BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN VOL. 42 3592—3593 (1969)

Synthesis of a Fragment (Pentapeptide) of *E. Coli* Acyl Carrier Protein Apoprotein

Yoshiro MIURA and Shuichi SETO

Chemical Research Institute of Non-Aqueous Solutions, Tohoku University, Katahira-cho, Sendai

(Received May 23, 1969)

Various peptides have been synthesized rapidly and in higher yield by the solid phase method.[1-3] We have synthesized by this method, a nonapeptide of melittin[4] and a heptapeptide[5]. Acyl carrier protein has recently been found to be essential for saturated fatty acid biosynthesis, amino acid sequence of which has been reported.[6,7]

Acyl carrier protein holoprotein (holo-ACP) synthetase which transfers 4′-phosphopantetheine from reduced CoA to apo acyl carrier protein (apo-ACP) to form holo-ACP and 3′,5′-adenosine diphosphate has been shown in *E. Coli B*.[8] It seemed interesting to study the smallest fragment of apo-ACP to be utilized as a substrate of this enzyme, and we tried to synthesize a pentapeptide having a serine residue to which 4′-phosphopantetheine is bound. We wish to report the synthesis of a fragment of apo-ACP, H-Gly-L-Ala-L-Asp-L-Ser-L-Leu-OH, by the solid phase method. Synthesis of a pentapeptide was carried out according to the procedure described by Marshall and Merrifield[3] for the solid phase method, using an automatic instrument which we constructed[5].

The peptide was synthesized stepwise, beginning with 1.7 mmol of *t*-butyloxycarbonyl (*t*-Boc)-L-leucine esterified to 10 g of the cross-linked polystyrene resin (copolymerized with 2% divinylbenzene). *t*-Boc-amino acids with protected side chains were *O*-benzyl-L-serine and *β*-benzyl-L-aspartic acid.

Coupling was mediated by *N*, *N*′-dicyclocarbodiimide (DCC). *t*-Boc groups were removed with 1 N hydrogen chloride in glacial acetic acid. Neutralization of the hydrochloride was carried out with triethylamine in dimethylformamide (DMF).

The total time required for the sysnthesis was 28 hr. The peptide was cleaved from the resin with hydrogen bromide in trifluoroacetic acid. The reagent also cleaved the ether bond in the *O*-benzyl-L-serine residue and the ester bond in the *β*-benzyl-L-aspartic acid residue. Paper chromatography of the crude peptide showed one major ninhydrin positive spot and a few minor spots. The pure pentapeptide was obtained through purification of the crude peptide by gel filtration on a column of Sephadex G-10 using 0.1 M acetic acid as the solvent and gave the correct amino acid ratios on acid hydrolysis. The over-all yield from the first leucine residue was 19.6%. Its homogeneity was shown by paper chromatography using two different solvent systems and by electrophoresis.

Experimental

Solvents used for ascending paper chromatography on Toyo Roshi No. 50 paper were a) *n*-butanol - acetic acid - water(BAW) (4 : 1 : 1) and b) *n*-butanol - acetic acid - pyridine - water (BAPW) (15 : 3 : 10 : 12). Paper electrophoresis was carried out using a pH 7.76 buffer (KH_2PO_4-Na_2HPO_4), on Toyo Roshi No. 50 paper at 500V/38 cm for 2 hr.

t-Boc-L-leucyl Resin. A solution of 3.06 g (13.25 mmol) of *t*-Boc-L-leucine and 1.34 g (13.25 mmol) of triethylamine in 14 m*l* of absolute ethanol and 7 m*l* of chloroform was added to 10.0 g of chloromethylcopolystyrene-2% divinylbenzene which contained 6.61% chlorine. The reaction mixture was stirred at reflux temperature for 48 hr. The esterified resin was filtered off and washed with absolute ethanol (three times), water (three times), and methanol (three times). The esterified resin was then dried *in vacuo* over KOH pellets; yield, 11.5 g. Amino acid analysis of an acid hydrolysate (dioxane-12 N HCl, 1 : 1) showed the product to contain 0.602 mmol of leucine/g of esterified resin.

t-Boc-glycyl-L-alanyl-β-benzyl-L-aspartyl-O-benzyl-L-seryl-L-leucyl-Resin. The *t*-Boc-L-leucyl (2.82 g) was placed in the reaction vessel. The following cycle of de-protection, neutralization, and coupling was carried out for the introduction of each residue: (1) three washings with 26 m*l* portions of glacial acetic acid; (2) cleavage of the *t*-Boc group by treatment with 1 N HCl in glacial acetic acid (30 m*l*) for 30 min at room temperature; (3) three washings with 26 m*l* portions of glacial acetic acid; (4) three washings with 33 m*l* portions of absolute ethanol; (5) three washings with 24 m*l*

1) R. B. Merrified, *J. Am. Chem. Soc.*, **85**, 2149 (1963).
2) R. B. Merrified, *Biochemistry*, **3**, 1385 (1964).
3) G. R. Marshall and R. B. Merrified, *ibid.*, **4**, 2394 (1965).
4) Y. Miura, M. Toyama and S. Seto, *Sci. RITU.* **A-Vol. 20**, 41 (1968).
5) H. Sugiyama, Y. Miura and S. Seto, *Yuki Gosei Kagaku Kyokai Shi (J. Synthetic Org. Chem. Japan)*, **26**, 1010 (1968).
6) T. C. Vanaman, S. J. Wakil and R. L. Hill, *J. Biol. Chem.*, **243**, 6409 (1968).
7) T. C. Vanaman, S. J. Wakil and R. L. Hill, *ibid.*, **243**, 6420 (1968).
8) J. Elovson and P. R. Vagelos, *J. Biol. Chem.*, **243**, 3603 (1968).

portions of DMF; (6) neutralization of the hydrochloride salt with 2.6 m*l* of triethylamine in 26 m*l* of DMF; (7) three washings with 24 m*l* portions of DMF; (8) three washings with 28 m*l* portions of methylene chloride; (9) addition of 6.80 mmol of the appropriate *t*-Boc-amino acid dissolved in 20 m*l* of methylene chloride and mixing for 10 min; (10) addition of 6.80 mmol of DCC in 16 m*l* of methylene chloride, followed by a reaction period of 3.0 hr at room temperature. For the *t*-Boc-*O*-benzyl-L-serine cycle the reaction time was 3.5 hr; (11) three washings with 28 m*l* portions of methylene chloride; (12) three washings with 33 m*l* portions of absolute ethanol.

After step 12 following the incorporation of the *t*-Boc-glycine, the protected peptide resin compound was further washed with methylene chloride, absolute ethanol and dried *in vacuo* over KOH pellets overnight; yield, 3.7 g.

Crude Glycyl-L-alanyl-L-aspartyl-L-seryl-L-leucine. The protected peptide resin (2.90 g) was suspended in 20 m*l* of trifluoroacetic acid, and a stream of hydrogen bromide was bubbled through the suspension with occasional shaking for 90 min. The resin was removed by filtration and washed with 4 m*l* of trifluoroacetic acid. The combined filtrates were evaporated on a rotary evaporator *in vacuo* at 25°C and the syrupy product obtained was triturated with dry ether. Ether was removed by decantation and the residue was washed with ether. The amorphous powder was collected; yield, 810 mg.

Paper chromatography in BAW showed a major spot at R_f 0.21 when sprayed with ninhydrin reagent and traces of materials at R_f 0.11, R_f 0.42, and R_f 0.62, and also in BAPW, a major spot at R_f 0.45 and traces of materials at R_f 0.52 and R_f 0.79.

Paper electrophoresis showed two spots when sprayed with ninhydrin reagent.

Purification of Crude Pentapeptide. A portion (18 mg) of the crude peptide was placed on a 1.2×68 cm column of Sephadex G-10 equilibrated with 0.1 M acetic acid. The column was then developed with the same solvent. Aliquots (0.1 m*l*) from 2 m*l* fractions were alanyzed by ninhydrin reagent. Fractions 6-19 contained only the major compound and were pooled and lyophilized; yield, 2.7 mg. This presents an overall yield 19.6% based on the amount of leucine initially esterified to the resin.

By paper chromatography we obtained R_f 0.20 (BAW), R_f 0.45 (BAPW). Paper electrophoresis of the pentapeptide showed a single ninhydrin positive spot. Amino acid ratios found were: Asp, 0.95; Ser, 0.98; Gly, 1.02; Ala, 1.00; Leu, 1.03. $[\alpha]_D^{23}$ −59.3 (*c* 0.73, H_2O).