HYDROLYSIS OF CYCLIC DEPSIDIPEPTIDES BY TRYPSIN OR CHYMOTRYPSIN

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1. Introduction

Trypsin (EC 3.4.21.4) and α-chymotrypsin (EC 3.4.21.1) hydrolyze a peptide bond containing a carbonyl group of sensitive amino acids, however, these enzymes do not hydrolyze a peptide bond in cyclic dipeptides even if the sensitive amino acid residue is present [1,2]. It is well known that these enzymes hydrolyze an ester bond far faster than a corresponding peptide bond. Therefore, we have been interested in studying whether cyclic depsidipeptides composed of a sensitive amino acid and a hydroxy acid are susceptible to these enzymes.

Here we report the syntheses and susceptibilities of *cyclo*(–Lys–Hpp–) (I) and *cyclo*(–Trp–Hmp–) (II), which are depsipeptide analogs of *cyclo*(–Lys–Phe–) and *cyclo*(–Trp–Leu–), respectively; Hpp, 2-hydroxy-3-phenylpropanoic acid; Hmp, 2-hydroxy-4-methylpentanoic acid. In order to study stereospecificity of substrates, four stereoisomers of I (LL, LD, DL, DD) and two of II (LL, LD) were synthesized. Trypsin hydrolyzed ILL and ILD rapidly but IDL and IDD very slowly. Chymotrypsin hydrolyzed IILL and IILD in appreciable rates. Compounds IILL and IILD are strongly bitter to taste. Depsipeptide H–Asp–Hpp–OMe (III) [4], an analog of the sweet peptide H–Asp–Phe–OMe [5], also is bitter to taste.

Abbreviations: according to IUPAC–IUB Commission (1972) J. Biol. Chem. 247, 977–983; other abbreviations; CDI, *N,N*-carbonyl diimidazole; HONSu, *N*-hydroxy-succinimide; TLC, thin layer chromatography

Nomenclature: Residues in peptides and the corresponding subsites in enzyme are numbered according to [3]

2. Materials and methods

Trypsin (2 × crystallized) and α-chymotrypsin (3 × crystallized) were supplied by Nutritional Biochem. Corp. (OH) and by Worthington Biochem. Corp. (NJ), respectively.

2.1. *Peptide synthesis*

The protected depsidipeptide, Boc–L-Lys(*Z*)–L-Hpp–N_2H_2Boc (1.93 g, 3 mmol) prepared by coupling of Boc–L-Lys(*Z*)–OH with H–L-Hpp–N_2H_2Boc by use of CDI, was treated with HCl in HCOOH and H–L-Lys(*Z*)–L-Hpp–N_2H_3·2 HCl obtained was converted to the azide by the action of isopentyl nitrite. The azide was treated with pyridine (3 mM) to give *cyclo*(–L-Lys(*Z*)–L-Hpp–), which was hydrogenated to give ILL·HCl (810 mg, 86%). Boc–L-Trp–L-Hmp–OBzl (491 mg) was obtained by use of CDI and hydrogenated to give the corresponding acid, which was converted to the succinimide ester. After deblocking, the depsidipeptide active ester was cyclized in pyridine to give IILL (81 mg, 28%). *Z*–L-Asp(OBzl)–L-Hpp–OMe (520 mg) prepared from *Z*–L-Asp(OBzl)–OH and H–L-Hpp–OMe by use of CDI was hydrogenated to give IIILL (140 mg, 50%). Authentic samples of H–L-Lys–L-Hpp–OH, H–L-Hpp–L-Lys–OH, H–L-Trp–L-Hmp–OH, and H–L-Hmp–L-Trp–OH were synthesized similarly. All the crystalline peptide derivatives showed one spot in TLC and satisfactory elemental analyses. Details of the synthesis will be reported elsewhere.

2.2. *Enzyme assays*

Esterase activities of trypsin and α-chymotrypsin were measured at 30°C, 0.01 M substrate. Proteolytic

Table 1
Proteolytic coefficients of *cyclo*(–Lys–Hpp–) by trypsin and of *cyclo*(–Trp–Hmp–) by chymotrypsin

Substrate	Enzyme[a]	*C*
cyclo(–L-Lys–L-Hpp–) (ILL)	trypsin[b]	112
cyclo(–L-Lys–D-Hpp–) (ILD)	trypsin[b]	34
cyclo(–D-Lys–L-Hpp–) (IDL)	trypsin[c]	0.34
cyclo(–D-Lys–D-Hpp–) (IDD)	trypsin[c]	0.11
Ac–L-Lys–OEt	trypsin[b]	44
cyclo(–L-Trp–L-Hmp–) (IILL)	chymotrypsin[c]	0.16
cyclo(–L-Trp–D-Hmp–) (IILD)	chymotrypsin[c]	0.065
Ac–L-Trp–OMe	chymotrypsin[d]	5.8

[a] Solvent in the case of trypsin, 0.1 M Tris buffer containing 5 mM Ca^{2+} (pH 7.8). Solvent in the case of chymotrypsin, 0.1 M Tris buffer containing 40% dimethyl sulfoxide (pH 7.8)
[b] Enzyme, 0.005 mg/ml
[c] Enzyme, 1.5 mg/ml
[d] Enzyme, 0.15 mg/ml

coefficients of I and II were calculated from the initial rates of hydrolysis by the method in [6] and are shown in table 1. Proteolytic coefficients (C) were estimated from $C = K/E$, where $K = \log[100/(100-\%\text{hydrolysis})]\cdot\text{min}^{-1}$, and E is the protein concentration in mg protein nitrogen/ml test solution. Each of compound I was slowly hydrolyzed spontaneously, but this spontaneous degradation did not appreciably influence the measurement of the initial rate of tryptic hydrolysis.

2.3. *Determination of bitterness*

The degree of bitterness was determined with a sensory test by several volunteers. Table 2 shows threshold values of the peptides.

3. Results and discussion

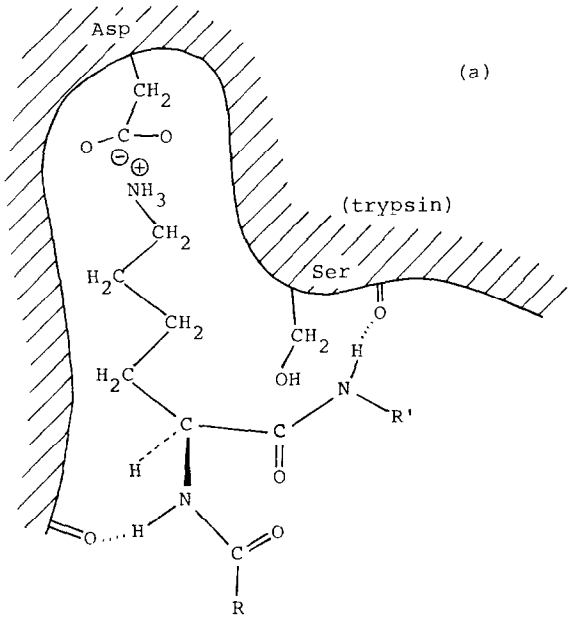
Comparison of TLCs of an incubation mixture with either of H–L-Hpp–L-Lys–OH, H–L-Lys–L-Hpp–OH, H–L-Hmp–L-Trp–OH or H–L-Trp–L-Hmp–OH showed that the enzymes hydrolyzed the supposed ester linkages. Different from cyclic dipeptides, cyclic depsidipeptides are hydrolyzed by proteolytic enzymes. Unexpectedly, trypsin hydrolyzed ILL 2.5-times faster than Ac–L-Lys–OEt. Change in configuration of the P_1 residue from L to D (ILL to IDL and ILD to IDD) decreased C to 1/300. On the other hand, change in configuration of the P_1' residue resulted in decrease in the coefficient to only 1/3 (ILL to ILD and IDL to IDD). It would be noteworthy that racemic 2-phenylthiazol-5-ones, which are of 5-membered cyclic compounds containing a –CO–S– linkage, of arginine and lysine were hydrolyzed by trypsin and yielded corresponding thiobenzoyl L-amino acids [7].

Table 2
Threshold values of bitterness and R_f values of *cyclo*(–Trp–Hmp–), H–Asp–Hpp–OMe and related compounds

Compound	Threshold value (mg/ml)	R_f[a]
cyclo(–L-Trp–L-Hmp–) (IILL)	0.03	0.92
cyclo(–L-Trp–D-Hmp–) (IILD)	0.015	0.94
H–L-Asp–L-Hpp–OMe (IIILL)	2.5	0.69
H–L-Asp–D-Hpp–OMe (IIILD)	> 20	0.67
cyclo(–L-Trp–L-Leu–)	0.015	0.91
H–L-Asp–L-Phe–OMe	> 20[b]	0.65

[a] TLC with the solvent system, BuOH:AcOH:water (4:1:2, v/v/v)
[b] Practically no bitterness

Figure 1 shows a possible model of interaction



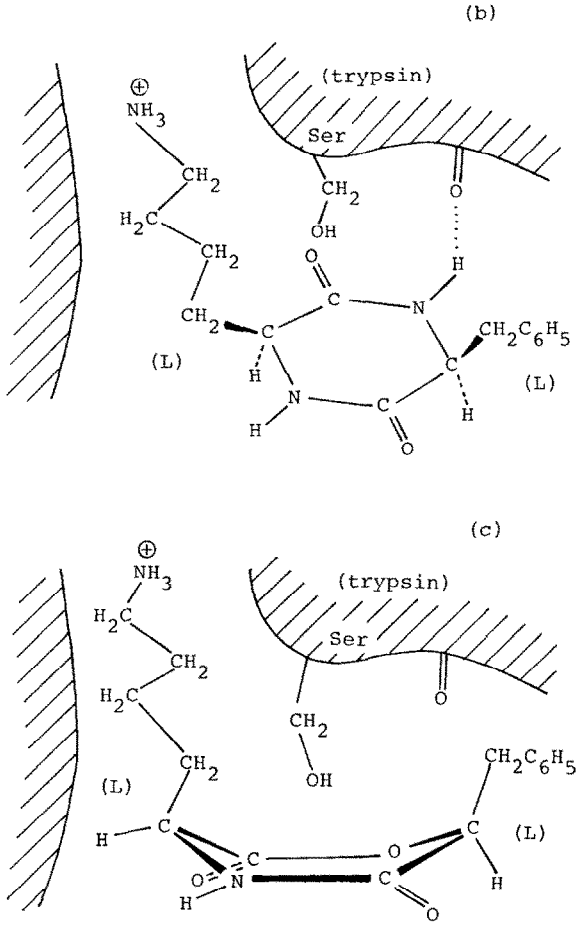


Fig.1. Supposed interaction between trypsin and substrates.

between trypsin and substrates. When the side chain of the L-lysine residue of a peptide substrate binds to the specific binding site S_1, the residue P_1' may interact with its binding site S_1' with an intermolecular hydrogen bond (fig.1a). If a cyclic dipeptide, e.g., *cyclo*(–L-Lys–L-Phe–), binds to the enzymes, the susceptible carbonyl group of the lysine residue should direct differently, hindering interaction with active serine, because of *cis* diketopiperazine structure (fig.1b). When ILL binds to trypsin, its hydroxy acid residue at P_1' site does not form hydrogen bonding with S_1' site. This possibly makes orientation of the susceptible ester linkage labile and allows suitable interaction with active serine to result in successful hydrolysis.

Compound *cyclo*(–L-Trp–L-Leu–), isolated from *B. subtilis* alkaline protease digest of casein is known to be strongly bitter [8]. It was noted [9] that this bitter taste is due to its hydrophobic nature. Depsipeptides IILL and IILD showed nearly the same bitterness and hydrophobicity to *cyclo*(–L-Trp–L-Leu–), compatible with the proposal [9]. Interestingly, the taste of compound IIILL, a depsipeptide analog of the sweet peptide H–L-Asp–L-Phe–OMe, is not sweet but bitter, although its diastereomer IIILD shows no bitterness. Table 2 shows that the differences in the taste of strongly bitter II (LL, LD) and *cyclo*(–L-Trp–L-Leu–), bitter IIILL, tasteless IIILD, and sweet H–L-Asp–L-Phe–OMe are apparently parallel to the R_F values of these compounds.

References

[1] Izumiya, N., Kato, T., Fujita, Y., Ohno, M. and Kondo, M. (1964) Bull. Chem. Soc. Jap. 37, 1809–1816.
[2] Ohno, M. and Izumiya, N. (1965) Bull. Chem. Soc. Jap. 38, 1831–1840.
[3] Schechter, I. and Berger, A. (1967) Biochem. Biophys. Res. Commun. 27, 157–162.
[4] Chorev, M., Willson, C. G. and Goodman, M. (1977) in: Peptides, Proc. 5th Am. Peptide Symp. (Goodman, M. and Meienhofer, J. eds), pp. 572–574, John Wiley and Sons (reports synthesis and no sweetness of this compound).
[5] Mazur, R. H., Schlatter, J. M. and Goldkamp, A. H. (1967) J. Am. Chem. Soc. 91, 2684–2691.
[6] Hestrin, S. (1949) J. Biol. Chem. 180, 249–261.
[7] Colletti-Previero, M.-A. and Axelrud-Cavadore, Cl. (1975) Biochem. Biophys. Res. Commun. 62, 844–848.
[8] Minamiura, N., Matsumura, Y. and Yamamoto, T. (1972) J. Biochem. (Tokyo) 72, 841–848.
[9] Shiba, T. and Nunami, K. (1974) Tetrahedron Lett. 509–512.