Synthesis and Conformational Studies of Model Histones. Sequential and Random Polypeptides with the Composition L-Lysyl-L-alanyl-glycine†

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ABSTRACT: The sequential polytripeptide $(Lys\text{-}Ala\text{-}Gly)_n$ and the random polypeptide $(Lys^{37.6}Ala^{33.8}Gly^{28.6})_n$ were prepared as model histones to be used in studying the structure and interactions of nucleohistone complexes. The circular dichroism (CD) spectra of $(Lys\text{-}Ala\text{-}Gly)_n$ in aqueous solutions, at both pH 7 and 11, as well as in MeOH, trifluoroethanol, and hexafluoroisopropyl alcohol solutions, indicated that this polypeptide predominately existed in the random-coil conformation. In aqueous solution, the CD spectra of the random polytripeptide indicated that this polymer undergoes a pH-dependent helix–coil transition as the pH is raised from 7 to 11. This polymer was approximately 90% helical in organic solvents (listed above) as judged from CD spectra. Polarized infrared spectra on oriented films of both polymers cast from aqueous and organic solutions were characteristic of random-coil polypeptides. The observation that the sequential polymer failed to assume a regular, asymmetric structure is probably due to the periodicity of the glycyl residue. The stability of the helical conformation of the random polypeptide may be explicable in terms of a clustering of lysyl and alanyl residues in the N-terminal portion of the polymer since the kinetics of polymerization of glycine *N*-carboxyanhydride indicate a faster incorporation of Gly than that of either Lys or Ala. It is concluded that $(Lys\text{-}Ala\text{-}Gly)_n$ is a plausible model for histone fraction f-1 as their CD spectra are very similar.

In the cell nuclei of eucaryotes DNA is found complexed with histones, non-histone proteins, and other constituents. The association of the histones with DNA in all higher organisms has led to two suggestions concerning their functions; they may have a regulatory role in transcription from DNA, as well as a structural role in packing the DNA; the evidence has been reviewed elsewhere (Elgin *et al.*, 1971). For a fundamental understanding of these roles and to differentiate between them, a detailed knowledge of the molecular structure and interactions of this nucleic acid–protein complex is essential. This laboratory has been approaching this problem by studying the interaction of calf thymus DNA with various homologous histone fractions: lysine-rich histone fraction f-1 (Fasman *et al.*, 1970b; Adler *et al.*, 1971, 1973a; Adler and Fasman, 1971; Fasman *et al.*, 1971), histone IV (Shih and Fasman, 1971, 1972; Adler *et al.*, 1974) and chromatin (Shih and Fasman, 1970; Slayter *et al.*, 1972).

Another approach to the understanding of the structure of nucleohistone complexes is the study of model systems. Model polynucleotide–polypeptide systems (poly(A)–poly(Lys)) (Davidson and Fasman, 1969, 1971) have offered insight into such complexes. Other polypeptide–nucleic acid studies (*e.g.*, Shapiro *et al.*, 1969; Clark and Felsenfeld, 1972) also offer promise. In this paper the use of model polypeptides having amino acid compositions resembling those of natural histones is proposed. In particular, the use of sequential polypeptides would facilitate the systematic investigation of the influence of a regular, repeating sequence of amino acid residues on the formation of DNA–histone complexes. Such probes might elucidate whether any specificity relationships exist between histone and DNA depending upon amino acid composition and sequence in relation to defined base sequences which might determine a code for nucleic acid–protein interaction. Although the primary interest is in histones in the present investigation, the relevance of such a code might be applicable to studies of ribosomes, repressors, initiation factors, polymerases, etc.

Since histone fraction f-1 contains about 28% lysine and relatively large amounts of alanine and glycine (Hnilica *et al.*, 1971), the sequential polytripeptide $(Lys\text{-}Ala\text{-}Gly)_n$ was prepared as a model of histone f-1. The random sequence polymer $(Lys,Ala,Gly)_n$ was also synthesized to serve as a comparison for the conformational studies. The study of the solution conformation of both polymers would allow a clearer assessment of the contribution of a repeating sequence.

The synthesis of polypeptides with a defined repeating sequence has been an active area for several years. Extensive lists of the various sequential polymers that have been synthesized can be found in DeTar (1967) and Jones (1969–1972.) The most widely used and the best general method for the synthesis of sequential–polypeptides is *via* the peptide active ester salts (DeTar *et al.*, 1963).

Tripeptide *N*-hydroxysuccinimidyl active esters have been successfully used to form sequential polymers (*e.g.*, Fridkin *et al.*, 1969; Shibnev *et al.*, 1969, 1970; Doyle *et al.*, 1970; Ramachandran *et al.*, 1971; Lorenzi *et al.*, 1971). One advantage of *N*-hydroxysuccinimide esters over both *p*-nitrophenyl and pentachlorophenyl esters is that they are very water soluble and hence easily separated from the products by simple extraction. Therefore, the *N*-hydroxysuccinimide active

† This is Publication No. 935 from the Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02154. *Received July 2, 1973.* This research was generously supported in part by Grants from the U. S. Public Health (GM 17533), National Science Foundation (GB 29204X), American Heart Association (71-111), and the American Cancer Society (P-577).

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ester of N^{ϵ}-Z-Lys-Ala-Gly[1] was chosen for polymerization to form the sequential polymer $(Lys\text{-}Ala\text{-}Gly)_n$.

The random sequence tricopolymer $(Lys,Ala,Gly)_n$ was synthesized by polymerization of the appropriate *N*-carboxyanhydrides. In this paper the synthesis and characterization of both the random and repeating sequence polypeptides are described in detail.

Synthesis. The synthetic route to $(Lys\text{-}Ala\text{-}Gly)_n$ is given in Figure 1. Alanine was converted to Boc-Ala (I) using *tert*-butyl azidoformate, following a slight modification of the method described by Schnabel (1967). Boc-Ala (I) was coupled to *N*-hydroxysuccinimide in the presence of dicyclohexylcarbodiimide to yield Boc-Ala-OSu (II) (Anderson *et al.*, 1964). Boc-Ala-OSu (II) was coupled to glycine under alkaline conditions in a dioxane–water mixture to form Boc-Ala-Gly (III). The dipeptide was deprotected with 98% formic acid at room temperature (Halpern and Nitecki, 1967) which resulted in the formation of Ala-Gly (IV). The formate salt was not isolated and was presumably destroyed by proton transfer of the carboxyl proton to the formate anion and subsequent removal of formic acid *in vacuo*.

N^{ϵ}-Z-Lys (V) was synthesized by the method of Neuberger and Sanger (1943). Treatment of V with *tert*-butyl azidoformate in the pH-Stat (Schnabel, 1967) gave N^{α}-Boc-N^{ϵ}-Z-Lys as an oil. The oil was converted into a crystalline salt of N^{α}-Boc-N^{ϵ}-Z-Lys dicyclohexylamine (VI) (Schnabel, 1967), and reconverted to the free acid by employing a molar equivalent amount of Dowex 50 ion-exchange resin (hydrogen ion form). An excess of resin was avoided as cleavage of the Boc group might be anticipated, as this technique has been used to cleave this group (Gray and Koujah, 1969). The doubly protected lysine derivative was converted to the *N*-hydroxysuccinimide ester (VII) by the addition of dicyclohexylcarbodiimide and *N*-hydroxysuccinimide.

The protected tripeptide free acid, N^{α}-Boc-N^{ϵ}-Z-Lys-Ala-Gly (VIII), was formed under alkaline conditions by the condensation of VII and IV. The product of this coupling was found to be slightly impure by thin-layer chromatography. This material was purified by undergoing a cycle of conversion to the dicyclohexylamine salt and then back to the free acid form by extraction of an ethyl acetate solution of the dicyclohexylamine salt with a citric acid solution. Compound VIII was found to be chromatographically homogeneous in several solvent systems.

The fully protected tripeptide *N*-hydroxysuccinimide ester IX was synthesized in a procedure analagous to the formation of II and VII. Since the tripeptide has glycine as the C-terminal amino acid residue, there was no possibility of racemization occuring during the formation of the tripeptide active ester. For optimal yields of polymer, it was found necessary to use freshly prepared fully blocked tripeptide active ester in the subsequent reactions and to carry out these reactions in as short a time as possible. The salts of *N*-hydroxysuccinimide esters of peptides cannot be kept for a long time because of their reactivity (Shibnev *et al.*, 1969). The best results were obtained when all the stages of the polymerization were done on the same day.

The fully blocked polymerization monomer IX was partially deblocked by the selective removal of the N^{α}-Boc group by trifluoroacetic acid (Schwyzer *et al.*, 1960). Excess trifluoroacetate ions were removed by repeated reprecipitation of

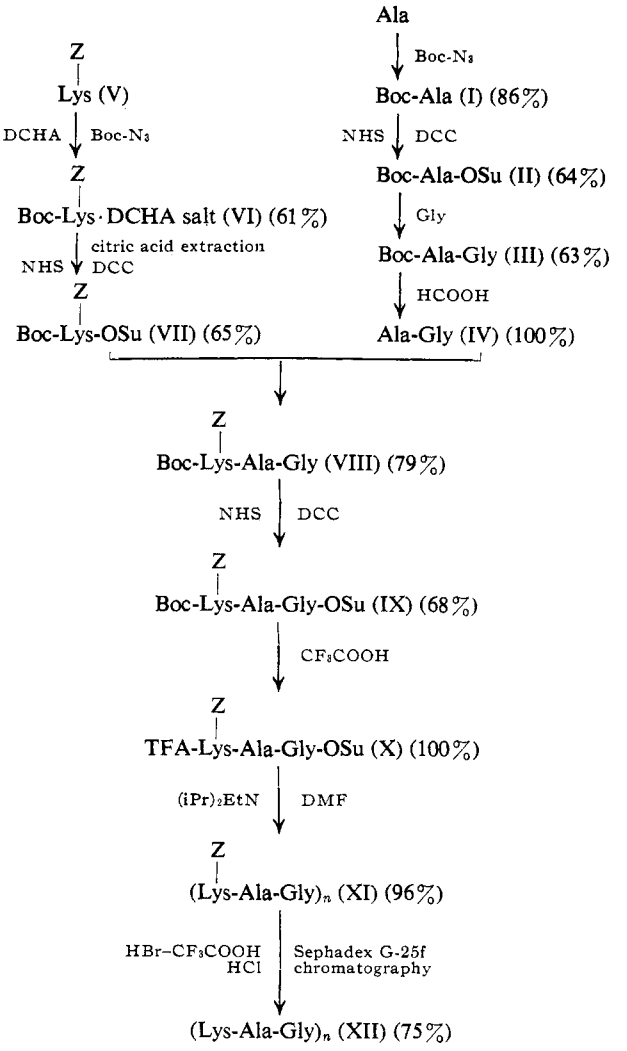

FIGURE 1: Synthetic route to $(Lys\text{-}Ala\text{-}Gly)_n$. Numbers in parentheses are the yields. Abbreviations used are: DCHA, dicyclohexylamine; DCC, dicyclohexylcarbodiimide; NHS, *N*-hydroxysuccinimide; DMF, dimethylformamide; TFA, CF_3CO–.

N^{ϵ}-Z-Lys-Ala-Gly-OSu trifluoroacetate salt (X). The polymerization of X was carried out in a concentrated solution in order to minimize the formation of cyclic peptides (DeTar *et al.*, 1963). *N,N*-Dimethylformamide was used as the solvent and the CF_3COOH ions present were neutralized with *N,N*-diisopropylethylamine.[2] After 4 days, the polymer was isolated from the reaction mixture by precipitation with diethyl ether. The N^{ϵ}-Z groups were removed from XI by the action of anhydrous hydrogen bromide in CF_3COOH. The deblocked polymer was converted to the hydrochloride salt and was fractionated by gel filtration through a column of Sephadex G-25f eluted with 0.01 N hydrochloric acid. The major fraction was found to be eluted with the void volume of the column. This material was pooled and lyophilized to yield the sequential polypeptide XII in 72% yield based on the amount of fully blocked monomer active ester IX used. The molecular weight obtained was $\bar{M}_w = 45{,}800$, $\bar{M}_n = 38{,}000$.[3]

[1] The following abbreviations are used in this paper: Boc, *tert*-butyloxycarbonyl; Gdn·HCl, guanidine hydrochloride; OSu, *N*-hydroxysuccinimidyl ester; Z, benzyloxycarbonyl.

[2] The tertiary amine base diisopropylethylamine was used in preference to triethylamine because the use of it in polymerization results in higher yields of polymers and no detectable racemization (G. T. Young, private communication, 1970).

[3] $\bar{M}_w$, weight-average molecular weight; $\bar{DP}_w$, weight-average degree of polymerization; $\bar{M}_n$, number-average molecular weight; $\bar{DP}_n$, number-average degree of polymerization.

While this work was in progress the preparation of a similar polymer with the repeating sequence $(Lys\text{-}Ala\text{-}Gly)_n$ was reported by Johnson (1968). The polymer was formed in dilute solution by the condensation of the tripeptide pentachlorophenyl ester on a small amount of partially blocked monomer. The number-average molecular weight for the $(Lys\text{-}Ala\text{-}Gly)_n$ hydrobromide so produced was obtained by membrane osmometry and was estimated to be 13,100 (Johnson, 1968). No physical characterization was reported.

The synthetic route to the random polymer, $(Lys,Ala,Gly)_n$, followed conventional strategy. The *N*-carboxyanhydrides of N^{ϵ}-Z-Lys, Ala, and Gly were prepared using the Fuchs–Farthing method (for a review, see Katchalski and Sela, 1958). These *N*-carboxyanhydrides were polymerized in dioxane by initiation with sodium methoxide. The polymer $(N^{\epsilon}\text{-Z-Lys,-Ala,Gly})_n$ (XIII) was deblocked with anhydrous hydrogen chloride and hydrogen bromide. The polymer was purified by dialysis against 0.01 N HCl and then lyophilized, to yield the hydrochloride salt of $(Lys,Ala,Gly)_n$ (XIV) in 74% yield. The $\bar{M}_w$ of the polypeptide was 12,000.[3]

Experimental Section

Reagents and Solvents. The amino acids, except for glycine, were of the L configuration. Alanylglycine was a product of Sigma Chemical Co., St. Louis, Mo. N^{α}-*tert*-Butyloxycarbonyl N^{ϵ}-benzyloxycarbonyllysine *N*-succinimidyl ester was a product of Miles Laboratories, Inc., Kankakee, Ill. Benzyl chloroformate was obtained from Schwarz/Mann, Orangeburg, N. Y. *tert*-Butyloxycarbonyl azide was obtained from Aldrich Chemical Co., Inc. Milwaukee, Wis. *N*-Hydroxysuccinimide was synthesized according to Wünsch and Jaeger (1966).

Dicyclohexylcarbodiimide was obtained from Eastman Organic Chemicals, Rochester, N. Y., and was purified by dissolving the crude carbodiimide in diethyl ether and removing the insoluble materials by filtration. The solvent was stripped from the filtrate using a rotary evaporator. Dicyclohexylcarbodiimide was sublimed from the residual solid at 100° and 1-mm pressure to give colorless crystals, mp 33–34°.

Dicyclohexylamine (Eastman) was fractionally distilled at 7-mm pressure and the fraction boiling at 107–107.5° was used.

1,2-Dimethoxyethane (Eastman) was refluxed over calcium hydride for 5 hr, and was then fractionally distilled at atmospheric pressure. The fraction boiling at 83° was used.

Acetonitrile (Fisher) was refluxed over phosphorus pentoxide for 4 hr, and was then fractionally distilled at atmospheric pressure. The fraction boiling at 82° was used.

N,N-Dimethylformamide (Fisher) was purified by treatment with solid sodium hydrogen carbonate overnight to remove formic acid. It was then decanted and treated with phosphorus pentoxide for 2–3 hr to remove traces of water. Dimethylformamide was then fractionally distilled from phthalic acid under vacuum. The forerun was discarded and the middle fraction boiling at 40° at 9-mm pressure was used.

Trifluoroacetic acid (Eastman) was fractionally distilled from phosphorus pentoxide at atmospheric pressure. The fraction boiling at 71° was collected and stored in a desiccator over calcium chloride–calcium sulfate.

Triethylamine (Eastman) was refluxed over calcium oxide for 4 hr, and then fractionally distilled at atmospheric pressure. The fraction boiling at 88–88.5° was used.

N,N-Diisopropylethylamine (Aldrich) was fractionally distilled and the fraction boiling at 127° was used.

The other solvents were of reagent grade and were used without further purification.

Sephadex G-25f was from Pharmacia Fine Chemicals Inc., Piscataway, N. J. Bio-Gel A-5m was from Bio-Rad Laboratories, Richmond, Calif.

Analysis and Characterization of the Intermediate and Final Products. Melting points were determined on a Mel-Temp apparatus and are uncorrected. Optical rotations were obtained with a Cary Model 60 spectropolarimeter. Infrared spectra were taken on thin films or Nujol mulls with sodium chloride plates using either a Perkin-Elmer Model 137 or Model 621 spectrophotometer. Proton magnetic resonance spectra were recorded with a Varian A60A spectrometer with trimethylsilane as external standard. Elemental analyses were performed by either Schwarzhopf Microanalytical Laboratory, Woodside, N. Y., or Amherst Microanalytical Laboratory, Inc., Amherst, Mass.

Ascending paper chromatography was performed with Whatman No. 1 paper. The solvent systems used were $R_F{}^1$, 1-butanol–acetic acid–water (4:1:5, v/v, top phase), and $R_F{}^2$, 1-butanol–acetic acid–water (4:1:1, v/v). Thin-layer chromatography was performed on precoated silica gel plates (Eastman Type K 301R2, Eastman Kodak Co., Rochester, N. Y.; Quanta/Gram Type Q1, Quantum Industries, Fairfield, N. J.). Solvent systems used were $R_F{}^{I}$, 1-butanol–acetic acid–water (3:1:1, v/v), $R_F{}^{II}$, 1-butanol–pyridine–acetic acid–water (30:20:6:24), $R_F{}^{III}$, acetone–methanol (1:1, v/v), $R_F{}^{IV}$, methanol–chloroform (10:90, v/v), $R_F{}^{V}$, methanol–chloroform (15:85, v/v), and $R_F{}^{VI}$, acetone. Compounds were visualized with a ninhydrin spray, with the sodium hypochlorite–*o*-tolidine–potassium iodide method (Stahl, 1965), or with iodine (Tanderath, 1966).

Circular Dichroism Measurements. CD measurements were taken on a Cary Model 60 recording spectropolarimeter fitted with a Model 6001 circular dichroism attachment. The slit width was programmed to maintain a 15-Å half-bandwidth. Fused quartz, jacketed cells (1.000–0.030 mm) (Optical Cell Co., Beltsville, Md.) were used. Variable temperatures, from 23 to 82°, were kept to within ±0.2° with a Tamson refrigerated circulator (Neslab Instruments, Inc., Durham, N. H.). The spectropolarimeter was calibrated with *d*-10-camphorsulfonic acid using the method of Adler *et al.* (1973b).

The mean residue ellipticity, $[\theta]_\lambda$, expressed as (deg cm^2) dmole^{-1}, was calculated according to the relationship

$$[\theta]_\lambda = \theta_\lambda \times 10/lc$$

where θ_λ is the observed ellipticity in degrees at wavelength λ, l is the cell path length in centimeters, and c is the concentration of amino acid residues in mol l.$^{-1}$.

The solutions for CD measurements were prepared as follows. An aliquot of a stock solution of polymer of approximately 0.2 M residue concentration by weight was diluted 20-fold with the appropriate solvent. The pH of the solution was adjusted, if necessary, with 1 M NaOH added from a 0.1-ml microburet (Digipet, Manostat Corp., N. Y.). The solution was filtered through a Millipore filter (HAWP 1300, 0.45 μ pore; Bedford, Mass.) into the optical cell for CD measurements. The remaining filtrate was retained for pH measurements and determination of polymer concentration by a modified Nessler nitrogen analysis (Lang, 1958).

pH Measurements. A Radiometer Model 25 pH meter with a Radiometer Type GK 2302 G combination glass electrode was used to measure pH. The pH meter was calibrated using standard buffers (Fisher Scientific Co.) of pH 7.00 and 10.00, accurate to ±0.02 pH unit.

Infrared Measurements. Polarized and unpolarized spectra were obtained with a Perkin-Elmer Model 621 infrared spectrophotometer equipped with a common-beam wire grid polarizer accessory.

Polypeptide samples were dissolved in either distilled water or deuterium oxide (Merck, Sharp & Dohme of Canada, Ltd., 99.7% minimum isotopic purity). Bound water was exchanged for D_2O by keeping the D_2O solutions in a desiccator for 20 hr and then evaporating the solutions by evacuating the desiccator. This procedure was repeated twice; 90% 2,2,2-trifluoroethanol solutions were obtained by adding trifluoroethanol to the H_2O or D_2O solutions. A drop or two of solution was placed on a silver chloride plate and the sample was stroked unidirectionally to prepare oriented films.

Amino Acid Analysis. The amino acid composition of the polymers was determined on a Beckman Model 120 automatic amino acid analyzer (Spackman *et al.*, 1958) after hydrolysis in 6 N HCl, under *vacuo* at 110° for 22 hr.

Synthesis

Synthesis of Sequential Polymer

tert-Butyloxycarbonylalanine (I). It was prepared following a modification of the procedure of Schnabel (1967); the pH of the reaction was controlled manually and not with a pH-Stat. The yield was 86%: mp 82–84°, $[\alpha]_D^{22.5}$ −26.5° (*c* 2.17, acetic acid) [lit. Schnabel (1967), 96% yield: mp 80–82°, $[\alpha]_D^{25}$ −22.4° (*c* 2.095, acetic acid)].

tert-Butyloxycarbonylalanine N-Succinimidyl Ester (II). It was prepared according to Anderson *et al.* (1964) in 64% yield: mp 162–164°, $[\alpha]_D^{24}$ −50.6° (*c* 3, dioxane) [lit. Anderson *et al.* (1964) 71% yield, mp 143–144 and 167°, $[\alpha]_D^{25°}$ −49° (*c* 2, dioxane)].

tert-Butyloxycarbonylalanylglycine (III). Glycine (1.332 g, 0.0177 mol) and sodium hydrogen carbonate (2.980 g, 0.0354 mol) were dissolved in water (70 ml) at room temperature. Boc-Ala-OSu (II) (4.500 g, 0.0157 mol) was added and the reaction mixture was stirred at room temperature for 48 hr. The solvent was then removed under reduced pressure on a rotary evaporator (water bath temperature was 40°). The residual oil was dissolved in water (20 ml), and the solution was cooled to 0°. After the pH of the solution was adjusted to 4 with solid citric acid, the product precipitated. The solid was collected by filtration and washed with cold water. It was dissolved in ethyl acetate, dried with sodium sulfate, and then crystallized from ethyl acetate–carbon tetrachloride. Additional product was obtained by an ethyl acetate extraction of the acidified aqueous phase after saturation with sodium chloride. The product was recrystallized from ethyl acetate–carbon tetrachloride to yield 2.43 g (63%): mp 124–125°; $[\alpha]_D^{24}$ −38.4° (*c* 2.00, dioxane); R_F^2 0.93, R_F^{III} 0.70; ν_{max} (Nujol) 1715, 1667, 1623, 1565 cm^{-1}; τ (Me_2SO-d_6) 2.14 (1 H, triplet, J_{AD} = 6 Hz, peptide NH), 3.33 (1 H, doublet, J_{BC} = 7 Hz, urethane NH), 6.03 (1 H, doublet, J_{BC} = 7 Hz, Ala α-CH), 6.34 (2 H, doublet, J_{AD} = 7 Hz, Gly α-CH_2), 8.74 (9 H, singlet, Boc CH_3), 8.92 (3 H, doublet, J_{CF} = 7 Hz, Ala β-CH_3). *Anal.* Calcd for $C_{10}H_{18}N_2O_5$ (mol wt 246.26): C, 48.77; H, 7.37; N, 11.38. Found: C, 49.01; H, 7.18; N, 11.01.

Alanylglycine (IV). A solution of III (1.40 g, 0.00546 mol) in 98% formic acid (70 ml) was stirred at room temperature for 2.5 hr. The formic acid was removed under reduced pressure on the rotary evaporator (water bath 30°). The residual oil solidified upon being triturated with methanol to yield crystalline product: 0.73 g (100%); mp 231–233° dec; $[\alpha]_D^{24}$ 50.0° (*c* 3.00, water); R_F^2 0.30; ν_{max} (Nujol) 1715, 1667 cm^{-1}; τ (D_2O) 5.89 (1 H, quartet, J_{AC} = 7 Hz, Ala α-CH), 6.21 (2 H, singlet, Gly α-CH_2), 8.47 (3 H, doublet, J_{AC} = 7 Hz, Ala β-CH_3) [lit. (Bailey, 1950) mp 232–235° dec; $[\alpha]_D^{22°}$ +49.1° (*c* 10, water); (Zervas, 1956) $[\alpha]_D^{23}$ +51.3° (*c* 2.5, water); (Beecham and Ham, 1968) τ (D_2O) 5.89 (quartet, J = 7 Hz), 8.47 (doublet, J = 7 Hz), deleted singlet for methylene].

A sample of Ala-Gly purchased from Sigma (lot 60C-0680) had R_F^I 0.10, R_F^{II} 0.05; $[\alpha]_D^{23}$ +50.5° (*c* 2.73, water).

N^ϵ-Benzyloxycarbonyllysine (V). It was prepared according to Neuberger and Sanger (1943): mp 246–247.5°; $[\alpha]_D$ +14.2° (*c* 1, 2 N HCl); $[\alpha]_D^{23}$ +16.5° (*c* 2.93, $CHCl_2COOH$); R_F^1 0.59; R_F^I 0.49, R_F^{II} 0.61, R_F^V 0.04 [lit. (Neuberger and Sanger, 1943) $[\alpha]_D$ +14.4° (*c* 1.6, 2 N HCl); (Costopanagiotis *et al.*, 1968) $[\alpha]_D$ +14° (*c* 1, 2 N HCl)].

N^α-tert-Butyloxycarbonyl-N^ϵ-benzyloxycarbonyllysine Dicyclohexylamine Salt (VI). It was prepared by the method of Schnabel (1967) and the oily product was converted to the dicyclohexylammonium salt: mp 111.5°; $[\alpha]_{578}^{24}$ −4.99° (*c* 2, glacial acetic acid); τ (Me_2SO-d_6) 2.66 (5 H, singlet, aromatic H), 2.90 (2 H, singlet, NH_2), 4.04 (2 H, singlet, urethane NH), 5.01 (2 H, singlet, benzyl methylene), 6.40 (1 H, singlet, Lys α-CH), 7.12 (4 H, multiplet, ϵ-methylene, cyclohexyl methines), 8.69 (35 H, multiplet, cyclohexyl methylenes, β, γ, δ methylenes, and *t*-Boc methyls) [lit. (Schnabel, 1967) mp 110–111°; $[\alpha]_{587}$ −9.3° (*c* 2, glacial acetic acid)]. *Anal.* Calcd for $C_{19}H_{28}N_2O_6 \cdot C_{12}H_{23}N$ (mol wt) 561.77): C, 66.28; H, 9.15; N, 7.48. Found: C, 66.48; H, 9.36; N, 7.39.

N^α-tert-Butyloxycarbonyl-N^ϵ-benzyloxycarbonyllysine N-Succinimidyl Ester (VII). A mixture of N^α-Boc-N^ϵ-Z-Lys-OH (5.70 g, 0.015 mol) (prepared from VI in 95% yield by batch treatment with 1 equiv of Dowex 50W-X2 (H^+ form) ion-exchange resin in 60% ethanol) and *N*-hydroxysuccinimide (1.73 g, 0.015 mol) in acetonitrile (120 ml) was cooled to 0°. Dicyclohexylcarbodiimide (3.40 g, 0.0165 mol) was added and the reaction was stirred for 2 hr at 0° and 72 hr at 4°. Dicyclohexylurea was removed by filtration and the filtrate was concentrated under reduced pressure at 40°. The residue was dissolved in ethyl acetate (50 ml) and glacial acetic acid (0.05 ml) was added to destroy any dicyclohexylcarbodiimide remaining in the solution. After 3 hr, a trace of dicyclohexylurea was removed by filtration and the filtrate was extracted with 5% sodium hydrogen carbonate (twice with 50 ml) which had been precooled to 0°. The organic phase was dried with magnesium sulfate and concentrated under reduced pressure to yield an oil which solidified after trituration with cold petroleum ether (bp 30–60°). The product was crystallized from ethyl acetate–petroleum ether to yield 4.60 g (65%): mp 99–102°; $[\alpha]_D^{24}$ −10.1° (*c* 1.5, chloroform); R_F^I 0.64; ν (Nujol) 1805, 1780, 1683 cm^{-1}; τ ($CDCl_3$) 2.52 (5 H, singlet, aromatic), 4.56 (1 H, singlet, NH), 4.68 (1 H, singlet, NH), 4.79 (2 H, singlet, benzyl methylene), 5.38 (1 H, singlet, α-CH), 6.72, (2 H, doublet, J = 6 Hz, ϵ-methylene), 7.18 (4 H, singlet, succinimido), 8.42 (15 H, multiplet, β, γ, δ methylenes, Boc methyls). *Anal.* Calcd for $C_{23}H_{31}N_3O_8$ (mol wt 477.52): C, 57.85; H, 6.54; N, 8.80. Found: C, 57.91; H, 6.41; N, 8.88.

Most samples of VII purchased from Miles–Yeda had a melting point and $[\alpha]_D$ identical with those values listed above. It was sometimes necessary to recrystallize to constant $[\alpha]_D$ before using their material.

N^α-tert-Butyloxycarbonyl-N^ϵ-benzyloxycarbonyllysylalanylglycine (VIII). A solution of VII (5.000 g, 0.01047 mol) in dioxane (50 ml) was added to a solution of IV (1.722 g, 0.01178 mol) and sodium hydrogen carbonate (1.979 g, 0.024 mol)

in water (50 ml). After stirring at room temperature overnight, the reaction mixture was concentrated under reduced pressure at 40°. The residue was dissolved in water (50 ml) and the pH was adjusted to 4 with solid citric acid. The acidified solution was extracted with ethyl acetate (three 100-ml portions). The combined organic phases were washed with water (three 100-ml portions), dried with sodium sulfate, and concentrated under reduced pressure. The product solidified after trituration with *n*-hexane–petroleum ether (1:1). The product was crystallized from ethyl acetate–petroleum ether (1:20) to yield 4.445 g (84%); mp 120°. Thin-layer chromatography indicated the product was impure; R_F^{I} 0.66 (major spot), 0.78 and 0.30 (minor spots); R_F^{II} 0.63 (major spot), 0.77 and 0.47 (minor spots); and R_F^{IV} 0.00 (major spot), 0.26 (minor spot).

Impure VIII (4.445 g, 0.00874 mol) was dissolved in methanol (10 ml) and insoluble material was removed by filtration. The filtrate was diluted with ether (50 ml) and a solution of dicyclohexylamine (1.74 ml, 0.00874 mol) in ether (150 ml) was added. After standing overnight at −20°, the solid was collected and dried *in vacuo* (crude weight 5.6 g, 90%). The solid was reprecipitated from methanol (50 ml) with ether (1000 ml) in the cold (−20°), collected, and dried *in vacuo* (2.964 g). A second crop was obtained from the filtrate after concentration under reduced pressure and the addition of petroleum ether (2.292 g). After being dried for 24 hr under high vacuum at 75°, both crops showed identical melting points and R_F values: mp 152–154°; R_F^{I} 0.59, 0.67 (slight trace); R_F^{II} 0.66, R_F^{V} 0.00.

A solution of N^{α}-Boc-N^{ϵ}-Z-Lys-Ala-Gly dicyclohexylamine salt (4.95 g, 0.0072 mol) in ethyl acetate (300 ml) was extracted with 1 N citric acid (three 150-ml portions), washed with a saturated sodium chloride solution (two 200-ml portions), dried with sodium sulfate, and concentrated under reduced pressure. The tripeptide free acid crystallized after the addition of petroleum ether. The solid was collected and dried *in vacuo* to give 3.412 g (94%): mp 121–122°; R_F^{I} 0.66, R_F^{II} 0.74, R_F^{V} 0.06; $[\alpha]_D^{22}$ −30.0° (*c* 2.6, methanol); $[\alpha]_D^{24}$ −11.1° (*c* 2.5, dimethylformamide); ν (Nujol) 1706, 1672, 1618 cm^{-1}; τ (Me_2SO-d_6) 1.40 (1 H, doublet, J = 5 Hz, glycyl amide), 2.30 (1 H, doublet, J = 7 Hz, alanyl amide), 2.78 (5 H, singlet, aromatic), 3.04 (1 H, doublet, J = 7 Hz, lysyl amide), 3.37 (1 H, doublet, J = 7 Hz, lysyl ϵ-NH), 5.12 (2 H, singlet, benzyl methylene), 5.80 (1 H, triplet, J = 7 Hz, lysyl α-CH), 6.20 (1 H, singlet, alanyl α-CH), 6.38 (2 H, doublet, J = Hz, glycyl α-CH_2), 7.16 (2 H, doublet, J = 5 Hz, lysyl ϵ-methylene), 8.92 (18 H, multiplet, lysyl β, γ, δ methylenes, Boc methyls, alanyl β-methyl) [lit. (Johnson, 1968) mp 122°; $[\alpha]_D^{27}$ −12.2° (*c* 6.8, dimethyformamide)]. *Anal.* Calcd for $C_{24}H_{36}N_4O_8$ (mol wt 508.58): C, 56.68; H, 7.14; N, 11.02. Found: C, 56.63; H, 7.16; N, 10.97.

N^{α}-tert-Butyloxycarbonyl-N^{ϵ}-benzyloxycarbonyllysylalanylglycine N-Succinimidyl Ester (IX). Dicyclohexylcarbodiimide (1.430 g, 0.00693 mol) was added to a solution of VIII (3.204 g, 0.00630 mol) in dimethyformamide (20 ml) which had been cooled to 0°. After 5 min, *N*-hydroxysuccinimide (0.725 g, 0.00630 mol) was added and the reaction mixture was stirred at 0° for 2 hr and at 4° overnight. The reaction mixture was acidified with several drops of glacial acetic acid and stored in the cold for several hours. Dicyclohexylurea was removed by filtration and the filtrate was concentrated under reduced pressure at 40°. The residue was dissolved in ethyl acetate (250 ml) and the solution was washed with water, 1 N citric acid, and a saturated sodium chloride solution. The organic phase was dried with sodium sulfate and concentrated under reduced pressure. The product was crystallized twice from chloroform with ether. The product was collected and dried *in vacuo* to give 2.595 g (68%); mp 116–117°; $[\alpha]_D^{22}$ −26.3° (*c* 2.98, chloroform); R_F^{VI} 0.66; ν (Nujol) 1838, 1795, 1721, 1689, 1647 cm^{-1}; τ ($CDCl_3$) 2.30 (1 H, singlet, glycyl NH), 2.66 (5 H, singlet, aromatics), 2.77 (1 H, singlet, alanyl NH), 4.42 (2 H, multiplet, lysyl NH's), 4.90 (2 H, singlet, benzyl methylene), 5.32 (1 H, multiplet, lysyl α-CH), 5.66 (2 H, doublet, J = 5 Hz, glycyl α-CH_2), 5.94 (1 H, multiplet, alanyl α-CH), 6.86 (2 H, doublet, J = 5 Hz, lysyl ϵ methylene), 7.24 (4 H, singlet, succinimido), and 8.55 (18 H, multiplet, lysyl β, γ, δ methylenes, Boc methyls, alanyl β-CH_3). *Anal.* Calcd for $C_{28}H_{39}N_5O_{10}$ (mol wt 605.65): C, 55.53; H, 6.49; N, 11.56. Found: C, 55.89; H, 6.92; N, 11.56.

N^{ϵ}-Benzyloxycarbonyllysylalanylglycine N-Succinimidoester Trifluoroacetate Salt (X). Compound IX (2.000 g, 0.0033 mol) was dissolved in freshly distilled trifluoroacetic acid (10 ml) and the resulting solution was kept at room temperature for 45 min. Excess trifluoroacetic acid was removed under reduced pressure with exclusion of moisture. The residue was solidified by trituration with cold anhydrous ether. The solvent was decanted and the solid was reprecipitated twice from ethyl acetate with ether. The hygroscopic product was collected by filtration and dried *in vacuo* for a brief time. The product ("X") was used directly in the next reaction.

$(N^{\epsilon}$-Benzyloxycarbonyllysylalanylglycyl$)_n$ (XI). Compound X (0.0033 mol) was dissolved in dimethylformamide (3.3 ml) by briefly heating the mixture in a 50° water bath. The trifluoroacetate ions were neutralized with diisopropylethylamine (0.575 ml, 0.0033 mol). After a few minutes the reaction mixture solidified and additional dimethylformamide (5.0 ml) was added. Polymerization was allowed to procede for 4 days. The reaction mixture was diluted with ether (500 ml) and the solid was collected by centrifugation. The product ("XI") was washed with ether and dried *in vacuo* to yield 1.243 g (96%). *Anal.* Calcd for $C_{19}H_{27}N_4O_5$ (mol wt 391.45): C, 58.30; H, 6.95; N, 14.31. Found: C, 56.12; H, 6.52; N, 14.25.

(Lysylalanylglycyl)$_n$ Hydrochloride Salt (XII). Compound XI (1.183 g, 0.003 mol) was dissolved in anhydrous trifluoroacetic acid. Hydrogen bromide gas was bubbled through this solution with the exclusion of moisture for 30 min with the reaction mixture cooled to 4° and for 30 min at room temperature. The hydrogen bromide gas was passed through two gas washing bottles containing tetrahydronaphthalene, a cold trap at −20°, a bottle containing phosphorus pentoxide, and a safety bottle before entering the flask containing the reaction mixture. Excess trifluoroacetic acid and hydrogen bromide were removed under reduced pressure with the exclusion of moisture. The residue was triturated with ether; the solid was collected by centrifugation and washed with ether. The product was dissolved in water. The pH of the solution was adjusted to *ca.* 9 with 1 N sodium hydroxide and then to *ca.* 4 with 1 N hydrochloric acid. The solution was lyophilized to yield 1.162 g (solid contained *ca.* 200 mg of salts).

[Lys(HCl)-Ala-Gly]$_n$ (1.162 g) was dissolved in 0.010 N hydrochloric acid (10.0 ml) and an aliquot of 2.0 ml was placed on top of a column (2.4 × 42 cm) filled with Sephadex G-25f. The column was eluted with 0.010 N hydrochloric acid and fractions of 4 ml were collected. The fractions were monitored by absorbance at 240 nm. Three peaks were found; the major peak emerged with the void volume of the column. The fractions corresponding to the major peak from five column runs were combined and lyophilized to yield 0.663 g of XII (75%). *Anal.* Calcd for $C_{11}H_{21}ClN_4O_3 \cdot 1.5H_2O$ (mol wt 319.79): C, 41.32; H, 7.56; Cl, 11.09; N, 17.52. Found: C,

41.21; H, 7.33; Cl, 11.96; N, 16.61. Amino acid composition as determined by analysis was $Lys_{1.00}Ala_{1.02}Gly_{0.98}$; $\bar{M}_w$ = 45,800; $\bar{M}_n$ = 38,000.

Molecular Weight Determination. The weight-average molecular weight of the polymer was estimated by gel filtration in 6 M Gdn·HCl using 6% agarose as the gel filtration medium. The column was prepared, operated, and calibrated using the procedure of Fish *et al.* (1969).

The number-average molecular weight was measured in 0.500 M sodium chloride–0.005 M sodium phosphate (pH 6.80) at 30° using a Hewlett Packard Model 502 high-speed membrane osmometer and an S & S B-20 membrane. The concentration of polymer in the solutions used was determined by a modified Nessler nitrogen analysis (Lang, 1958).

Verification of the Optical Purity of (Lysylalanylglycyl)$_n$ by Examination of the Optical Rotation of the Hydrolysate. Hydrolysis of the polymer (29.3 mg, 0.10 mmol) with 6 N hydrochloric acid in a sealed, evacuated tube for 22 hr at 110° followed by evaporation and dissolution of the residue in 2.0 ml of 1 N hydrochloric acid gave a solution with $[\alpha]_{500}^{23}$ +24.0°, $[\alpha]_{400}^{23}$ +46.9°, and $[\alpha]_{300}^{23}$ +137.1°. The concentration of the solutions was determined by a modified Nessler nitrogen analysis (Lang, 1958). Treatment of a control mixture of lysine monohydrochloride (18.30 mg, 0.01 mmol), alanine (8.90 mg, 0.10 mmol), and glycine (7.50 mg, 0.10 mmol) under precisely the same conditions gave a solution with $[\alpha]_{500}^{23}$ +24.9°, $[\alpha]_{400}^{23}$ +46.2°, and $l\alpha]_{300}^{23}$ +136.8°. This corresponds to an optical purity of 99.3%; assessment of optical purity by this method is subject to errors of 2–3% (DeTar and Estrin, 1966; Kovacs *et al.*, 1966).

Synthesis of Random Polymer

N-Carboxyanhydrides. The *N*-carboxyanhydrides of N^{ϵ}-Z-Lys, Ala, and Gly were prepared according to the Fuchs–Farthing method (Katchalski and Sela, 1958). The anhydrides were purified several times by recrystallization from a mixture of ethyl acetate and *n*-hexane. The final products were white crystals and the melting points were 98–98.5° for N^{ϵ}-Z-lys-*N*-carboxyanhydride, 89–90° for Ala, and 99–100° for Gly-*N*-carboxyanhydride [lit. N_{α}^{ϵ}-Z-Lys-*N*-carboxyanhydride, mp 101° (Fasman *et al.*, 1961); Ala-*N*-carboxyanhydride, mp 90° (Bailey, 1950); Gly-*N*-carboxyanhydride, mp 100° dec (Farthing, 1950)].

(Lys$^{37.6}$Ala$^{33.8}$Gly$^{28.6}$)$_n$ Hydrochloride Salt (XIV). N^{ϵ}-Z-Lys-*N*-carboxyanhydride (0.519 g, 0.00169 mol), Ala-*N*-carboxyanhydride (0.1960 g, 0.00170 mol), and Gly-*N*-carboxyanhydride (0.168 g, 0.00168 mol) were dissolved in freshly distilled dioxane (90 ml) with stirring. The polymerization was initiated by the addition of sodium methoxide (0.5778 ml of 0.351 N solution, 0.000203 mol; the molar ratio of total anhydride to initiator was ~25) and the reaction mixture was allowed to stand at room temperature for 24 hr. From an aliquot (5 ml) of the reaction mixture, the polymer (N^{ϵ}-Z-Lys,Ala,Gly)$_n$ (XIII) was precipitated with ether and the solid was collected by centrifugation, dissolved in dioxane, lyophilized, and dried *in vacuo* at 53° to yield 0.044 g; η_{sp}/c = 1.01 (2% in $CHCl_2COOH$). The polymer in the original polymerization solution was diluted with 85 ml of $CHCl_3$ and was deblocked by passage of HCl gas for 10 min and then HBr gas through the reaction mixture for 40 min. After standing at room temperature for 4 hr, nitrogen was passed through the reaction mixture for 1 hr to remove excess HCl and HBr. Water (100 ml) was added, the pH of the solution was adjusted to 8 with 1 M NaOH, and the basic solution was extracted with ether. The pH was lowered to 2 with 1 N HCl and the solution was dialyzed against three changes of 0.01 N HCl over a period of 3 days. The dialysate was filtered through a sintered glass funnel, M porosity, lyophilized, and dried *in vacuo* at 53° for 24 hr to yield the product XIV: 0.365 g (74%); $[\eta]$ = 0.26 (0.5% in 1 M NaCl, pH 2.82). The molecular weight $\bar{M}_{w'}$ = 12,000, based on the data of Appelquist and Doty (1962). Amino acid composition determined by a Beckman amino acid analyzer 120C was $Lys_{1.00}$, $Ala_{0.90}$,$Gly_{0.77}$.

Results

Synthesis. The series of reactions leading to the sequential polytripeptide (Lys-Ala-Gly)$_n$ (XII) is shown in Figure 1. The partially blocked polymer (N^{ϵ}-Z-Lys-Ala-Gly)$_n$ (XI) was formed by polymerization of a concentrated solution of the CF_3COOH salt of N^{ϵ}-Z-Lys-Ala-Gly-OSu (X) in dimethylformamide after neutralization of the CF_3COOH with *N,N*-diisopropylethylamine. Compound XI was isolated in 96% yield, based on the starting monomer, N^{α}-Boc-N^{ϵ}-Z-Lys-Ala-Gly-OSu (IX). Compound XI was then quantitatively converted to the hydrobromide salt XII, by treatment with HBr in CF_3COOH. This was purified by gel filtration through a column of Sephadex G-25f with 0.01 N HCl elution. Three peaks were found in the effluent by monitoring the optical density at 230 nm and were present in relative amounts of 100:10:1. The major peak emerged with the void volume and the other peaks at two and three column volumes. The high molecular weight major peak was isolated (0.663 g, 75% yield) and characterized. The other peaks were not investigated.

The $\bar{M}_w$ of XII was estimated by gel filtration through a 6% agarose column eluted with 6 M Gdn·HCl to be 45,800.[3] This corresponds to a $\overline{DP}_w$ of 429 amino acid residues. The $\bar{M}_n$, as measured by membrane osmometry, was 38,000, which corresponds to a $\overline{DP}_n$ of 357 amino acid residues.[3] The ratio $\bar{M}_w/\bar{M}_n$ for XII was 1.20, indicating minimal heterogeneity. The amino acid composition of an acid hydrolysate of XII was found to be $Lys_{1.00}Ala_{1.02}Gly_{0.98}$. The optical rotatory dispersion spectrum of this acid hydrolysate was the same as that of a control hydrolysate, indicating that no detectable racemization occurred during the synthesis of XII.

The random sequence tricopolymer (N^{ϵ}-Z-Lys,Ala,Gly)$_n$ (XIII) was synthesized by the polymerization in dioxane of the *N*-carboxyanhydrides of N^{ϵ}-Z-Lys, Ala, and Gly, with sodium methoxide as initiator. The N^{ϵ}-Z-Lys-blocked polymer XIII was converted to the hydrochloride salt [Lys(HCl),-Ala,Gly]$_n$ (XIV) by treatment with HBr followed by dialysis against 0.01 N HCl. Compound XIV was then filtered, lyophilized, and dried to constant weight *in vacuo*. The $[\eta]$ at 1 M NaCl (pH 2.8) of XIV was 0.26. This corresponds to a $\bar{M}_w$ of about 12,000, based on the behavior of (Lys-HCl)$_n$ (Appelquist and Doty, 1962), and represents a $\overline{DP}_w$ of about 120 amino acid residues.[3] The amino acid composition of an acid hydrolysate of XIV was found to be Lys, 37.6%; Ala, 33.8%; Gly, 28.6% ($Lys_{1.00}Ala_{0.90}Gly_{0.77}$).

Circular Dichroism Studies. The solution conformations of polymers XII and XIV were investigated by circular dichroism spectroscopy. In aqueous solution at pH 7 and 11, over a range of ionic strengths (0–0.5 M NaF), the sequential polymer XII behaved as a random coil with a small negative band at ~225 nm and a large negative band at ~197 nm (Adler *et al.*, 1973b). There was no ionic strength dependence of the CD spectra. The CD band positions and magnitudes of XII in various solvents are summarized in Table I. In Figure 2 are shown the CD spectra of XII in 0.2 M NaF as a function

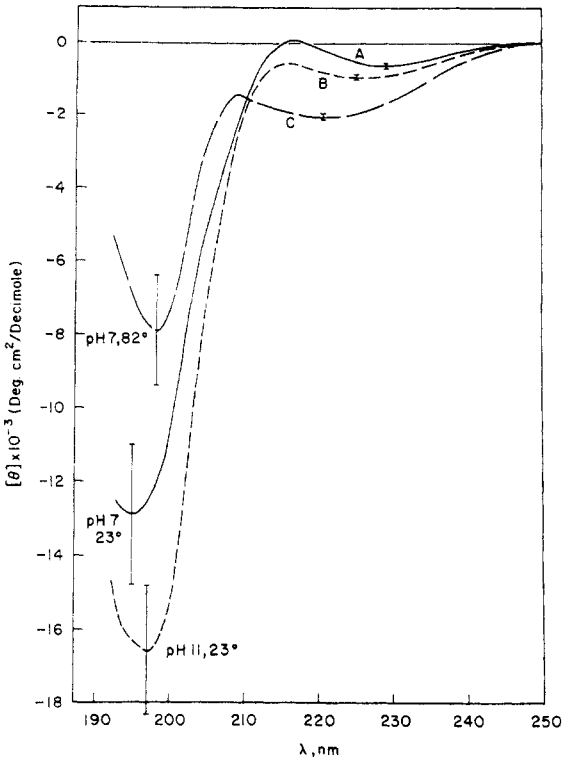

FIGURE 2: Circular dichroism spectra of $(Lys\text{-}Ala\text{-}Gly)_n$ in 0.2 M NaF as a function of pH and temperature. Curve A (–), pH 7.0, 23°; curve B (---), pH 7.0, 82°; curve C (– –), pH 11.0, 23°. The vertical bars represent the standard deviation of ellipticity values for replicate experiments. The cell path length used was 1 mm for the wavelength range 250–210 nm and 0.1 mm for wavelengths below 210 nm. The mean residue concentration of the solutions ranged from 0.008 to 0.02 M (0.08–0.2%).

of pH and temperature. In 0.2 M NaF, pH 7, 23° (Figure 2, curve A), the CD spectrum of XII had a small, broad negative band centered at 229 nm ($[\theta]^{23}_{229}$ −612°) and a large negative band at 195 nm ($[\theta]^{23}_{195}$ −12,800°). As the temperature of the solution was raised to 82°, the polymer exhibited a typical random-coil temperature-dependent CD spectra (Yaron *et al.*, 1971); the small, broad band became larger, more broad, and blue shifted ($[\theta]^{82}_{217}$ −2240°) while the larger band became smaller and red shifted ($[\theta]^{82}_{197}$ −7930°) (Figure 2, curve B). The CD spectrum of XII in 0.2 M NaF, pH 11, 23° (Figure 2, curve C) had a small, broad band at 225 nm ($[\theta]^{23}_{225}$ −954°) and a large negative band at 197 nm ($[\theta]^{23}_{197}$ −16,600°).

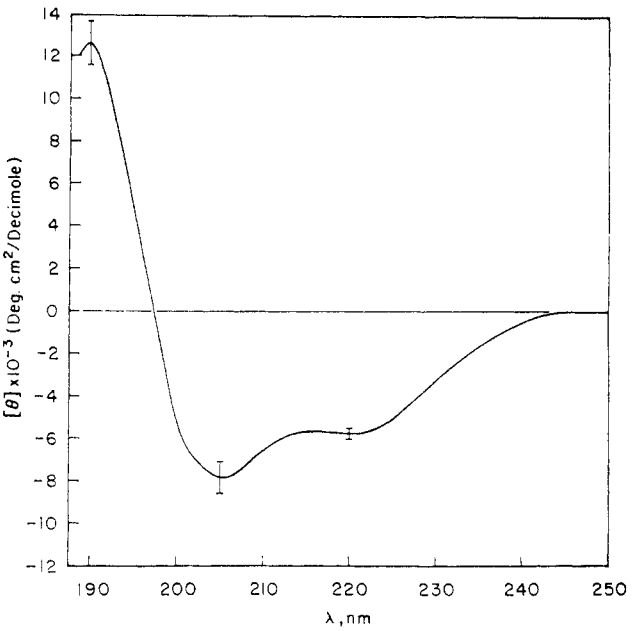

FIGURE 3: Circular dichroism spectrum of $(HCl\text{-}Lys\text{-}Ala\text{-}Gly)_n$ in trifluoroethanol–H_2O (9:1, v/v), 23°. Conditions were the same as in Figure 2.

When the polymer was dissolved in 1 M sodium dodecyl sulfate, pH 7, 23°, the CD spectrum indicated that XII seemed to assume a more ordered conformation (possibly some α-helical structure). When the polymer XII was dissolved in the organic solvents hexafluoroisopropyl alcohol, trifluoroethanol, and methanol, bands were found in the CD spectra at 222 and ~205 nm typical of helical polypeptides (Adler *et al.*, 1973b). However, the small magnitudes of the $[\theta]_\lambda$ values indicated that XII had become only partially helical. In Figure 3 the CD spectra of XII in trifluoroethanol–H_2O (90:10, v/v) is shown. The negative bands are at 220 nm ($[\theta]^{23}_{220}$ −5740°) and 205 nm ($[\theta]^{23}_{205}$ −7860°) and the positive band is at 189 nm ($[\theta]^{23}_{189}$ 12,700°). In an attempt to quantitate the conformational change which had occurred, a computer analysis of the experimental CD spectrum was performed to find the best fit of this spectrum to the CD spectra of $(Lys)_n$ in terms of the fractions of α helix, β structure, and irregular structure (Greenfield and Fasman, 1969). The computer analysis of this spectrum indicated the presence of about 25% α helix and 75% irregular structure. The CD spectra of XII in various hexafluoroisopropyl alcohol–water mixtures are

TABLE I: Circular Dichroism, $[\theta]_\lambda$, Values of $(Lys\text{-}Ala\text{-}Gly)_n$ in Various Solvents.

Solvent	Conditions	Band Position (nm) and Magnitude ($[\theta]_\lambda \times 10^{-3}$)					
		λ	$[\theta]_1$	λ	$[\theta]_3$	λ	$[\theta]_2$
0.2 M NaF	pH 7, 23°	229	−0.61			195	−12.8
	pH 7, 82°	217	−2.24			197	−7.93
	pH 11, 23°	225	−0.95			197	−16.6
0.5 M NaF	pH 7, 23°	229	−0.54			195	−13.2
H_2O	pH 7, 23°					195	−12.0
MeOH–H_2O[a]	95:5 (v/v), 23°	222	−2.5	205	−5.2	<195	(+)
TFE–H_2O[b]	90:10 (v/v), 23°	220	−5.74	205	−7.86	189	12.7
HFIP–H_2O[c]	95:5 (v/v), 23°	222	−2.1	200	−3.1	<195	(+)
	80:20 (v/v), 23°	217	−3.5	203.5	−4.5	<195	(+)
	50:50 (v/v), 23°	220	−4.2	202	−6.8	<195	(+)

[a] MeOH, methanol. [b] TFE, trifluoroethanol. [c] HFIP, hexafluoroisopropyl alcohol.

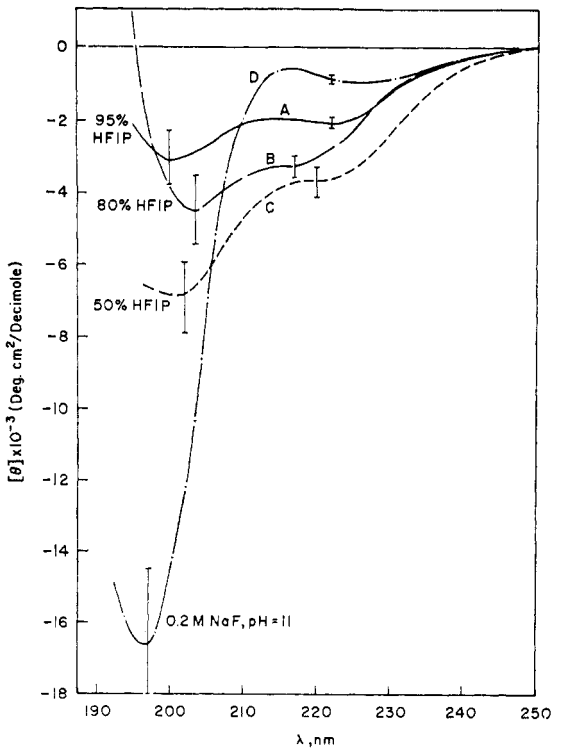

FIGURE 4: Circular dichroism spectrum of $(HCl\text{-}Lys\text{-}Ala\text{-}Gly)_n$ in various hexafluoroisopropyl alcohol (HFIP)–H_2O mixtures. Curve A (–), 95% HFIP–5% H_2O; curve B (– –), 80% HFIP; curve C (----), 50% HFIP; curve D (-·-·), 0.2 M NaF, pH 11.0, 23°. Conditions were the same as in Figure 2.

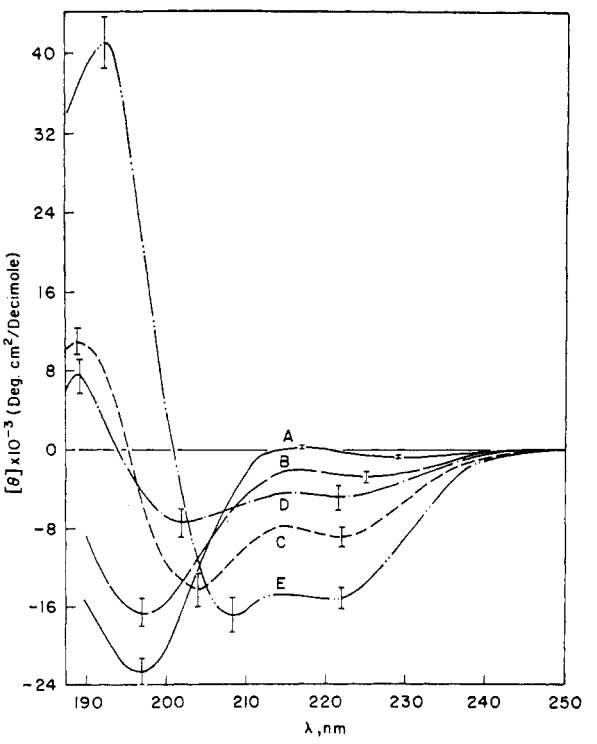

FIGURE 5: Circular dichroism spectra of $(Lys^{37.6}Ala^{33.8}Gly^{28.6})_n$ in 0.2 M NaF as a function of pH and temperature. Curve A (–), pH 7.0, 23°; curve B (– –), pH 7.0, 84°; curve C (---), pH 11.0, 23°; curve D (-·-·), pH 11.0, 81°; curve E (-·-·-·), 1.0 M sodium dodecyl sulfate, pH 7.0, 23°. The cell path length used was 1 mm for the wavelength range 250–210 nm and 0.1 mm for wavelengths below 210 nm. The mean residue concentrations of the solutions ranged from 0.0085 to 0.021 M (0.087–0.22%).

given in Figure 4. As the volume percentage of hexafluoroisopropyl alcohol increases, the CD spectra begin to resemble the CD spectra of helical polypeptides. However, even in 95% hexafluoroisopropyl alcohol, XII is only about 10% helical.

The CD band positions and magnitudes of the random sequence polymer XIV in various solvents are summarized in Table II. In Figure 5 are shown the CD spectra of XIV in 0.2 M NaF as a function of pH and temperature. In 0.2 M NaF, pH 7, 23°, the CD spectrum (Figure 5, curve A) of XIV had a small negative band at 229 nm ($[\theta]^{23}_{229}$ −740°), a small positive band centered at 217 nm ($[\theta]^{23}_{217}$ 270°), and a large negative band at 197 nm ($[\theta]^{23}_{197}$ −23,700°). When the temperature of this solution was increased to 81°, the polymer showed a typical random-coil high-temperature spectrum (Figure 5, curve B) with a decreased negative band at 197 nm ($[\theta]^{81}_{197}$ −15,700°) and a shoulder centered at 225 nm ($[\theta]^{81}_{225}$ = −2,800°). The CD spectrum of XIV in 0.2 M NaF, pH 11, 23° (Figure 5, curve C), resembled the CD spectrum of partially helical poly(α-amino acids) with two negative bands at 222 and 202 nm and a positive band at 189 nm ($[\theta]^{23}_{222}$ −8,340°, $[\theta]^{23}_{202}$ −13,700°, $[\theta]^{23}_{189}$ 11,200°). Raising the temperature of this solution to 81° resulted in the melting out of some of the helical structure (Figure 5, curve D) ($[\theta]^{81}_{222}$ = −4750°, $[\theta]^{81}_{202}$ =

TABLE II: Circular Dichroism, $[\theta]_\lambda$ Values of $(Lys^{37.6}Ala^{33.8}Gly^{28.6})_n$ in Various Solvents.

Solvent	Conditions	Band Position (nm) and Magnitude ($[\theta]_\lambda \times 10^{-3}$)					
		λ	[θ]	λ	[θ]	λ	[θ]
0.2 M NaF	pH 7, 23°	229	−0.74	217	0.27	197	−23.7
	pH 7, 84°	225	−2.8			197	−15.7
	pH 11, 23°	222	−8.34	202	−13.7	189	11.2
	pH 11, 81°	222	−4.75	202	−7.37	189	7.6
0.5 M NaF	pH 7, 23°					196	−27.8
H_2O	pH 7, 23°					197	−23.8
MeOH–H_2O[a]	95:5 (v/v), 23°	222	−24.6	208	−30.5	191	66.0
TFE–H_2O[b]	95:5 (v/v), 23°	220	−22.8	207	−29.6	190	62.5
HFIP–H_2O[c]	90:10 (v/v), 23°	220	−15.4	206	−23.2	190	39.5
	50:50 (v/v), 23°	220	−14.9	207	−20.7	190	34.2
	25:75 (v/v), 23°	220	−10.6	204	−18.2	190	20.4
1 M SDS[d]	pH 7, 23°	222	−14.3	207	−16.1	192	41.0

[a] MeOH, methanol. [b] TFE, trifluoroethanol. [c] HFIP, hexafluoroisopropyl alcohol. [d] SDS, sodium dodecyl sulfate.

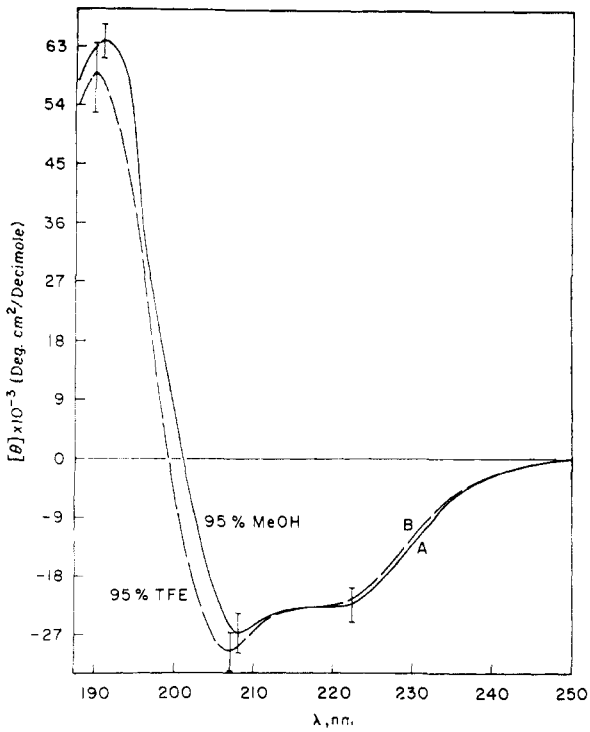

FIGURE 6: Circular dichroism spectra of (HCl-Lys$^{37.6}$Ala$^{33.8}$Gly$^{28.6}$)$_n$ in 95% methanol, curve A (—); 95% TFE, curve B (---). The cell path length used was 0.102 mm. The mean residue concentrations of the solutions ranged from 0.008 to 0.010 M (0.081–0.102%).

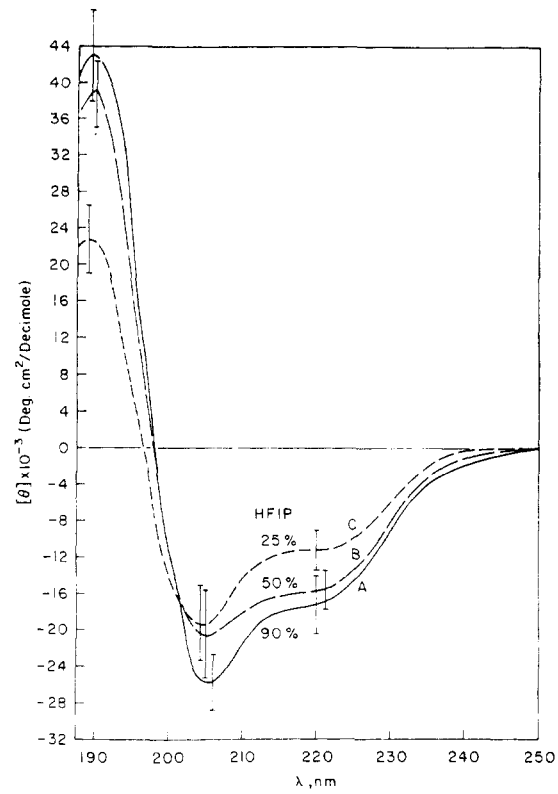

FIGURE 7: Circular dichroism spectra of (HCl-Lys$^{37.6}$Ala$^{33.8}$Gly$^{28.6}$)$_n$ in various hexafluoroisopropyl alcohol–H_2O mixtures. Curve A (—), 90% HFIP; curve B (– –), 50% HFIP; curve C (----), 25% HFIP. Conditions were the same as in Figure 6.

$-7370°$, $[\theta]^{81}_{189} = 7600°$). In 1 M sodium dodecyl sulfate, pH 7, the random sequence polymer XIV assumed a more ordered conformation (Figure 5, curve E) ($[\theta]_{222} = -14{,}300°$, $[\theta]_{207} = -16{,}100°$, $[\theta]_{192} = 41{,}000°$). The best fit of this spectrum to the (Lys)$_n$ CD spectra indicated that XIV in 1 M sodium dodecyl sulfate, pH 7, could be ~50% α helical, ~20% β structure, and ~30% random coil.

When the polymer XIV was dissolved in methanol or trifluoroethanol, the CD spectra closely resembled those of helical poly(α-amino acids). In Figure 6 the CD spectra of XIV in 95% methanol and 95% trifluoroethanol are shown. In these solvents, the two negative bands have ellipticities of $\sim -25{,}000°$ and $\sim -30{,}000°$ at 222 and 207 nm, respectively, while the positive band has $[\theta]_{191} = 66{,}000°$ which indicates that the polymer XIV is about 90% helical in these solvents. The CD spectra of XIV in various hexafluoroisopropyl alcohol–water mixtures are given in Figure 7. As the volume percentage of hexafluoroisopropyl alcohol decreases, the CD spectra show that the polymer becomes less helical. However, even in 75% water–25% hexafluoroisopropyl alcohol, the polymer is only about 45% helical.

Infrared Spectroscopy. In the solid state, the positions of the amide I and II bands in the ir spectra of the sequential polymer XII, cast as a thin film from water or deuterium oxide solutions, indicated the polymer was random (Susi *et al.*, 1967; Miyazawa, 1967): from H_2O, amide I, 1656 cm^{-1}, amide II, 1530 cm^{-1}; from D_2O, amide I′, 1648 cm^{-1}, amide II′, 1530 cm^{-1}. The polarized ir spectra of an oriented film of XII cast from trifluoroethanol–D_2O (9:1) solution showed no dichroism in either the amide I′ band (1650 cm^{-1}) or amide II′ band (1552 cm^{-1}), indicating that the polymer was in the random structure in the solid state or extremely difficult to orient, probably the former. The polarized ir spectra of XIV cast from trifluoroethanol–H_2O (9:1) also showed no dichroism in either the amide I′ (1655 cm^{-1}) or amide II′ (1535 cm^{-1}) bands.

Discussion

Synthesis herein of optically pure (Lys-Ala-Gly)$_n$ (XII) in high yield of relatively high molecular weight polymer with a low degree of polydispersity, readily soluble in water, is another example of the usefulness of the active ester method of preparing sequential polypeptides. The number-average molecular weight of XII, $\bar{M}_n$ = 38,000, was almost three times larger than that of a similar polypeptide previously reported by Johnson (1968). Johnson's (1968) method of preparing polypeptides utilizes a dilute solution of the polymerizing unit in the presence of a partially blocked monomer. Utilizing a concentration of 122 mmol/l. during polymerization, the polymer so obtained gave a 23% overall yield (based on the polymerizing unit). In the synthesis reported herein, a yield of 72% resulted after carrying out the polymerization at a concentration of 370 mmol/l.

The solution conformation of XII was investigated by circular dichroism spectroscopy (Table I). In aqueous solution at pH 7 and 11, over a range of ionic strengths (0.0–0.5 M NaF), the polymer had a CD spectra (Figure 2) similar to that of a random coil (Adler *et al.*, 1973b) with a small, negative shoulder at 225–227 nm and a trough at 195–197 nm. There was no ionic strength dependence found under these conditions. Poly(L-lysine) (Applequist and Doty, 1962) or copolymers of Lys with other amino acids [*e.g.* (Lys,Leu)$_n$ (Snell and Fasman, 1972), (Lys,Ala)$_n$ (Privat, 1970), (Lys-Ala-Ala)$_n$ (Yaron *et al.*, 1972; Cowell and Jones, 1972)] usually undergo a random coil–helix conformational change on raising the pH from 7 to 11. As a result of decreasing the charge on the ϵ-amino group, the repulsion of like charges is reduced, and the polypeptides fold into their most thermodynamically stable conformation, the α helix. With the sequential tripeptide polymer XII studied herein however,

removal of charge has no effect, and the polymer remains a random coil at pH 11. It appears that the charge distribution of the repeating trimer was not solely responsible for the random conformation at pH 7.0. This conclusion is further strengthened by the fact that the CD spectra show no ionic strength dependence.

The temperature dependence of the circular dichroism spectra was also typical of a random coil (Yaron *et al.*, 1971); the magnitude of the trough decreased and the band red shifted while the higher wavelength shoulder broadened, became larger in magnitude, and blue shifted. An interesting observation from these CD studies is that this polymer has smaller mean residue ellipticities at the extrema than those usually found for random-coil poly(α-amino acids). The $[\theta]_{197}$ values for poly(α-amino acids) range from about −26,400° [N^5-2-(hydroxyethyl-L-glutamine)$_n$] in water (Adler *et al.*, 1968) to about −36,900° (L-glutamic acid)$_n$ in water at pH 7.5 (Adler *et al.*, 1968), and (L-lysine-HCl)$_n$ in water at pH 5.7 (Greenfield and Fasman, 1969). The $[\theta]_{197}$ value for (Lys-Ala-Gly)$_n$ is ∼−13,000 in 0.2 M NaF (pH 7.0). The shape of the CD spectra of XII in aqueous solution more closely resemble the random-coil CD spectra found for thin films of polypeptides and denatured proteins (Fasman *et al.*, 1970a; Dearborn and Wetlaufer, 1970) than the CD spectra found for the polyelectrolytes, (Lys)$_n$ and (Glu)$_n$, in the random-coil form.

The polypeptide XII can be forced into a more ordered or regular conformation by lowering the dielectric constant of the solvent, *e.g.*, dissolving it in 95% methanol, trifluoroethanol, or hexafluoro-2-propanol (Table I). The CD spectra of XII in these solvents resembles those of partially helical polypeptides (Figures 3 and 4). There is no evidence to indicate that this polypeptide can assume the β structure. The CD spectra of XII in aqueous solutions at pH 7 and 11, at room temperature and at 80°, as well as in 1 M sodium dodecyl sulfate (pH 7) do not exhibit any of the ellipticity bands characteristic of the β structure. The β structure can be produced by similar treatment of poly(Lys), *i.e.*, heating poly(Lys) at pH 11 (Davidson and Fasman, 1967, and references cited therein) and by the addition of sodium dodecyl sulfate to poly(Lys) at pH 7.0 (Sarkar and Doty, 1966; Li and Spector, 1969). In the solid state, oriented films of XII cast from either aqueous or organic solutions had ir spectra indicative of an irregular conformation (Susi *et al.*, 1967; Miyazawa, 1967).

Relatively few conformational studies have been undertaken with sequential polypeptides. The most thoroughly studied group are those investigated as collagen models, containing either proline or hydroxyproline (for a review, see Traub and Piez, 1971). An extensive conformational study of (Tyr-Ala-Glu)$_n$ (Schechter *et al.*, 1971) demonstrated the importance of the ionic environment in the determination of conformation. A cursory study of the sequential polypeptide (Lys-Ala)$_n$ has been reported (Privat, 1970) and a thorough investigation of (Lys-Ala-Ala)$_n$ has been described (Yaron *et al.*, 1972). The most extensively studied sequence is (Ala$_x$-Gly$_y$)$_n$, with x and y varying from one to three residues. Recently, Doyle *et al.* (1970) reported that their (Ala$_2$-Gly)$_n$ could be fractionated into water soluble ($\bar{M}_w$ ∼2200) and insoluble ($\bar{M}_w$ ∼4500) fractions. The CD spectrum of the low molecular weight material in ethylene glycol–water (2:1, v/v) had features characteristic of a random or structureless conformation ($[\theta]_{228}$ −400°, $[\theta]_{198}$ ∼−3800°). The CD spectrum of the higher molecular weight material in hexafluoro-2-propanol ($[\theta]_{219}$ ∼−4000°, $[\theta]_{203}$ −6250°, and a positive peak below 195 nm) was interpreted to indicate that the polymer was in a "modified" α helix. The low ellipticity values were due to an appreciable amount of random structure. In ethylene glycol–hexafluoro-2-propanol (2:1, v/v), this fraction of (Ala$_2$-Gly)$_n$ had a CD spectrum thought to be due to the presence of 60% β structure and 40% random structure.

Iio and Takahashi have studied the conformation of the series (Ala$_x$-Gly$_y$)$_n$ with x and y = 1, 2; 2, 1; 3, 1, in $CHCl_2$-COOH–chloroform (Takahashi, 1969; Iio and Takahashi, 1970; Iio, 1971). Their polymers were not water soluble, presumably due to the higher molecular weight materials synthesized ($\bar{M}_w$ 20,000–35,000). From their ORD studies they found, in agreement with Fraser *et al.* (1967) and Block and Kay (1967), that the amino acid sequence of a sequential polypeptide exerts a significant influence on the stability of the α helix.

Rippon and Walton (1971) found that (Ala-Gly$_2$)$_n$ forms either 3_1 or poly(glycine) II helices. This sparingly water-soluble polymer, with $\bar{M}_w$ = 14,100, had a CD spectrum with a $[\theta]_{213}$ of +6000° and a $[\theta]_{192}$ of −22,000°. When the temperature was raised to 60°, the $[\theta]_{196}$ decreased to −2000°.

The careful and thorough study of (Ala$_x$-Gly$_y$)$_n$ sequential polypeptides by Brack and Spach (1972) indicated that these polymers, in the solid state, can exist in the random, α-helical, antiparallel β structure, or poly(glycine) II helix, depending on the sequence and on the manner of handling the sample. The ORD spectra of these polymers were measured in organic solvents. In CF_3COOH, all polymers behaved as random coils and only (Ala$_2$-Gly)$_n$ was partially helical in $CHCl_2COOH$. Addition of $CHCl_3$ to $CHCl_2COOH$ solutions of all the polymers resulted in the formation of helical structure. The stability of the helix increased regularly with increasing alanyl residue content in the polypeptide and did not follow the order suggested by Iio (1971). (Ala$_2$-Gly)$_n$ was not found to form any poly(glycine) II helical structures (Brach and Spach, 1972).

Since Ala is known to form stable α helices (Gratzer and Doty, 1963; Yaron *et al.*, 1972; Cowell and Jones, 1972), it would appear that the Gly residue is responsible for destabilizing the helical structure of (Ala$_x$-Gly$_y$)$_n$ polymers. However, the inclusion of one Gly residue in a repeating tripeptide sequence apparently does not make the helical conformation thermodynamically impossible. The conformation then depends on the helical stabilization effects of the other residues. This conclusion from these experimental studies agrees well with analysis of Chou and Fasman (1974) on the helix normalization factors derived from a study of 15 proteins whose conformation was established through X-ray crystallography. Ala was found to have a high value for the helix conformational parameter (P_α = 1.45), *e.g.*, helix promoting, while Lys (P_α = 1.07) has marginal helical stability, and Gly (P_α = 0.53) is a helix destabilizer (P_α = 1.00 is interpreted to mean a 50% probability of being helical). Although the flexibility of the glycine residue and its lack of a side chain do not prevent helix formation, the fact that (Lys-Ala-Gly)$_n$ does not assume any regular, repeating conformation must be due, in considerable measure, to the glycine residue. The conformational properties of (Lys-Ala-Gly)$_n$ in aqueous solutions are a reflection of the consequences of the periodic inclusion of glycine residues.

In contrast to the sequential polymer, the random sequence polymer (Lys,Ala,Gly)$_n$ (XIV) more readily assumed the α-helical structure even though it was of lower molecular weight ($\bar{M}_w \simeq$ 12,000). The CD spectra of XIV were measured under the same conditions used for the sequential polymer. At pH 7 this polypeptide behaved as a random coil with a

TABLE III: Approximate Analysis of Composition *vs.* Time during the Polymerization of (Lys-Ala-Gly)$_n$.[a]

Time after Initiation (min)	% Completion of Polymerization	% of Initial Concn Incorp in Polymer			Amino Acid Composition at Time t		
		Lys	Ala	Gly	Lys	Ala	Gly
20	27.2	11	11	60	0.134	0.135	0.731
60	50.7	29	29	94	0.193	0.194	0.612
180	76.5	65	65	100	0.283	0.284	0.434
1440	100.0	100	100	100	0.333	0.335	0.332

[a] Equations used for this analysis may be found in Shalitin and Katchalski (1960). Specific rate constants were obtained from Katchalski and Sela (1958).

small negative band at 229 nm, a small positive band at 217 nm, and a large trough at 197 nm (Figure 5). The magnitude of the $[\theta]_{197}$ band was about as large as that found for (Glu)$_n$ (Adler *et al.*, 1968) and twice the size of the band found for (Lys-Ala-Gly)$_n$ (XII). The 197 nm band showed a slight ionic strength dependence; the value increased 17% as the ionic strength was increased from 0.0 to 0.5 M NaF. The polymer also had a temperature dependent CD spectrum typical of random-coil poly(α-amino acids) (Yaron *et al.*, 1971). At pH 11 the polymer became partially helical. Upon heating a solution of the polymer at pH 11.0 to ~80°, it underwent a partial helix to random-coil transition. The high-temperature CD spectrum did not have any noticeable β-conformation features. In 1 M sodium dodecyl sulfate, pH 7, XIV exhibited a CD spectrum that was resolved by computer-fitting techniques into α-helical (~50%), β-structure (~20%), and random (~30%) components. In 95% methanol, trifluoroethanol, or hexafluoro-2-propanol, the CD spectra (Figure 6) of XIV resemble the CD spectra of (Lys)$_n$ with about ~90% helix content. The stability of the helix formed by XIV is emphasized by the fact that even in hexafluoro-2-propanol–H_2O (1:3, v/v) (Figure 7) the polymer is about 45% helical. This is in contrast to the behavior of (Lys-Ala-Gly)$_n$ which assumed only about 10% helical structure in 95% hexafluoro-2-propanol.

The stability of the helical conformation of XIV might be explicable in terms of a nonrandom sequential distribution of residues along the polypeptide backbone. This clustering of residues could be formed during polymerization if the kinetics of polymerization of the *N*-carboxyanhydrides were dissimilar. Although kinetic analyses of polymerization have not yet been performed for tricopolypeptides, considerable work has been carried out on copolypeptides with two different residues, as well as with homopolymers (Katchalski and Sela, 1958; Shalitin and Katchalski, 1960; Nylund and Miller, 1965). These studies have demonstrated that nonrandom sequences are formed during polymerization when one of the *N*-carboxyanhydrides has a much larger polymerization rate constant than the other. Indeed, the compositional distribution in the copolymers can be calculated once the rate coefficients of copolymerization are known. An approximate mathematical analysis of the copolymerization of (Lys,Ala,Gly)$_n$ was carried out using the specific rate constants for the *N*-carboxyanhydrides of N^ϵ-Z-Lys, Ala, and Gly given by Katchalski and Sela (1958). The assumptions used were that the relative polymerization kinetics observed for the *N*-carboxyanhydrides in dioxane with *n*-hexylamine initiation would not change drastically with sodium methoxide initiation. Since the available data indicate that N^ϵ-Z-Lys-*N*-carboxyanhydride and Ala-*N*-carboxyanhydride have very similar polymerization kinetics, it was assumed that they were identical during the formation of (Lys,Ala,Gly)$_n$. The equation used to follow the change in the composition with time of a growing polymer is taken from Shalitin and Katchalski (1960)

$$\frac{[\text{Gly}]'}{[\text{Lys} + \text{Ala}]'} = \frac{[\text{Gly}]_0 - [\text{Gly}]_0 e^{-k_G[\text{I}]_0 t}}{[\text{Lys} + \text{Ala}]_0 - [\text{Lys} + \text{Ala}]_0 e^{-k_{L+A}[\text{I}]_0 t}} \quad (1)$$

where [Gly]′ and [Lys + Ala]′ are the amino acid residue concentrations in the growing polymer at time t; $[\text{Gly}]_0$, $[\text{Lys} + \text{Ala}]_0$, and $[\text{I}]_0$ are the initial concentrations of the *N*-carboxyanhydrides of Gly, Lys, and Ala and the initiator; and k_G and k_{L+A} are the specific rate constants of polymerization for Gly and both Lys and Ala. The values used were $[\text{Gly}]_0 = 1.87 \times 10^{-2}$ M, $[\text{Lys}]_0 = 1.88 \times 10^{-2}$ M, $[\text{Ala}]_0 = 1.89 \times 10^{-2}$ M, $[\text{I}]_0 = 2.25 \times 10^{-3}$ M, $k_G = 34$ l. mol^{-1} sec^{-1}, $k_{L+A} = 4.3$ l. mol^{-1} sec^{-1}. The calculated results of composition *vs.* time are given in Table III. These calculations indicate that the C-terminal sequence of the polymer could contain excess Gly and the N-terminal quarter of the molecule would have almost no Gly residues. This putative clustering or higher density of Lys and Ala residues in the N-terminal portion of the random sequence polymer could account for its greater propensity to form helical segments and the greater relative stability of such helices.

In conclusion, this study of sequential and random sequence polypeptides of the same amino acid composition (Lys,Ala,Gly) has demonstrated the influence that the periodic inclusion of glycine residues has upon the conformation of these polymers. The sequential polymer remained in a random conformation in aqueous solutions regardless of the pH, temperature, or ionic strength. In 95% methanol, trifluoroethanol, or hexafluoroisopropyl alcohol, this polymer could be forced into a partially helical conformation (~10%). The random polymer, however, more readily assumed the α-helical structure. On raising the pH from 7 to 11, the random polymer underwent a partial random-coil to helix conformational change. In 95% methanol, trifluoroethanol, or hexafluoroisopropyl alcohol, this polypeptide assumed a 90% α-helical conformation. The sequential polypeptide (Lys-Ala-Gly)$_n$ has conformational characteristics similar to those found for histone fraction f-1 (Fasman *et al.*, 1970b). The CD spectrum of f-1 histone is independent of ionic strength and pH between 5 and 9 and corresponds to that of a random conformation with $[\theta]_{199} = -18{,}900°$ at pH 7, 0.14 M NaF. The sequential polypeptide (Lys-Ala-Gly)$_n$ is a plausible model of histone f-1 for use in studying the structure and interactions of nucleohistone complexes. Such studies are under way.

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The Folded Conformation of the Encephalitogenic Protein of the Human Brain†

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ABSTRACT: The encephalitogenic protein from human myelin was shown to have a nonrandom structure in aqueous solution on the basis of its intrinsic viscosity and from the observed change in the far-ultraviolet circular dichroism between a solution of the protein in 6 M guanidine hydrochloride and in the absence of denaturing agents. This nonrandom structure was shown to be stable to large variations of pH on the basis of circular dichroism and viscosity, suggesting that this protein has a specific folded structure. A folded structure is also expected on the basis of a β-bend analysis. Viscosity, sedimentation velocity, low-angle X-ray scattering, and electron microscopy results are all consistent with a prolate ellipsoid model for the shape of this protein with approximate dimensions of 15 × 150 Å.

There are a small number of major protein components of the myelin membrane. One of these proteins, the encephalitogenic protein, accounts for approximately 30% of the protein content of myelin. An injection of as little as 1 μg of this protein in complete Freund's adjuvant into guinea pigs will induce experimental autoimmune encephalomyelitis, a demyelinating disease (Oshiro and Eylar, 1970). The encephalitogenic protein is thus of interest because of its potent biological activity and because of its importance as a major constituent maintaining the structure of the myelin membrane.

Until now it has been assumed by most workers that the encephalitogenic protein is largely devoid of an ordered structure (Eylar and Thompson, 1969; Choa and Einstein, 1970). This conclusion was based mainly on the high intrinsic viscosity of the protein and on its rotatory properties. This protein was found to be an excellent substrate for a neural acid proteinase (Einstein *et al.*, 1972). A theory of myelin breakdown was advanced (Einstein *et al.*, 1970, 1972) in which this protein in a highly open structure was readily hydrolyzed by neural acid proteinase.

The intrinsic viscosity and optical rotatory data (Eylar and Thompson, 1969; Choa and Einstein, 1970) are also consistent with a highly ordered protein having a specific tertiary structure which is asymmetrical and relatively devoid of α helical or β structures. It is the purpose of the present study to distinguish between these two alternatives, *i.e.*, between a largely random, highly solvated structure and an ordered, compact and asymmetric structure.

Methods

Isolation of the Acid-Soluble Encephalitogen. Myelin was prepared from normal human white matter and the acid-soluble encephalitogen was isolated (Lowden *et al.*, 1966). The protein was further purified by chromatography on a Celex-P column (Eylar *et al.*, 1969).

Concentration and Purity of Protein Solutions. Concentrations of protein solutions were determined by amino acid analysis with a Beckman 120C analyzer using an internal standard of norleucine.

Viscosity Measurements. Viscosities were measured with Cannon-Ubbelohde semimicro viscometers having flow times of 70 and 250 sec with solvent at 25.000 ± 0.005°. Protein solutions of concentrations of 0.2–1% were used.

Circular Dichroism Measurements. These measurements were performed with a Cary, Model 61, spectropolarimeter calibrated according to the values given by Cassim and Yang (1969). The temperature of the sample was controlled by means of a thermostatable cell holder attached to a circulating constant-temperature bath. A mean residue weight of 108.5 was calculated from the amino acid composition (Eylar, 1970; Carnegie, 1971).

Ultracentrifugal Studies. These studies were performed at 20° with a Spinco Model E ultracentrifuge using sapphire windows. A double-sector, capillary-type, synthetic boundary cell was used for the sedimentation velocity runs with the schlieren optical system and a speed of 60,000 rpm.

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