Synthesis, Circular Dichroism Spectra, and Immunological Properties of the Sequential Polypeptide, Poly(Tyr-Ala-Glu-Gly)†

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ABSTRACT: A sequential polypeptide, poly(*O*-Bzl-Tyr-Ala-γ-Bzl-Glu-Gly), was synthesized by a stepwise condensation of *N*-hydroxysuccinimide esters of *tert*-butoxycarbonyl amino acids. Polymerization of the monomer, *O*-benzyltyrosylalanyl-γ-benzylglutamylglycine pentachlorophenyl ester, was as a dispersion in benzene. After deprotection and dialysis, the weight average molecular weight of poly(Tyr-Ala-Glu-Gly) was found to be 33 000. The circular dichroism spectra of the polymer in 0.15 M phosphate buffered NaF, pH 7.2 and 11.8, and that of the monomer at pH 7.2 indicate that only the polymer exists in an α-helical configuration at neutral pH. Poly(Tyr-Ala-Glu-Gly) was immunogenic in two of four rabbits. The specificity of antibodies was measured by direct binding using ^{125}I-labeled polypeptides of similar amino acid composition, and by inhibition of the radiolabeled antigen-antibody reaction with unlabeled polypeptides. The data obtained showed the presence of at least two classes of antibody. One class, which consisted of a majority of the antibodies (≈80%), recognized poly(Phe-Ala-Glu-Gly) but not poly(Tyr-Glu-Ala-Gly); another class, which consisted of a minority of the antibodies (6–7%), recognized poly(Tyr-Glu-Ala-Gly). Neither class showed recognition of poly(Tyr-Ala-Glu) nor of any of several random polypeptides having similar amino acids. The antibody specificities are discussed in light of molecular models of the tyrosine-containing sequential polypeptides.

Polypeptides of repeating amino acid sequences are protein models of less complexity. The repeating forces present in these polypeptides may lead to limited secondary structures which could be studied in order to evaluate the conformational preferences of the constituent amino acids. In addition, the limited primary and secondary structures may result in restricted antigenic determinants, which may be defined by specificity studies at both the humoral and cellular levels. Advances in the methodology of peptide polymerization now make possible the synthesis of high molecular weight sequential polypeptides of related amino acid composition (Spach and Brack, 1967; Brack and Spach, 1970; Zeiger et al., 1973, 1975) which can be used for systematic conformational and immunological studies.

Two related polypeptides, poly(Tyr-Ala-Glu) (Ramachandran et al., 1971) and poly(Tyr-Glu-Ala-Gly) (Zeiger et al., 1975) have been previously synthesized and shown to be α helical at physiological pH. Circular dichroism (CD)[1] spectra of the two were similar. We now report the synthesis and CD properties of a third polypeptide of related composition, poly(Tyr-Ala-Glu-Gly).

Both poly(Tyr-Ala-Glu) (Schechter et al., 1971b) and poly(Tyr-Glu-Ala-Gly) (Johnson and Trask, 1970) have been shown to be immunogenic in rabbits. In addition, poly(Tyr-Glu-Ala-Gly) has been shown to be immunogenic in inbred strain 13 guinea pigs but not in inbred strain 2 guinea pigs (Maurer et al., 1973). Recent studies indicate that poly(Tyr-Ala-Glu-Gly) is immunogenic in strain 2 guinea pigs but not in strain 13 guinea pigs (Zeiger and Maurer, 1976) and in mice of H-2^b, H-2^f, and H-2^r haplotypes (Merryman et al., 1977). In this paper, the immunogenicity of poly(Tyr-Ala-Glu-Gly) in rabbits is presented. A comparison is made between the ability of antibodies and that of CD to distinguish between antigens of related amino acid composition and secondary structure.

Experimental Procedure

All melting points are corrected. Optical rotations were measured with a Perkin-Elmer Model 141 polarimeter at the 580-nm sodium line. Elemental analyses were performed by the Alfred Bernhardt Microanalytical Laboratories, Engelskirchen, West Germany. The buffer used in these studies was 0.15 M phosphate buffered NaCl except for the CD studies, for which 0.15 M phosphate buffered NaF was employed. Viscosity measurements were determined at pH 7.2 with a Cannon-Ubbelohde viscometer, Model 150. Analytical ultracentrifugation was carried out in a Spinco Model E ultracentrifuge at 20 °C on several concentrations of the polymer at pH 7.2. The Svedberg (S) value was determined using a Kegeles cell at 52 000 rpm on polymer solutions of 3.40 mg/mL and 2.28 mg/mL. The molecular weight was estimated from the Scheraga–Mandelkern equation (Scheraga and Mandelkern, 1953) using a value of 2.2×10^6 for β and 0.72 for the partial specific volume. Amino acid analyses were performed on samples which had been hydrolyzed for 16 h in evacuated, sealed tubes at 110 °C. CD measurements were made on a Jasco ORD/UV-5 spectropolarimeter using cells of 0.1, 1.0, and 10 mm path lengths. Samples of approximately 1 mg/mL in the NaCl buffer, pH 7.2, were diluted with the NaF buffer. All solutions were filtered before use and concentrations subsequently determined by amino acid analysis.

The following peptide moieties were detected by thin-layer chromatography using commercially available silica gel plates (Brinkmann): free amine, 0.1% ninhydrin spray followed by heating; *tert*-butoxycarbonylamine, hydrochloride vapor for 15 min followed by the procedure to detect free amine; benzyloxycarbonylamine, iodine vapor; peptide bond, 1% sodium hypochlorite spray followed by 1% potassium iodide–1% soluble starch spray. R_f values on these plates refer to the fol-

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[1] Abbreviations used are: CD, circular dichroism; DMF, *N,N*-dimethylformamide; Boc, *tert*-butoxycarbonyl; OBzl, benyzl ester or benzyl ether; ONSu, *N*-hydroxysuccinimide ester; PCP, pentachlorophenyl ester; TLC, thin-layer chromatography.

lowing solvent systems: (A) methanol–ethyl acetate (1:2 v/v); (B) *n*-butyl alcohol–acetic acid–pyridine–water (4:2:1:1 v/v); (C) *n*-butyl alcohol–acetic acid–water (4:1:5 v/v). Protected amino acid monomers and the dipeptide, L-alanylglycine, were purchased from Fox Chemical Co. (Los Angeles, Calif.) and were homogeneous by thin-layer chromatography.

Synthesis of Poly(Tyr-Ala-Glu-Gly). *N*-α-*tert*-Butoxycarbonyl-γ-benzylglutamylglycine (I). To 4.40 g (58.4 mmol) of glycine dissolved in 140 mL of water was added 5.00 g (58.5 mmol) of sodium bicarbonate. The *N*-hydroxysuccinimide ester of *N*-α-butoxycarbonyl-γ-benzylglutamic acid (12.80 g, 29.56 mmol), dissolved in 60 mL of dioxane, was added at 4 °C and the solution was stirred overnight. The solution was evaporated to dryness and the residue was brought up in a mixture of 100 mL of 1 M sodium bicarbonate and 100 mL of ethyl acetate. The organic layer was extracted twice more with 100-mL portions of 1 M sodium bicarbonate. The aqueous fractions were pooled, acidified to pH 2.9, and extracted three times with 100-mL portions of ethyl acetate. The organic layers were pooled, dried with anhydrous sodium sulfate, and concentrated to an oil, which crystallized upon refrigeration: yield, 9.86 g (86%); mp 117–118 °C; $[\alpha]^{23}_D$ −33.3 (*c* 1.35, methanol); TLC R_fA 0.39, R_fB 0.75, R_fC 0.60.

Anal. Calcd for $C_{19}H_{26}N_2O_7$ (394.43): C, 57.86; H, 6.64; N, 7.10. Found: C, 57.64; H, 6.51; N, 7.26.

N-α-*tert*-Butoxycarbonylalanyl-γ-benzylglutamylglycine (II). Compound I (1.33 g, 3.07 mmol) was dissolved in 20 mL of 4.1 N HCl in dioxane for 15 min. The product, γ-benzylglutamylglycine hydrochloride, was precipitated with ether and was hygroscopic (R_fA origin; R_fB 0.41). To this residue dissolved in 35 mL of distilled water was added 684 mg (8.15 mmol) of sodium bicarbonate. Then at 4 °C, 1.17 g (4.10 mmol) of the *N*-hydroxysuccinimide ester of *N*-α-*tert*-butoxycarbonylalanine dissolved in 35 mL of dioxane was added. After 16 h, the solution was evaporated and the oil was partitioned between 1 M sodium bicarbonate (50 mL) and ethyl acetate (100 mL). The aqueous layer was removed and the ethyl acetate layer was further extracted with two 50-mL portions of 1 M sodium bicarbonate. The sodium bicarbonate solutions were pooled, acidified to pH 2.5, and extracted three times with 50-mL aliquots of ethyl acetate. The pooled ethyl acetate fractions were dried and concentrated. Ether was added and 1.17 g (82%) of II was obtained: mp 157–159 °C; $[\alpha]^{23}_D$ −9.4 (*c* 1.39, methanol); TLC R_fA 0.27, R_fB 0.73, R_fC 0.63.

Anal. Calcd for $C_{22}H_{31}N_3O_8$ (465.51): C, 56.76; H, 6.71; N, 9.03. Found: C, 56.57; H, 6.63; N, 8.96.

N-α-*tert*-Butoxycarbonyl-*O*-benzyltyrosylalanyl-γ-benzylglutamylglycine (III). Compound II (2.7 g, 5.8 mmol) was dissolved in 15 mL of 4.4 N HCl in dioxane for 15 min. Ether was added and the product was filtered and desiccated to dryness. The yield of alanyl-γ-benzylglutamylglycine hydrochloride was 2.18 g (87%); mp 193–195 °C dec; TLC R_fA, R_fC, origin, R_fB, 0.40. To 0.85 g of sodium bicarbonate (10.1 mmol) in 50 mL of water was added 2.04 g (5.1 mmol) of alanyl-γ-benzylglutamylglycine hydrochloride. The solution was cooled to 4 °C and 2.8 g (5.9 mmol) of the *N*-hydroxysuccinimide ester of *N*-α-*tert*-butoxycarbonyl-*O*-benzyltyrosine dissolved in 50 mL of dioxane was added. After 16 h at 4 °C, the solution was concentrated to about 40 mL on a rotary evaporator. Approximately 50 mL of 1 M sodium bicarbonate and 100 mL of ethyl acetate were added. Thin-layer chromatography indicated that the majority of the tetrapeptide was in the organic layer. The latter was extracted once more with 50 mL of 1 M sodium bicarbonate, water, three times with 0.5 M citric acid and water. The ethyl acetate solution was dried over anhydrous sodium sulfate, filtered, and concentrated. Yield was 1.90 g (52%) in two crops; mp 143–145 °C; TLC R_fA −0.39, R_fB −0.73, R_fC −0.68.

Anal. Calcd for $C_{38}H_{46}N_4O_{10}$: C, 63.50; H, 6.45; N, 7.79. Found: C, 63.65; H, 6.35; N, 7.85.

N-α-*tert*-Butoxycarbonyl-*O*-benzyltyrosylalanyl-γ-benzylglutamylglycine Pentachlorophenyl Ester (IV). To a solution of compound III (1.80 g, 2.64 mmol) dissovled in 13 mL of distilled *N,N*-dimethylformamide (DMF) were added, at 4 °C, 0.77 g (2.9 mmol) of pentachlorophenol and 0.60 g (2.9 mmol) of *N,N'*-dicyclohexylcarbodiimide. After 16 h, 5 mL more of DMF was added, and the mixture was warmed and filtered. The filtrate was concentrated, 50 mL of ethyl acetate was added and the product filtered. Yield was 2.18 g (84%) in two crops; mp 207.5–209 °C; TLC R_fA −0.82.

Poly(*O*-benzyltyrosylalanyl-γ-benzylglutamylglycine) (V). Compound IV (1.50 g, 1.62 mmol) was suspended in 30 mL of 4.3 N HCl in dioxane with stirring. After 12 min, 100 mL of ether was added and the mixture was filtered. The yield of *O*-benzyltyrosylalanyl-γ-benzylglutamylglycine pentachlorophenyl ester hydrochloride was 1.21 g (81%). The product was desiccated exhaustively over P_2O_5 and suspended in 13 mL of benzene which had been dried with CaH_2. Triethylamine (0.46 mL, 3.3 mol) was added at 4 °C with stirring and the reaction was allowed to proceed for 2 weeks at 4 °C. Ether was added and the product filtered. The powder was washed with ether, methanol, and water and desiccated to dryness: yield, 0.81 g (100%).

Poly(tyrosylalanylglutamylglycine). Compound V (200 mg) was deprotected by bubbling HBr in 7 mL of dried benzene for 3 h. The product was filtered and desiccated. The powder was suspended in 10 mL of phosphate-buffered NaCl, pH 7.0, and went into solution when the pH was raised to 9.9. The volume was brought up to 15 mL with this buffer to a pH of 8.8 and was dialyzed against distilled water. The sac contents were lyophilized. Amino acid analysis showed the yield of dry neutral polymer to be 83 mg. Amino acid ratios were $Tyr_{0.90}Glu_{0.96}Ala_{1.08}Gly_{1.06}$.

Other Polypeptides. Random polypeptides were prepared as described (Katchalski and Sela, 1958). Poly(Tyr-Ala-Glu) was a kind gift of Dr. B. Schechter and Dr. M. Sela. Poly(Phe-Ala-Glu-Gly) was synthesized in a similar manner to poly(Tyr-Ala-Glu-Gly) and will be reported elsewhere. The molecular weight of the fraction of poly(Phe-Ala-Glu-Gly) used in this study was 24 000.

Immunization Methods. Four New Zealand white rabbits were injected intramuscularly with 2 mg of poly(Try-Ala-Glu-Gly) in complete Freund's adjuvant. Two weeks later, they were bled and boosted with 1 mg of poly(Tyr-Ala-Glu-Gly) in complete Freund's adjuvant. After 2 weeks, bleedings were taken twice a week for several months. The potent sera from rabbits R-1374 and R-1375 were pooled individually.

Quantitative Precipitin Reaction. Antigen in 50 μL of buffer was added to 40 μL of sera and incubated at 37 °C for 1 h and overnight at 4 °C. Buffer was added to a total volume of 0.5 mL and the samples were centrifuged and washed 3× with buffer. Precipitates were analyzed with the Folin–Ciocalteau reagent as described (Maurer et al., 1964).

Radioimmunoassay. Poly($Glu^{60}Ala^{40}$) and poly(Phe-Ala-Glu-Gly) were tyrosylated (≈0.8%) as described (Maurer and Merryman, 1974). Polypeptides were iodinated with ^{125}I according to the Chloramine-T procedure of Hunter (1969). The labeled polymers were dialyzed extensively against buffer and had initial specific activities of 0.5–1.0 mCi/mg.

For the radioimmunoassay, aliquots of immune (A) or normal (B) rabbit sera were diluted 1:2 with 1% bovine serum

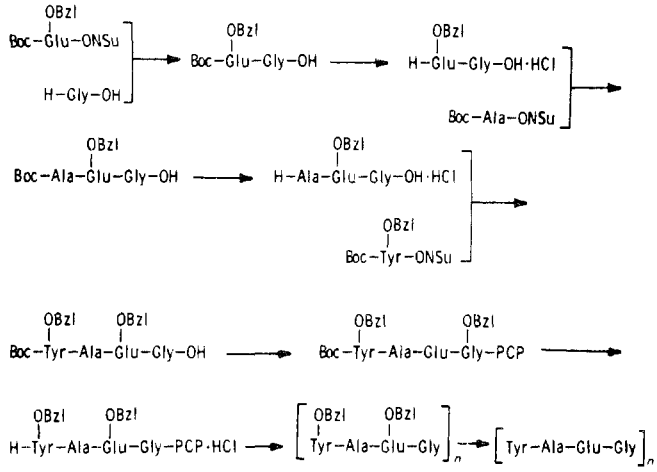

FIGURE 1: Synthetic route of poly(Tyr-Ala-Glu-Gly). Boc, *tert*-butoxycarbonyl; OBzl, benzyl ester or benzyl ether; ONSu, *N*-hydroxysuccinimide ester; and PCP, pentachlorophenyl ester.

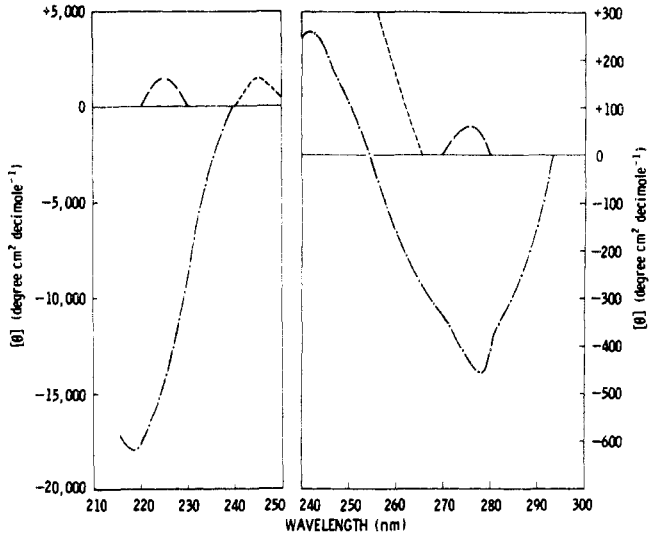

FIGURE 2: CD spectra. Poly(Tyr-Ala-Glu-Gly) in 0.15 M phosphate buffered NaF at pH 7.2 (—·—·—); at pH 11.8 (- - -); Tyr-Ala-Glu-Gly in 0.15 M phosphate buffered NaF at pH 7.2 (— — —).

albumin in buffer. To 25 μL of each of the diluted sera was added 25 μL of radiolabeled antigen (7.5 ng) in buffered serum albumin. After 1 h at 37 °C, 500 μL of pretitered polyvalent sheep anti-rabbit globulin was added. After 2 h at 37 °C, the samples were incubated at 4 °C for 30 min and centrifuged. Aliquots of the supernatants were counted in a well type of scintillation counter (Nuclear Chicago Corp., Des Plaines, Ill.). Values of the percent antigen bound, (B-A)/(B-background) × 100, were the average of duplicate samples.

Inhibition of the reaction of R-1374 sera and ^{125}I-labeled poly(Tyr-Ala-Glu-Gly) with unlabeled polypeptides was done as follows: R-1374 serum was diluted 1:2 with buffered serum albumin and 1:128 with nonimmune rabbit serum. To 25 μL of diluted serum was added 10 μL of unlabeled polypeptide. After 1 h at 37 °C, 7.5 ng of ^{125}I-labeled poly(Tyr-Ala-Glu-Gly) in 20 μL of buffered serum albumin was added. After 1 h at 37 °C, pretitered goat anti-rabbit serum (500 μL) was added and the mixture was incubated 2 h at 37 °C, and 30 min at 4 °C. The samples were centrifuged and aliquots of the supernatant counted. The control (C) containing no inhibitor (10 μL of buffer) was done in triplicate. Percent inhibition was obtained from values of (sample-C)/(B-C) × 100.

Results

Synthesis of Poly(Tyr-Ala-Glu-Gly). The synthesis of the monomer, Boc-*O*-Bzl-Tyr-Ala-γ-Bzl-Glu-Gly-OH, was effected by the stepwise addition of the *N*-hydroxysuccinimide esters of the amino acid to an amino acid or peptide unprotected at the α-amine and α-carboxylic acid (Figure 1). The monomer was esterified with pentachlorophenol and the amino protecting group was removed with HCl. Polymerization was as a dispersion in benzene. After deprotection in HBr, the dialyzed fraction of poly(Tyr-Ala-Glu-Gly) had a sedimentation coefficient of 1.7 S and an intrinsic viscosity of 0.244 dL/g. This corresponds to a molecular weight of 33 000 using the method of Scheraga and Mandelkern (1953).

Circular Dichroism Studies. The CD spectrum of poly(Tyr-Ala-Glu-Gly) of molecular weight 33 000 in the NaF buffer at pH 7.2 is shown in Figure 2. At 278 nm there was a minimum of −460 deg cm^2 dmol^{-1}. There was also a shoulder at 283 nm. The spectrum crossed zero at 255 nm and reached a maximum at 242 nm of +260 deg cm^2 dmol^{-1}. It crossed zero again at 238 nm and attained a minimum at 220 nm of −18 000 deg cm^2 dmol^{-1}. At pH 11.8, the CD spectrum of poly(Tyr-Ala-Glu-Gly) was quite different, having only a single maximum at 242 nm of +2000 deg cm^2 dmol^{-1}. The monomer, Tyr-Ala-Glu-Gly, had two small maxima at pH 7.2, one at 275 nm and one at 225 nm.

Immunological Studies. Four New Zealand white rabbits were immunized and boosted with a total of 3 mg of poly(Tyr-Ala-Glu-Gly). Only two of the four (R-1374 and R-1375) yielded antibody responses as measured by the precipitin reaction or with a radioimmunoassay. Both R-1374 and R-1375 had antibody levels as high as 1.2 mg/mL. In the radioimmunoassay, the same bleedings showed about 60% binding of radiolabeled antigen (8 ng) at 1:500 dilution. Sera of high antigen-binding capacities from each rabbit were pooled. The binding of a constant amount (7.5 ng) of ^{125}I-labeled poly(Tyr-Ala-Glu-Gly) by serial dilutions of pooled R-1374 sera is shown in Figure 3. Binding of almost 95% was achieved at a 1:8 dilution of sera. Approximately half of this binding occurred at a 1:128 dilution.

Antibody specificities of R-1374 and R-1375 sera were probed with radioiodinated polypeptides of similar amino acid composition. Both poly(Tyr-Glu-Ala-Gly) and poly(Phe-Ala-Glu-Gly) (Tyr)$^{0.8}$ were recognized by these sera. Binding of the former polypeptide to these antisera ranged from 50 to 75% at a 1:4 dilution (Figure 3). The degree of binding of poly(Tyr-Glu-Ala-Gly) dropped as rapidly with serial dilution as did that of the homologous polymer, poly(Tyr-Ala-Glu-Gly). Binding of poly(Phe-Ala-Glu-Gly) (Tyr)$^{0.8}$, on the other hand, never reached 35%. Nevertheless, this binding was fairly constant until those dilutions (>1:64) where binding of the homologous polymer to the sera also decreased. No significant cross-reactions to R-1374 and R-1375 sera occurred with the sequential polypeptide, poly(Tyr-Ala-Glu), nor with the random sequence polypeptides, poly(Glu^{50}Tyr^{50}), poly(Glu^{60}Ala^{40}) (Tyr$^{0.8}$) and poly(Glu^{60}Ala^{30}Tyr^{10}).

The antibody specificities were also probed with unlabeled polypeptides by inhibiting the binding of 7.5 ng of ^{125}I-labeled poly(Tyr-Ala-Glu-Gly) to a 1:128 dilution of R-1374 sera (Figure 4). The homologous polymer, poly(Tyr-Ala-Glu-Gly), and poly(Phe-Ala-Glu-Gly) were very potent inhibitors at low concentrations (1-5 ng), where inhibition was below 70%. At concentrations greater than 5 ng, poly(Tyr-Ala-Glu-Gly) consistently gave about 15% more inhibition than poly(Phe-Ala-Glu-Gly). By contrast, poly(Tyr-Glu-Ala-Gly) gave no significant inhibition (<5%) at concentrations ranging from 7.5 ng to 7.5 μg.

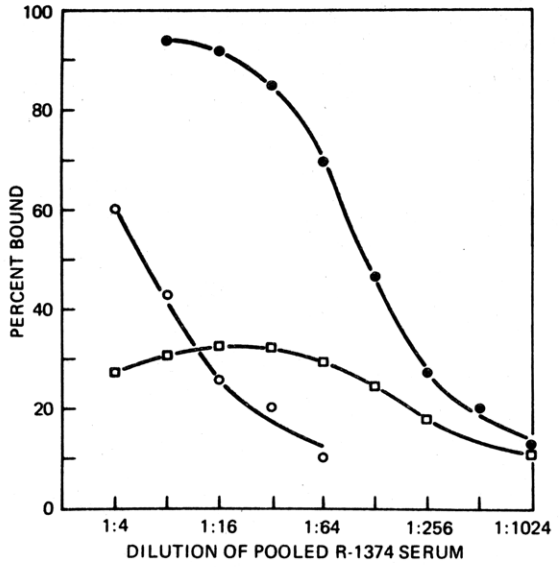

FIGURE 3: Direct binding of R-1374 serum with ^{125}I-labeled polypeptides. Serial dilutions of R-1374 serum with nonimmune rabbit serum (25 μL) were incubated with 7.5 ng of ^{125}I-labeled polypeptides (25 μL) at 37 °C for 1 h, followed by precipitation with 500 μL of pretitered goat anti-rabbit serum at 37 °C for 2 h and at 4 °C for 30 min. (●) Poly(Tyr-Ala-Glu-Gly); (○) poly(Tyr-Glu-Ala-Gly); (□) poly(Phe-Ala-Glu-Gly). Rabbit R-1375 serum yielded similar profiles.

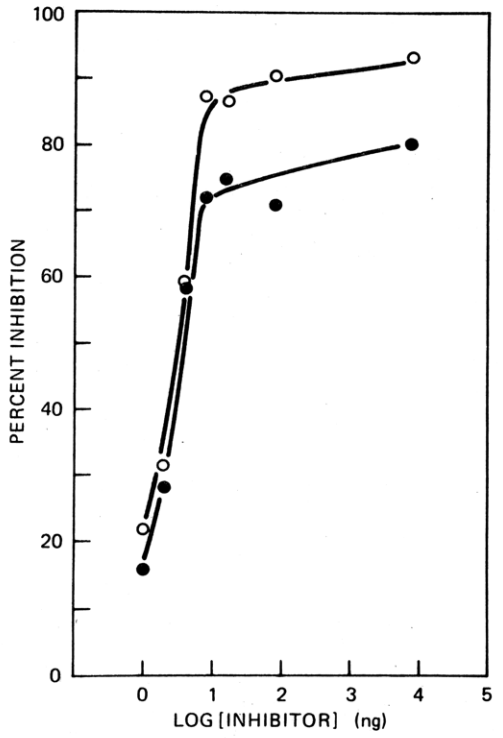

FIGURE 4: Inhibition of the binding of ^{125}I-labeled poly(Tyr-Ala-Glu-Gly) to R-1374 serum. Dilutions of unlabeled polypeptide (10 μL) were added to R-1374 serum (25 μL), which had been diluted 1:2 with buffered serum albumin and 1:128 with nonimmune rabbit serum. After 1 h at 37 °C, 7.5 ng of ^{125}I-labeled poly(Tyr-Ala-Glu-Gly) (20 μL) was added. After 1 h at 37 °C; pretitered goat anti-rabbit serum (500 μL) was added and the mixture was incubated 2 h at 37 °C and 30 min at 4 °C: (○) poly(Tyr-Ala-Glu-Gly); (●) poly(Phe-Ala-Glu-Gly).

Discussion

The synthesis of poly(Tyr-Ala-Glu-Gly) using established procedures proceeded smoothly. Intermediates were of high purity and were generally obtained in good yield. The overall yield of the polymer fraction used in these studies from compound IV was 48%, which is lower than the 71% yield obtained from the monomer used in the polymerization of poly(Tyr-Glu-Ala-Gly) (Zeiger et al., 1975), but which still is high enough to accumulate sufficient material for the structural and immunological studies reported here.

The CD spectrum of the deprotected dialyzed fraction in buffer at physiological pH was virtually identical with that of poly(Tyr-Glu-Ala-Gly) (Zeiger et al., 1975) and markedly similar to that of poly(Tyr-Ala-Glu) (Schechter et al., 1971a), both polymers which have been characterized as α helices. If poly(Tyr-Ala-Glu-Gly) is in an α helix at physiological pH, it may be expected that, at basic pH, where both Glu and Tyr are ionized, charge repulsion may result in a helix-to-coil transition. In fact, the two major CD minima found in poly(Tyr-Ala-Glu-Gly) at pH 7.2 were not found at pH 11.8. Also consistent with the assignment of helical structure to poly(Tyr-Ala-Glu-Gly) at pH 7.2 is the vastly different CD spectrum of the monomer from that of the polymer. The former spectrum is remarkably similar to what has been observed for the oligopeptides, Tyr-Ala-Glu (Schechter et al., 1971a) and Tyr-Glu-Ala-Gly (Zeiger et al., 1975). Thus, it is apparent that, at physiological pH, the tyrosine residues in poly(Tyr-Ala-Glu-Gly) are in a similar environment to those in the α-helical polypeptides, poly(Tyr-Glu-Ala-Gly) and poly(Tyr-Ala-Glu).

A space filling model (Cortauld Atomic Models) of poly(Tyr-Ala-Glu-Gly) in an α-helical configuration has been made (Figure 5). A comparison with the model assembled for poly(Tyr-Glu-Ala-Gly) (Zeiger et al., 1975) shows points of similarity and dissimilarity. Both polymers have a given amino acid repeating every four residues, giving essentially a "peppermint stripe" effect; i.e., each amino acid side chain independent of its neighbors winds around the helix axis in a right-handed super helix. The spatial relationship between residues of a given amino acid, such as tyrosine, is very similar in the two. The spatial relationship between adjacent different amino acids, however, is dependent on the amino acid sequence differences between the two polypeptides. The Gly-Tyr sequence is common to both polytetrapeptides, whereas the other three dipeptide sequences are different. As reported by Ramachandran et al. (1971), the tyrosine residues in poly(Tyr-Ala-Gly) wind in a left-handed superhelix. Thus, the spatial relationship between the residues of a given amino acid in this polytripeptide is different from that found in the polytetrapeptides. However, poly(Tyr-Ala-Glu) and poly(Tyr-Ala-Glu-Gly) do have two dipeptides and a tripeptide sequence in common.

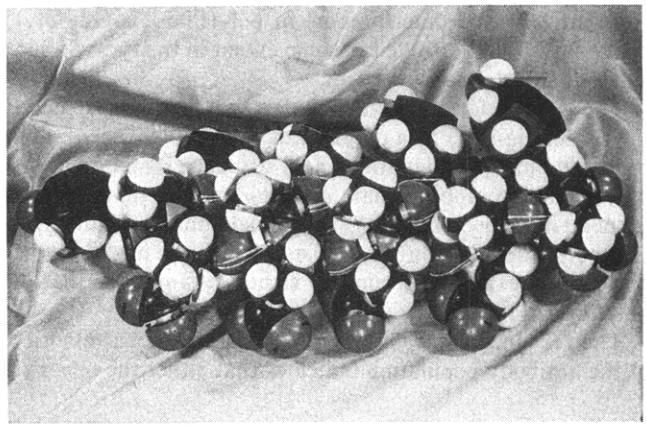

FIGURE 5: A space-filling model of poly(Tyr-Ala-Glu-Gly), showing the side chains of tyrosine, alanine, and glutamic acid.

Only two out of four outbred rabbits responded to poly(Tyr-Ala-Glu-Gly). This suggests that the immune responsiveness in rabbits to this antigen is under genetic control. Genetic control to poly(Tyr-Ala-Glu-Gly) has already been shown in inbred guinea pigs (Zeiger and Maurer, 1976) and mice (Merryman et al., 1977).

The specificity of antibodies produced by R-1374 and R-1375 in response to poly(Tyr-Ala-Glu-Gly) was probed by direct binding studies and inhibition studies using related polypeptides. The binding of poly(Tyr-Glu-Ala-Gly) appeared to be limited to a minor antibody population. The dilution binding profile was similar to that of the homologous antigen, although the midpoint was at a 1:8 dilution rather than 1:128. If the binding affinities of the two polypeptides were similar, this would represent a cross-reacting antibody population of 6-7%. The inhibition data support the presence of a minor antibody population recognizing poly(Tyr-Glu-Ala-Gly) in that the latter was not able to inhibit significantly the antibody–homologous antigen reaction even at a 1000-fold excess. It is of interest that the majority of antibodies were able to distinguish between these closely related polypeptides, whereas CD, which has often been utilized as a probe of structure, could not be used in this way.

The binding of poly(Phe-Ala-Glu-Gly) by R-1374 and R-1375 was significantly different from that of poly(Tyr-Glu-Ala-Gly). Direct binding studies indicated that radiolabeled poly(Phe-Ala-Glu-Gly) $(Tyr)^{0.8}$ was not bound as well by the antibodies as the homologous polymer. However, inhibition by the former polypeptide was very effective up to levels of 70–75%, indicating that the majority of the antibody population was able to recognize poly(Phe-Ala-Glu-Gly). The poorer affinity of the antibodies for poly(Phe-Ala-Glu-Gly) could be due to some specificity of the antibodies for the phenolic hydroxyl moieties present in the homologous antigen. Alternatively, the determinants in poly(Phe-Ala-Glu-Gly) could have been destroyed to some extent in the tyrosylation and iodination procedures.

The recognition of poly(Phe-Ala-Glu-Gly) but not poly(Tyr-Glu-Ala-Gly) by a majority of the antibody population probably indicates that amino acid sequence is an important part of the immunodominant determinant and that tyrosine is not an immunodominant residue. In addition, the data imply that poly(Phe-Ala-Glu-Gly) exists or might be forced by the antibody to exist in a secondary structure similar to that of poly(Tyr-Ala-Glu-Gly).

The recognition of poly(Tyr-Glu-Ala-Gly) by a minority of the antibody population indicates that either the Gly-Tyr sequence or a particular "peppermint stripe" is recognized by these antibodies. Further studies are continuing to determine the specificities of these classes of antibody at the molecular level.

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