WATER-SOLUBLE HELICAL POLYPEPTIDES[1,2]

Sir:

We report here the synthesis and some physical-chemical properties of high molecular weight water-soluble polypeptides capable of assuming either random or helical conformations in aqueous solutions. The new polypeptides are derivatives of poly-α,L-glutamic acid and certain copolymers thereof where the γ-carboxyl group has reacted with hydrophilic amino compounds to form γ amido derivatives. The synthesis makes use of amide-ester interchange.[3] This reaction has been applied to polypeptides[4a] and some rotatory data have been reported recently.[4b] A typical preparation of such a water-soluble polypeptide is given.

Poly-γ-benzyl-α,L-glutamate (($\eta_{sp/c}$) = 2.0, c = 0.2% in dichloroacetic acid; $MW_w \cong 350,000$) 4.38 g. (0.02 mole) was dissolved in 10.4 g. (0.08 mole) of 2-(N-morpholinyl)-ethylamine and heated in an oil bath at 70° for 70 hr. The solution at this state was completely miscible with water and showed no ester absorption in its infrared spectrum. The solution was dissolved in 50 ml. of water, dialyzed for 48 hr. with four changes of water, lyophilized and dried *in vacuo* at 50°; yield 3.6 g. (75%) Calcd. for $(C_{11}H_{19}N_3O_3)_n$· C, 54.74; H, 7.95; N, 17.40. Found: C, 55.05; H, 7.65; N, 17.01. The poly-γ-N-[2-morpholinylethyl]-α-L-glutamamide, I, had an intrinsic viscosity of 0.82 in 0.2 M aqueous NaCl. If the amide-ester interchange is run at higher temperatures or in a sealed tube, lower molecular weight products are obtained. Similar reactions were employed to prepare the copolypeptides described below. It has been found that, using the above procedure, poly-γ-methyl-α,L-glutamate also undergoes aminolysis to yield I. I is completely soluble in water from pH 1.5 to 13 and is also soluble in various ratios of methyl alcohol or dioxane and water. Lower molecular weight preparations of I are soluble in methyl alcohol alone.

Measurements of the rotatory dispersion of I in various methanol–water solutions have been made over the wave length region 313–578 mμ and the data calculated using the Moffitt equation,[5] from which b_0 values have been obtained. A plot of these b_0 values as a function of solvent composition is shown in Fig. 1. Assuming that the magnitude of b_0 in the range 0 to ~ 630 indicates the percentage helix present in solution,[6,7] it is apparent that I undergoes a transformation from a random form in water solution to a helical conformation in water solutions containing more than about 50% of methanol. When I was dissolved in a water solution containing 2 M NaCl, it showed a b_0 of −180 indicating a helix content of ~30%. Measurement of the short wave length optical rotatory dispersion of I in 95% methanolic solution showed the negative 225 mμ Cotton effect[8] and the positive 190 mμ Cotton effect[9] characteristic of the α-helical conformation. The infrared spectra of oriented films of I, obtained from either water or methanol–water solutions, showed infrared dichroism characteristic of the α-helical conformation.[10]

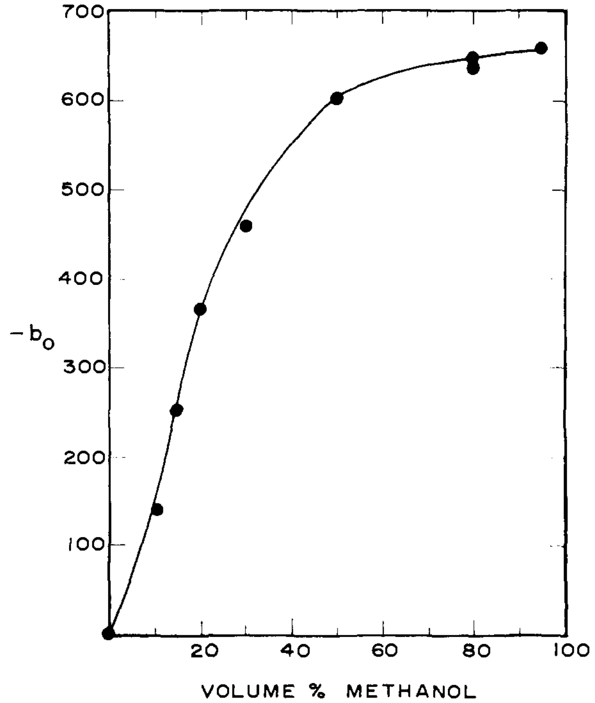

Fig. 1.—The dependence of b_0 (calculated from the Moffitt equation[5] over the wave length range 313 to 578 mμ using λ_0 = 212 mμ) upon the solvent composition for water–methanol solutions of poly-γ-N-[2-morpholinylethyl]-α,L-glutamamide.

Since, until this time, there has been no reported preparation of a synthetic high molecular weight non-ionic water-soluble polypeptide containing only one optical isomer and having an α-helical conformation in water, it was of some interest to see whether helix formation in water solution could be obtained through the preparation of suitable copolypeptides of I. Copolypeptides of γ-benzyl-L-glutamate with L-alanine, L-leucine and L-methionine in various mole ratios were prepared and treated in the manner described above to replace the γ-ester group by 2-morpholinylethylamine. Those containing up to 10 mole per cent. leucine, or up to 40 mole per

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[1] This is Polypeptides. XXXIX. For the preceding paper in this series see ref. 9.

[2] This work was supported in part by the Office of the Surgeon General, Department of the Army.

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[9] E. R. Blout, I. Schmier and N. S. Simmons, *J. Am. Chem. Soc.*, **84**, 3193 (1962).

[10] See for example C. H. Bamford, A. Elliott and W. E. Hanby in "Synthetic Polypeptides," Academic Press, Inc., New York, N. Y., 1956, p. 147, and T. Miyazawa and E. R. Blout, *J. Am. Chem. Soc.*, **83**, 712 (1961).

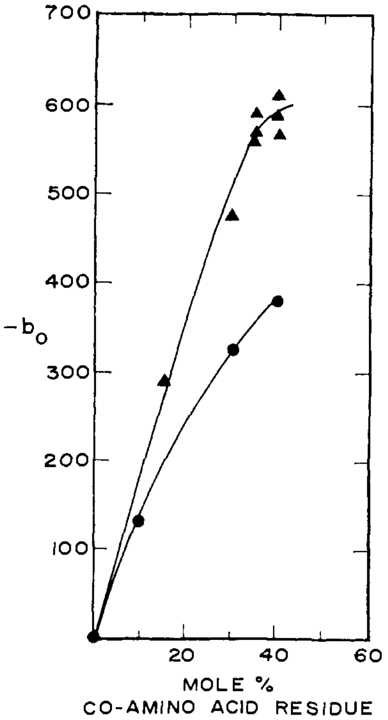

Fig. 2.—The dependence of b_0 (calculated from the Moffitt equation[5] over the wave length range 313 to 578 mμ using λ_0 = 212 mμ) upon amino acid composition for a series of high molecular weight copolypeptides derived from γ-N-[2-morpholinylethyl]-α,L-glutamamide and L-alanine, ●-●-●; poly-γ-N-[2-morpholinylethyl]-α,L-glutamamide and L-methionine, ▲-▲-▲. The solvent in all cases was water containing 0.2 *M* NaCl. All measurements were performed at 25 ± 1° except those on the copolypeptides containing 30, 35 and 40% L-methionine with which compounds the measurements were made at 5 ± 1°.

cent. alanine or methionine were water-soluble. Some rotatory dispersion data of the water-soluble copolypeptides are shown in Fig. 2, where b_0 is plotted as a function of mole per cent. coamino acid residue content. The solubility of the copolypeptides containing 30 or more per cent. L-methionine is increased at lower temperatures so the dispersion measurements on these compounds were made at 5°. The data reveal that, by incorporation of ~40% L-methionine in I, it is possible to obtain an essentially completely helical water-soluble synthetic polypeptide.

Other experiment were performed with the copolypeptide containing 30 mole percent L-alanine which, in 0.2 *M* NaCl, had a b_0 value of −331 indicating slightly more than 50% helix content. When this copolypeptide's rotatory dispersion was measured in 8 *M* urea or in 8.6 *M* lithium bromide solution, the b_0 value was found to be zero.

From the experiments and the data reported here we can conclude the following. The helical form of poly-γ-N-[2-morpholinylethyl]-α,L-glutamamide is not stable in water solution but this polypeptide may be converted to a helical conformation by changing to less polar solvents such as methanol or dioxane. In certain water soluble polypeptides L-methionine residues are more effective in promoting helix formation in aqueous solution than L-alanine residues. Also, it is clear that polypeptide helix formation in water solution depends not only on the formation of intramolecular peptide hydrogen bonds but also is aided by "hydrophobic bond" and dispersion force stabilizations[11] provided by residues such as methionine and alanine. Further, these new water-soluble polypeptides having high helix contents, can be transformed by reagents known to denature proteins into completely random conformations.

(11) For a discussion see W. Kauzmann in "Advances in Protein Chemistry," Vol. 14, C. B. Anfinsen, Jr., M. L. Anson, K. Bailey and J. T. Edsall, Editors, Academic Press, Inc., New York, N. Y., 1959.

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FLUORINE N.M.R. SPECTROSCOPY. XII. PROOF OF OPPOSITE SIGNS FOR THE "DIRECT" CARBON-13 COUPLING CONSTANTS TO HYDROGEN AND TO FLUORINE

Sir:

The use of "spin decoupling" (double resonance)[1] for the determination of relative signs of coupling constants in fluorocarbon derivatives[2-4] and in proton compounds[5,6] has become important as an experimental check on theoretical predictions. The work of Lauterbur and Kurland[6] was particularly significant, since it was a test of the proposal[7] that "direct" couplings of C^{13} to protons have the same sign as vicinal proton–proton couplings.

The predictions of signs and magnitudes of couplings involving fluorine, though less certain than those for protons, nevertheless represent a further development of the theory. It is of prime importance to test the supposition that the "direct" couplings, $J(C^{13}F)$ and $J(C^{13}H)$, have the same sign. This has now been done for the particularly simple case of CHCl$_2$F, dichlorofluoromethane.

Experimental.—The basic n.m.r. spectrometer and techniques were as previously described.[8,9] For the double resonance work a Model SD-60 Spin Decoupler[10] was employed. It was equipped with modules designed for strong irradiation of fluorine nuclei at 37.65 mc./sec., while observing protons at 40.000 mc./sec. (symbolized as H{F} decoupling[1]) and for F{H} decoupling with irradiation at 42.50 mc./sec. With this equipment some ten watts of radiofrequency power is available to the probe; however, far less than this is required since the F–H coupling constant in CHCl$_2$F is

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(10) Manufactured by the Nuclear Magnetic Resonance Specialties Co., Inc., Box 145, Greensburg Road, New Kensington, Pa.