Note

Specific Inhibition of Dipeptidyl Aminopeptidase IV by a New Synthetic Inhibitor, L-Thiopropylthiazolidine

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Received August 17, 1990

Dipeptidyl aminopeptidase IV [EC 3.4.14.5] has been purified from various tissues of animals^{1–7)} and microorganism.^{8,9)} In spite of the wide distribution of the enzyme in organs of various mammals, its physiological roles are still unclear. To elucidate the physiological role of dipeptidyl aminopeptidase IV in organisms, the finding and uses of an inhibitor specific for the enzyme are very important.

Dipeptidyl aminopeptidases IV were purified from porcine kidney and *Flavobacterium meningosepticum*.⁹⁾ The enzyme activity was assayed using Gly-L-Pro- β -naphthylamide (Gly-Pro-2-NNap) as a substrate.

L-Thiopropylthiazolidine hydrochloride was synthesized as follows: Z-L-thiopropylthiazolidine¹⁰⁾ (1.35 g, 4 mmol) was added dropwise to 25% HBr-AcOH solution (8 ml) at 0°C, and the mixture was stirred for 2 hr at room temperature, then concentrated *in vacuo*. The residue was dissolved in H₂O (10 ml) and the solution was washed with ether. The aqueous layer was neutralized with 4N NaOH, and extracted with CH₂Cl₂ (10 ml $\times$ 3). The extracts were combined, dried over anhydrous Na₂SO₄, and concentrated. The resulting oily product was dissolved in 5% HCl-MeOH (6 ml), then concentrated *in vacuo*. The resulting solid was recrystallized from MeOH to give 0.45 g (46.7%) of L-thiopropylthiazolidine hydrochloride as colorless needles, mp 174–180°C. $[\alpha]_D^{20}$ –164.36 (*c* = 1, MeOH). IRV_{max} cm^{–1} 1660 (*c* = 0). Anal. Calcd. for C₇H₁₃ClN₂O₂: C, 34.91; H, 5.44; N, 11.64; Cl, 14.73; S, 26.63%. Found: C, 34.85; H, 5.28; N, 11.44; Cl, 14.62; S, 26.51%, MS *m/z* 205 (*M*⁺ + 1), 175, 157, 143, 88.

Inhibition by several proline-containing peptides of dipeptidyl aminopeptidase IV was examined. IC₅₀ values are shown in Table I. The enzyme was inhibited by a few dipeptides that had proline as the C-terminal residue: Val-Pro, Leu-Pro and Phe-Pro. However, no inhibition was observed by Gly-Pro, pyroglutamylproline (Pyr-Pro), His-Pro, Arg-Pro, Met-Pro, Pro-Pro, and Z-Thiopropyl-

thiazolidine even at concentrations above 1 mM of these dipeptides. We have reported that the amino terminal protecting dipeptide, *i.e.*, Z-Gly-Pro, acts as an inhibitor for prolyl endopeptidase while a non-protecting compound of it acts as an inhibitor for dipeptidyl aminopeptidase IV.⁷⁾ In addition, we have shown that introduction of sulfur to the proline residue of the substrate analog causes a great increase in inhibitory activity for prolyl endopeptidase.¹⁰⁾ Taking into account these results, we examined the inhibitory activity of L-thiopropylthiazolidine (Thioprop-thiazolidine) on dipeptidyl aminopeptidase IV. As shown in Table I, dipeptidyl aminopeptidases IV from porcine kidney and *F. meningosepticum* were inhibited by this compound with IC₅₀ value of 20 μ M and 31 μ M, respectively.

Both enzymes were inhibited competitively by Thioprop-thiazolidine and the inhibition constants, *K_i*, for the enzymes from porcine kidney and *F. meningosepticum* were 18 μ M and 36 μ M, respectively. When Thioprop-thiazolidine was incubated at 37°C for 10 min with dipeptidyl aminopeptidase IV in 20 mM Tris-HCl buffer, pH 7.0, and was followed by dialysis against the same buffer, the enzyme activity was restored almost completely. This result indicates that Thioprop-thiazolidine is a reversible inhibitor.

To evaluate the specificity of Thioprop-thiazolidine as an inhibitor for enzymes, the inhibitory activity of this compound was tested for several proline-specific peptidases (Table II). Thioprop-thiazolidine was completely inert

Table I. INHIBITORY ACTIVITIES OF VARIOUS PEPTIDES CONTAINING PROLINE RESIDUES ON DIPEPTIDYL AMINOPEPTIDASE IV FROM PORCINE KIDNEY AND *F. meningosepticum*

Compounds P3 P2 P1	IC ₅₀ (μ M)	
	Porcine kidney	<i>F. meningosepticum</i>
Val-Pro	50	90
Leu-Pro	130	350
Phe-Pro	80	630
Gly-Pro	>1,000	>1,000
Pyr-Pro	>1,000	>1,000
His-Pro	>1,000	>1,000
Arg-Pro	>1,000	>1,000
Met-Pro	>1,000	>1,000
Pro-Pro	>1,000	>1,000
Z-Thioprop-thiazolidine	>1,000	>1,000
Thioprop-thiazolidine	20	31
	18 (<i>K_i</i>)	36 (<i>K_i</i>)

Pyr: pyroglutamyl.

Table II. INHIBITORY ACTIVITIES OF THIOPRO-THIAZOLIDINE TOWARD VARIOUS PROLINE-SPECIFIC PEPTIDASES

The reaction mixture with the enzyme (0.3 units) and peptide in 20 mM Tris-HCl buffer, pH 7.0, was incubated at 37°C. After 10 min, remaining activity was measured.

Enzyme	Substrate	IC ₅₀ (μM)
Dipeptidyl aminopeptidase IV		
from porcine kidney	5 mM Gly-Pro-2-NNap	20
from <i>F. meningosepticum</i>	5 mM Gly-Pro-2-NNap	31
Prolyl endopeptidase		
from bovine brain	5 mM Z-Gly-Pro-2-NNap	>1,000
from <i>F. meningosepticum</i>	5 mM Z-Gly-Pro-2-NNap	>1,000
Proline iminopeptidase	5 mM Pro-2-NNap	>1,000
Prolidase	25 mM Gly-Pro	>1,000
Aminopeptidase P	2 mM Gly-Pro-2-NNap	>1,000

toward prolyl endopeptidases from bovine brain and *F. meningosepticum*, proline iminopeptidase from *B. coagulans*, prolidase from porcine kidney, and aminopeptidase P from *E. coli* HB 101. This compound was not hydrolyzed by these enzymes.

Harada *et al.*¹¹⁾ reported that proline-containing dipeptides act as inhibitors for dipeptidyl aminopeptidase IV. We also found Val-Pro, Leu-Pro, and Phe-Pro are competitive inhibitors (Table I). Since a dipeptide, X-Pro, is a digestion product of the enzyme, it seems to act as the product inhibitor located at S2 and S1 subsites and is the substrate for prolidase.

Umezawa *et al.*¹²⁾ isolated a new peptide named "diprotin A" from *B. cereus*, and showed that this peptide is a potent inhibitor of dipeptidyl aminopeptidase IV from rat kidney. However, diprotin A was hydrolyzed by the enzymes (data not shown). Since the diprotin A, Ile-Pro-Ile, is sufficient as a substrate of the enzyme, it will compete with Gly-Pro-2-NNap, the substrate used.

Schorn *et al.*¹³⁾ synthesized Ala-Pro-O-(4-nitrobenzoyl)hydroxylamine as an inhibitor for dipeptidyl aminopeptidase IV, which was used for the study of T cells, but it seems that the compound is a substrate for aminopeptidase P.

Thioprop-thiazolidine is a new specific inhibitor for dipeptidyl aminopeptidase IV and is not hydrolyzed by other proline-specific enzymes. Therefore, it will be useful for studying the biological roles of the enzyme.

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