SYNTHESIS OF REGULAR POLYPEPTIDES INCLUDING L-LYSINE AND GLYCINE*

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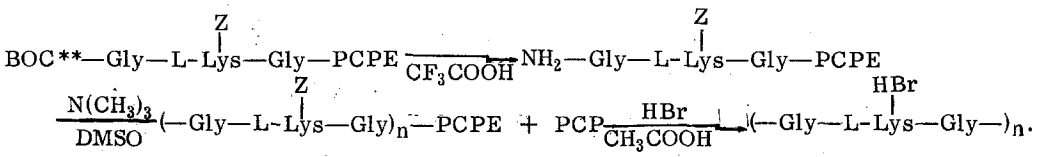
The synthesis of regular polypeptides including neutral and basic amino acids is of dual interest. By studying the behavior of such polypeptides in solution it is possible to obtain information on the influence of the charge distribution on the macromolecular properties of a polypeptide chain. The other aspect of the study of polypeptides including basic amino acids is the investigation of their interaction with nucleic acids. At present, it has been established that electrostatic interactions between DNA and histones play an important role in the structure of the deoxyribonucleoprotein (DNP) on which the structure of the chromosomes of eucaryotes is based.

This paper describes the synthesis of two regular polypeptides with the composition $(\text{—L-LyS—L-Lys—Gly})_n$ and $(\text{Gly—L-Lys—Gly})_n$. The synthesis of the polypeptides was effected by the following scheme:



For the α-amino group we selected BOC protection, which is readily eliminated in the presence of a benzyloxycarbonyl group. Pentachlorophenyl ethers of tripeptides were condensed [1–3]. The polypeptides were subjected to gel filtration through columns of Bio-Gel P-10 in order to obtain polymers more homogeneous with respect to their molecular weights, and also in order to obtain a preliminary evaluation of their molecular weights. The eluent was 0.01 N HCl in order to prevent sorption in the columns. The results of this fractionation for poly(—L-Lys—L-Lys—Gly—) and reference substances, the total histone of calf thymus (mean mol wt 20 000) and the protamine stellin (mean mol wt 4500),*** are given in Fig. 1, and the results of the gel filtration of the polymer $(\text{—Gly—L-Lys—Gly—})_n$ and of the repeated gel filtration of the polymer fraction extracted from the first portion of the eluate are shown in Fig. 2. The shift in the elution maximum shows the reality of fractionation with respect to molecular weight. An evaluation of the molecular weights of the polypeptides based on the results of gel filtration gave figures of the order of 3000.

The molecular weights of the polypeptides were also determined by Archibald's method [5] using a "Spinco E" ultracentrifuge. The polymer samples were dissolved in 1% NaCl solution to a final concentration of 5 mg/ml and were centrifuged at 35 000 rpm. The specific partial volume of the polypeptide was calculated from the specific partial volume of the amino acid residues: glycine 0.64 and lysine 0.82 [6]. The sedimentograms were treated in the usual way [7]. This gave a mol wt of 2400 for $(\text{L-Lys—L-Lys—Gly—})_n$ and 3500 for $(\text{—Gly—L-Lys—Gly—})_n$. For the fraction of the (Gly—L-Lys—Gly) polymer obtained by rechromatography the mol wt was ~5000. These results are in good agreement with the values obtained by the gel filtration method. The completeness of the removal of the benzyloxycarbonyl group (99%) was checked spectrophotometrically.

EXPERIMENTAL

Synthesis of monomers for polycondensation. A) Methyl ester of N^{α}-tert-butoxycarbonyl-N^{ε}-benzyloxycarbonyl-

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**BOC) tert-butoxycarbonyl group; Z) benzyloxycarbonyl group; PCP) pentachlorophenol; PCPE) pentachlorophenyl ether; DMSO) dimethyl sulfoxide; DMF) dimethylformamide.

***The stellin was kindly given to us by E. D. Kaverzneva, and the histone was obtained in our laboratory [4].

L-lysyl-N$^{\varepsilon}$-benzyloxycarbonyl-L-lysine (I). A solution of 8.16 g of N$^{\alpha}$-tert-butoxycarbonyl-N$^{\varepsilon}$-benzyloxycarbonyl-L-lysine [8] in 100 ml of absolute chloroform was treated with 3 ml of triethylamine and the mixture was cooled to −10° C. Then, with stirring, 2.86 ml of isobutyl chloroformate was added, and after 40 min the temperature was raised to −5 to −3° C and kept at this level for 15 min, and then it was again lowered to −15° C. Then a suspension, cooled to −15° C, containing 6.80 g of the hydrochloride of the methyl ester of N$^{\varepsilon}$-benzyloxycarbonyl-L-lysine [9], 3 ml of triethylamine, and 100 ml of absolute chloroform was added to the reaction mixture. After this it was kept at 0° C for 1 hr and at +15° C for 15 min. Then it was extracted with cold water (2 × 30 ml), with a saturated solution of citric acid (2 × 30 ml), with 0.5 N sodium bicarbonate solution (3 × 30 ml), and again with water (1 × 30 ml). The chloroform solution was dried with Na_2SO_4 and evaporated in vacuo. The yield of oil was 9 g (63.7%), R_f 0.84 [system 1: sec-butanol–3% ammonia (100 : 44) on silica gel with iodine as the developer]. Found, %: C 62.13; H 7.29. Calculated for $C_{34}H_{48}O_9$, %: C 62.19; H 7.47.

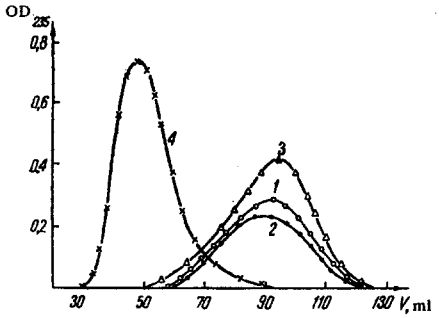

Fig. 1. Fractionation of 1) (—Gly—L-Lys—Gly—)$_n$ hydrobromide, 2) (—Gly—Lys—Lys—)$_n$ hydrobromide, 3) stellin sulfate, and 4) histone hydrobromide on a column of Bio-Gel P-10.

B. N$^{\alpha}$-tert-Butoxycarbonyl-N$^{\varepsilon}$-benzyloxycarbonyl-L-lysyl-N$^{\varepsilon}$-benzyloxycarbonyl-L-lysine (II). A solution of 3.55 g of the methyl ester I in 6 ml of acetone was treated at room temperature with 6 ml of 1 N NaOH and left for 1 hr. The acetone was evaporated off in vacuo at room temperature and 6 ml of water was added, and then, to eliminate the unsaponified ester, the reaction mixture was extracted with ethyl acetate (3 × 15 ml). The aqueous layer was acidified with a solution of citric acid to pH 2.0 and extracted with ethyl acetate (4 × 15 ml). The extract was dried with Na_2SO_4 and the solvent was distilled off in vacuo. Yield 2.5 g (72%), R_f 0.07 (system 1).

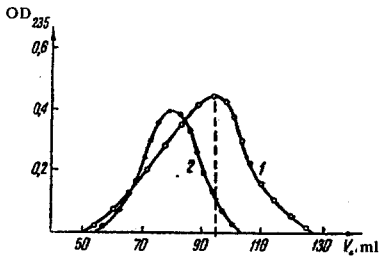

Fig. 2. Chromatography (1) and rechromatography (2) of the polymer (—Gly—Lys—Gly—)$_n$ on a column of Bio-Gel P-10 (the broken line shows the separation into fractions I and II after the first chromatographic cycle).

C) Pentachlorophenyl ether of the N$^{\alpha}$-trifluoroacetate of N$^{\varepsilon}$-benzyloxycarbonyl-L-lysyl-N$^{\varepsilon}$-benzyloxycarbonyl-L-lysylglycine (IV). A solution of 1.5 g of III in 0.234 ml of absolute trifluoroacetic acid was kept at room temperature for 1.5 hr. The solvent was driven off by evaporation in vacuo, and the residue was treated with absolute benzene (10 ml) and evaporated twice. The yield of IV was 0.9 g (71%), R_f 0.6 (system 1), $[\alpha]_D^{20}$ − 5.3° (c 0.1 DMF). The characteristics and methods of synthesis of the other compounds are given in the table.

Polycondensation. A solution of 0.42 g of IV in 0.42 ml of absolute dimethyl sulfoxide was treated with 0.07 ml of triethylamine and the mixture was left at room temperature for 4 days. Then 5 ml of absolute methanol was added and the precipitate was separated off and washed with ether. Yield 0.1 g (30%), $[\alpha]_D^{20}$ − 6.8° (c 1 DMF). The

benzyloxycarbonyl group was removed with HBr/CH_3COOH. Yield 88%. The hydrobromide of $(—Gly—L\text{-}Lys—Gly—)_n$ was obtained similarly. The yield at the polycondensation stage was 50%.

Compound	Method of Preparation	Yield, %	Mp, °C	$[\alpha]_D^{20}$, deg (c 1, DMF)	R_f	Elementary analysis		
						found, %	formula	calculated, %
Z Z BOC–L-Lys–L-Lys–Gly–PCPE (III)	A	65	102	–11,2	0.9	C 51,90; H 5.42	$C_{41}H_{48}O_{10}N_5Cl$	C 51,92; H 5.06
Z BOC–Gly–L-Lys–OCH_3 (V)	A	50	—	—	0,88	C 58,10; H 7,59	$C_{22}H_{33}O_7N_3$	C 58,07; H 7,31
Z BOC–Gly–L-Lys–OH (VI)	Б	85	—	—	0.65	—	—	—
Z BOC–Gly–L-Lys–Gly–PCPE (VII)	A	66	155	–0,22	0.78	C 46,77; H 4,23 Cl 24,01	$C_{29}H_{33}O_8N_4Cl_5$	C 46,90; H 4,46; Cl 23,87
N^{α}-trifluoroacetyl–Gly–L-Lys–Gly–PCPE (IX)	B	83	170	–0,17	0,54	—	—	—

Fractionation of the polypeptides on columns of Bio-Gel P-10. Columns 2 cm in diameter and 5 cm long, with a volume of Bio-Gel of 120 ml, were used. The polymers were dissolved in 0.01 N HCl before deposition on the column and were eluted with the same solvent. All the experiments were carried out in a single column. The rate of elution was 60 ml/hr and the volume of each of the fractions collected was 3 ml. The load of polymer or protein in 3 ml was 30–80 mg. Within this range of concentrations the position of the elution maximum does not depend on the load. The yield of polypeptides and proteins from the column was checked from the UV absorption at 235 mμ. One of the test-tubes at the beginning of the issue of a peak was used for checking purposes.

CONCLUSIONS

The synthesis of two regular polypeptides with the composition $(—Gly—L\text{-}Lys—Gly)_n$ and $(—L\text{-}Lys—L\text{-}Lys—Gly—)_n$ with molecular weights of 250 and 5000 has been effected.

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