Linear Polypeptide of a Known Sequence of Amino Acids: Poly-(L-phenylalanyl-L-glutamyl-L-alanylglycyl)glycine Methyl Ester

Keyphrases ☐ Poly-(L-phenylalanyl-L-glutamyl-L-alanylglycyl)glycine methyl ester—synthesis ☐ Polypeptide, linear—known sequence of amino acids ☐ Immunochemical properties—linear polypeptide, known sequence of amino acids

Sir:

Random copolymers containing varying amounts of the amino acid residues, alanine, glutamic acid, and tyrosine, have been shown to be antigenic (1–3). However, due to the unknown primary sequence of these random polymers, it is difficult to describe the locus of the active site of these antigenic polymers. To overcome this difficulty, the use of linear polypeptides with a known sequence of amino acids has been suggested. For this purpose, poly-(L-tyrosyl-L-glutamyl-L-alanylglycyl)glycine-1-^{14}C ethyl ester was recently synthesized (4, 5). This polypeptide was found to be antigenic (6), giving a precipitin reaction with its antiserum. To ascertain the role of the various amino acid residues in this antigenic polypeptide, it was necessary to prepare a series of polymers that differ from each other with respect to the following parameters: primary sequence of amino acids, variations in the steric requirements of the hydrophobic side chains, and the presence or absence of the phenolic hydroxyl group.

To investigate this latter point, we wish to report the synthesis and immunochemical properties of poly-(L-phenylalanyl-L-glutamyl-L-alanylglycyl)glycine methyl ester (Compound 1, Scheme I).

N-tert-Butyloxycarbonyl-L-phenylalanine pentachlorophenyl ester (7) was coupled to γ-*tert*-butyl-L-glutamyl-L-alanylglycine methyl ester hydrochloride (8) to give *N-tert*-butyloxycarbonyl-L-phenylalanyl-γ-*tert*-L-glutamyl-L-alanylglycine methyl ester (Compound 2), m.p. 191–193°, $[\alpha]_D^{29}$ −15.3° (c 1.50 in dimethylformamide).

Anal.—Calcd. for $C_{29}H_{44}N_4O_9$: C, 58.8; H, 7.5; N, 9.5. Found: C, 59.0; H, 7.3; N, 9.5.

Saponification of Compound 2 with *N* NaOH yielded the tetrapeptide free acid *N-tert*-butyloxycarbonyl-L-phenylalanyl-γ-*tert*-butyl-L-glutamyl-L-alanylglycine (Compound 3), m.p. 132.5°, $[\alpha]_D^{29}$ −12° (c 1.46 in dimethylformamide).

Anal.—Calcd. for $C_{28}H_{42}N_4O_9$: C, 58.15; H, 7.3; N, 9.7. Found: C, 58.1; H, 7.25; N, 9.6.

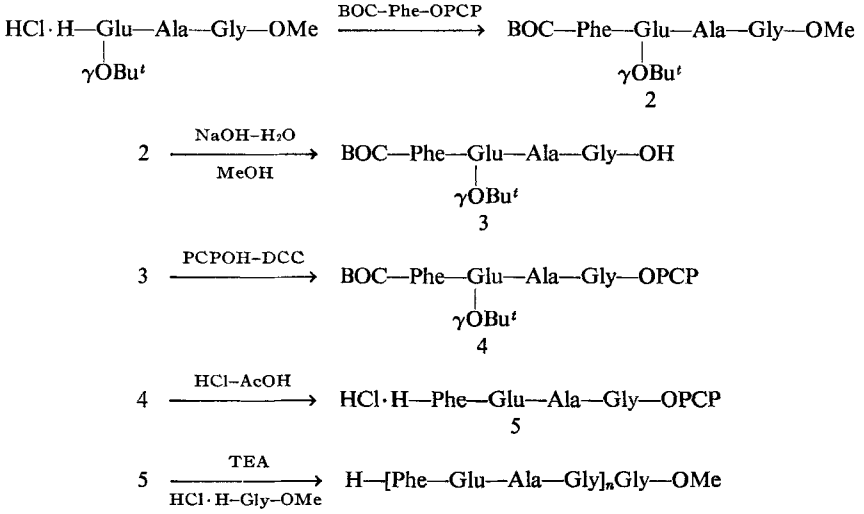
Coupling Compound 3 with pentachlorophenol, using 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide methotoluene-*p*-sulfonate, yielded the tetrapeptide activated ester, *N-tert*-butyloxycarbonyl-L-phenylalanyl-γ-*tert*-butyl-L-glutamyl-L-alanylglycine pentachlorophenyl ester (Compound 4), m.p. 210°.

Anal.—Calcd. for $C_{34}H_{41}Cl_5N_4O_9$: C, 49.4; H, 5.0; N, 6.8. Found: C, 49.3; H, 5.0; N, 6.8.

Treatment of Compound 4 with dry hydrogen chloride in glacial acetic acid removed the *N-tert*-butyloxycarbonyl and *tert*-butyl ester protecting groups to give L-phenylalanyl-L-glutamyl-L-alanylglycine pentachlorophenyl ester hydrochloride (Compound 5), m.p. 186° dec., $[\alpha]_D^{29}$ 2.5° (c 1.01 in dimethylformamide).

Anal.—Calcd. for $C_{25}H_{26}Cl_6N_4O_7$: C, 42.5; H, 3.5; N, 7.9. Found: C, 42.7; H, 3.8; N, 7.85.

The polymerization of Compound 5 was conducted under dilute conditions in the presence of the preformed monomer glycine methyl ester hydrochloride. This established polymerizing procedure has been shown to yield high molecular weight polypeptides (4, 5, 8–10). Thus, the polymerizing unit, Compound 5, dissolved in dimethyl sulfoxide was added to a solution of glycine methyl ester hydrochloride containing the total amount (3.5 equivalents) of triethylamine such that the final concentration of reactants was never more than 100 mmoles/l. The polymerization was allowed to proceed for a week, after which it was acidified and dialyzed against distilled water. The precipitated polymer, poly-(L-phenylalanyl-L-glutamyl-L-alanylglycyl)glycine methyl ester, was collected by centrifugation, washed with methanol, dried, converted to its sodium salt, and extensively dialyzed. Final purification was achieved by passage through a column of Sephadex G-100 (2.5 ×



Scheme I

40 cm.) using a buffer (0.05 *M* KH_2PO_4 corrected to pH 8.0) as eluent. Acidification and dialysis of the high molecular weight fractions yielded the pure polypeptide, Compound 1.

Anal.—Calcd. for $C_{19}H_{24}N_4O_6$: C, 55.3; H, 6.1. Found: C, 55.0; H, 6.0.

Filtration of the polymer through a calibrated column (11) of Sephadex G-100 (2.5 × 45 cm.), using a solution of 0.05 *M* KH_2PO_4 corrected to pH 8.0 as eluent, indicated a molecular weight of at least 1×10^5.

Two rabbits were immunized with poly-(phe.glu.ala.gly)gly methyl ester (Compound 1) using the same protocol as that previously described (6). To aliquots of the sera obtained from each rabbit was added up to 500 mcg. of the synthetic polypeptide (Compound 1). No precipitin reaction was observed. Thus, substitution of the tyrosyl residue in the antigen poly-(tyr.glu.ala.gly)gly-1-^{14}C ethyl ester with the phenylalanyl moiety caused a loss of antigenicity, as shown by the precipitin reaction. It has been concluded that it is the phenolic hydroxy group of tyrosine that confers antigenicity to the molecule.

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Portal Vein Blood Sampling in Intestinal Drug Absorption Studies

Keyphrases ☐ Portal vein sampling—intestinal drug absorption ☐ Cannulation method—portal vein

Sir:

In an attempt to study the *in situ* absorption of drugs from the rat small intestine, a method of sampling portal vein blood was devised.

Generally, *in situ* drug absorption from the small intestine has been studied by determining the reduction in concentration of drug in the lumen (1–3). To complete the picture of intestinal absorption, simultaneous measurements of drug appearance in the portal blood and in the bile are required.

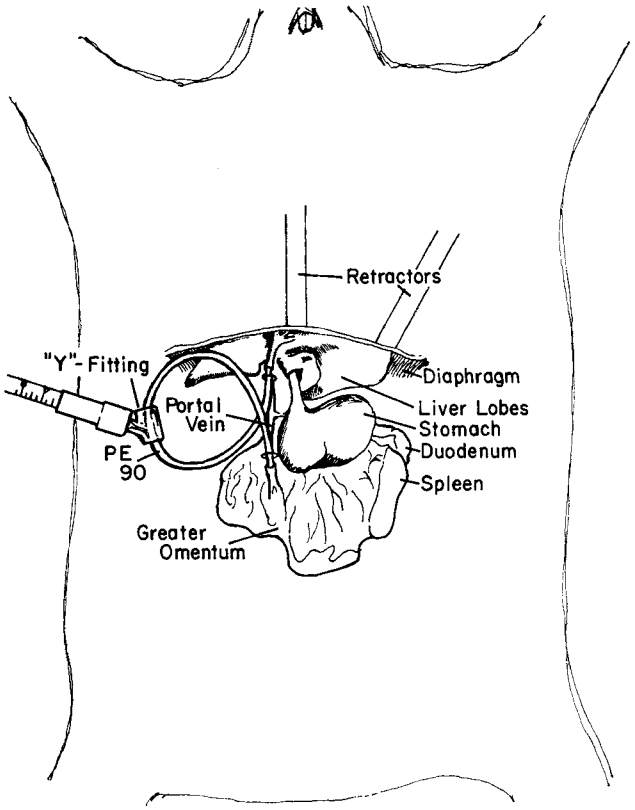

Figure 1—*Portal vein cannulation. Stomach, liver, and mesenteries were retracted to show the cannulation.*

In this communication, we report a method for sampling portal blood. The methods presented in previous work on dogs (4, 5) and rabbits (6) are not practical for the portal vein of a 200-g. rat since this portal vein is quite fragile. We tried direct venous puncture, using a needle point with tubing attached for sampling. The major problems were placing and securing the needle among the liver lobes, the mesenteries, and the small intestine, all of which should remain in their normal positions. The method described here consists of a shunt of polyethylene tubing cannulated in opposite directions in the portal vein. The surgery is delicate, requiring speed and accuracy to prevent excessive engorgement of the intestinal veins and acute loss of blood in the liver.

The viscera were exposed by midline abdominal incision. The portal vein was exposed by gentle retraction of the stomach just above the pyloric sphincter. The distal cannulation was prepared first. A Johns Hopkins bulldog clamp was placed around the portal vein, deep in the greater omentum. Fat and mesentery were included in the clamp to cushion the vein, thus preventing its collapse. The vein should not be cleaned of connective tissue. A cut in the vessel was then made approximately 3 mm. proximal to the clamp, and a slightly beveled length of PE 90 polyethylene tubing [i.d. 0.086 cm. (0.034 in.), o.d. 0.13 cm. (0.050 in.), length 27.9 cm. (11 in.)],[1] containing heparin solution, was inserted

[1] PE 90 Intramedic, Clay Adams.