Table II

	$[\alpha]_D$ (H_2O), deg
H-Gly-L-Pro-L-Ala-OH·0.52H_2O	−140
H(-Gly-L-Pro-L-Ala-)$_2$OH	−200
H(-Gly-L-Pro-L-Ala-)$_4$OH·4H_2O	−234
H(-Gly-L-Pro-L-Ala-)$_n$OH	−206

The specific rotations of the Gly-L-Pro-L-Ala oligomers are considerably more negative than those of the analogous Gly-L-Pro-Gly peptides reflecting the substantial rotatory contribution of the L-alanyl residue. If the prolyl residue contribution is taken as that observed with the Gly-L-Pro-Gly peptides then from the above data, in theory, one should be able to obtain the alanyl residue contribution. Such calculations have been made and indicate either that the alanyl residue contribution is greater than that generally assumed for a random polypeptide chain ($\sim -110°$)[25] *or* that there are residue–residue interactions which substantially affect the rotations.

(25) P. Urnes and P. Doty, *Advan. Protein Chem.*, **16**, 401 (1961).

On the Structure of Gly-Pro-Gly and Gly-Pro-Ala Oligopeptides and Sequential Polypeptides[1-3]

P. J. Oriel[4] and E. R. Blout

Contribution from the Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts 02115. *Received January 20, 1966*

Abstract: Physical–chemical studies of the chain regularity and interaction of Gly-L-Pro-X oligopeptides and sequential polypeptides are described. It is shown that association of single chains of Gly-L-Pro-Gly polymer stabilizes the formation of partly helical structures, whereas Gly-L-Pro-L-Ala polymers do not show regular structure in solution.

Conformational studies of single-chain synthetic homopolypeptides and amino acid copolymers have helped to elucidate the influences of hydrogen bonding, side-chain steric hindrance, electrostatic interactions, and other short-range interactions on the formation of ordered polypeptide chain structures in solution. Many of these same forces serve to stabilize protein structures, but additional stabilization of structure in proteins appears to come from disulfide bonds and specific short-range interactions which frequently occur on widely separated parts of the peptide chains or on altogether different chains. Since the studies on polypeptides heretofore have been for the most part confined to homopolyamino acids or random copolyamino acids, it is of considerable interest to examine polypeptide models in which the amino acid contents and sequences more closely approximate those found in proteins.

In this communication, we describe conformational studies of ordered sequences of oligomers and polymers of (glycyl-L-prolyl-X)$_n$, where X is glycine or alanine. These sequences were selected to serve as models of collagen since collagen contains approximately 30–35% glycine, 20–25% of imino acids (L-proline and L-hydroxyproline), and approximately 10% L-alanine.[5] Collagen proteins apparently have large portions of the sequence Gly-L-Pro-X,[6] although X is in many cases an amino acid other than glycine or alanine or an imino acid. It will be shown in this communication that the oligomers and polymers investigated appear to have no structural regularity as single chains, but upon association with other chains form structures resembling collagen in many respects. In addition, the choice of the third amino acid in the trimer sequence has a marked influence on the ability of the polymer to associate to regular structures.

Experimental Section

Materials. The syntheses of the oligomers and polymers used in this study are described in the accompanying paper.[2] All optically active amino acids used in the syntheses were the L isomers. All oligomers and the Gly-Pro-Ala polymer were water soluble. Solutions of these materials were made by dissolving the lyophilized material directly in distilled water. For the Gly-Pro-Gly polymer which is acid soluble, a high concentration of acid was used for initial solvation with subsequent dilution with distilled water. Concentrations were calculated from the weight of material used and were checked for internal consistency with ultraviolet spectra using the 190–195-mμ maxima. The dichloroacetic acid (DCA) used was freshly distilled. Other solvents used were reagent or spectral grade.

Optical Rotatory Dispersion. Optical rotatory dispersion measurements were made with a Cary 60 recording spectropolarimeter. In most measurements dispersions were taken using Opticell cuvettes with optical path lengths from 0.1 mm to 5 cm. The temperature of the cell compartment was 25–28°. Temperature variation and kinetic studies utilized jacketed Opticell cuvettes. The temperature of the solution was measured by means of a calibrated thermistor probe. Refractive index corrections were not made because of the paucity of refractive index dispersion data for mixed solvents at short wavelengths.

(1) This is Polypeptides LI. For the previous paper in this series see ref 2.

(2) S. M. Bloom, S. K. Dasgupta, R. P. Patel, and E. R. Blout, *J. Am. Chem. Soc.*, **88**, 2035 (1966).

(3) This work has been supported in part by the Office of the Surgeon General, Department of the Army.

(4) Postdoctoral Research Fellow of the Public Health Service.

(5) K. A. Piez and J. Gross, *Biochem. Biophys. Acta*, **34**, 24 (1959).

(6) W. F. Harrington and P. H. von Hippel, *Advan. Protein Chem.*, **16**, 1 (1961).

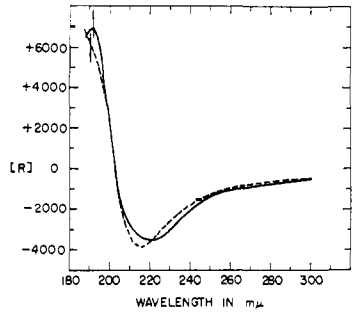

Figure 1. Optical rotatory dispersions of Gly-Pro-Gly oligomers in water: - - - -, Gly-Pro-Gly; ———, $(Gly\text{-}Pro\text{-}Gly)_2$ and $(Gly\text{-}Pro\text{-}Gly)_4$.

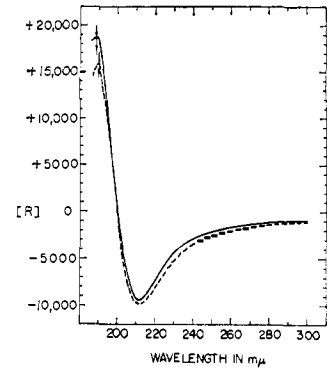

Figure 2. Optical rotatory dispersions of Gly-Pro-Ala oligomers and polymer in water: ———, Gly-Pro-Ala; - - - -, $(Gly\text{-}Pro\text{-}Ala)_2$ and poly(-Gly-Pro-Ala-).

Viscosity. Ubbelhode-type viscometers with flow times of approximately 2 min were used for viscometric measurements. Temperature was maintained at 25.0° by means of a thermostated bath.

Molecular Weight Determinations. Determinations of molecular weight were made with both the sedimentation and Archibald approach-to-equilibrium techniques.[7,8] Single-sector cells were used for sedimentation measurements, and Kegeles-type synthetic boundary cells were used to determine c_0.[9] For highly acid solutions, a Kegeles synthetic boundary cell was constructed from a 2° single-sector Kel-F cell. Checks on the methods and alignment of the centrifuge were made with raffinose in water.

X-Ray Measurements. Gels for diffraction studies were made by overnight preparative ultracentrifugation of the polymer in 14 *M* acetic acid at 30,000 rpm in a Spinco No. 40 rotor. The gel was washed with distilled water and dried at room temperature. Powder-type diagrams were obtained using a microcamera. Spacings were measured with a microcomparator using a silicon powder reference to obtain the sample-to-film distance.

Discussion and Results

Optical Rotatory Dispersion of Oligomers. Optical rotatory dispersion (ORD) measurements of glycyl-L-prolylglycine (Gly-Pro-Gly) and glycyl-L-prolyl-L-alanine (Gly-Pro-Ala) oligomers are shown in Figures 1 and 2. For the Gly-Pro-Gly oligomers the rotatory dispersion of the dodecamer (four Gly-Pro-Gly units) was identical with that of the hexamer within experimental error, indicating that there were no structural changes that affected the small negative $\pi^0 \rightarrow \pi^-$ Cotton effect. The constancy of the rotatory dispersion with oligomer length and the agreement of the measured $[\alpha]_D$ $-253 \pm 17°$ (corrected for weight fraction of proline)[2] with accepted values of prolyl random chain values[6] of $-250°$ indicate that the oligomers of Gly-Pro-Gly exist in solution as random chains. The slight differences observed in the rotatory dispersion of the trimer and hexamer are undoubtedly due to end effects, since the trimer dispersion also differed in the ethyl ester hydrochloride of the trimer. The peaks observed in the rotatory dispersion curves of all random chain oligomers below 190 mμ were reproducible, but may be artifactual due to stray light conditions in this spectral region.

Increased levorotations are shown with the introduction of the asymmetric L-alanine residue in the Gly-Pro-Ala oligomers. The ultraviolet rotatory dispersion properties observed with the Gly-Pro-Ala trimer, hexamer, and higher molecular weight polypeptides were all similar, suggesting that these compounds all possessed random chain structures.

Although neither the Gly-Pro-Gly nor the Gly-Pro-Ala oligomers appear to show effects from structural regularity, they are of interest from two standpoints. First, the rotation of the Gly-Pro-Gly hexamer and dodecamer can be attributed primarily to the prolyl residue, since the rotation induced in the optically inactive glycine from prolyl interactions should be relatively small in the random chain. This prolyl rotation can then be used to estimate the prolyl residue contribution to proline-containing proteins in the random chain form, *e.g.*, denatured collagen. Using this prolyl rotation, for instance, in conjunction with rotatory dispersion measurements of random chain polypeptides,[10] one obtains a close approximation of the Gly-Pro-Ala oligomer rotatory dispersion, verifying the conclusion that the Gly-Pro-Ala oligomers are also random. Secondly, it appears that unlike α-helix forming amino acid oligomers,[11] not enough structural stability is afforded from interresidue interaction to form ordered structures in the oligomers, containing 33% proline, which were examined.

Optical Rotatory Dispersion of Polymers. The rotatory dispersion of the Gly-Pro-Ala polymer in H_2O (see below for molecular weight) was found to be identical with that of the hexamer within experimental error and it is therefore also in a random conformation (see Figure 2).

The Gly-Pro-Gly polymers were not soluble in water but dissolved in aqueous organic acid solutions. These polymers were soluble in 14 *M* acetic acid and remained in solution when this solvent was diluted with water to 1.4 *M* acetic acid. Upon dilution, association occurred (see below), but the polymer remained in solution and gave reproducible ORD measurements over several weeks. Some rotatory dispersion results are shown in Figure 3.

Marked differences are apparent in comparing the Gly-Pro-Gly polymers with the random chain oligomers. The magnitude of the small peak at 233 mμ varied markedly with acetic acid concentration and with the addition of alcohols. The magnitude of the large trough near 202 mμ was more constant, remaining between $-20,000$ and $-21,500°$. Changes in levorota-

(7) W. J. Archibald, *J. Phys. Colloid Chem.*, **51**, 1204 (1947).
(8) H. K. Schachman in "Methods in Enzymology," Vol. IV, S. P. Colowick and N. O. Kaplan, Ed., Academic Press Inc., New York, N. Y., 1957.
(9) G. Kegeles, *J. Am. Chem. Soc.*, **74**, 5532 (1952).
(10) E. R. Blout, I. Schmier, and N. S. Simmons, *ibid.*, **84**, 3193 (1962).
(11) M. Goodman and G. Rosen, *Biopolymers*, **2**, 537 (1964).

tion at the 202-mμ trough were of the same relative magnitude as observed at the peak, but opposite in sign, suggesting a small positive Cotton effect with a peak near 230 mμ and a trough near 200 mμ superimposed on a larger 202-mμ trough. The changes in the smaller Cotton effect seem to be correlated with the amount of association as evidenced by the slight turbidities observable with long path lengths and the slow changes observed in rotation with time, but such a correlation was not definitively established.

Lot-to-lot variations of the ORD were, in contrast, very small even with lots of widely differing molecular weight. In acetic acid concentrations from 1 to 14 *M*, the dispersions fit a one-term Drude equation down to 275 mμ with λ_c 186 mμ in spite of the observed changes in the ultraviolet Cotton effects. The large trough at 202 mμ is similar in magnitude to that found for collagen at 207 mμ and for polyproline II at 216 mμ.[12] The Cotton effects giving rise to these troughs probably arise from $\pi^0 \rightarrow \pi^-$ transitions of this peptide absorption band. The origin of the small positive Cotton effect estimated to be centered at 215–220 mμ is not clear. It could be either a component of a split $\pi^0 \rightarrow \pi^-$ transition or an $n \rightarrow \pi^0$ transition. However, the wavelength appears very long for the $\pi^0 \rightarrow \pi^-$ exciton component predicted for polyproline II or collagen structures.[13] A similar small positive Cotton effect has been reported at 221 mμ in the ORD of polyproline II.[14] Since the far-ultraviolet ORD of poly(Gly-L-Pro-Gly) differs from those of the known structures, collagen and polyproline II, it is impossible to calculate the amount of either structure present in poly(Gly-L-Pro-Gly) at present. However, the ORD of poly(-Gly-L-Pro-Gly-) is consistent with the structure inferred from the X-ray data (*vide infra*).

In highly protic solvents such as DCA or 16.5 *M* formic acid, the rotation of the Gly-Pro-Gly polymer could be measured only to 230 mμ and was found to fit the rotatory dispersion of the random oligomers. Moreover, if the material was recovered and dissolved in 1.4 *M* acetic acid, only 15–18% of the structural rotation was recovered after 24 hr, as estimated at 202 mμ, with little further change with time. The viscosity in 16.5 *M* formic acid was constant over several days, indicating that hydrolysis of the polymer was not occurring to any appreciable extent even under these highly acid conditions.

Ultracentrifugation Studies. Archibald measurements of the Gly-Pro-Ala polymer yielded a molecular weight of 14,000 ± 500 in 0.2 *N* aqueous NaCl and in 5.13 *M* formic acid, using an assumed $\bar{V}$ of 0.75. The Gly-Pro-Gly dodecamer in water and formic acid also showed the same molecular weight (800 ± 50) in these two solvents if a $\bar{V}$ of 0.74 was assumed. These results indicate that these random chains have little tendency to associate in the manner observed for the Gly-Pro-Gly polymers.

The molecular weights obtained for the Gly-Pro-Gly polymers were markedly dependent upon solvent. In 14 *M* acetic acid molecular weights between 40,000 and 100,000 were obtained and in 1.4 *M* acetic acid fast sedimentation occurred at very low speed indicating very highly associated material. In formic acid solutions of 5.13 *M* and above, where random coil rotatory dispersions were found, greatly reduced molecular weights were obtained, reflecting breakup of the aggregates and probably reflecting the true molecular weights of the polymer. Since partial specific volume determinations by pycnometry were inaccurate in these high density solvents, a $\bar{V}$ of 0.74 in 5.13 *M* formic acid was calculated from an Archibald run on the Gly-Pro-Gly dodecamer. It was assumed that this value could be used with the dissociated polymer and that increased formic acid concentration would not change this value by more than a few per cent. Resulting molecular weight values for early preparations of the polymer yielded weight-average molecular weights (from approach-to-equilibrium) in the range 800 ± 200. A later lot (P-1) using improved synthesis methods gave a weight of 6000 ± 300.[2] The interesting breakup of aggregates in formic acid and dichloroacetic acid, with concomitant loss of secondary structure, is perhaps attributable to partial protonation of the amide groups.[15]

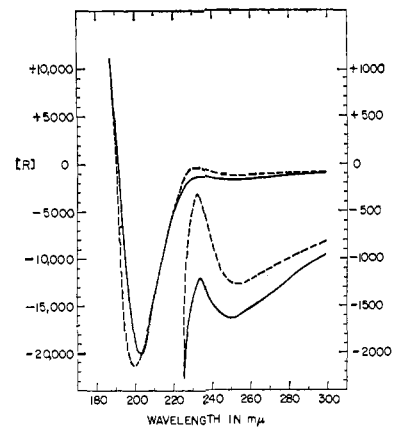

Figure 3. Optical rotatory dispersions of Gly-Pro-Gly polymer: ———, poly(-Gly-Pro-Gly-) in 1.4 *M* aqueous acetic acid; – – – –, poly(-Gly-Pro-Gly-) in 1.4 *M* aqueous acetic acid–methanol (50%, volume). The curves in the lower right portion of the figure are drawn scaled to the right-hand ordinate.

X-Ray Diffraction. X-Ray diffraction studies of the Gly-Pro-Gly polymer were carried out to determine if the collagen-like packing recently observed by Andreeva, *et al.*, for a Gly-Pro-Hypro polymer[16] in the solid state would also be found for the Gly-Pro-Gly polymer. Attempts were made to draw fibers from gels made by preparative ultracentrifugation from 14 *M* acetic acid, but no success was achieved. Instead, the gel was washed with water, dried at room temperature, and used for powder pattern determinations. Three reflections of medium to strong intensity were observed, yielding spacings of 3.1 (medium), 4.4 (intense), and 13.4 A (intense). A weaker X-ray pattern from the polymer lyophilized from water gave the latter two spacings, indicating that any solvent occlusion in the gel did not alter the spacings. On the basis of this information one cannot hope to establish the detailed ordering of the polymer, but some correlations may be

(12) E. R. Blout, J. P. Carver, and J. Gross, *J. Am. Chem. Soc.*, **85**, 644 (1963).

(13) (a) L. Stryer, unpublished data; (b) W. D. Gratzer, W. Rhodes, and G. D. Fasman, *Biopolymers*, **1**, 319 (1963).

(14) J. P. Carver, E. Shechter, and E. R. Blout, *J. Am. Chem. Soc.*, in press.

(15) S. Hanlon and I. M. Klotz, *Biochemistry*, **4**, 37 (1965).

(16) N. S. Andreeva, M. I. Millionova, and Yu. N. Chiradze, in "Aspects of Protein Structure," G. N. Ramachandran, Ed., Academic Press Inc., New York, N. Y., 1963.

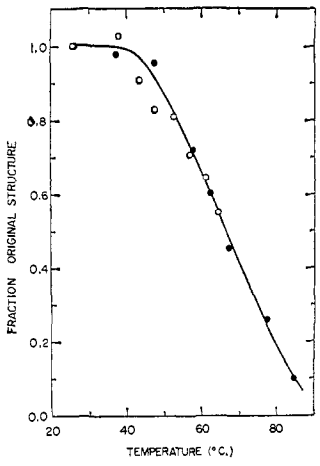

Figure 4. Temperature transition of 0.974% poly(-Gly-Pro-Gly-) (wt/vol) in 1.4 *M* acetic acid: O, at 233 mμ; ●, at 202 mμ. In both cases the 26.0° rotation was taken as that of the original structure.

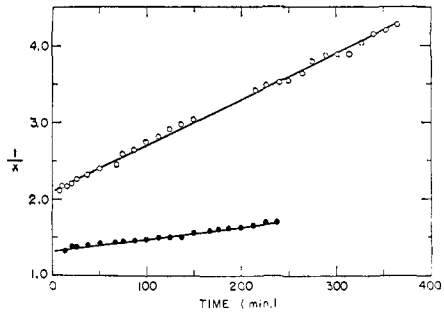

Figure 5. Refolding kinetics of 0.0433% (wt/vol) poly(Gly-Pro-Gly) in 0.7 *M* acetic acid at 233 mμ: ●, heated at 85°; O, heated at 67°.

made with the wealth of X-ray data of collagen-related structures. First, the rather sharp reflections observed with poly(-Gly-Pro-Gly-) indicate that much of the polymer is in an ordered form, and that the structure of the individual chains may be like that of the polyglycine or polyproline II helix, since the 3.1-A reflection of the axial residue translation is present.[17,18] Secondly, assuming that the 13.4-A reflection is equatorial, this is likely to be a spacing between groups of chains, analogous to the 11-A spacing in collagen. Thirdly, the 4.4-A spacing may be related to the distance between single chains in a multichain structure. We note that the interchain distance is 4.4 A in the collagen model of Ramachandran.[19] These suggestions, however, can only be regarded as tentative pending further experimentation. It is clear, nonetheless, that the Gly-Pro-Gly polymer structure in the solid state may be made up, at least in part, of polyproline II or polyglycine type single helices which are packed in a regular but different manner from either of these structures.

Temperature Transition. Studies of the temperature variation of optical rotation were undertaken to determine if the Gly-Pro-Gly polymer underwent a cooperative helix-random transition. Results are shown in Figure 4. Both the 233-mμ peak and the 202-mμ trough showed the same rather broad transition with a midpoint at 67°. It is of interest that the transition starts with zero slope, implying that, although the estimated helix content of this polymer is only part of that observed with collagen, a stable limiting structure is apparently obtained below about 40°.

Kinetics. To further investigate the nature of the temperature transition, the kinetics of refolding of the Gly-Pro-Gly polymer was measured. This was done by heating the solution in the jacketed polarimeter cell until rotation was constant (2 to 2.5 hr), then quickly equilibrating at 25.0° with a new bath and following the change in optical rotation with time. The initial heating was carried out (in different experiments) at 67 and at 85°, and the kinetic constants were determined.

A comparison of the kinetic constants then could be used to deduce information as to the complexity of the refolding reaction. The kinetic results, obtained when the solution was quenched from 85°, did not fit a first-order equation but fit very well a second-order equation. Figure 5 shows the data plotted according to the equation[20] $1/x = kC_0t + 1/x_0$, where x is the fraction of random chain remaining, x_0 is that fraction of random chain at $t = 0$, and C_0 is the concentration of the polymer. The value of k for refolding after quenching from 67° is 16.2×10^{-3} l./residue sec, while that obtained after quenching from 85° is 4.4×10^{-3} l./residue sec. The observed difference in the constants may be due in part to the difference in the initial concentration of random chains.

Second-order plots, such as those in Figure 5, have also been observed for collagen renaturation.[20-22] Although the mechanism of collagen renaturation is not completely understood, the reaction has been regarded as a pseudo-second-order propagation of helix along single chains.[22,23] Recent experiments[20,24] have suggested that association between chains may be important in this step, however.

Measurements of the 202-mμ rotation of a solution of the Gly-Pro-Gly polymer which had been heated at 67° showed that the rotation returned to 96% of its original (preheating) value after several weeks. From these measurements it is concluded that 96% of the original structure was reformed after heat treatment and quenching. Another experiment in which a solution of the polymer was heated at 85° before quenching to 25° showed that only 75% of the original rotation value was regained even after several weeks. These results indicate heating to 85° converted some of the polymer into a state from which refolding was either very slow or impossible.

To determine the effect of chain interaction of the Gly-Pro-Gly polymer on the refolding process, the same type of heating and quenching experiments were carried out and the solutions measured viscometrically at 25°. Upon quenching the solution heated to 67°, it was found that the reduced viscosity had dropped to 93% of its original value of 2.83 dl/g with no subsequent change with time. The viscosity of the solution heated to 85° and quenched to 25° dropped to 71% of its

(17) H. F. C. Crick and A. Rich, *Nature*, **176**, 780 (1955).
(18) P. M. Cowan and S. McGavin, *ibid.*, **176**, 501 (1955).
(19) G. N. Ramachandran in "Aspects in Protein Chemistry," G. N. Ramachandran, Ed., Academic Press Inc., New York, N. Y. 1963.
(20) M. P. Drake and A. Veis, *Biochemistry*, **3**, 135 (1964).
(21) C. R. Smith, *J. Am. Chem. Soc.*, **41**, 135 (1919).
(22) W. F. Harrington and P. H. von Hippel, *Arch. Biochem. Biophys.*, **92**, 100 (1961).
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original value also with no further change with time. These viscometric experiments suggest that, once the polymer has undergone the change associated with this loss in viscosity, this conversion cannot be reversed. The optical rotation experiments, on the other hand, suggest that the change in structure associated with them is essentially reversible. Thus there appear to be two transitions, the first responsible for a reversible change in conformation, the second for a further and irreversible change in hydrodynamic shape.

This mechanism is similar to that suggested for collagen denaturation. As a result of an interesting series of light scattering studies on collagen, Engel suggested[25] that collagen first unfolds to associated random chains and then dissociates (in the random chain form)

$$\text{associated helices} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{associated random chains} \underset{k_4}{\overset{k_3}{\rightleftharpoons}} \text{dissociated random chains}$$

Applying such an interpretation to our work with poly(-Gly-Pro-Gly-), k_2 would be obtained from the optical rotation kinetics described above. The absence of viscosity increase after quenching suggests that k_4 is very small. k_3, associated with the hydrodynamic change, must be highly temperature dependent. None of the measurements reported here gives the value of k_1 or k_3. Added support for this suggestion is given by the slow and incomplete refolding of poly(-Gly-Pro-Gly-) in acetic acid after complete dissociation and unfolding in concentrated formic acid.

Conclusions

As a result of these studies, it appears that the repeating sequence Gly-Pro-Gly polymer serves as a useful, but not complete, model for collagen structure and association. The chain interaction revealed by the physical–chemical measurements suggests that the parallel is perhaps closest between this polymer and the α strands of collagen which renature into a more randomly associated structure containing approximately 60% of the initial helical character of collagen.[24] It has been suggested by Piez and Carrillo[24] that in the case of α strands, re-formation is initially between two strands with pyrrolidine-rich sections of one chain acting as a guide for pyrrolidine-poor sections of the adjacent chain. From the work reported here it appears that adjacent proline residues are not required for helix formation in structures stabilized by interchain interaction, but may be important in the more specific ordering of collagen. Further studies of interactions of pyrrolidine-rich with pyrrolidine-poor organized sequence polymers may increase understanding in this area.

The interesting lack of ordered structure in the Gly-Pro-Ala polymer, assuming little or no racemization has occurred,[2] can be attributed either to an increased stability of the random chain alanyl residue compared to the glycyl residue in water or to a decreased stability of the alanyl residue in the corresponding polyproline II type helix. In view of the lack of knowledge of solvation effects of this kind, it is difficult to make a definite choice between the two possibilities. However, with the knowledge that model studies show that alanyl residues can fit into the polyproline II or collagen helix with little hindrance and that alanine shows little hydrophobic character,[26] it appears that solvation of the random chain may be a critical factor in determining the resultant structures of the polymers.

Acknowledgments. We thank Dr. L. Bethune for help and advice in the ultracentrifugation work, Dr. C. Cohen for valuable assistance in the X-ray studies, and Dr. E. R. Simons for critical and helpful comments during the writing of this manuscript.

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Communications to the Editor

Tetraphosphorus Hexaoxide–Diborane

Sir:

In a recent communication, Riess and Van Wazer[1] reported that P_4O_6 will replace one CO from $Ni(CO)_4$ to give $Ni(CO)_3P_4O_6$; they also indicated that P_4O_6 can behave as a polydentate ligand. In order to determine how diborane is cleaved by P_4O_6, we have recently examined the reaction of P_4O_6 and B_2H_6 and have isolated the solid $P_4O_6 \cdot B_2H_6$. Two possible structures for this solid might be suggested. The first one, arising from nonsymmetrical cleavage of the diborane molecule[2] and utilizing the polydentate characteristics of P_4O_6, would be represented as $[H_2BP_4O_6^+][BH_4^-]$. The second structure, arising from symmetrical cleavage of diborane, would involve direct coordination of BH_3 groups to two of the four phosphorus atoms in P_4O_6. The latter structure is somewhat analogous to the structure of P_4O_{10} and suggests the analogy between BH_3 and O used earlier to interpret the reaction of F_3PBH_3 with ammonia.[3] The ^1H nmr spectrum of the compound offers strong support for the second formulation, $H_3BP_4O_6BH_3$, indicating symmetrical cleavage of the diborane molecule by P_4O_6.

All reactions were carried out in a standard high-vacuum system. Liquid P_4O_6, contained in an evacuated tube attached to the vacuum line, was exposed for several hours at room temperature to gaseous diborane

(1) J. G. Riess and J. R. Van Wazer, *J. Am. Chem. Soc.*, **87**, 5506 (1965).

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