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Enzymatic Synthesis of a CCK-8 Tripeptide Derivative

Li GUO[1]*, Zi Min LU[2], Heiner ECKSTEIN[3]

[1]West China School of Pharmacy, Sichuan University, Chengdu 610041
[2]Tongji Medical College, Huazhong Science & Technology University, Wuhan 430030
[3]Institute of Organic Chemistry, University of Tuebingen, 72076 Tuebingen, Germany

Abstract: The enzymatic synthesis of CCK-8 tripeptide derivative Phac-Met-Asp(OMe)-Phe-NH_2 is reported. Starting with Phac-Met-OCam, we have successfully synthesized the target tripeptide with three free or immobilized enzymes, α-chymotrypsin, papain and thermolysin in reasonable yields. The key steps in this synthesis were the coupling of Phac-Met-OCam and H-Asp$(OMe)_2$ to form Met-Asp peptide bond catalyzed by α-chymotrypsin and the selective hydrolysis of α-ester of Phac-Met-Asp$(OMe)_2$ catalyzed by papain.

Keywords: Enzymatic synthesis, CCK-8, tripeptide derivative.

Enzymes have proved to be an attractive alternative method to the peptide synthesis[1,2]. We are interested in enzymatic synthesis of biological active peptides, such as Phac-Met -Asp(OMe)-Phe-NH_2, which corresponds to a fragment of cholecystokinin[3] C-terminal octapeptide CCK-8[Asp-Try(SO_3)-Met-Gly-Trp-*Met-Asp-Phe-*NH_2]. The phenylacetyl (Phac) group, which can be cleaved with penicillin G amidase without effecting the peptide bonds[4]. So it was used to protect the amino group of methionine. The aim of this investigation is to demonstrate the possibility of synthesis of this peptide by enzymatic methods.

We have provided a successful enzymatic synthesis of the target tripeptide derivative with three free enzymes(α-chymotrypsin, papain and thermolysin) in reasonable yields as shown in **Figure 1**. The key steps in this synthesis were the formation of Met-Asp peptide bond and the selective hydrolysis of α-ester of **1**. Usually, it is not easy to form peptide bond between Met and Asp catalyzed by enzymes [5]. We carried out this key reaction through the coupling of Phac-Met-OCam(Cam, carboxamidomethyl) and H-Asp$(OMe)_2$ in the presence of α-chymotrypsin to form **1** in more than 63% yield. The reaction proceeded in ethyl acetate containing 0.05 mol/L Tris-HCl buffer (1.5%, v/v, pH 9.0). It should be noted that no product **1** could be obtained when the reaction carried out in dry ethyl acetate or acetonitrile with the same enzyme. **2** was obtained by saponification of **1** in 92% yield catalysed by papain in 0.2 mol/L KH_2PO_4 buffer (pH 6.0) containing $HSCH_2CH_2OH$. In this condition only α-methyl aspartate was hydrolysed, while β-methyl aspartate remained. The resulted

*E-mail: rosaguo2000@yahoo.com

dipeptide **2** could bond H-Phe-NH_2 with thermolysin as a catalyst in H_2O(pH 7.0) to give **3** in high yield(>82.6%).

Procedure: The enzyme was added to the reaction medium containing the acyl donor(1 mmol) and nucleophile(1.5~1.7 mmol). The reaction was monitored by HPLC. When the reaction was completed(for **1**, 15 mg α-chymotrypsin the mixture was added, then was shaken 2 h at 25°C; for **2**, 12 mg papain was added, then the mixture was shaken 2 h at 25°C; for **3**, 3 mg thermolysin was added, then the mixture was kept 0.5 h at 40°C), the mixture was extracted with organic solvent. The organic phase was washed with 5% citric acid, 5% $NaHCO_3$ (except **2**), saturated NaCl, subsequently, dried over Na_2SO_4. The solvent was evaporated to afford the crude product, which was purified by preparative reversed phase chromatography(MPLC) on Polygosil C18 60-200, eluted with 20~80% MeOH, containing 0.005mol/L NH_4OAc. After evaporation of MeOH, the residue was submitted to lyophilisation. The obtained peptides were verified by FD-MS or FAB-MS[6].

HPLC analysis: Column: Nucleosil 100 RP-18, 5 μm and 100x2 mm column (Macherey-Nagel). Mobile Phase: (System I): 0.05 mol/L NH_4OAc (pH 6.5), 80% of MeOH/0.05 mol/L NH_4OAc, elution gradient from 45% to 85%; (System II): H_2O (0.1% TFA), 80% acetonitrile (0.1% TFA), elution gradient from 30% to 70%; Flow rate 0.3 mL/min; UV detection at 260 nm.

Figure 1 Enzymatic Synthesis of CCK-8 Tripeptide Derivative

Phac-Met-OCam
α-Chymotrypsin↓ + H-Asp(OMe)-OMe
Phac-Met-Asp(OMe)$_2$ **1**
Papain↓
Phac-Met-Asp(OMe)-OH **2**
Thermolysin↓ + H-Phe-NH_2
Phac-Met-Asp(OMe)- Phe-NH_2 **3**

Meanwhile, we have also applied the immobilized enzymes (α-chymotrypsin/ Celite -545, papain/ VA-Epoxy，thermolysin/ Celite-545) in the synthesis of the tripeptide, to obtain **1**(yield 72%) and **3**(yield 86%, calculated from **1**) without separation of the intermediate **2**. The result showed that this procedure was even better than above reported ones. We will give the report elsewhere.

References and Notes

1. G. A. Sellek, J.B. Chaudhuri, *Enzyme Microb. Technol.,* **1999**, *25* (6), 471.
2. P. Liu, G. L. Tain, Y. H. Ye, *Chem. J. Chin. Uni.,* **2001**, *22* (8), 1342.
3. C. A. Bails, C. L. Mclaughlin, M. A. Della-Fera, *Phys. Rev.,* **1986**, *66*, 172.
4. H. Waldmann and D. Sebastian, *Chem. Rew.*, **1994**, *94*, 911.
5. Kullman W., *Enzymatic peptide synthesis,* CRC Press: BOCA RATON, FL, **1987**.
6. All compounds were characterized by elemental analysis, FAB or FD-MS. Compound.**1**, mp. 128~129°C, FAB-MS: 411(M+1), $C_{19}H_{26}N_2O_6S$(410); Compound.**2**, mp. 141~144°C, FD-MS: 396(M^+), $C_{18}H_{24}N_2O_6S$(396); Compound.**3**, mp. 210~213°C, FAB-MS: 543 (M+1), $C_{27}H_{34}N_4O_6S$ (542).

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