

Enzymatic Synthesis of a CCK-8 Tripeptide Derivative

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Abstract: The enzymatic synthesis of CCK-8 tripeptide derivative Phac-Met-Asp(OMe)-Phe-NH₂ 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-NH₂, which corresponds to a fragment of cholecystokinin³ C-terminal octapeptide CCK-8[Asp-Try(SO₃)-Met-Gly-Trp-Met-Asp-Phe-NH₂]. The phenylacetyl (Phac) group, which can be cleaved with penicillin G amidase without effecting the peptide bonds⁴. 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 enzymes⁵. 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 KH₂PO₄ buffer (pH 6.0) containing HSCH₂CH₂OH. In this condition only α -methyl aspartate was hydrolysed, while β -methyl aspartate remained. The resulted

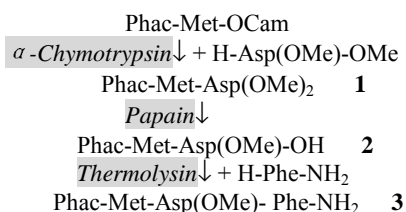
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dipeptide **2** could bond H-Phe-NH₂ with thermolysin as a catalyst in H₂O (pH 7.0) to give **3** in high yield (>82.6%).

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% NaHCO₃ (except **2**), saturated NaCl, subsequently, dried over Na₂SO₄. 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 0.005 mol/L NH₄OAc. After evaporation of MeOH, the residue was submitted to lyophilisation. The obtained peptides were verified by FD-MS or FAB-MS⁶.

HPLC analysis: Column: Nucleosil 100 RP-18, 5 μ m and 100x2 mm column (Macherey-Nagel). Mobile Phase: (System I): 0.05 mol/L NH₄OAc (pH 6.5), 80% of MeOH/0.05 mol/L NH₄OAc, elution gradient from 45% to 85%; (System II): H₂O (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



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

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6. All compounds were characterized by elemental analysis, FAB or FD-MS. Compound **1**, mp. 128~129°C, FAB-MS: 411(M+1), C₁₉H₂₆N₂O₆S(410); Compound **2**, mp. 141~144°C, FD-MS: 396(M⁺), C₁₈H₂₄N₂O₆S(396); Compound **3**, mp. 210~213°C, FAB-MS: 543 (M+1), C₂₇H₃₄N₄O₆S (542).

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