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Enzymatic Synthesis of a CCK-8 Tetrapeptide Fragment

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Abstracts: The enzymatic synthesis of a tetrapeptide Phac-Met-Gly-Trp-Met-OEt is reported. It was synthesized by coupling Phac-Met-OEt with Gly-OMe · HCl, Trp-OMe and Met-OEt successively, catalyzed by α-chymotrypsin, papain and α-chymotrypsin respectively. The results of FAB-MS showed that the products had the correct molecular mass.

Keywords: CCK-8, enzymatic method, α-chymotrypsin, papain.

Many efforts have been devoted to the synthesis of CCK-8, its derivatives and analogues. Among the different methods, the enzymatic method is the most effective one, it has many advantages over conventional chemical methodologies: enzyme specifically suppresses side reactions and ensures the production of chemically and chirally pure peptides. Thus, peptide synthesis using enzymes becomes an important compensation for chemical methods.

In the past few years, our group also has engaged in studying the synthesis of CCK-4[1]. Here we reported the synthesis of the tetrapeptide (Phac-Met-Gly-Trp-Met-OEt) fragment by enzymatic methods. It was obtained through the following route:

$$\text{Phac}-\text{Met}-\text{OMe}+\text{Gly}-\text{OMe}\bullet\text{HCl}\xrightarrow{\text{i}}\text{Phac}-\text{Met}-\text{Gly}-\text{OMe}\xrightarrow{\text{ii}}\text{Phac}-\text{Met}-\text{Gly}-\text{OH}\xrightarrow{\text{iii}}$$
$$\text{Phac}-\text{Met}-\text{Gly}-\text{OCs}\xrightarrow{\text{iv}}\text{Phac}-\text{Met}-\text{Gly}-\text{OCam}\xrightarrow{\text{v}}\text{Phac}-\text{Met}-\text{Gly}-\text{Trp}-\text{OMe}$$
$$\xrightarrow{\text{vi}}\text{Phac}-\text{Met}-\text{Gly}-\text{Trp}-\text{Met}-\text{OEt}$$

Reagents and conditions:(i)α-chymotrypsin,CH_3CN; (ii)acetone/H_2O, NaOH; (iii) EtOH, H_2O, Cs_2CO_3; (iv)DMF, 2-chloroacetamide; (v)Trp-OMe, papain, EtOAc; (vi) Met-OEt, Na_2CO_3, $Na_2CO_3 \cdot H_2O$, α-chymotrypsin.

As shown in the above route, the dipeptide Phac-Met-Gly-OMe was obtained by coupling Phac-Met-OEt with Gly-OMe·HCl. According to Kullman's reports[2], we first tried to use papain as catalyst, the test was failed to obtain the dipeptide. A better result was obtained by α-chymotrypsin catalyzation. Our results showed that α-chymotrypsin can effectively catalyze the formation of the dipeptide in Tris·HCl buffer. The optimized

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reaction conditions are: the ratio of Phac-Met-OEt to Gly-OMe·HCl was 1 to 5, the reaction solvent was mixture of Tris·HCl buffer and acetonitrile (1:4), the pH of the reaction solvent was around 8.3-8.5 by adjusting with 4 mol/L NaOH.

In order to obtain the tripeptide Phac-Met-Gly-Trp-OMe, we first converted Phac-Met-Gly-OMe to Phac-Met-Gly-OCam by the cesium salt method [3]. Calvet[4] reported that papain-mediated coupling of Z-Gly-OCam with Trp-OMe can give quantitative dipeptide in acetonitrile containing 4% of boric acid-borax buffer. So we initially also coupled Phac-Met-Gly-OCam with Trp-OMe under these conditions, but the conversion rate was no more than 60%. The reason for this might be that the reactants could not be dissolved in this solvent system sufficiently. By changing acetonirile to ethyl acetate, the reactants can dissolve entirely and the conversion rate was almost 100%. The optimized reaction solvent system was ethyl acetate containing 5% boric acid-borax buffer.

To couple Phac-Met-Gly-Trp-OMe with Met-OEt, we selected papain and α-chymotrypsin as catalysts according to their primary specificity. The yield was less than 20% in case of papain and could not be improved by changing the reaction conditions including the buffer contents and the organic solvents. Capellas[5] obtained satisfying results using α-chymotrypsin to catalyze the coupling Z-Gly-Trp-OCam with Met-OEt. At first, they had to convert Z-Gly-Trp-OMe to Z-Gly-Trp-OCam. However, we tried directly couple Phac-Met-Gly-Trp-OMe with Met-OEt by the solvent-free method, according to a procedure reported by Cerovsky[6], and obtained satisfactory result. This method was much more convenient in comparison to the method of Capellas. We also observed by-products which contained more than one methionine residue as reported by Capellas. Under carefully controlled reaction conditions, the percentage of the by-products could be kept as low as 3%. One of the by-products was isolated and characterized as Phac-Met-Gly-Trp- Met-Met-OEt by FAB-MS, *m/z* 801.2, $[M+H]^+$, 823.2, $[M+Na]^+$.

Experimental

Synthesis of Phac-Met-Gly-OMe

Phac-Met-OEt (1.185 g, 4 mmol) and Gly-OMe · HCl (2.5 g, 20 mmol) were dissolved in a mixture of acetonitrile (10 mL), Tris · HCl (0.1 mol/L, pH8.1, 40 mL) and NaOH (4 mol/L, 4 mL). To this solution, 250 mg of α-chymotrypsin were added, the reaction was monitored by HPLC. After 1 h, the conversion rate of the reaction was 63.4%, then the solvent was evaporated under reduced pressure. The residue was dissolved in 100 mL ethyl acetate and washed successively with critic acid 5% (w/v) (3×100 mL), $NaHCO_3$ 10% (w/v) (2×100 mL), and saturated NaCl (2×100 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated to dryness under vacuum, the pure dipeptide was obtained, 0.72 g, 53%, mp:124-126°C, FAB-MS:*m/z* 340.1$[M+H]^+$, 362.1$[M+Na]^+$.

Synthesis of Phac-Met-Gly-Trp-OMe

Phac-Met-Gly-OCam (1.09 g, 2.86 mmol, prepared by the cesium salt method according to S. S. Wang[3]) and Trp-OMe (0.872 g, 4 mmol) were dissolved in 30 mL EtOAc, containing borax buffer (0.1 mol/L, pH 8.2, 1.5 mL), β-mercaptoethanol (0.15 mL) and EDTA (0.1 mg/mL, 0.25 mL). To this solution, papain (25 mg) was added. After 4 h , the reaction conversion rate measured by HPLC exceeded 95%. The product was worked up as described above, and the residue was lyophilized yielding a white solid, 1.18 g (2.25 mmol), yield 78.7%, mp:106-110°C, FAB-MS:*m/z*: 525.2 $[M+H]^+$, 547.1 $[M+Na]^+$.

Synthesis of Phac-Met-Gly-Trp-Met-OEt

Phac-Met-Gly-Trp-OMe (0.524 g, 1 mmol) and Met-OEt (0.708 g, 4 mmol) were triturated with Na_2CO_3 (2.544 g, 24 mmol) until a fine powder was obtained, then $Na_2CO_3 \cdot 10 H_2O$ (0.286 g, 1 mmol) and α-chymotrypsin (60 mg) were added and mixed again thoroughly. After 1 h, the reaction conversion rate, measured by HPLC , was 53%. After extraction of the reaction mixture with 100 mL EtOAc, the solution was treated as described above. The residue was dissolved in 30% MeOH and separated by MPLC with 30% MeOH/0.05 mol/L $AcONH_4$. The protected tetrapeptide was obtained as a white solid, 0.301 g(0.45 mmol), yield 45%. mp: 128-131°C, FAB-MS:*m/z* 670.1 $[M+H]^+$, 692.1 $[M+Na]^+$.

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