Conformations of Prolyl Residues in Oligopeptides

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The structures of the prolyl residues as found in the crystals of various oligopeptides were studied, and a few interesting structural features were found. (i) The C^{α}–C′–N angle of the peptide group formed by the N atom of the pyrrolidine ring and the C^{α} and C′ atoms of the preceding residue is widened to about 118° from the usual value of 114° due to the steric repulsion between the C^{α} and the two hydrogen atoms bonded to the C^{δ} atom of the pyrrolidine ring. (ii) The C^{γ} atoms of some pyrrolidine rings have some sort of disorder, and the C^{β}–C^{γ} and C^{γ}–C^{δ} bonds seem to be shortened and the C^{β}–C^{γ}–C^{δ} angles seem to be widened than usual. (iii) Almost half of the pyrrolidine rings have an approximate C_2 (half-chair) symmetry, the twofold axis passing through the N atom and the midpoint of the C^{β}–C^{γ} bond. The others have an approximate C_S (envelope) symmetry, the mirror plane passing through either the C^{β} or C^{γ} atom with equal probabilities. In this case the $NC^{\alpha}C^{\gamma}C^{\delta}$ or $NC^{\alpha}C^{\beta}C^{\delta}$ groups are almost planar, respectively. (iv) The conformation of C^{γ}-*exo* against the C′ atom bonded to the C^{α} atom is mainly found in the residues having the α-helix type torsion angles, while the C^{γ}-*endo* is in those having the collagen type torsion angles.

Proline is a unique imino acid which imposes a certain restriction on the conformations of proteins. The restriction is due to the formation of the pyrrolidine ring, and also to the bulky methylene group linked to the imino nitrogen atom, to which only a hydrogen atom is linked in all the other amino acids. The conformations of the pyrrolidine rings as observed in crystal structures were reviewed by Balasubramanian *et al.*,[1] however, they could include in the review only five examples of the prolyl residues in peptides. Recently, the structures of seven prolyl residues in three oligopeptides were established in our laboratory. The present paper deals with the conformations and the dimensions of ten prolyl and one hydroxyprolyl residues in six crystals.

Residues **1′** *and* **1″**: Pyrrolidine rings of C(4) C(5) C(6′) C(7) N(2) and C(4) C(5) C(6″) C(7) N(2) of L-Leu–L-Pro–Gly, numbering as in the original report.[2] R=12.9, mean e.s.d. of the bond distances σ=0.015 Å.

Residues **2** *and* **3**: Pro and Hyp of tosyl-L-Pro–L-Hyp.[3] R=9.3, σ=0.016 Å.

Residue **4**: Pro of *p*-bromo-Z–Gly–L-Pro–L-Leu–Gly.[4,5] R=4.3, σ=0.02 Å.

Residues **5** *and* **6**: Pro(1) and (2) of *o*-bromo-Z–Gly–L-Pro(1)–L-Leu–Gly–L-Pro(2).[6] R=11.6, σ=0.03 Å.

Residues **7** *and* **8**: Pro(1) and (2) of Z–Gly–L-Pro(1)–L-Leu–Gly–L-Pro(2).[7] R=5.8, σ=0.008 Å.

Residues **9**, **10**, *and* **11**: Pro(1), (2), and (3) of Aoc–L-Pro(1)–L-Pro(2)–L-Pro(3).[8] R=6.0, σ=0.008 Å.

Bond Lengths and Angles

The nomenclature and the definition of the atoms, bonds and torsion angles[9] are given in Fig. 1, and the bond lengths and angles are summarized in Table 1. In Fig. 2 their mean values of the peptide groups are compared with the standard values for the usual peptide groups given by Corey and Pauling[10] and by Marsh and Donohue.[11] The torsion angles of the peptide main chain are listed in Table 2. All these values were recalculated from the published coordinates of the crystal structures.

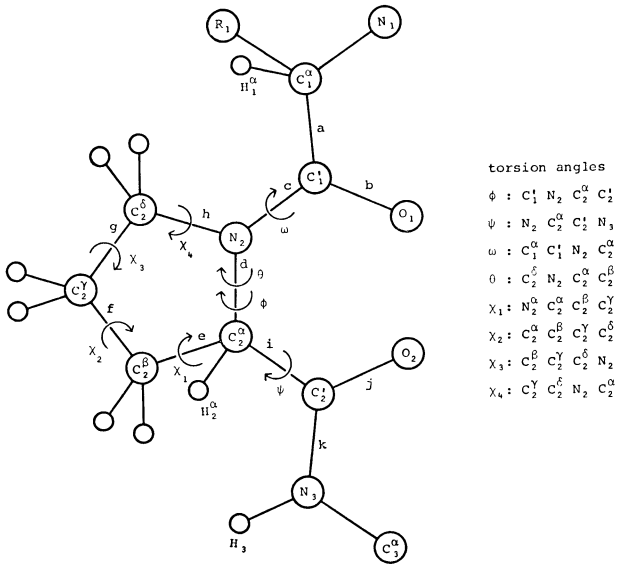

Fig. 1. The nomenclature and the definition of the atoms, bonds and torsion angles. The convention given by IUPAC-IUB Commission on Biochemical Nomenclature[9] is adopted.

Peptide Groups. The mean C^{α}–C′–N angle (ac) of 118° is significantly larger than 114° given by Corey and Pauling[10] and 116° by Marsh and Donohue.[11] While the O–C′–N (bc) of 120.5° is less than 125° or 123.5°, respectively. This is certainly due to the steric repulsion between the C^{α} atom of the preceding residue and the bulky C^{δ} methylene group of the pyrrolidine ring, in particular two hydrogen atoms bonded to the C^{δ} atom. In the cases of **4** to **8** the preceding residues are glycines, but the same tendency is observed. Thus this discrepancy from the standard values is not affected by the sizes of the side groups of the preceding residues.

A similar tendency is also found in the bond angles of C^{α}–C′–N (ik) and O–C′–N (jk) of the peptide groups between the prolyl and the following residues. However, if the residues **2**, **9**, and **10**, which are followed by prolyl residues, are not taken into account, the mean value of the C^{α}–C′–N angles of 116° is quite close to the standard value. Thus the presence of the pyr-

Table 1. Bond lengths (Å) and angles (degrees) in prolyl residues in oligopeptides

Residue[a]	1[b]	2	3	4	5	6	7	8	9	10	11	Mean
	Bond Lengths											
a	1.50		1.52	1.51	1.47[c]	1.52	1.53	1.51		1.53	1.51	1.516
b	1.27		1.23	1.26	1.26	1.27	1.24	1.24	1.22	1.22	1.25	1.246
c	1.34		1.33	1.37[c]	1.33	1.32	1.34	1.33	1.34	1.33	1.33	1.332
d	1.45	1.48	1.47	1.46	1.50[c]	1.46	1.47	1.45	1.45	1.46	1.46	1.461
e	1.50	1.54	1.54	1.55	1.62[c]	1.54	1.53	1.52	1.55	1.54	1.53	1.534
f	1.51	1.50	1.55	1.41[d]	1.54	1.46[c]	1.44[d]	1.50	1.52	1.49[d]	1.52	1.520
g	1.50	1.54	1.48[c]	1.49[d]	1.54	1.58[c]	1.45[d]	1.53	1.53	1.43[d]	1.52	1.521
h	1.46	1.47	1.47	1.46	1.46	1.55[c]	1.48	1.48	1.47	1.47	1.48	1.470
i	1.52	1.52	1.50	1.49	1.57[c]	1.51	1.52	1.53	1.53	1.51	1.53	1.516
j	1.24	1.23		1.22	1.22		1.21		1.22	1.25		1.227
	Bond Angles											
ab	119		123	122	123	122	122	122		121	120	121.6
ac	119		116	119	118	119	118	117		118	118	118.0
bc	122		121	120	120	119	120	121		120	122	120.6
cd	121		120	122	120	125[c]	121	121	124	118[c]	121	121.3
ch	126		130[c]	124	126	121[c]	127	126	121[c]	128	127	126.3
dh	113	113	111	114	114	114	112	113	114	113	112	113.0
di	111	111	110	117[c]	113	110	114	111	111	110	110	111.1
de	104	102	103	102	103	103	103	104	105	104	104	103.4
ei	113	109	110	115[c]	106[c]	111	111	110	111	112	111	110.9
ef	107	108	104	108	103	105	108	105	102	105	104	105.4
fg	106	102	101[c]	109[d]	108	108	111[d]	105	109	111[d]	104	106.0
gh	103	104	107	104	105	98[c]	104	102	102	104	103	103.8
ij	121	123		122	119		121		121	120		121.0
ik	115	116		116	116		117		118	118		116.6
jk	123	121		122	125		122		120	122		122.1

a) References: residue **1**, Ref. 2; **2** and **3**, Ref. 3; **4**, Ref. 5; **5** and **6**, Ref. 6; **7** and **8**, Ref. 7; **9**, **10** and **11**, Ref. 8. b) The bond lengths and angles for the residue **1** are the means of the two models **1′** and **1″**. c) The values are not included in estimating the mean values because of the large discrepancies from the others. d) The values are not included in estimating the mean values because of the anomalies of the C^{γ} atoms.

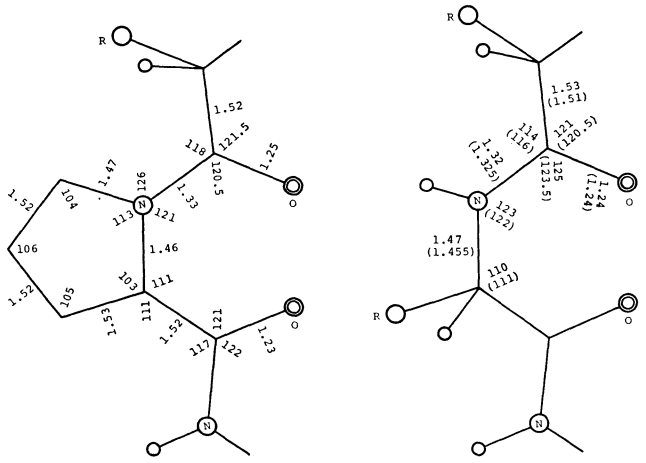

Fig. 2. *left*: the mean bond distances and angles in the prolyl residues in oligopeptides. *right*: the bond distances and angles of the peptide bond given by Corey and Pauling[10] and those (in brackets) given by Marsh and Donohue.[11]

rolidine ring affects significantly the shape of the peptide group preceding, but does not affect the one following. This fact should be considered in the conformational studies on proteins or peptides, especially collagen, polyproline and polyhydroxyproline.

Table 2. The torsion angles of the prolyl residues in the oligopeptides (in degrees)

Residue	ϕ	ϕ	ω
1	−68	162	175
2		157	
3	−51		176
4	−58	−33	186
5	−65	−26	191
6	−72		178
7	−63	−23	184
8	−67		181
9	−59	155	
10	−56	139	172
11	−63		181

Pyrrolidine Ring. The pyrrolidine rings of the residues **4**, **7**, and **10** show significant and systematic anomalies in the C^{β}–C^{γ} and C^{γ}–C^{δ} lengths and the C^{β}–C^{γ}–C^{δ} angles. The bond lengths are shorter, while the angles are wider than usual. This is clearly due to some sort of anomaly associated with the locations of the C^{γ} atoms.

In the analysis of Leu–Pro–Gly, Leung and Marsh[2] found a similar behavior of the C^{γ} atom, and they placed successfully two half C^{γ} atoms on both sides of the pyrrolidine plane, the two models being the residues

1′ and **1″** now discussed in this paper. On the other hand in *p*-bromo-Z–Gly–Pro–Leu–Gly,[5] Z–Gly–Pro–Leu–Gly–Pro,[7] and Aoc–(Pro)$_3$,[8] the C$^\gamma$ atoms of the residues **4**, **7**, and **10** gave unusually large vibrational amplitudes in the direction perpendicular to the pyrrolidine planes, but no anomalies were found in the vicinities of the C$^\gamma$ sites in the difference electron density maps. Although the possibilities of the disordered structure as postulated for Leu–Pro–Gly should be taken into account, the refinements employing usual anisotropic temperature factors seemed to be reasonably converged. The models thus obtained give the shorter bond lengths of C$^\beta$–C$^\gamma$ and C$^\gamma$–C$^\delta$ and the wider C$^\beta$–C$^\gamma$–C$^\delta$ angles than the true values. DL-Proline HCl shows also a similar tendency.[12]

Puckering of Pyrrolidine Ring

The torsion angles of all the five bonds of the pyrrolidine ring are listed in Table 3. Balasubramanian *et al.* classified the proline ring structures into two classes, A and B.[1] Conformation A is characterized by negative χ_1, and C′ and C$^\gamma$ are on opposite side of the roughly planar NC$^\alpha$C$^\beta$C$^\gamma$ plane (C$^\gamma$-*exo*), while conformation B has positive χ_1, and the two atoms are on the same side of the plane (C$^\gamma$-*endo*).

Among newly determined structures of the pyrrolidine rings in peptides, several residues, such as **5**, **9**, and **10**, are reported to have more planar NC$^\alpha$C$^\gamma$C$^\delta$ groups rather than NC$^\alpha$C$^\beta$C$^\delta$. Also in two of four rings in tetra-proline[13] the C$^\beta$ atoms are displaced mostly from the ring planes. Therefore, a further study of the puckering of the rings is made from a slightly different viewpoint from that of Balasubramanian *et al.*[1]

The structures viewed along the C′–N bonds of the peptide linkages, more strictly, along the bisectors of the C$^\alpha$–N–C$^\delta$ angles are presented in Fig. 3. It is clearly shown that almost half of the structures have roughly a C$_2$ (half-chair) symmetry[14] with the twofold axis passing through the N atom and the midpoint of the C$^\beta$–C$^\gamma$ bond. In this structure neither the NC$^\alpha$C$^\beta$C$^\delta$ nor NC$^\alpha$C$^\gamma$C$^\delta$ groups are planar. This structure may be designated as C$_2$-C$^\gamma$-*exo* (C$^\beta$-*endo*) or C$_2$-C$^\gamma$-*endo* (C$^\beta$-*exo*) depending on whether C$^\gamma$ is displaced on the same side as C′ or on the opposite side of the NC$^\alpha$C$^\delta$ plane. Both of these structures have been actually found so far. The others have an approximate C$_s$ (envelope) symmetry, the mirror plane passing through either the

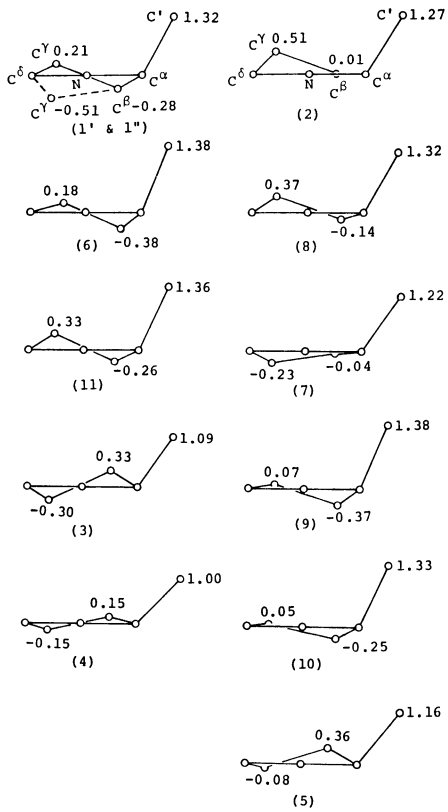

Fig. 3. The conformations of the pyrrolidine rings viewed along the bisectors of the C$^\alpha$NC$^\delta$ angles. The displacements of the atoms from the NC$^\alpha$C$^\delta$ planes are shown in Å unit. *left*: the rings of approximate C$_2$ symmetry, *right*: the rings of approximate C$_s$ symmetry.

Table 3. The conformations of the pyrrolidine rings in the oligopeptides

Residue	Conformation	Shift (Å) from NC$^\alpha$C$^\delta$-plane			Torsion angles (degrees)				
		C$^\beta$	C$^\gamma$	C′	θ	χ_1	χ_2	χ_3	χ_4
	C$^\beta$-*endo*, C$^\gamma$-*exo*, (C$^\delta$-*endo*) (conformation A)								
1″	C$_s$-C$^\delta$-*endo*	−0.28	−0.51	1.32	−11	−3	16	−22	21
3	C$_2$-C$^\gamma$-*exo*	0.33	−0.30	1.09	13	−31	37	−30	12
4	C$_2$-C$^\gamma$-*exo*	0.15	−0.15	1.00	6	−16	21	−17	6
5	C$_s$-C$^\beta$-*endo*	0.36	−0.08	1.16	13	−24	27	−19	3
7	C$_s$-C$^\gamma$-*exo*	−0.04	−0.23	1.22	−2	−7	14	−14	9
	C$^\beta$-*exo*, C$^\gamma$-*endo* (conformation B)								
1′	C$_2$-C$^\gamma$-*endo*	−0.28	0.21	1.32	−11	26	−31	24	−8
2	C$_s$-C$^\gamma$-*endo*	0.01	0.51	1.27	0	21	−32	31	−20
6	C$_2$-C$^\gamma$-*endo*	−0.38	0.18	1.38	−15	32	−38	27	−7
8	C$_s$-C$^\gamma$-*endo*	−0.14	0.37	1.32	−5	24	−33	29	−14
9	C$_s$-C$^\beta$-*exo*	−0.37	0.07	1.38	−14	25	−27	19	−3
10	C$_s$-C$^\beta$-*exo*	−0.25	0.05	1.33	−10	17	−20	14	−2
11	C$_2$-C$^\gamma$-*endo*	−0.26	0.33	1.36	−10	29	−38	31	−13

The conformation of **6** is an intermediate of C$_2$-C$^\gamma$-*endo* and C$_s$-C$^\beta$-*exo*.
The conformation of **8** is an intermediate of C$_s$-C$^\gamma$-*endo* and C$_2$-C$^\gamma$-*endo*.

C^β or C^γ atoms. If the mirror plane passes through C^β, χ_4 is close to zero and the atoms $C^\alpha C^\gamma C^\delta N$ are coplanar. This structure may be designated as C_s-C^β-*endo* or C_s-C^β-*exo* depending on the direction of the displacement of the C^β atom from the $NC^\alpha C^\gamma C^\delta$ plane. If the C^γ atom is on the mirror plane, θ is close to zero, the four atoms $C^\alpha C^\beta C^\delta N$ are coplanar, and the structure may well be characterized by C_s-C^γ-*exo* or C_s-C^γ-*endo*. All the four C_s structures have also been found so far. In Fig. 3 it is clearly shown that notwithstanding the approximate symmetry the displacement of the C′ atom from the $NC^\alpha C^\delta$ plane is larger for C^γ-*endo* (C^β-*exo*) than for C^γ-*exo* (C^β-*endo*) due to a steric requirement.

The above discussion shows that the displacement alone of the C^γ atom from the $NC^\alpha C^\beta C^\delta$ plane is not necessarily appropriate to describe the conformation of the pyrrolidine ring. The five torsion angles or the displacements of the C^β and C^γ atoms from the $NC^\alpha C^\delta$ plane are the necessary informations to describe the structure. The notation suggested in this report, such as C_2-C^γ-*endo*, gives more directly an approximate picture of the prolyl ring. The conformations of the prolyl residues in peptides classified on these lines are summarized in Table 3, where the displacements of the C^β and C^γ atoms and the five torsion angles are also listed. Among these examples residue **6** is an intermediate of C_2-C^γ-*endo* and C_s-C^β-*exo*, and residue **8** is an intermediate of C_s-C^γ-*endo* and C_2-C^γ-*endo*. Residue **1″** cannot be classified into anyone of these structures, but is a unique example of C_s-C^δ-*endo*. According to the classification by Balasubramanian *et al.*,[1] conformation A includes C^β-*endo* and/or C^γ-*exo* irrespective of the symmetry of the ring, and B includes C^β-*exo* and/or C^γ-*endo*.

The pyrrolidine rings in crystals of amino acids are also classified in this way; L-proline[15] is C_2-C^γ-*endo*, L-hydroxyproline[16] and proline in copper DL-proline dihydrate[17] are C_s-C^γ-*exo*, and the pyrrolidine ring in acetyl-L-proline-*N*-methylamide[18] is an intermediate of C_2-C^γ-*endo* and C_s-C^γ-*endo*. The pyrrolidine ring of DL-proline HCl[12] might be termed C_s-C^α-*exo*, really a rare example.

A theoretical treatment of the conformations of the pyrrolidine rings by a potential energy calculation showed that C^γ-*endo* is somewhat stabler than C^γ-*exo*, but the energy difference between the two conformations is only marginal, and may not be so decisive in determining the conformations.[19,20] Among 12 examples, 7 are C^γ-*endo*, while 5 are C^γ-*exo*.

In such discussions the rotation of the C^α–C′ bond should be taken into consideration.[21] The prolyl residues are classified into two groups according to the rotation angle ψ as shown in Table 2. One is the collagen type with large positive ψ (residues **1**, **2**, **9**, **10**), and the other is the α-helix type with small negative ψ (residues **4**, **5**, **7**). A model building shows easily that in the α-helix type the NH group of the following residue is in contact with the H atoms bonded to the C^γ and C^δ atoms of the ring, and the contact may be too close if the C^γ atom is in *endo* against the C′ atom. Each of the three residues of this type is C^γ-*exo* (conformation A). While in collagen type, since all the four residues are C^γ-*endo* or C^β-*exo*, the O atom of the following peptide group may be in appropriate nonbonding (van der Waals) contact with the ring atoms of conformation B. Thus, although the number of examples may not be large enough, it may be concluded that the α-helix type residues take conformation A while the collagen type residues take conformation B. The relation, however, may not be so explicit, since a simple molecule of acetyl-L-proline-*N*-methylamide[18] is an α-helix type with $\psi=-16°$, but C^γ is in the *endo* position. The intermolecular interactions may play a key role in this structure rather than the intramolecular interactions mentioned above.

The last point is that the disordered C^γ atom mentioned earlier has another effect on the shape of the pyrrolidine ring, that is, the ring having a disordered C^γ atom, such as residues **4**, **7**, and **10**, is significantly more planar than the others. All the torsion angles of the bonds of the ring are close to zero, and the displacements of the C^β and C^γ atoms from the $NC^\alpha C^\delta$ plane are smaller than the others. The C^γ atom has usually two stereochemically possible sites on each side of the ring, if the rest of the ring is fixed. For instance, in residue **10** the distance between the two possible sites is about 0.8 Å. The actually located site of the C^γ atom in this residue is on the midway of the line joining the two possible sites, 0.2 Å to the closer site and 0.6 Å to the other. Thus, the actually located site is significantly closer to the plane of the pyrrolidine ring than the stereochemically ideal one.

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4) Abbreviations: Z, benzyloxycarbonyl; Aoc, *t*-amyloxycarbonyl.

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20) In Ref. 1 it is reported inconsistently that C^{γ}-*exo* is stabler than C^{γ}-*endo*.
21) The possibility of a relation between ϕ and the ring conformation is suggested by a referee, to whom the authors' thanks are due.