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Note

Substrate Specificity of Aminopeptidase from the Mid-gut Gland of the Scallop (*Patinopecten yessoensis*)

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An action for various peptides and a kinetic study for amino acid *p*-nitroanilides (*p*NAs) and 4-methylcoumaryl-7-amides (MCAs) were performed with purified aminopeptidase from the mid-gut of the scallop. The enzyme preferred dipeptides having Ala, Met, and Phe in the amino-terminal or the penultimate position from the amino-termini. The catalytic efficiencies, k_{cat}/K_m values for Ala-*p*NA and MCA were the highest in the tested substrates, and those for *p*NA and MCA substrates having Met or Phe were the next highest. The enzyme was found to be a new alanine-specific aminopeptidase.

Key words: scallop; mid-gut gland; aminopeptidase; substrate specificity; kinetic parameter

Aminopeptidases (α-aminoacyl-peptides hydrolase, EC 3.4.11.1-15) have the ability to liberate various amino acid residues from the amino-termini of peptide substrates and are classified according to preference for amino-terminal amino acid of substrates, their location, sensitivity to inhibitors, and the need for metal, such as zinc or cobalt, for their enzyme activities.[1)] There are substantial reports concerning substrate specificity, biological function, and structure of aminopeptidases in prokaryotes and mammals.[2,3)]

Among various aminopeptidases, the alanyl aminopeptidases (EC 3.4.11.2, 3.4.11.14), which preferentially liberate amino-terminal amino acids, such as Ala, Met, Leu, and Tyr of peptides, are widely distributed in mammalian tissues and body fluids,[4–9)] and in plant tissues[10)] and cyanobacteria,[11)] as a membrane or cytosolic type. These enzymes from mammals are believed to participate in the metabolism of hormones and neurotransmitters.[12–14)]

In a previous paper,[15)] we demonstrated that an aminopeptidase, isolated and purified from the mid-gut gland of the scallop (*Patinopecten yessoensis*), is an enzyme with a MW of 61,000 by SDS-polyacrylamide gel electrophoresis and an isoelectric point of 5.2. The enzyme preferred substrates having Ala or Met as an amino acid residue from the results of relative hydrolysis rates of amino acid-*p*NAs and MCAs. The enzyme appeared to be classifiable as a cytosol alanyl aminopeptidase (EC3.4.11.14) with respect to its substrate specificity, localization, and the need for Zn^{2+} for enzyme activity, but differs from cytosol alanyl aminopeptidase in molecular weight and sensitivity to puromycin, SH-blocking reagents, and divalent metal ions.

This paper describes an action of scallop aminopeptidase on various peptide substrates and a kinetic study for amino acid-*p*NAs and MCAs.

Ala-*p*NA, Leu-*p*NA, Ala-MCA, Arg-MCA, Leu-MCA, Lys-MCA, Met-MCA, Phe-MCA, 7-amino-4-methylcoumarin (AMC), Gly-Phe, $(Gly)_3$, and Ala-Ser-Thr-Thr-Asn-Tyr-Tyr-Gly were purchased from the Peptide Institute, Inc. (Osaka, Japan). Asp-MCA, Ser-MCA, Tyr-MCA, His-Ala, Met-Tyr-Phe, Ala-Gl-Gly, $(Ala)_4$, and Ala-Phe-Ser-Ser-Trp-Gly were purchased from Bachem Inc. (Bubendorf, Switzerland), and all other amino acid-*p*NAs and peptides from Sigma. The aminopeptidase was purified from the mid-gut gland of the scallop and assayed using Ala-*p*NA as a substrate as in our previous experiment.[15)] One katal was the amount of enzyme required to liberate 1 mol *p*-nitroaniline per second from Ala-*p*NA at pH 7.0 and 30 °C. The fluorometric assay was done as in our previous exprriment[15)] by the use of amino acid-MCAs as substrates.

The substrate specificity of the scallop aminopeptidase was examined with various peptide substrates (Table 1). The purified enzyme from scallop had a remaining activity of 91% of its original activity in the absence of substrate after incubation at pH 7.0 and 30 °C for 24 h. The enzyme acted preferentially on Phe-Met, Ala-Phe, Met-Phe, Ala-Ala, and Ala-Leu, and then moderately on Met-Ala, Gly-Phe, and Met-Gly. However, the enzyme scarcely hydrolyzed dipeptides having His, such as Ala-His or His-Ala, and those having

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Table 1. Relative Hydrolysis Rates of Various Peptides by the Action of Aminopeptidase from Scallop

Substrate	Relative activity* (%)
Phe-Met	158
Ala-Phe	137
Ala-*p*NA	100
Met-Phe	94
Ala-Ala	92
Ala-Leu	89
Met-Ala	32
Gly-Phe	30
Met-Gly	12
Ala-His	tr
His-Ala	tr
Ala-Pro	tr
Met-Pro	tr
Ala-Ala-Ala	32
Met-Ala-Ser	18
Ala-Gly-Gly	14
Gly-Gly-Gly	8
Met-Tyr-Phe	tr
Ala-Phe-Tyr-Glu	20
Ala-Ala-Ala-Ala	tr
Ala-Phe-Ser-Ser-Trp-Gly	0
Ala-Ser-Thr-Thr-Asn-Tyr-Tyr-Gly	0

*The rate of hydrolysis of Ala-*p*NA=100. tr., trace.
The enzyme (1 nkat/ml) and peptide (250 nmol/ml) in 100 mM ammonium hydrogen carbonate (pH 7.0) were incubated at 30 °C for 24 h and the liberated amino acids were analyzed by an amino acid analyzer.

Table 2. Kinetic Parameters of Aminopeptidase from Scallop for Amino Acid-*p*NAs and MCAs

Substrate	K_m (μM)	k_{cat} (sec^{-1})	k_{cat}/K_m ($sec^{-1} \cdot mM^{-1}$)
Ala-*p*NA*	32.2	29.5	921
Met-*p*NA	34.1	6.24	184
Gly-*p*NA	80.6	3.66	46
Pro-*p*NA	24.9	0.87	35
Leu-*p*NA	71.9	0.68	9.4
Ala-MCA*	11.1	9.49	854
Phe-MCA	1.12	0.706	628
Met-MCA	8.70	3.84	441
Ser-MCA	40.4	15.1	377
Arg-MCA	3.92	0.680	173
Tyr-MCA	2.11	0.274	130
Leu-MCA	8.33	0.269	32.3
Asp-MCA	16.0	0.108	6.8
Lys-MCA	16.7	0.049	2.9

*Data from reference (15).
The enzyme activities for amino acid-*p*NAs and MCAs were measured in MacIlvaine buffer (pH 7.0) at 30 °C. Enzyme concentration was estimated from the absorbance at 280 nm by the extinction coefficient, $A^{1\%}_{1cm}$ value of 23.0.[15] Each experiment was done at five different substrate concentrations. The values of K_m and k_{cat} were determined graphically from Lineweaver-Burk plots.

imidopeptide bond, such as Ala-Pro or Met-Pro. Among tripeptide substrates, $(Ala)_3$ was the most rapidly hydrolyzed. The hydrolysis rates of Met-Ala-Ser, Ala-Gly-Gly, and $(Gly)_3$ slowed in that order, and Met-Tyr-Phe was scarcely attacked by the enzyme. Among peptides longer than tripeptide, Ala-Phe-Tyr-Glu was hydrolyzed with a relative hydrolysis of 20% compared to Ala-*p*NA, whereas the other peptides tested were scarcely hydrolyzed. These findings show that the enzyme prefers substrates having Ala, Met, and Phe in the amino-terminal or the penultimate position from the amino-termini. Compared with the actions for Ala-Ala, $(Ala)_3$, and $(Ala)_4$, the hydrolysis rate of Ala-Ala was faster and that of $(Ala)_3$ slower. $(Ala)_4$ was not hydrolyzed by the enzyme. The enzyme strongly cleaved Ala-Phe, whereas Ala-Phe-Ser-Ser-Trp-Gly were scarcely attacked. These results show that the enzyme hydrolyzes most rapidly dipeptide and tirpeptides slowly, and acts with difficulty on peptides longer than tripeptide.

Table 2 shows the kinetic parameters of the scallop aminopeptidase for amino acid-*p*NAs and MCAs. The k_{cat} and K_m values were affected by amino acid residues of the substrates. In the *p*NA substrates, the values of K_m were in the range of 24.9–80.6 μM, and those of k_{cat} varied in the range of 0.68–29.5 sec^{-1}. In the MCA substrates, the values of K_m and k_{cat} varied dramatically in the range of 1.12–40.0 μM and 0.049–15.1 sec^{-1}, respectively. The catalytic efficiencies, k_{cat}/K_m values for Ala-*p*NA and Ala-MCA[15] were 921 and 854 $s^{-1} \cdot mM^{-1}$, respectively. These values were highest in the *p*NA and MCA substrates. In the *p*NA substrates, the k_{cat}/K_m value for Met-*p*NA was second highest and those for the substrates having Gly, Pro, and Leu decreased in that order. In the MCA substrates, the enzyme had second highest k_{cat}/K_m values of 628 and 441 $s^{-1} \cdot mM^{-1}$ for Phe- and Met-MCA, respectively. When Ser, Arg, Tyr, Leu, Asp, and Lys were amino acid residues in the MCA substrates, the k_{cat}/K_m values decreased in that order. The k_{cat}/K_m values for various *p*NA and MCA substrates were of the same levels as those for MCA substrates in alanine aminopeptidase from guinea-pig brain,[9] for peptide substrates in rat-liver cytosol,[8] and for *p*NA substrates and dipeptides in *Aeromonas caviae* aminopeptidase.[16]

The results of the action for various peptides and the kinetic study for *p*NA and MCA substrates showed that scallop aminopeptidase is specific for alanine in the amino-termini of substrates and resembles mammal cytosol alanyl aminopeptidases[4–6,8,9] with respect to substrate specificity. Our previous paper[15] demonstrated, however, that the enzyme differs from mammal cytosol alanyl aminopeptidase in molecular weight and sensitivity to puromycin, SH-blocking reagents, and divalent metal ions. Judging by these overall results, the enzyme from the mid-gut gland of the scallop may be classified into a new category different from cytosol alanyl aminopeptidase. Further investigation concerning primary or higher order structure will be required to verify the category of the scallop enzyme.

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