δ 1.74. These results required a C_{14}-enediyne triol since the secondary hydroxyl groups could be placed

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