mixt was dild with EtOH, and the ppts were collected by filtration and dried. Recrystn from Me_2CO gave brown powder, mp 220° dec. *Anal.* ($C_{17}N_{13}N_3O_6$) C, H, N.

Linear Polypeptides of a Known Primary Structure. Synthesis and Immunochemical Studies of Poly(*O*-methyl-L-tyrosyl-L-glutamyl-L-alanylglycyl)glycine-*1*-^{14}C Ethyl Ester

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A recent investigation of the immunochemical properties of poly(Tyr-Glu-Ala-Gly)Gly-*1*-^{14}C-OEt,[1,2] has shown that the polypeptide is antigenic, eliciting antibodies in rabbits.[3] In order to ascertain the role of the phenolic OH on the antigenicity of the polypeptide we wish to report the synthesis and immunochemical properties of poly(*O*-Me-Tyr-Glu-Ala-Gly)Gly-*1*-^{14}C-OEt (**1**).

Chemistry.—The polymerizing unit *O*-Me-Tyr-γ-*tert*-Bu-Glu-Ala-Gly-OC_6Cl_5·HCl (**4**) 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 shown to produce linear high molecular weight polypeptides.[1,2,4-9] Following the established procedure the insol polymer, poly(*O*-Me-Tyr-γ-*tert*-Bu-Glu-Ala-Gly)Gly-*1*-^{14}C-OEt was prepared; from which the protecting *tert*-Bu groups were removed by the use of 90% F_3CCO_2H to yield poly(*O*-Me-Tyr-Glu-Ala-Gly)Gly-*1*-^{14}C-OEt (**1**). After extensive dialysis, the polymer was purified and fractionated by passage through calibrated columns of Sephadex G-100[10] and Corning CPG 10-240 glass granules. By this means the mol wt of the polypeptide was found to be at least 1×10^5.

Immunochemistry.—Two rabbits were immunized with poly(*O*-Me-Tyr-Glu-Ala-Gly)Gly-*1*-^{14}C-OEt (**1**), using the same protocol as that previously described.[3] To aliquots of the pooled sera were added incremental amounts of the polypeptide **1**. A precipitin reaction was observed. The total amount of protein pptd was estimated by analysis for N (Kjeldahl). From these results the precipitin curve shown in Figure 1 was obtained. For comparative purposes the precipitin curve for poly(Tyr-Glu-Ala-Gly)Gly-*1*-^{14}C-OEt[3] is also shown.

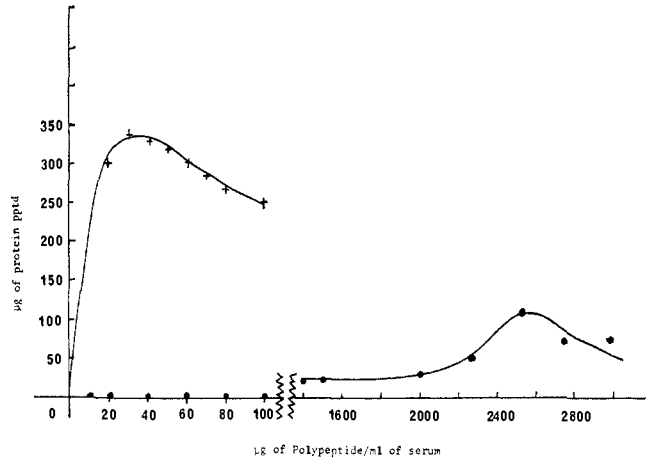

Figure 1.—Precipitin curve: +, poly(Tyr-Glu-Ala-Gly) *vs.* its antisera;[2] ●, poly(*O*-Me-Tyr-Glu-Ala-Gly) *vs.* its antisera.

Conclusions

Conversion of the phenolic OH groups of the tyrosyl residues in the parent antigen, poly(Tyr-Glu-Ala-Gly)-Gly-*1*-^{14}C Et, to their Me ethers still gave a molecule that was antigenic. Thus, it has been concluded that the presence of the phenolic OH groups is not a necessary prerequisite for antibody formation in this system. However, the equivalence points of the precipitin curves of the two polymers are not the same. Thus it would appear that the antigenic determinates of the polypeptide **1** are different from those of the parent antigen, poly(Tyr-Glu-Ala-Gly)Gly-*1*-^{14}C-OEt.

Experimental Section

Melting points were taken with a Mel-Temp apparatus and are uncorrected. Optical rotations were taken with a Carl Zeiss precision polarimeter.

Z-*O*-Me-Tyr-γ-*tert*-Bu-Glu-Ala-Gly Me† (**2**).—To a mixt of 17 g (58 mmoles) of Z-*O*-methyltyrosine in 400 ml of CH_2Cl_2 and 16 g (58 mmoles) of pentachlorophenol was added 13.2 g (64 mmoles) of *N,N*-dicyclohexylcarbodiimide. The soln was stirred at room temp for 12 hr, filtered, and then concd *in vacuo*. The residue was dissolved in EtOAc and washed with H_2O, $NaHCO_3$ soln, and H_2O, then dried (Na_2SO_4) and concd *in vacuo* to give the activated ester, 15 g (45%). To 10.5 g (18.2 mmoles) of this pentachlorophenyl ester in 250 ml of CH_2Cl_2 was added 6.5 g (17 mmoles) of γ-*tert*-Bu-Glu-Ala-Gly-OMe·HCl and 1.75 g (17 mmoles) of Et_3N. The reaction mixt was stirred overnight at room temp and then concd *in vacuo*. The residue was dissolved in EtOAc and washed with H_2O, 10% citric acid soln, and H_2O, then dried (Na_2SO_4), and concd *in vacuo*. The crude product was chromatographed on Silicar-CC-7, eluted with $CHCl_3$ and crystd from EtOAc–hexane to yield 7 g (63%): mp 152–154°; $[\alpha]^{31}D$ −24.4° (*c* 1.04, DMF). *Anal.* ($C_{33}H_{44}N_4O_{10}$) C, H, N.

Z-*O*-Me-Tyr-γ-*tert*-Bu-Glu-Ala-Gly-OC_6Cl_5·HCl (**3**).—A soln of 7 g (10.7 mmoles) of **2** in 250 ml of MeOH was treated with 10.7 ml of 1 *N* NaOH with stirring for 2 hr and then concd under reduced pressure. The residue was flooded with H_2O, acidified with 1 *N* HCl, and extd into EtOAc. The EtOAc soln was dried (Na_2SO_4) and concd under reduced pressure to give the tetrapeptide free acid as an oil. To this material in 200 ml of CH_2Cl_2 was added 2.85 g (10.7 mmoles) of pentachlorophenol and 5.0 g (11.8 mmoles) of 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate. The mixt was shaken for 48 hr at room temp. The solvent was removed *in vacuo* and the residue was washed with H_2O and crystd from MeOH to yield 3.6 g (37%): mp 202–205°; $[\alpha]^{31}D$ −21.2° (*c* 1.09 DMF). *Anal.* ($C_{38}H_{41}Cl_5N_4O_{10}$) C, H, N.

***O*-Me-Tyr-γ-*tert*-Bu-Glu-Ala-Gly-OC_6Cl_5·HCl** (**4**).—To a fine suspension of 3.5 g (3.93 mmoles) of the tetrapeptide pentachlorophenyl ester **3** and 0.5 g of 10% Pd/C in 500 ml of MeOH was added 0.144 g (3.93 mmoles) of dry HCl in MeOH, and the suspension was hydrogenated for 2 hr. The reaction mixt was filtered and the filtrate was concd. The residue was crystd from MeOH–

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(8) B. J. Johnson, *ibid., C*, 1412 (1969).
(9) B. J. Johnson, *ibid., C*, 3008 (1968).
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† Z = benzyloxycarbonyl.

Et_2O to give 2.3 g (77%): mp 185°; $[\alpha]^{31}D$ −0.55° (*c* 0.92, DMF). *Anal.* ($C_{30}H_{36}Cl_6N_4O_5$) C, H, N.

Poly(*O*-Me-Tyr-Glu-Ala-Gly)Gly-*1*-^{14}*C*-OEt (1).—To a soln of 1 mg (7.6 mmoles) of glycine-*1*-^{14}*C*-OEt·HCl (specific activity 3.15 mCi/mmole) and 1.53 g (15.1 mmoles) of Et_3N in 5 ml of DMSO was slowly added a soln of 2.3 g (2.9 mmoles) of the polymerizing unit **4** in 15 ml of DMSO. The transfer vessels were washed with 9.5 ml of DMSO which was added to the reaction mixt giving a final concn of 100 mmoles/l. The reaction mixt was shaken for 6 days and then centrifuged to yield the product which was washed with three 35-ml portions of H_2O and three 35-ml portions of Et_2O and dried to give the blocked polymer. This material was dissolved in 50 ml of 90% F_3CCO_2H and stirred for 1 hr, and then concd under reduced pressure to yield the crude polypeptide 1. This material was washed with Et_2O, suspended in 20 ml of H_2O, and dissolved by the addition of 1 *N* NaOH to pH 7.8. The soln was dialyzed against distilled H_2O for 12 hr, acidified to pH 2.5 with HCl, and dialyzed for 3 days. The pptd polypeptide was collected by centrifugation and then lyophilized to yield 0.3 g (24%): radioassay, 35.2 nCi/mg indicates 47% incorporation of the radioactive label. *Anal.* ($C_{20}H_{26}N_4O_7 \cdot H_2O$) C, H, N.

Mol Wt Determination.—Calibrated columns of Sephadex G-100 (2.5 × 38.5 cm) and of Corning CPG 10-240 glass granules (2.0 × 28 cm) were employed for the mol wt detn. Using 0.1 *M* NaCl–0.05 *M* KH_2PO_4 corrected to pH 8.0 as eluent, 4.2 mg of poly(*O*-Me-Tyr-Glu-Ala-Gly)Gly-*1*-^{14}*C*-OEt was passed through each of these columns. The polypeptide was eluted from each column in a vol equiv to that corresponding to a mol wt of at least 1 × 10^5.

Immunochemical Results.—Two rabbits were treated at weekly intervals with 500 μg of poly(*O*-Me-Tyr-Glu-Ala-Gly)Gly-*1*-^{14}*C*-OEt 1. The first 2 weeks they were injected intradermally using complete Freunds adjuvant as suspension medium and the 3rd week they were injected sc. The injection on the 4th week was done iv using buffered saline. Bleedings were conducted on the following week and the serum from each animal was found to give a precipitin reaction with the antigenic polypeptide 1. The preimmunized sera under the same conditions gave a negative precipitin reaction. The quantitative determination of the total precipitate was obtained by the addition of dils of poly(*O*-Me-Tyr-Glu-Ala-Gly)Gly-*1*-^{14}*C*-OEt (1) to 2-ml samples of the pooled rabbit sera. The samples were incubated at 37° for 1 hr and then stood at 4° for 48 hr. The ppts were collected by centrifugation, washed twice with buffered saline, and collected. The total amount of protein precipitated was estimated by analysis for N (Kjeldahl). From these results the precipitin curve shown in Figure 1 was obtained.

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Analogs of Vitamin B_6 with Reactive Groups[1]

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Pyridoxol analogs that have alkylating groups in appropriate positions may react irreversibly with cofactor sites of appropriate apoenzymes, or, more generally, with any of the receptor sites that are available for the various biologically active forms of vitamin B_6.[2,3] In order to obtain an analog with dimensions comparable to those of the cofactor forms of vitamin B_6 (pyridoxal or pyridoxamine phosphates), we synthesized the chloro ketone V (Scheme I) and have also attempted to obtain the lower homologs of this compd. The latter were considered as potential irreversible inhibitors, primarily for enzymes that have nonphosphorylated forms of vitamin B_6 as substrates.

As the starting materials for the syntheses of the α-chloro ketones, we used the homologous carboxylic acids (I, VI, XIII),[4] which were protected with an α^4,3-*O*-isopropylidene group. The higher homologs (I and VI) could be readily converted to the corresponding acid chlorides by carefully reacting the acids with $SOCl_2$ in C_6H_6, but we could not obtain an acid chloride from the lowest homolog XIII by this procedure. Variations of reaction conditions with different chlorinating reagents did not give the desired result.[5]

Addition of the acid chlorides II and VII to a large excess of CH_2N_2 in Et_2O resulted in the formation of the diazo ketones III and VIII. The higher homolog II also gave an appreciable amt of the α-chloro ketone IV, which was isolated; the mixt of III and IV, however, gave the desired α-chloro ketone V on treatment with HCl, as indicated by a positive Baker's test[6] (a test for active halogen, applied as a tlc spray reagent), and by ir and nmr spectra. The lower homolog VIII, on being similarly treated with HCl, gave a mixt of products, with a negative Baker's test. Treatment of the diazo ketone VIII with ethereal HCl resulted in the formation of the blocked α-chloro ketone IX, as indicated by a positive Baker's tests. Mild hydrolysis of the protecting isopropylidene group gave exactly the same mixt of products as was obtained directly from the diazo ketone VIII by treatment with HCl. The 2 products were separated by preparative tlc, and the main product was shown to be the bicyclic hemiacetal X of the α-chloro ketone, and was further characterized as the diacetyl derivative XII. The minor component of the reaction mixt was probably formed by dehydration of the major compd X, and it was indicated by nmr to be XI (see Experimental Section). The small amt of the sample precluded the further work necessary for making an unequivocal structural assignment.

The α-chloro ketone V was tested as an inhibitor of several enzymes. It did not inhibit apotryptophanase[7,8] at 5 × 10^{-4} *M;* but when the concn was raised to 4 × 10^{-2} *M*, 99% of the enzyme activity was abolished. After being filtered through a Sephadex G-25 column,

(1) Pyridoxine Chemistry. 25. (a) Preceding paper in this series: W. Korytnyk, H. Ahrens, and N. Angelino, *Tetrahedron*, **26**, 5415 (1970); (b) a brief report of this study has appeared: 157th National Meeting of the American Chemical Society, Minneapolis, Minn., April 1969, MEDI 11.

(2) For a review of the synthesis and biological activity of vitamin B_6 analogs see W. Korytnyk and M. Ikawa, *Methods Enzymol.*, **18A**, 524 (1970).

(3) Two vitamin B_6 analogs that have bifunctional alkylating groups in the 5 position have been described earlier and found to have weak antitumor effects: N^6,$N^{6'}$-bis(2-chloroethyl)-O^4-methylisopyridoxamine (methoxypyridoxyl N mustard) and N^6,$N^{6'}$-bis(2-chloroethyl)-4-deoxyisopyridoxamine (4-deoxypyridoxyl N mustard) [C. C. Stock, S. Buckley, K. Suguira, and C. P. Rhoads, *Cancer Res.* **11**, 432 (1951)].

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(5) Attempted preparation of the acid chloride XIV from the acid XIII according to the method of I. Tomita, H. G. Brooks, and D. E. Metzler [*J. Heterocycl. Chem.*, **3**, 178 (1966)], gave the hydrochloride of the starting material XIII. Reaction with oxalyl chloride was also negative. Compare the unsuccessful efforts at obtaining acid chlorides from pyridine carboxylic acids by E. Wenkert, F. Haglid, and S. L. Mueller, *J. Org. Chem.*, **34**, 247 (1969).

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(8) The assay method was that of W. A. Newton and E. E. Snell, *Proc. Nat. Acad. Sci. U. S.*, **48**, 1431 (1962).