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Full enzymatic synthesis of a precursor of bioactive pentapeptide OGP(10-14) in organic solvents

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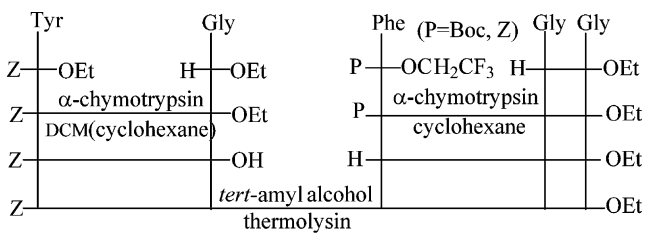
Abstract—Full enzymatic synthesis of a fragment of osteogenic growth peptide (OGP), a precursor of bioactive pentapeptide OGP(10-14) (Z-TyrGlyPheGlyGlyOEt), was accomplished by papain, α-chymotrypsin, and thermolysin via 2+3 or 3+2 synthetic routes in organic solvents for the first time. The factors influencing the enzymatic synthesis of fragments of OGP(10-14) were systematically studied.

Enzymatic synthesis of peptides has drawn much attention because of enzyme stereospecificity, mild reaction conditions, minimum side-chain protection and avoidance of racemization. Enzymatic synthesis of peptides in organic solvents offers special advantages. The enzymes are more stable in organic solvents where the formation of peptide bonds is more favorable than the hydrolysis of the peptide. Most organic compounds are soluble in organic solvents and so on.[1–4] Enzymatic synthesis of peptides has mostly been focused on the synthesis of dipeptides and tripeptides. To our knowledge, there are only a few papers dealing with enzymatic synthesis of pentapeptides or longer peptides. In our previous study, we reported the enzymatic synthesis of a Leu-enkephaline precursor.[2] Herein we describe the enzymatic synthesis of a precursor of bioactive pentapeptide OGP(10-14) in organic solvents.

The osteogenic growth peptide (OGP) is a naturally occurring tetradecapeptide identical to the C-terminal amino acid sequence 89-102(H-Ala-Leu-Lys-Arg-Gln-Gly-Arg-Thr-Leu-Tyr-Gly-Phe-Gly-Gly-OH) of histone H4.[5,6] Endogenous OGP is a proteolytic cleavage product of PreOGP translated from H4 mRNA via alternative translational initiation at a downstream initiation codon.[7] OGP increases bone formation and density when administered to rats.[5] OGP regulates not only proliferation, alkaline phosphatase activity and matrix mineralization but also hematopoiesis.[5,8–11] The OGP C-terminal pentapeptide, H-Tyr-Gly-Phe-Gly-Gly-OH [OGP(10-14)], shares the OGP-like in vitro mitogenic effect and in vivo stimulation of osteogenesis and hematopoiesis. It is the minimal OGP-derived sequence that retains the peptides full proliferative activity.[5]

The synthesis of the precursor of bioactive pentapeptide OGP(10-14), i.e. Z-TyrGlyPheGlyGlyOEt, by catalysis using protease has not been reported before. We have now successfully completed the full enzymatic synthesis of OGP(10-14) precursor via 2+3 (Scheme 1) or 3+2 (Scheme 2) synthetic routes in organic solvents for the first time. In this paper, the factors influencing the synthesis of the fragments of OGP(10-14) were also studied.



Scheme 1. (2+3) Synthetic route of the precursor of bioactive pentapeptide OGP(10-14).

Keywords: full enzymatic synthesis; OGP(10-14); protease; organic solvent.

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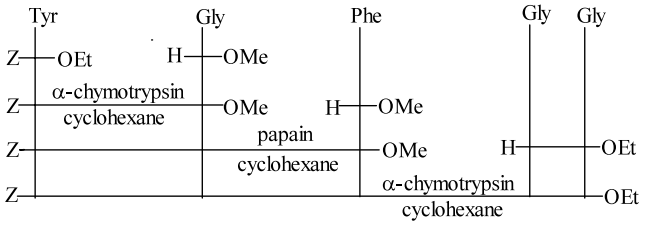

Scheme 2. (3+2) Synthetic route of the precursor of bioactive pentapeptide OGP(10-14).

The yields of the fragments of OGP(10-14) synthesized by α-chymotrypsin depended on their solubility in organic solvents. For the synthesis of Z-PheGlyGlyOEt, the yield in cyclohexane was about double that in DCM. This could be explained by the fact that the solubility of the product in cyclohexane was so poor that the product precipitated from the reaction solution, which shifted the reaction equilibrium toward peptide formation. However, the solvents had little effect on the synthetic yield of Z-TyrGlyOEt because the solubility of the product was poor in both cyclohexane and DCM.

The water content of the cyclohexane influenced the yield of Z-PheGlyGlyOEt synthesized using α-chymotrypsin. The expected product was not obtained when no water was added to the reaction system as water is essential to make the enzyme active. The yield of the product increased with the increasing water content from 0 to 0.5%, but the yield decreased when the water content was above 0.5% due to the increase of hydrolysis of the product. So the optimal water content for the synthesis of Z-PheGlyGlyOEt using α-chymotrypsin in cyclohexane was around 0.5% (Fig. 1).

The effect of the amount of Et_3N added to the reaction system on the synthesis of Z-PheGlyGlyOEt was also studied. The observed pH of the reaction solution would be much higher than 7 when the equivalent amount of Et_3N was added to the reaction system to neutralize the hydrochloride salt of the amino component. This is because the hydrochloride salt of the amino component could not be completely neutralized by Et_3N due to the poor solubility of amino component (GlyGlyOEt·HCl) in cyclohexane. Therefore, it was difficult to investigate the effect of pH on the synthesis of Z-PheGlyGlyOEt. The result indicated that the amount of Et_3N had little effect on the synthesis of Z-PheGlyGlyOEt when the amount of Et_3N was 1∼2 times that of the amino component. The yield of Z-PheGlyGlyOEt was a little higher when the amount of Et_3N was 1.5 times the amino component concentration (Fig. 2).

In addition, the effect of Z and Boc protecting groups on the yield of P-PheGlyGlyOEt (P = Z, Boc) was ignored (Table 1, entries 4 and 7). In comparison with *N*-protected amino acid ethyl ester, the coupling yield of the corresponding trifluoroethyl ester as the carboxyl component was considerably improved (Table 1, entries 6 and 7).

Full enzymatic synthesis of the precursor of bioactive pentapeptide OGP (10-14) (Z-TyrGlyPheGlyGlyOEt) was achieved using α-chymotrypsin, papain and thermolysin via 2+3 or 3+2 synthetic routes in organic solvents for the first time (Table 1, entries 9 and 10). The yield of the above precursor obtained by thermodynamically controlled peptide synthesis via the 2+3 synthetic route was higher than that obtained by kinetically controlled peptide synthesis via the 3+2 synthetic route, but the number of the synthetic steps in the 2+3 route was more than that in the 3+2 route. In the 2+3 route, we also tried to synthesize the precursor of OGP(10-14) using papain as catalyst in several organic solvents including DCM, ethyl acetate, methanol, *tert*-amyl alcohol. The carboxyl component was Z-TyrGlyOR(R = H, Et, Me), the amino component was PheGlyGlyOEt, but the expected product was not obtained.

In conclusion, the precursor of bioactive pentapeptide OGP(10-14) (Z-TyrGlyPheGlyGlyOEt) was synthesized by a full enzymatic method via two different synthetic routes. Some important factors, such as organic solvents, water content, the amount of Et_3N added and so on, were systematically optimized. Our study broadens the range of the application of proteases in the synthesis of peptide and provided some useful information for enzymatic synthesis of peptides, especially for longer peptides.

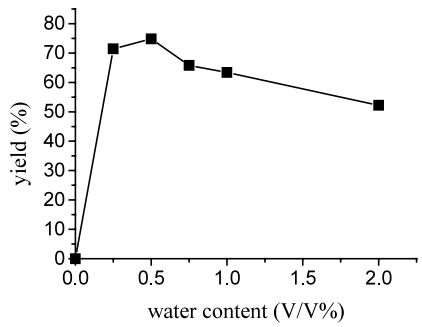

Figure 1. The effect of water content on the yield of Z-PheGlyGlyOEt catalyzed by α-chymotrypsin in cyclohexane.

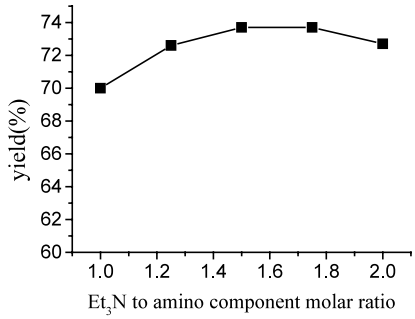

Figure 2. The effect of the amount of Et_3N on the yield of Z-PheGlyGlyOEt catalyzed by α-chymotrypsin in cyclohexane.

Table 1. Yields of *N*-protected OGP(10-14) and its fragments synthesized by catalysis using protease in organic solvents

Entry	Carboxyl component[a]	Amino component[a]	Product[b]	Yield (%) (solvent[c])	Mp (°C)	$[\alpha]_D^{20}$ (*c*, solvent)
1	Z-TyrOEt	GlyOEt	Z-TyrGlyOEt	54 (D)[d]	168–170	−23.5 (1, DMF)
2	Z-TyrOEt	GlyOEt	Z-TyrGlyOEt	63 (C)[e]	169–171	−23.3 (1, DMF)
3	Z-PheOCH$_2$CF$_3$	GlyGlyOEt	Z-PheGlyGlyOEt	32 (D)[d]	92–93.5	+4.6[h]
4	Z-PheOCH$_2$CF$_3$	GlyGlyOEt	Z-PheGlyGlyOEt	75 (C)[e]	91–93	+4.4[h]
5	Z-TyrOEt	GlyOMe	Z-TyrGlyOMe	58 (C)[e]	138–140	−24.5 (1, DMF)
6	Boc-PheOEt	GlyGlyOEt	Boc-PheGlyGlyOEt	37 (C)[e]	98.5–100	+9.0 (1, DMF)
7	Boc-PheOCH$_2$CF$_3$	GlyGlyOEt	Boc-PheGlyGlyOEt	75 (C)[e]	98–100	+8.9 (1, DMF)
8	Z-TyrGlyOMe	PheOMe	Z-TyrGlyPheOMe	61 (C)[f]	79–81	23.1 (1, CHCl$_3$)
9	Z-TyrGlyOH	PheGlyGlyOEt	Z-TyrGlyPheGlyGlyOEt	70 (T)[g]	159–161	−23.8 (0.5, DMF)
10	Z-TyrGlyPheOMe	GlyGlyOEt	Z-TyrGlyPheGlyGlyOEt	51 (C)[e]	159–161	−23.8 (0.5, DMF)

[a] Carboxyl component:amino component = 0.2:0.2 (mmol). As for 8, carboxyl component:amino component = 0.2:0.3 (mmol). As for 9, 10, carboxyl component:amino component = 0.1:0.3 (mmol).

[b] All products were confirmed by FAB-MS. Three (product 6 (or 7), 8, 9 (or 10)) are new compounds and their structures were confirmed by ^{1}H NMR, High Resolution-SIMS or elemental analysis. The physical data of other products were consistent with literature values.

[c] D: DCM; C: cyclohexane; T: *tert*-amyl alcohol.

[d] The products were synthesized using 3 mg α-chymotrypsin with 0.25% H_2O(v/v), pH 10 at 20°C.

[e] Et_3N:amino component = 1.25:1. The other reaction conditions were the same as in footnote d.

[f] The product was synthesized using 3.5 mg papain with 0.25% H_2O (v/v), 1.7% (v/v) $HSCH_2CH_2OH$ at 37°C, Et_3N:amino component = 1.25:1.

[g] The product was synthesized using 5 mg thermolysin with 8% $Ca(OAc)_2$ buffer (v/v), pH 8.5 at 40°C.

[h] *c* = 1, $CHCl_3$:MeOH = 4:1.

Acknowledgements

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References

1. Ye, Y. H. *Univ. Chem.* **1992**, *7*, 17–21.
2. Ye, Y.-H.; Tian, G.-L.; Xing, G.-W.; Dai, D.-C.; Chen, G.; Li, C.-X. *Tetrahedron* **1998**, *54*, 12585–12596.
3. Xing, G.-W.; Tian, G.-L.; Ye, Y.-H. *J. Peptide. Res.* **1998**, *52*, 300–304.
4. Basso, A.; Martin, L. D.; Ebert, C.; Gardossi, L.; Linda, P. *Chem. Commun.* **2000**, 467–468.
5. Chen, Y. C.; Bab, I.; Mansur, N.; Muhlrad, A.; Shteyer, A.; Namdar-Attar, M.; Gavish, H.; Vidson, M.; Chorev, M. *J. Peptide Res.* **2000**, *56*, 147–156.
6. Bab, I.; Gazit, D.; Chorev, M.; Muhlrad, A.; Shteyer, A.; Greenberg, Z.; Namdar, M.; Kahn, A. *EMBO J.* **1992**, *11*, 1867–1873.
7. Bab, I.; Smith, E.; Gavish, H.; Attar-Namdar, M.; Chorev, M.; Chen, Y. C.; Muhlrad, A.; Birnbaum, M. J.; Gary, S.; Frenkel, B. *J. Biol. Chem.* **1999**, *274*, 14474–14481.
8. Greenberg, Z.; Chorev, M.; Muhlrad, A.; Shteyer, A.; Namdar, M.; Mausm, N.; Bab, I. *Biochim. Biophys. Acta* **1993**, *1178*, 273–280.
9. Robinson, D.; Bab, I.; Nevo, Z. *J. Bone. Miner. Res.* **1995**, *10*, 690–696.
10. Greenberg, Z.; Gavish, H.; Chorev, M.; Muhlrad, A.; Shteyer, A.; Attar-Namdar, M.; Tartakovsky, A.; Bab, I. *J. Cell. Biochem.* **1997**, *65*, 359–367.
11. Gurevitch, O.; Slavin, S.; Muhlrad, A.; Shteyer, A.; Gazit, D.; Chorev, M.; Vidson, M.; Namdar-Attar, M.; Berger, E.; Bleiberg, I.; Bab, I. *Blood* **1996**, *88*, 4719–4724.