properties of poly(L-proline) exhibit two minima for rotation about ψ.[45] The effect of calcium chloride on the intrinsic viscosity of poly(L-proline)[8,15] can be accounted for if 20% or more of the L-prolyl residues have ψ near 130° in concentrated calcium chloride solution.[46] The ir spectrum of poly(L-proline) in aqueous calcium chloride is also ambiguous concerning isomerization about ψ or ω.[47,48] If the salt effect on poly(L-proline) is due to isomerization about ψ the ^{13}C nmr chemical shifts would have to be similar for isomerization about ψ and about ω.[44] Inspection of molecular models does not provide any basis for ruling out such a coincidence. In summary, it is clear that at present an unequivocal description of the structure of the B isomer cannot be made from the evidence in hand.

Acknowledgments. We thank Drs. D. Torchia and A. Tonelli for making their results available to us prior to publication.

(45) W. L. Mattice, K. Nishikawa, and T. Ooi, *Macromolecules*, **6**, 443 (1973).
(46) T. Ooi, D. Clark, and W. L. Mattice, unpublished data.
(47) C. A. Swenson, *Biopolymers*, **10**, 2591 (1971).
(48) N. Johnston and S. Krimm, *Biopolymers*, **10**, 2597 (1971).

Evidence for β-Turn Analogs in Proline Peptides in the Solid State. An Infrared Study

Charles M. Deber

Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts 02115.
Received June 19, 1973

ABSTRACT: The solid-state infrared spectra of a group of proline-containing di- and tripeptide carboxylic acids and esters are interpreted in terms of peptide secondary structure. When an L-Pro-D-Pro, L-Pro-Gly, or Gly-L-Pro sequence occurs in the positions nearest the C-terminal acid, the peptides are proposed to fold into 10-membered hydrogen-bonded structures analogous to β turns. Experimental evidence for this folding is obtained from observation of consistent shifts of appropriate carbonyl bands due to hydrogen-bond formation. Confirmation of assignments of amide I bands in *t*-Boc-Gly-L-Pro-OBz and *t*-Boc-Gly-L-Pro-OH is obtained through a comparison of the infrared spectra of natural abundance samples of these peptides with samples enriched 60% with ^{13}C at the Gly carbonyl carbon atom. Additional structures deduced from solid-state infrared spectra are proposed for peptides containing L-Pro-L-Pro, D-Pro-D-Pro, or L-Pro-Sar sequences nearest the C-terminal acid. Infrared spectra in chloroform and dioxane solutions indicated the absence of any shifted bands which could be correlated with specific intra- or intermolecular structures.

The folding of a peptide chain into a β turn (also called β bend, or hairpin bend), producing a hydrogen bond between the first and fourth residues of the chain, and causing a 180° reversal in chain direction, has been recognized as an essential feature of protein secondary structure.[1-3] Experimental observations of β turns have come largely from X-ray crystallographic determinations of peptide[4a,b,5] and protein structure,[6] as well as from nuclear magnetic resonance studies of solution conformations of a variety of naturally occurring[7-9] and synthetic[10-13] cyclic peptides.

The present report provides evidence from solid-state infrared spectra for the formation in linear peptide carboxylic acids of structures analogous to β turns. These structures are observed when the sequence Gly-L-Pro, L-Pro-Gly, or L-Pro-D-Pro occurs in the two residues nearest the C terminus of the peptide chain.

Results

A study was made of the carbonyl regions of the infrared spectra of the peptide acids listed in Table I, and their corresponding benzyl esters. These regions (1600–1800 cm^{-1}) include bands attributable to ester, carboxylic acid, urethane, and peptide amide I functional groups. All acids were studied as solids in KBr disks; some (noncrystalline) esters were examined as smears between NaCl plates.

In the absence of specific interactions, these multifunctional compounds would have carbonyl frequencies the same as those observed for corresponding monofunctional compounds. Such "normal" frequencies are observed for peptide benzyl esters. The ester carbonyl position in each case was 1740–1745, appropriate for a benzyl ester carbonyl in simple organic esters.[14] The band for the *tert*-butyloxycarbonyl group (*t*-Boc) occurred at 1685–1695, in the region previously noted for secondary and tertiary urethanes (carbamates).[14] The peptide amide I bands[15] oc-

(1) P. N. Lewis, F. A. Momany, and H. A. Scheraga, *Proc. Nat. Acad. Sci. U. S.*, **68**, 2293 (1971).
(2) I. D. Kuntz, *J. Amer. Chem. Soc.*, **94**, 4009 (1972).
(3) J. L. Crawford, W. N. Lipscomb, and C. G. Schellman, *Proc. Nat. Acad. Sci. U. S.*, **70**, 538 (1973).
(4a) T. Ueki, T. Ashida, M. Kakudo, Y. Sasada, and Y. Katsube, *Nature (London)*, **216**, 1205 (1967).
(4b) T. Ueki, S. Bando, T. Ashida, and M. Kakudo, *Acta Crystallogr., Sect. B*, **27**, 2219 (1971).
(5) A. D. Rudko, F. M. Lovell, and B. W. Low, *Nature (London), New Biol.*, **232**, 18 (1971).
(6) R. E. Dickerson, T. Takano, D. Eisenberg, O. Kallai, L. Samson, A. Cooper, and E. Margoliash, *J. Biol. Chem.*, **246**, 1511 (1971).
(7) Yu. A. Ovchinnikov, V. T. Ivanov, V. F. Bystrov, A. I. Miroshnikov, E. N. Shepel, N. D. Abdullaev, E. S. Efremov, and L. B. Senyavina, *Biochem. Biophys. Res. Commun.*, **39**, 217 (1970).
(8) D. W. Urry and R. Walter, *Proc. Nat. Acad. Sci. U. S.*, **68**, 956 (1971).
(9) C. H. Hassall and W. A. Thomas, *Chem. Brit.*, **7**, 145 (1971).
(10) D. A. Torchia, A. diCorato, S. C. K. Wong, C. M. Deber, and E. R. Blout, *J. Amer. Chem. Soc.*, **94**, 609 (1972).
(11) D. A. Torchia, S. C. K. Wong, C. M. Deber, and E. R. Blout, *J. Amer. Chem. Soc.*, **94**, 616 (1972).
(12) R. Schwyzer, C. Grathwohl, J. P. Meraldi, A. Tun-kyi, R. Vogel, and K. Wuthrich, *Helv. Chim. Acta*, **55**, 2545 (1972).
(13) L. G. Pease, C. M. Deber, and E. R. Blout, *J. Amer. Chem. Soc.*, **95**, 258 (1973).
(14) N. B. Colthup, L. H. Daly, and S. E. Wiberley, "Introduction to Infrared and Raman Spectroscopy," Academic Press, New York, N. Y., 1964.
(15) H. Susi in "Structure and Stability of Biological Macromolecules," S. N. Timasheff and G. D. Fasman, Ed., Marcel Dekker, Inc., New York, N. Y., 1969, p 575.

Table I
Assignments (in cm^{-1}) of Carbonyl Regions of Proline Peptides in the Solid State[a]

Peptide	Carboxylic Acid	*t*-Boc Urethane	Peptide Amide I (C Terminal)	Peptide Amide I (Central)
t-Boc-L-Pro-Gly-OH	1771	*1662*	1662	
t-Boc-Gly-L-Pro-OH	1741	*1660*	1645	
t-Boc-L-Pro-D-Pro-OH	1746	*1652*	1645	
t-Boc-L-Pro-L-Pro-OH	1747	1684	1606	
t-Boc-D-Pro-D-Pro-OH	1747	1684	1606	
t-Boc-L-Pro-Sar-OH	1750[b]	1688	1604	
t-Boc-L-Pro-L-Pro-L-Pro-OH	1742	1686	1645[c]	1645[c]
t-Boc-D-Pro-D-Pro-D-Pro-OH	1742	1686	1645[c]	1645[c]
t-Boc-L-Pro-L-Pro-D-Pro-OH	1738	1696	1642	*1602*

[a] Assignments of carbonyl frequencies of proline peptide carboxylic acids in the region 1600–1800 cm^{-1}. Spectra were recorded as KBr disks. In tripeptides, the "central" peptide bond is the one twice removed from the C terminus. Estimated uncertainty in band positions: ±1.5 cm^{-1}. Sar = sarcosine (*N*-methylglycine). *t*-Boc = *tert*-butyloxycarbonyl. Bands shifted due to involvement of carbonyl group in β-turn formation are italicized. [b] Slightly split band, centered at 1750 cm^{-1}. [c] Broad band, centered at 1645 cm^{-1}.

curred generally at 1640–1650, accounting for either one (dipeptides) or two (tripeptides) peptide bonds. This position is found to be typical for X—Pro peptide bonds (where X is any residue), and is 10–20 cm^{-1} lower than the usual amide I position for peptide bonds of the type X-amino acid (*e.g.*, the Pro—Gly bond in *t*-Boc-L-Pro-Gly-OBz, 1660 cm^{-1}). This shift to lower wave numbers of X-Pro bonds may reflect the increased basicity[16] of the prolyl tertiary nitrogen atom, which would increase the double-bond character of the C—N portion of the peptide bond, but weaken the C=O portion.

By contrast, several shifts to lower frequency are evident in the carboxylic acid series (Table I), a phenomenon likely arising from hydrogen-bonded structures involving the C-terminal acid OH proton. In the acids, the COOH frequency itself appears at *ca.* 1740–1750, somewhat high for aliphatic carboxylic acids, but at the frequency often observed for un-ionized carboxylic acid groups[17] on carbon atoms also having electronegative substituents (in this case, α-amino groups).[18] Note, however, that (a) the urethane band of *t*-Boc-L-Pro-Gly-OH occurs at 1662; (b) the urethane band of *t*-Boc-Gly-L-Pro-OH occurs at 1660; and (c) the urethane band of *t*-Boc-L-Pro-D-Pro-OH is similarly shifted to lower wave numbers (1652 cm^{-1}), while that of *t*-Boc-L-Pro-L-Pro-OH retains its "normal" position (1684 cm^{-1}).

Assignment of Amide I Bands by Isotopic Substitution with Carbon-13. Experimental verification of the amide I band assignments in *t*-Boc-Gly-L-Pro-OBz and *t*-Boc-Gly-L-Pro-OH was obtained by examining the infrared spectra of samples which had been enriched *ca.* 60% with ^{13}C at the glycine carbonyl carbon atom. From the relationship of vibration frequency to the reduced mass of the vibrating atoms,[14] the magnitude of the shift expected due to isotopic substitution with ^{13}C can be calculated, assuming that amide I bands arise predominantly from carbon—oxygen stretching frequencies. The calculation predicts a shift from the normal band position (*i.e.*, 1640–1650) to lower frequency by about 40 cm^{-1} (*i.e.*, 1600–1610).

The infrared spectrum of the ^{13}C-enriched *t*-Boc-Gly-L-Pro-OBz (Figure 1) has the same ester, urethane, and amide I frequencies found for the unenriched material, but the ^{13}C-enriched component of its amide I peptide band is shifted from 1647 to 1606 cm^{-1}. Similarly, the carbonyl region of the enriched *t*-Boc-Gly-L-Pro-OH (Figure 2) displays bands at 1741 (acid) and 1660 (urethane), unchanged; 1645 (remaining unenriched amide I); and a new strong band at 1603 cm^{-1} (^{13}C-enriched amide I). In both enriched compounds, the only significant change detected in their infrared spectra was this shift of the amide I carbonyl frequency.

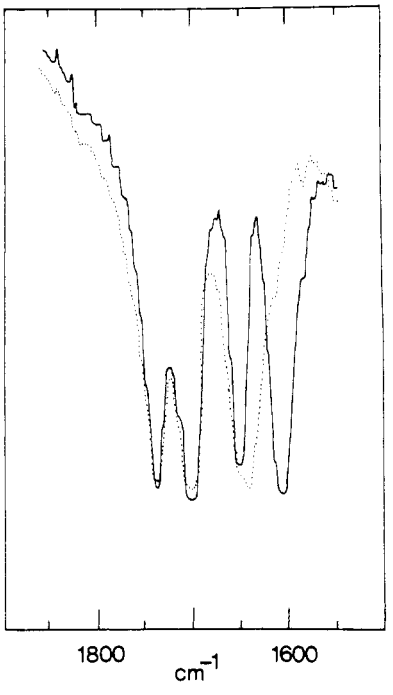

Figure 1. Carbonyl regions of the infrared spectra (KBr) of *t*-Boc-Gly-L-Pro-OBz: dotted line, natural abundance sample; solid line, Gly carbonyl carbon enriched *ca.* 60% with ^{13}C.

Discussion

β-Turn Analogs. Hydrogen-bond formation in various types of polypeptide secondary structure is known to shift amide I frequencies of those carbonyl groups involved to lower frequency.[15,17] Consistent with this, the absorption band (3560–3500 cm^{-1}) due to the free OH stretching of the carboxylic acid moieties is absent for all the peptides studied, while weak absorption bands appear in the 2500–2700-cm^{-1} region, corresponding to overtone and combination bands arising from a hydrogen-bonded OH species.[14] Although ambient moisture present during the preparation of KBr pellets was not excluded, elemental

(16) A. Veis and C. F. Nawrot, *J. Amer. Chem. Soc.*, 92, 3910 (1970).
(17) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," 2nd ed, Wiley, New York, N. Y., 1958.
(18) The carboxylic acid frequency for *t*-Boc-L-Pro-Gly-OH (1771 cm^{-1}) is unaccountably high. A similar but smaller shift to higher wave numbers is also observed for the ester frequency of the corresponding benzyl ester *t*-Boc-L-Pro-Gly-OBz (1755 cm^{-1}).

Figure 2. Carbonyl regions of the infrared spectra (KBr) of *t*-Boc-Gly-L-Pro-OH: dotted line, natural abundance sample; solid line, Gly carbonyl carbon enriched *ca.* 60% with ^{13}C.

analyses of peptides reported herein consistently showed the absence of specific incorporation of water molecules into the crystal lattices. Thus, it does not appear that the observed carbonyl shifts are arising from general H bonding to water. Rather, the shifts to lower wavenumbers of the urethan bands of *t*-Boc-L-Pro-Gly-OH, *t*-Boc-Gly-L-Pro-OH, and *t*-Boc-L-Pro-D-Pro-OH (bands in italic type in Table I) are likely to be due to the occurrence of specific (and probably similar) hydrogen-bonded structures in these acids. The data are accommodated by an intramolecular hydrogen bond between the C-terminal OH proton and the urethane carbonyl group in each of these peptides to give a 10-membered hydrogen-bonded ring. Such an "oxy analog" of a β turn is illustrated for *t*-Boc-L-Pro-Gly-OH (Figure 3). In this structure, the *t*-Boc group serves as "residue 1," and the C-terminal OH group serves as "residue 4" of the β turn.

A consideration of the categories of β turns[1,19,20] suggests that peptides such as *t*-Boc-L-Pro-Gly-OH, *t*-Boc-Gly-L-Pro-OH, and *t*-Boc-L-Pro-D-Pro-OH contain amino acid sequences favorable for folding into β turns. Firstly, each has a proline residue available to occupy either position 2 or 3 in the turn. Proline, having a fixed N—C_α dihedral angle of ~120°, fits particularly well into these β-turn corners. Secondly, each of the peptides has the configuration "LD" or "DL" (where Gly may be substituted for a D residue) in positions 2 and 3. This configuration allows the peptide to adopt the type II β turn, which is calculated to be the lower energy structure (*vs.* a type I β turn with a 2,3 LL configuration).[21] Figure 3 illustrates the type II β-turn analog proposed for *t*-Boc-L-Pro-Gly-OH. A similar structure, but with D-Pro in the 3 position, is proposed for *t*-Boc-L-Pro-D-Pro-OH. Finally, because it has a "DL" rather than "LD" sequence in positions 2 and 3, *t*-Boc-Gly-L-Pro-OH is proposed to take up the type II′ β

(19) C. M. Venkatachalam, *Biopolymers*, 6, 1425 (1968).
(20) D. W. Urry and M. Ohnishi, "Spectroscopic Approaches to Biomolecular Conformation," American Medical Association, Chicago, Ill., 1970, p 263.
(21) R. Chandrasekaran, A. V. Lakshminarayanan, U. V. Pandya, and G. N. Ramachandran, *Biochim. Biophys. Acta*, 303, 14 (1973).

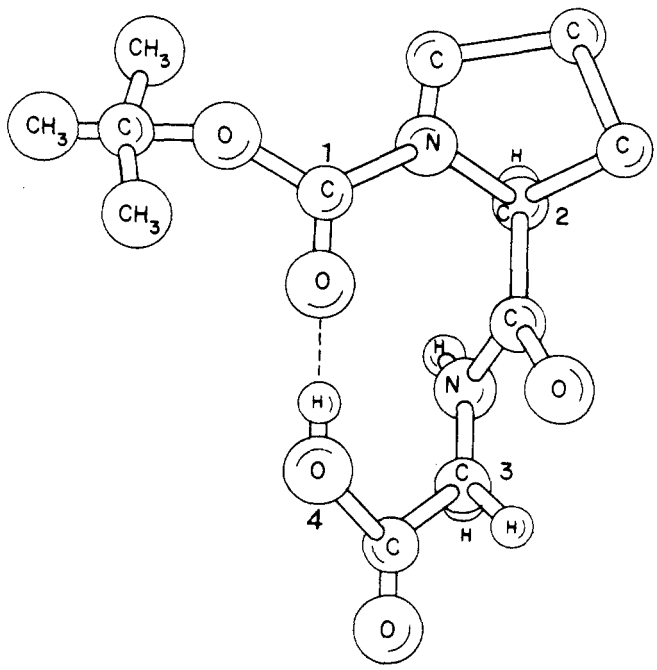

Figure 3. The type II β-turn analog structure proposed for *t*-Boc-L-Pro-Gly-OH, deduced from solid-state infrared spectra.

turn—the mirror image of the type II structure. This type II′ β turn is formally analogous to the β turn (with D-Phe-L-Pro in the 2,3 positions) found in the solution conformation of the cyclic decapeptide gramicidin S.[20] Models suggest that the proposed β-turn analogs have sets of backbone (ϕ,ψ) angles comparable to actual β turns in higher peptides.

If the formation of folded β-type structures correlates reliably with the shift to lower frequencies of the urethan carbonyl bands of the above three peptides, it may be expected generally that *β-turn formation will be signaled by a 20–40 cm⁻¹ shift to lower wave numbers of the band attributable to the carbonyl group twice removed from the C terminus*, due to involvement of this carbonyl in an intramolecular hydrogen bond. Extrapolation of these findings to the tripeptide series indicates that the band at 1602 cm^{-1} observed for one of the *t*-Boc-L-Pro-L-Pro-D-Pro-OH amide I carbonyl groups (but not for either of the *t*-Boc-L-Pro-L-Pro-L-Pro-OH amide I groups; see Figure 4 and Table I) can be assigned to a structure in which this carbonyl group is involved in an intramolecular hydrogen bond to the C-terminal OH group. In the case of the tripeptide, the carbonyl group twice removed from the C terminus is part of the first Pro—Pro peptide bond—not the urethan as in the dipeptides—and thus the H bonding lowers the amide I frequency of this peptide bond from its unperturbed position at 1642 to 1602 cm^{-1}. Therefore, it appears that *t*-Boc-L-Pro-L-Pro-D-Pro-OH has a type II oxy β turn analogous to that taken up by the dipeptide *t*-Boc-L-Pro-D-Pro-OH, but with the additional L-Pro unit replacing the *t*-Boc group as "residue 1."

Intermolecular packing effects in the crystals of these acids cannot be ruled out unequivocally as the source of shifted carbonyl frequencies. Nevertheless, several arguments can be advanced which suggest that the observed shifts may arise from intramolecular structures. First, the shifted band is the band twice removed from the C terminus, precisely the one which would be shifted if a 10-membered intramolecular β-type H-bonded ring were present. Second, this particular band shifts only when the configuration of the primary sequence is favorable for folding structures into the lowest energy β turns, as above

Table II
Assignments (in cm^{-1}) of Carbonyl Regions of Proline Peptides in Solution[a]

Peptide	Solvent	Carboxylic Acid	*t*-Boc Urethane	Peptide Amide I
t-Boc-Gly-L-Pro-OH	Chloroform	*Ca.* 1730 (sh)	1708	1656
	Dioxane	1750	1710	1665 and 1625 (^{13}C)[b]
t-Boc-D-Pro-D-Pro-OH	Chloroform	1756 (and 1720 (sh))	1687	1660
t-Boc-L-Pro-D-Pro-OH	Chloroform	1752	1695	1655
t-Boc-D-Pro-D-Pro-D-Pro-OH	Chloroform	1757 (and 1720 (sh))	1687	1656[c]
t-Boc-L-Pro-L-Pro-D-Pro-OH	Chloroform	1752	1687	1658, 1645 (sh)
	Dioxane	1744	1696	1657, 1649 (sh)

[a] Assignments of carbonyl frequencies of selected proline peptide carboxylic acids in solution, in the region 1600–1800 cm^{-1}. Spectra were recorded in sodium chloride (0.2-mm path length) or calcium fluoride (0.05 mm) cells in the solvents indicated. Concentration: *ca.* 4 mg/0.2 ml. Sh = shoulder. [b] Enriched sample of *t*-Boc-Gly-L-Pro-OH (60% ^{13}C at Gly carbonyl carbon) was used in dioxane. [c] Corresponds to two overlapping amide I bands.

discussed. Finally, several linear proline-containing peptides have been shown by X-ray crystallography to exist as intramolecularly H-bonded structures. These include *p*-bromocarbobenzoxy-Gly-L-Pro-L-Leu-Gly-OH and *p*-bromocarbobenzoxy-Gly-L-Pro-L-Leu-Gly-L-Pro-OH (both with L-Pro-L-Leu in positions 2 and 3),[4a,b] and the linear tail peptide of oxytocin, *S*-benzyl-L-Cys-L-Pro-L-Leu-Gly-NH_2.[5,22,22a] A β-turn analog has been proposed for the minimum energy conformation of thyrotropin-releasing factor, L-pyroglutamyl-L-histidyl-L-prolinamide,[24a] as well as for H-L-Pro-L-Leu-Gly-amide (MSH-release-inhibiting hormone) in the crystalline state.[24b]

Additional Structures in the Solid State. Many of the assignments given throughout this report differ from those previously suggested[25] for a series of *tert*-amyloxycarbonyl-L-proline oligomeric esters and acids. The assignments of Isemura *et al.*[25] may have been influenced by their observation of some unusually low (*ca.* 1610–1620 cm^{-1}) carbonyl bands, which they ascribed to ionized carboxylic acid functions. In the present work, three peptides have also been found to display this shifted band (Table I): *t*-Boc-L-Pro-L-Pro-OH (1606), *t*-Boc-D-Pro-D-Pro-OH (1606), and *t*-Boc-L-Pro-Sar-OH (1604). Since the other bands in the carbonyl regions of the three peptides are at the unshifted positions for un-ionized acid (1745–1750) and urethane (1685) (*vide infra*), the shifted band must be due to the peptide carbonyl. These amide I bands of low frequency could arise through the possible formation of an intermolecular species such as an antiparallel dimer. If this were the case, the OH proton of the acid function of each molecule would be hydrogen bonded to the peptide carbonyl group of its neighbor, forming a 14-membered ring between the two molecules, closed with two hydrogen bonds. Such a structure would shift the amide I carbonyl band to the observed position, while leaving urethan and acid carbonyl positions intact. The all-L (and all-D) tripeptides of proline also have broad amide I bands tending to lower wave numbers, suggesting that the phenomena observed for the dipeptides may be operative to some extent in the tripeptides as well.

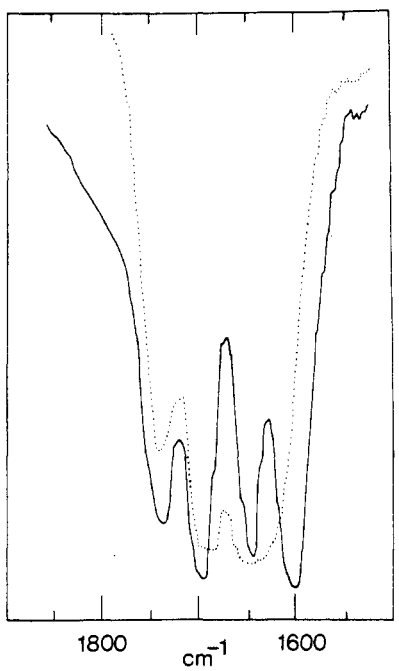

Figure 4. Carbonyl regions of the infrared spectra (KBr) of *t*-Boc-L-Pro-L-Pro-L-Pro-OH (dotted line) and *t*-Boc-L-Pro-L-Pro-D-Pro-OH (solid line). In one spectrum recorded as a fluorolube mull, the amide I region of *t*-Boc-D-Pro-D-Pro-D-Pro-OH could be resolved into two bands occurring at 1653 and 1637 cm^{-1}.

Alternatively, a γ turn, in which the C-terminal OH proton and the peptide carbonyl group nearest the C terminal are involved in a seven-membered ring containing a somewhat nonlinear hydrogen bond, could account for the observed amide I shift. Evidence for the importance of γ turns has emerged from conformational energy calculations[26-28] and experimental observations.[29-31] Since the relatively weak H bonds formed in γ turns may not be of

(22) A brief reference to carbobenzoxy-Gly-L-Pro-OH (Z-Gly-L-Pro-OH) in an earlier X-ray study[23] indicated it to be in an "extended form" in the crystal. Consistent with this result is the carbonyl region of this compound: 1736 (acid), 1680 (urethane), 1651 (amide I), which suggests the lack of intramolecular structure. However, the role of the aromatic ring in influencing crystal packing (*vs.* the *t*-Boc group) is apparently significant.

(22a) The discussion herein predicts that the L-Cys-L-Pro peptide bond of the oxytocin tail peptide *S*-benzyl-L-Cys-L-Pro-L-Leu-Gly-NH_2, found by X-ray crystallography[5] to be involved in a β turn, would be shifted from *ca.* 1640–1645 to *ca.* 1605–1610 cm^{-1}. Consistent with expectation, the infrared spectrum (KBr) of a crystalline sample of this peptide (kindly provided by Dr. Victor J. Hruby, University of Arizona, Tucson) displayed the following carbonyl region: a more intense band centered at 1660 cm^{-1} (assigned to L-Pro-L-Leu, L-Leu-Gly, and Gly-NH_2 amide I bands), and a less intense band at 1609 cm^{-1} (assigned to the shifted L-Cys-L-Pro amide I band).

(23) Y. Sasada, K. Tanoka, Y. Ogawa, and M. Kakudo, *Acta Crystallogr.*, 14, 326 (1961).

(24a) D. E. Blagdon, J. Rivier, and M. Goodman, *Proc. Nat. Acad. Sci. U. S.*, 70, 1166 (1973).

(24b) R. Walter, I. Bernal, and L. F. Johnson in "Chemistry and Biology of Peptides," J. Meienhofer, Ed., Ann Arbor Science Publishing Co., Ann Arbor, Mich., 1972, p 131.

(25) T. Isemura, H. Okabayashi, and S. Sakakibara, *Biopolymers*, 6, 307 (1968).

(26) B. Maigret, B. Pullman, and M. Dreyfus, *J. Theor. Biol.*, 26, 321 (1970).

(27) G. Nemethy and M. P. Printz, *Macromolecules*, 5, 755 (1972).

(28) V. Madison, *Biopolymers*, 12, 1837 (1973).

(29) M. Tsuboi, T. Shimanouchi, and S. Mizushima, *J. Amer. Chem. Soc.*, 81, 1406 (1959).

(30) B. W. Matthews, *Macromolecules*, 5, 818 (1972).

(31) H. E. Bleich, R. E. Galardy, and M. P. Printz, *J. Amer. Chem. Soc.*, 95, 2041 (1973).

sufficient strength to shift the carbonyl frequencies by the observed increments (*ca.* 40 cm^{-1}), it seems likely that the antiparallel dimers provide the better explanation of the data. In concert with the discussion above concerning β turns, a variety of linearly extended intermolecular structures can account for the observed shifts. However, it is believed that the consistent nature of the shifts again argues for the specific structures proposed.

Infrared Spectra in Solution. If intramolecular structures occur in a crystal, where opportunity for association with nearest neighbors is great, one might anticipate that such structures would also persist in dilute solution. However, as seen from preliminary data obtained from solution infra-red studies (Table II) in chloroform and dioxane—two of the least polar solvents which will dissolve the peptides—this appears *not* to be the case. There is a general trend of 10–15 cm^{-1} to higher wave numbers of all bands in solution *vs.* the solid state. However, bands whose shifts in the solid state could be correlated with specific secondary structures, whether intra- or intermolecular, occur in positions expected for unordered conformations.

Several examples from Table II illustrate these observations. For *t*-Boc-Gly-L-Pro-OH, the urethan band is near 1710 cm^{-1} in solution *vs.* 1660 in the solid state. For *t*-Boc-L-Pro-D-Pro-OH, the urethan band is at 1695 in solution *vs.* 1652 in the solid state. For *t*-Boc-L-Pro-L-Pro-D-Pro-OH in two solvents, the two amide I bands are barely resolved and are clustered around 1645–1655 cm^{-1}. This latter result is in contrast to the shifted amide I band observed for this peptide at 1602 cm^{-1} in the solid state. Even in the case of *t*-Boc-D-Pro-D-Pro-OH, where the shifted (1606 cm^{-1}) amide I band in the solid state might be due to intermolecular H-bonded structure, the solution spectrum in chloroform shows this band at 1660 cm^{-1}. Again, band assignments could be confirmed in part by employing ^{13}C-enriched *t*-Boc-Gly-L-Pro-OH; the amide I band of this sample in dioxane has two components: 1665 (unenriched) and 1625 cm^{-1} (enriched).

The only evidence for any specific structural feature obtained from solution spectra is the slightly shifted frequencies observed for the carboxylic acid band of *t*-Boc-Gly-L-Pro-OH in chloroform (*ca.* 1730 (sh)), and the shoulders at 1720 cm^{-1} observed for this band in both *t*-Boc-D-Pro-D-Pro-OH and *t*-Boc-D-Pro-D-Pro-D-Pro-OH. These shifts to lower frequency may correspond to simple carboxylic acid dimers. No bands due to OH stretching are visible in the 3550–2500 cm^{-1} regions in chloroform in any of the peptides examined, suggesting a hydrogen-bonded state for the acid OH proton. In *t*-Boc-Gly-L-Pro-OH in dioxane, a band at 3310 cm^{-1}, presumably corresponding to H-bonded Gly NH, may be discerned.

Experimental work reviewed by Susi[15] indicates the importance of competitive equilibria between amide–amide, amide–solvent, and solvent–solvent interactions, and indeed, amide–solvents interactions were shown to be significant in the "nonpolar" solvent chloroform. Polypeptides which have ordered secondary structure in solvents such as dioxane or chloroform often lose this structure in corresponding model oligopeptides of 4–6 residues. The results herein emphasize the importance of working in the least polar medium possible (*i.e.*, carbon tetrachloride or cyclohexane) when manifestations of (intra- or intermolecular) amide–amide interactions are sought. An understanding of the detailed nature of peptide–solvent interactions clearly requires further investigation.

Conclusions

Di- and tripeptides of proline containing an L-Pro-D-Pro, L-Pro-Gly, or Gly-L-Pro sequence adjacent to a C-terminal carboxylic acid have been proposed to form β-turn analogs in the solid state, as deduced from appropriate shifts to lower frequencies of hydrogen-bonded carbonyl groups. Isotopic labeling with ^{13}C at the Gly carbonyl carbon in *t*-Boc-Gly-L-Pro-OBz and *t*-Boc-Gly-L-Pro-OH provided experimental confirmation of certain band assignments. Bands shifted due to H-bonded structures in the solid state were not shifted in chloroform or dioxane solution.

Peptides where C-terminal carboxylic acid functions have been replaced with primary or secondary (*e.g.*, NH—CH_3) amides would be expected to display similar behavior. However, the carbonyl bands arising from C-terminal amides would occur in the region 1630–1670 cm^{-1} (*vs.* 1740–1750 for the acids), and unequivocal band assignments may be difficult, at least without ^{13}C labeling. The preference of the COOH moiety for the syn conformation (OH proton cis to carbonyl oxygen)[32,33] may have been a factor in the failure of these peptides to take up intramolecular structures in solution; the preference of secondary amides for the anti conformation may help to favor intramolecular species in solution.

X-Ray crystallography remains the method of choice for substantiation of the postulated intramolecularly hydrogen-bonded structures. Until this is done, caution should be used in any attempt to apply the spectral shifts and structural correlations presented herein to other peptide or protein systems.

Materials and Methods

The peptides reported in the tables were synthesized using mixed-anhydride-coupling procedures (employing *N*-methylmorpholine and isobutyl chloroformate). Hydrogenation to remove benzyl esters was carried out in *tert*-butyl alcohol with 10% palladium/charcoal as catalyst. Details of the syntheses of *t*-Boc-Gly-L-Pro-OH, *t*-Boc-L-Pro-L-Pro-OH, and *t*-Boc-L-Pro-L-Pro-L-Pro-OH, along with their corresponding benzyl esters, have been reported.[34] The remaining compounds were similarly prepared and characterized as described therein. Peptides were generally crystallized from ethyl acetate, chloroform, and ether, or mixtures thereof. Carbon-13 enriched glycine (*ca.* 60% at the glycine carbonyl carbon atom) was purchased from Merck & Co., Rahway, N. J. It was converted to *tert*-butyloxycarbonyl-glycine-OH and reacted with L-proline benzyl ester hydrochloride to produce the ^{13}C-enriched sample of *t*-Boc-Gly-L-Pro-OBz.

Infrared spectra were recorded on a Perkin-Elmer Model 521 infrared spectrometer.

Acknowledgment. The author is indebted to Professor Elkan R. Blout for advice and encouragement throughout the course of this investigation. Thanks are due also to Dr. Vincent Madison of this laboratory for numerous helpful discussions. Support, in part, by U. S. Public Health Service Grants AM-07300 and AM-10794, is gratefully acknowledged.

(32) T. Miyazawa and K. S. Pitzer, *J. Chem. Phys.*, **30**, 1076 (1959).
(33) V. Madison and J. Schellman, *Biopolymers*, **9**, 511 (1970).
(34) C. M. Deber, F. A. Bovey, J. P. Carver, and E. R. Blout, *J. Amer. Chem. Soc.*, **92**, 6191 (1970).