(c 0.5, 1 N AcOH). *Anal.* ($C_{47}H_{67}N_{13}O_{12}S_2 \cdot C_2H_4O_2 \cdot H_2O$) C, H, N. A sample was hydrolyzed in 6 N HCl at 110° for 24 hr and analyzed in the Beckman-Spinco amino acid analyzer.[24] The following molar ratios were found: Asp 1.1, Glu 1.1, Pro 1.1, Gly 1.0, Cys 2.0, Phe 1.0, Tyr 1.0, Lys 1.0, NH_3 1.9, and $MeNH_2$ 1.1.

Z-Cys(Bzl)-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-NMe$_2$. The nonapeptide resin I (2.0 g) was suspended in 10 ml of anhyd MeOH and about 20 ml of dry Me_2NH was condensed into the suspension at −70°. The reaction mixt was stirred in a sealed flask at room temp for 24 hr. Further treatment of the reaction mixt was analogous to that described for the monomethyl analog, except that the pptn from AcOH-hot EtOH was repeated a second time; yield 320 mg, mp 204-208°, $[\alpha]^{23}$D −40° (c 0.6, AcOH). *Anal.* ($C_{84}H_{101}N_{13}O_{16}S_3$) C, H, N.

Lysine-vasopressinoic Acid Dimethylamide. Z-Cys(Bzl)-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-N(Me)$_2$ (104 mg) was reduced with Na in NH_3, the resulting disulfhydryl compd was oxidized, and the product was desalted as described above to yield 67.4 mg of crude product. Chromatog on IRC-50 in 0.5 M NH_4OAc as described above, followed by development of Folin-Lowry color, revealed a major peak centered at fraction 21. The fractions corresponding to the center portion of this peak were pooled, desalted, and lyophilized to yield 28.5 mg of purified lysine-vasopressinoic acid dimethylamide; $[\alpha]^{25}$D −28.2° (c 0.5, 1 N AcOH). *Anal.* ($C_{48}H_{69}N_{13}O_{12}S_2 \cdot C_2H_4O_2 \cdot 2.5H_2O$) C, H, N. A sample was hydrolyzed in 6 N HCl at 110° for 24 hr and analyzed in the Beckman-Spinco amino acid analyzer. The following molar ratios were found: Asp 1.1, Glu 1.1, Pro 0.9, Gly 1.0, Cys 1.7, Phe 1.0, Tyr 1.0, Lys 1.0, and NH_3 1.9. The Me_2NH content of the hydrolysate was detd by chromatog analysis of dinitrophenyl derivs according to a method described earlier.[6] By this method Me_2NH was found to be present in the hydrolysate in a molar ratio of 1.0 mole of Me_2NH per mole of analog.

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Immunochemical Studies on Linear Antigenic Polypeptides with a Known Primary Structure. Specificity of Antibodies to Poly(L-tyrosyl-L-glutamyl-L-alanylglycyl)glycine-*1*-^{14}C Ethyl Ester†

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The synthesis of poly(Trp-Glu-Ala-Gly)Gly Me ester is described. The specificity of antibodies formed by rabbits in response to the antigenic challenge of poly(Tyr-Glu-Ala-Gly)Gly-*1*-^{14}C Et ester (**1**) has been studied with respect to the role of the tyrosyl residue. For this purpose a series of polypeptides was used in which the tyrosyl residue was replaced by one of the following: OMe-Tyr, Phe, Trp, and Ala. Using these polypeptides and antisera to **1** it has been found by cross-reaction studies that all of these polymers have the same conformation. Further, absorption studies have shown that antibodies to the antigen **1** possess specificities for the phenolic OH and also for the aromatic system of the tyrosyl residue.

The specificity of antibody formed in response to an antigen is dependent on both the antigenic determinants present in the antigen and also on the genetic capacity of the animal to differentiate between and respond to those determinants. Neither the mechanisms of specific antigen recognition nor the nature of its genetic control is well understood. With respect to antibody specificity we have been studying the antigen poly(Tyr-Glu-Ala-Gly)Gly-*1*-^{14}C Et ester[1-3] (**1**). It has been found that antibody to this antigen is dependent on the conformation of the antigen.[4-7] The next phase of this work has been to study the specificity of these antibodies pertaining to the role of the tyrosyl residue. In this paper we wish to report the characterization of the specificity of antisera produced by rabbits against the antigen **1** as studied by cross-reactions and absorption studies. For this purpose the following polymers have been used: poly(OMe-Tyr-Glu-Ala-Gly)Gly-*1*-^{14}C Et ester[8] (**2**), poly(Phe-Glu-Ala-Gly)Gly-*1*-^{14}C Et ester[9] (**3**), poly(Ala-Glu-Ala-Gly)Gly-*1*-^{14}C Et ester[5] (**4**), and poly(Trp-Glu-Ala-Gly)Gly Me ester (**5**).

Chemistry. The synthesis of polymers **1**, **2**, **3**, and **4** have been reported.[4,5,8,9] The polymerizing unit Trp-*tert*-Bu-Glu-Ala-Gly pentachlorophenyl ester·HCl (**6**) and the necessary intermediates for its preparation were synthesized as detailed in the Experimental Section. The polymerization was performed at a reagent concentration of 100 mmoles/l. in the presence of a preformed monomer since this has been

†All amino acids are of the L variety.

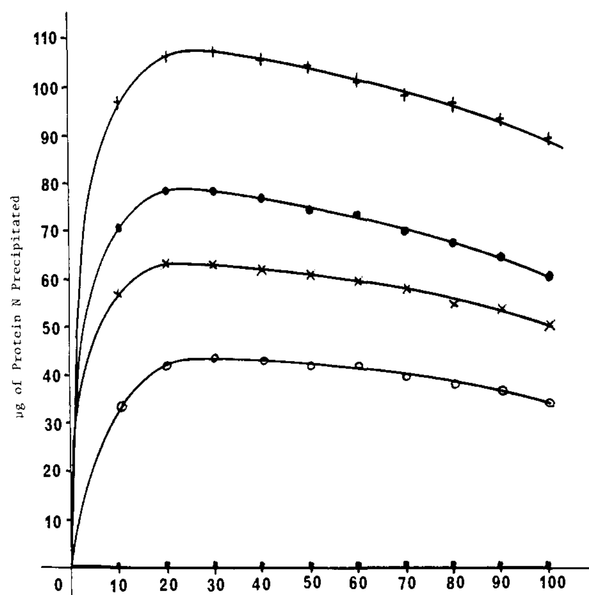

Figure 1. Micrograms of polypeptide per milliliter of anti-poly(Tyr-Glu-Ala-Gly)$_n$Gly-serum where + = poly(Tyr-Glu-Ala-Gly)$_n$Gly (1), • = poly(Ome-Tyr-Glu-Ala-Gly)$_n$Gly (2), x = poly(Phe-Glu-Ala-Gly)$_n$Gly (3), ○ = poly(Trp-Glu-Ala-Gly)$_n$Gly (5), ▪ = poly(Ala-Glu-Ala-Gly)$_n$Gly (4).

Table I

Polypeptide	μg of protein N pptd at equiv pt[a]	μg of protein N pptd by 1 after absorption[a]	% of protein N pptd by polypeptide[a]	% of inhibition of pptn reaction with 1[a]
(Tyr-Glu-Ala-Gly)$_n$Gly (1)	105	0	100	NA[c]
(Ome-Tyr-Glu-Ala-Gly)$_n$Gly (2)	77	28	73	NA
(Phe-Glu-Ala-Gly)$_n$Gly (3)	61	45	57	NA
(Trp-Glu-Ala-Gly)$_n$Gly (5)	42	64	40	NA
(Ala-Glu-Ala-Gly)$_n$Gly (4)	0	53[b]	0	50[b]

[a]per ml of anti-poly(Tyr-Glu-Ala-Gly)Gly-*1*-^{14}C ester-sera. [b]in the presence of 750 μg of the polypeptide 4. [c]NA = not applicable.

shown to produce linear high molecular weight polypeptides.[1,2,4-11] Following this established procedure, the insoluble polymer poly(Trp-*tert*-Bu-Glu-Ala-Gly)Gly Me ester was prepared; from which the protecting *tert*-Bu groups were removed by use of 90% CF_3CO_2H to yield poly(Trp-Glu-Ala-Gly)Gly Me ester (5). After extensive dialysis, the polymer was purified and fractionated by passage through a calibrated column of Sephadex G-50.

Immunochemistry. Eight rabbits were immunized against poly(Tyr-Glu-Ala-Gly)Gly-*1*-^{14}C Et ester (1) using the previously reported protocol.[3] It was found that each serum gave a positive precipitin reaction with the homologous polymer 1. The serum from each animal was pooled, it being assumed that in this time interval that each rabbit had responded to the same antigenic determinants.[12]

In separate experiments incremental amounts of each of the polypeptides 1, 2, 3, 4, and 5 were added to 1-ml aliquots of this pooled antisera. It was observed that four of the polypeptides, 1, 2, 3, and 5, cross-reacted with this serum. The relative precipitin curves are shown in Figure 1. Although the polypeptide 4 does not cross-react with antiserum to 1, it does inhibit the precipitin reaction between this antiserum and its homologous antigen such that 50% inhibition of the precipitin reaction is observed in the presence of 750 μg of 4.[5] It was noted that none of the heterologous polypeptides, 2, 3, and 5, precipitated as much antibody as the homologous polypeptide 1. In order to quantitate the amount of antibody not precipitated by these heterologous polypeptides a separate series of experiments was performed. Quantities equal to the equiv point amounts of each of the heterologous polypeptides were reacted with the pooled sera. After removal of the ppts, 30 μg of the homologous polypeptide 1 was added to the resulting supernatant liq. Further pptn was obtained in each case and quantitated by analysis for N (Kjeldahl). These results are illustrated in Table I.

Conclusions

Our results suggest that all of the heterologous polypeptides, 2, 3, 4, and 5, have the same conformation as the antigen 1. All of these polymers either cross-react with anti-1-sera of inhibit its precipin reaction. From this conclusion it has been assumed that the determinants of these heterologous polypeptides are in the same orientation as those of the antigen 1. Thus the observed differences in abilities of these heterologous polypeptides to bind with antibody to 1 are due only to the modifications of the Tyr residue. Using this rationale it has been concluded that the antibody-combining site shows a gradation of binding ability in the diminishing order: Tyr > OMe-Tyr > Phe > Trp > Ala. Thus it would appear that antibody to 1 has a specificity for the phenolic OH groups since the Phe-containing polymer, 3, does not ppt all of the antibody. The modification of Tyr to OMe-Tyr, polymer 2, causes a 27% loss of precipitating ability which could be due to either the steric inability to fit the combining site as the phenolic hydroxyl groups or the presence of at least two different populations of antibodies, one of which is specific for the phenolic OH group. However, both of these polymers, 2 and 3, do precipitate over half of the available antibody. The replacement of the Tyr residue with the Trp moiety, polymer 5, causes a 60% loss of precipitating ability, which is possibly due to the larger steric requirements of the tryptophanyl residue to fit the antibody-combining site. However, it is of interest that there is only a 17% difference in precipitating ability between the Phe- and Trp-containing polymers. This may be due to the abilities of their aromatic residues to fit the antibody-combining site to a similar extent, even though polymer 5 has a lower mol wt than 3. The complete removal of the aromatic moiety from the system by the replacement of the Tyr residue with the Ala moiety, polymer 4, causes a complete loss of precipitating ability with the antisera. The smaller size of the alanyl residue should allow it to fit into the antibody-combining site, however, since no cross-reaction was observed it has been concluded that antibody to poly(Tyr-Glu-Ala-Gly)Gly-*1*-^{14}C Et ester (1) has not only a specificity directed toward the phenolic OH group but also toward the aromatic system of the Tyr residue.

Experimental Section

Melting points were taken with a Mel-Temp apparatus and are uncorrected.

Z-Trp-γ-*tert*-Bu-Glu-Ala-Gly Me Ester (6).‡ To a soln of 5.52 g (0.0145 mole) of γ-*tert*-Bu-Glu-Ala-Gly Me ester·HCl[8] and 1.5 g (0.0149 mole) of Et_3N in 300 ml of CH_2Cl_2 was added 8.5 g (0.0145 mole) of Z-Trp pentachlorophenyl ester. The mixt was stirred over-

‡Z = benzyloxycarbonyl.

night at room temp, and concd, and the product was dissolved in EtOAc, washed with 10% citric acid soln and H_2O, then dried (Na_2SO_4), and concd under reduced pressure to give a solid. This material was chromatographed on a column of Silicar CC-7 using $CHCl_3$–EtOAc (1:1) as eluent, to give the fully blocked tetrapeptide; crystn from EtOAc–hexane yielded 6.5 g (67%): mp 150°, $[\alpha]^{29}D$ −23.0° (*c* 1.5, DMF). *Anal.* ($C_{34}H_{44}N_5O_9$) C, H, N.

Z-Trp-γ-*tert*-Bu-Glu-Ala-Gly (7). To a soln of 6.5 g (0.0098 mole) of **6** in 300 ml of MeOH was added 10 ml of 1 *N* KOH and the soln was stirred for 90 min at room temp and then concd under reduced pressure. The residue was flooded with H_2O, acidified with 10% citric acid soln, and extd into EtOAc. The EtOAc soln was dried (Na_2SO_4) and concd under pressure to give the tetrapeptide free acid, crystn from EtOAc–hexane yielded 6.1 g (95%): mp 93°. $[\alpha]^{29}D$ −16.9 (*c* 2.9, DMF). *Anal.* ($C_{33}H_{42}N_5O_9$) C, H, N.

Z-Trp-γ-*tert*-Bu-Glu-Ala-Gly Pentachlorophenyl Ester (8). To a soln of 6.0 g (0.0092 mole) of the tetrapeptide free acid **7** in 150 ml of DMF was added 2.66 g (0.01 mole) of pentachlorophenol and 4.7 g (0.011 mole) of 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate. The mixt was stirred overnight at room temp and then 400 ml of H_2O was added to it. The ppt was filtered and crystd from MeOH to yield 4 g (45%): mp 170° $[\alpha]^{29}D$ −22.1° (*c* 2.0, DMF). *Anal.* ($C_{39}H_{41}Cl_5N_5O_9$) C, H, N.

Trp-γ-*tert*-Bu-Glu-Ala-Gly Pentachlorophenyl Ester·HCl (9). A fine suspension of 4.0 g (0.0044 mole) of the tetrapeptide active ester **8** and 0.8 g of 10% Pd/C in 200 ml of MeOH was treated with 0.162 g (0.0044 mole) and dry HCl in MeOH, and the suspension was hydrogenated for 2 hr. The reaction mixt was filtered and the filtrate concd. The residue was triturated with Et_2O to give 1.5 g (45%): mp 202°, $[\alpha]^{29}D$ −17.8° (*c* 0.7, DMF). *Anal.* ($C_{31}H_{36}Cl_6N_5O_7$) N.

Poly(Trp-Glu-Ala-Gly)Gly Me Ester (5). To a soln of 0.83 g (0.008 mole) of Et_3N and 1 mg of Gly Me ester·HCl in 5 ml of DMSO was added a soln of 2.2 g (0.00274 mole) of the polymn unit **9** in 22.5 ml of DMSO. The mixt was shaken for 1 week and then centrifuged to yield the fully protected polymer which was washed with three 35-ml portions of H_2O, three 35-ml portions of MeOH, and three 35-ml portions of Et_2O and dried to give 0.62 g (47%) of the blocked polymer. This material was treated with 50 ml of 90% F_3CCO_2H stirred for 50 min, and then concd under reduced pressure to yield the crude polypeptide **5**. This material was dissolved by the addn of 1 *N* NaOH to pH 7.5. The soln was dialyzed against distd H_2O overnight, and then chromatogd on Sephadex G-10 using H_2O as eluent. The high mol wt fraction was collected and dialyzed against distd H_2O and then lyophilized to yield 0.27 g (22%). *Anal.* ($C_{21}H_{24}N_5O_6\cdot Na\cdot H_2O$) C, H, N.

Molecular Weight Determination. A calibrated column of Sephadex G-50 (2.5 × 39.0 cm) was employed for the mol wt detn. Using 0.15 *M* NaCl as eluent, 4 mg of the Na salt of poly(Trp-Glu-Ala-Gly)Gly Me ester was passed through it and the polypeptide was eluted in a vol equiv to that corresponding to a mol wt of 3×10^4.

Immunochemical Procedures. Eight rabbits were treated with poly(Tyr-Glu-Ala-Gly)Gly-*1*-^{14}C Et ester (**1**) at weekly intervals, using the immunization schedule previously described;[3] 25 days after the last injection all rabbits were bled using the std heart puncture technique. Serum from each rabbit was tested for the precipitin reaction with the homologous antigen **1**, serum from each animal gave a positive precipitin reaction. The serum from each animal was pooled and this combined serum was used for the following expts. It was assumed that antibody produced by each rabbit after the same time interval was directed against the same antigenic determinants of poly(Tyr-Glu-Ala-Gly)Gly-*1*-^{14}C Et ester (**1**).

Quantitative Precipitin Reactions. To 1-ml aliquots of the pooled rabbit serum was added incremental amts of the polypeptide **1**. Each tube was made up to a total of 2 ml with buffer (0.1 *M* NaCl–0.05 *M* $NaHCO_3$) and incubated for 1 hr at 37°, and then kept at 4° for 48 hr. The tubes were centrifuged in the cold and the ppts were washed twice with 1 ml of buffer (0.05 *M* K_2HPO_4–NaOH), pH 7.0. The total amount of protein ppt was estd by analysis for N (Kjeldahl). For each of the polypeptides **2**, **3**, **4**, and **5** quant precipitin reactions were performed using the pooled rabbit serum, which were identical with and run simultaneously with that used for the polypeptide **1**. The precipitin curves are shown in the figure.

Absorption Studies. The pooled rabbit serum was treated with quantities equal to the equiv pt amount of the heterologous polypeptides **2**, **3**, and **5**, as described above. The corresponding ppts were centrifuged out and the supernatant liquids were poured off into sep tubes. To each of these supernatant liquids was added 30 μg of the homologous antigen, poly(Tyr-Glu-Ala-Gly)Gly-*1*-^{14}C Et ester (**1**). The tubes were incubated at 37° for 1 hr, and then stood at 4° for 48 hr. The ppts were collected by centrifugation, and washed twice with 1 ml of buffer soln (0.05 *M* K_2HPO_4–NaOH), pH 7.0. The amount of protein ppt was estd by analysis for N (Kjeldahl). The amount of ppts obtained using this procedure are shown in the table. Controls in which the serum was first absorbed with the homologous antigen **1** ascertained that the homologous antigen pptd all of the antibody, since the supernatant liq gave no further precipitin reaction with 30 μg of **1** was added.

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Antimalarials. 1. 2-Quinolinemethanols†

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Ten substituted 2-quinolinemethanols were synthesized and tested against *Plasmodium berghei* in mice. Only α-di-*n*-butylaminomethyl-4-(4-chlorophenyl)-6,8-dichloro-2-quinolinemethanol was active at 640 mg/kg; the other 9 compounds were inactive.

As reported by Wiselogle,[1] the World War II antimalarial program generated 7 unsubstituted α-dialkylaminomethyl-2-quinolinemethanols wherein the amino alcohol group was varied. The synthesis of 4 of these was reported by Campbell and coworkers.[2] None of the 7 compounds possessed a quinine index higher than 0.2.[1] On the other hand, the unsubstituted 4-quinolinemethanols (ref **1**, p 142) were only marginally better, but highly active antimalarials were acquired by placing substituents in various positions in the quinoline nucleus.‡ The impact was most notable with

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‡For World War II data on the 4-quinolinemethanols see ref 2. Recent major references include Boykin, *et al.*,[3] and Saggiomo, *et al.*[4] A complete compilation of data is maintained at the Walter Reed Army Institue of Research, Washington, D. C.