Studies on Peptides. CXLIX. 1,2) Solid-Phase Synthesis of a Rabbit Stomach Peptide by Application of a New Polymer Support and a New Deprotecting Procedure

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(Received September 19, 1986)

A newly found rabbit stomach peptide, H-Pyr-Val-Asp-Pro-Asn-Ile-Gln-Ala-OH, was synthesized by the solid-phase method. A new polymer support, cross-linked polystyrene-polypropylene composite fiber (IONEX), was employed to facilitate multiple washing processes in chain elongation reactions. ß-Cycloheptyl aspartate, Asp(OChp), was employed for the first time in this solid-phase peptide synthesis. In the final step of the synthesis, the peptide was cleaved from the resin, together with other protecting groups employed, by treatment with 1 M trimethylsilyl trifluoromethanesulfonate-thioanisole in trifluoroacetic acid. The deprotected peptide was found to be identical with the sample obtained from the natural source.

Keywords—rabbit stomach peptide synthesis; new polymer support; polystyrene-polypropylene composite fiber; ß-cycloheptyl aspartate; trimethylsilyl trifluoromethanesulfonate deprotection; trifluoromethanesulfonic acid deprotection.

Recently, Lazarus et al.3) isolated a physalaemin-like immunoreactive peptide from the rabbit stomach (PHLIP-8) and its sequence, H-Pyr-Val-Asp-Pro-Asn-Ile-Gln-Ala-OH, was determined by mass-spectrometric analysis. In order to confirm this sequence, we synthesized this heptapeptide by the solid phase method (Fig. 1).

General methods employed here are essentially the same as described by Merrifield.4) However, several modifications have been made. As a support, a chloromethylated fiber-resin, cross-linked polystyrene-polypropylene composite fiber (IONEX),5) was adopted. This fiber (Fig. 2) has a higher mechanical strength and larger surface areas per unit of weight than the usual polystyrene-divinylbenzene bead-resin and its volume is not changed by organic solvents, such as methylene chloride, DMF and MeOH. The latter property facilitates the multiple washing procedures required for solid-phase peptide synthesis. A new amino acid ß-cycloheptyl aspartate was employed for the first time in the synthesis. In the final step of the synthesis, the peptide was cleaved from the resin, together with other protecting groups employed, by treatment with 1 M trimethylsilyl trifluoromethanesulfonate-thioanisole in trifluoroacetic acid. The deprotected peptide was found to be identical with the sample obtained from the natural source.

Fig. 1. Application of the TMSOTf Deprotecting Procedure to Solid-Phase Peptide Synthesis
acid derivative, Asp(OChp), was employed for the first time in solid-phase synthesis in combination with a new deprotecting reagent. In the final step of the synthesis, the octapeptide was cleaved from the resin, together with other protecting groups, Z(OMe) and Chp, by treatment with 1 M trimethylsilyl trifluoromethanesulfonate (TMSOTf)-thioanisole/TFA. Recently, we found that this reagent cleaved various Bzl and Tos-type protecting groups, and even the Chp group more readily than 1M TFMSA-thioanisole/TFA. The deprotected peptide was purified by gel-filtration, followed by preparative high performance liquid chromatography (HPLC) on a TSK-GEL LS-410KG column using isocratic elution with acetonitrile in 0.1% TFA aq. Alternatively, TFMSA deprotection was performed for comparison. The time required for TMSOTf deprotection was 60 min, while TFMSA deprotection required 120 min. The yield in the former experiment was 27.4% from the peptide resin, while that in the latter case was 15.8%. Both purified products exhibited an identical retention time, when examined by analytical HPLC on a Nucleosil SC18 column. Identity of the synthetic peptide with the natural peptide was established by mass-fragmentation studies.

By means of the above experiments, we confirmed the sequence of the natural peptide. In addition, this is the first example of application of the TMSOTf deprotecting procedure to solid-phase peptide synthesis. Establishment of the utility of the fiber-resin will require the accumulation of further results, especially in relation to syntheses of larger and more complex peptides.

Experimental

An automated solid-phase synthesizer, Vega Biotech. Coupler 250C was employed. HPLC was conducted with a Waters 204 compact model. Optical rotation and ultraviolet (UV) absorption were measured with a Union PM 101 instrument and a Hitachi model 100-20 spectrometer, respectively. Rf values on thin layer chromatography (TLC), performed on silica gel (Kieselgel G, Merck), refer to the following solvent systems: Rf, n-BuOH-AcOH-pyridine-H2O (4:1:1:2) and Rf2, n-BuOH-AcOH-AcOEt-H2O (1:1:1:1).

Solid-Phase Synthesis——Z(OMe)-Ala-OCH2-fiber-resin (Ala content = 0.61 mmol/g) was prepared by coupling Z(OMe)-Ala-OH to chloromethylated IONEX (Lot. No. A-503) by the standard procedure. Starting with 1.0 g of the Z(OMe)-Ala-resin, each of derivatized amino acids, corresponding to the sequence of PHLIP-8, was condensed successively onto the resin, i.e., Z(OMe)-Gln-OH, Z(OMe)-Ile-OH, Z(OMe)-Asn-OH, Z(OMe)-Pro-OH, Boc-Asp(OChp)-OH, Z(OMe)-Val-OH, and Z(OMe)-Pyr-OH. The Gln and Asn residues were introduced by the Np method and the rest of the residues by the symmetrical anhydride procedure. Deprotection, neutralization and washing were performed according to the schedule of Chang et al. Washing the resin was easily performed. Since this resin has a pale brown color, the ninhydrin test was not accurate enough, so after two coupling reactions, a part of the resin was subjected to acid hydrolysis to ensure the satisfactory incorporation of the respective amino acids. Thus, 1.38 g of protected PHLIP-8-resin was obtained.
Deprotection—(a) The peptide resin (0.1 g) was suspended in 1 M thioanisole/TFA (1 ml, 16 eq), and TMSOTf (to a final concentration of 1 M) and m-cresol (0.19 ml, 30 eq) were added. The mixture was stirred in an ice-bath for 60 min, then the solution was filtered and the resin was washed with TFA (2 ml). The filtrate and the washing were combined and concentrated in vacuo at a bath temperature below 15°C, then dry ether was added and the resulting powder was collected by centrifugation. The deprotected peptide was dissolved in ice-chilled 5% NH₄OH (2 ml) containing NH₄F (10 eq) at pH 8.0 to ensure the hydrolysis of trimethylsilyl compounds. After 10 min, the solution was neutralized with 1 N AcOH and applied to a column of Sephadex G-10 (2.8 × 85 cm), which was eluted with 0.01 M NH₄HCO₃ buffer (pH 7.8). The solvent of the desired fractions (10.5 ml each, tube Nos. 19–26, monitored by using the Folin-Lowry test) was removed by lyophilization to give a fluffy powder; yield 25 mg (47%). Subsequent purification of the crude product (6 mg each) was performed by reverse-phase HPLC on a TSK-GEL LS-410KG column (21.5 × 300 mm), by isocratic elution with 17% acetonitrile in 0.1% TFA aq. at a flow rate of 6.0 ml/min. The eluate corresponding to the main peak (retention time of 40.2 min, monitored by UV absorption measurement at 233 nm) was collected. The rest of the sample was similarly purified and the solvent of the combined eluates was removed by lyophilization to give a white fluffy powder; yield 14.5 mg (27.4%, based on the starting loading of Ala onto the resin); [α]₃⁰ D = -114.6 (c = 0.1, 0.01 M NH₄HCO₃), a single spot on TLC, Rf₁ 0.36, Rf₂ 0.48. HPLC on a Nucleosil 5C18 column (4 × 150 mm), with 17% acetonitrile in 0.1% TFA aq. as the mobile phase at a flow rate of 0.8 ml/min, resulted in a single peak with a retention time of 9.5 min. Amino acid ratios in a 6 N HCl hydrolysate (numbers in parentheses are theoretical): Asp 1.96 (2), Glu 1.94 (2), Pro 0.93 (1), Ala 1.00 (1), Ile 0.97, Val 0.94 (recovery of Ala, 81%).

(b) The peptide resin (0.50 g) suspended in 1 M thioanisole/TFA (10 ml) was similarly treated with TFMSA (to a final concentration of 1 M) in the presence of m-cresol (0.5 ml, 30 eq) in an ice-bath for 60 min, then at room temperature for 60 min. The deprotected peptide was treated with 5% NH₄OH (3 ml), then purified by gel-filtration on Sephadex G-10, followed by HPLC on a TSK-GEL LS-410KG column as described above; yield 41.8 mg (15.8% from the peptide resin); Rf₁ and Rf₂ were identical with those of the sample obtained in (a). A mixture of the samples obtained in (a) and (b) emerged from a Nucleosil 5C18 column (4 × 150 mm) as a single peak (retention time, 9.5 min), when eluted with 17% acetonitrile in 0.1% TFA aq. at a flow rate of 0.8 ml/min (Fig. 3). Synthetic PHLIP-8 yielded a mass fragmentation pattern identical with that of the natural peptide as reported. Amino acid ratios in a 6 N HCl hydrolysate: Asp 2.04, Glu 1.94, Pro 1.05, Ala 1.00, Ile 0.96, Val 0.94, (recovery of Ala, 88%). Anal. Calcd for C₃₇H₅₈N₁₀O₁₄·H₂O: C, 50.21; H, 6.83; N, 15.82. Found: C, 50.42; H, 6.70; N, 16.08.

References and Notes

2) The following abbreviations are used: Bzl = benzyl, Z(OMe) = p-methoxybenzyloxy carbonyl, Pyr = pyroglutamyl, Chp = cycloheptyl, DMF = dimethylformamide, TFA = trifluoroacetic acid, TFMSA = trifluoromethanesulfonyl acid.


