

## Energy Calculations on Di- and Poly-L-Proline

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Received March 30, 1972

The problem of distortion of a pyrrolidine ring in a prolyl residue is treated in terms of the rotational angle of  $\phi$  about N-C $\alpha$  bond.<sup>1)</sup> Assuming that the C $\gamma$  atom in the ring occupies one of the two representative positions, energy maps of poly-L-proline and di-peptide Pro-Gly can be calculated with the variables of  $\phi$ ,  $\psi$  and  $\omega$  ( $\omega$  is fixed at  $\pm 180^\circ$  for Pro-Gly). The energy maps of Pro-Gly resemble the usual dipeptide maps in the sterically allowable range of  $\phi$ . Polyproline in the trans-planar configuration has two minima in the lower energy contour area (II) involving polyproline II conformation, and in the cis-configuration has a single deep minimum (I). The energy difference between the minima for both configurations is about 2.5 Kcal/mol/residue. Two other areas (III, IV) of lower energies are found mainly in the ( $\omega$ ,  $\psi$ ) maps under the constant values of  $\phi$ . One of them lies on a line of  $\omega \doteq -165^\circ$  and the other on a line of  $\omega \doteq -30^\circ$ . The result that the minimum (III) has a lower energy than that of the latter (IV) is discussed in relation to the helical conformation and the possibility of their existence.

### INTRODUCTION

Energy calculations on poly-L-proline have been made by various authors<sup>2)</sup> on the assumption that the rotational angle about C $\alpha$ -N bond,  $\phi$ , is fixed because of the restriction by pyrrolidine ring. The fixed angle of  $\phi$  is usually employed either *ca.*  $-80^\circ$  or *ca.*  $-60^\circ$  according to the crystallographic data of the proline residue in oligopeptides.<sup>3)</sup> On the other hand, it is well-known that the pyrrolidine ring is not frozen in a fixed configuration but is able to move in a way, so called ring puckering. It is, therefore, reasonable to consider the freedom of the rotational angle  $\phi$  in a limited range. Ramachandran *et al.*<sup>4)</sup> have calculated the possible range of ( $\phi$ ,  $\psi$ ) in a proline residue, although they took only a part of the pyrrolidine ring into consideration. In the previous paper<sup>1)</sup> we calculated the distortion energies of the pyrrolidine ring under the assumption of the fixed bond lengths and bond angles in the ring, and determined the extreme limits of angle  $\phi$  with respect to two representative positions of C $\gamma$  atom (with the H atoms attached to it), because the positions were not determined uniquely from a fixed value of the angle  $\phi$ .

In this article we will describe the energy maps of poly-L-proline obtained by the use of the same treatment on pyrrolidine ring as in the previous report. The rotational angle about N-C $\gamma$  of peptide part ( $\omega$ ) along the backbone is treated also as a variable in order to take trans-cis interconversion of polyproline into account. All energy maps made are concerned with three variables  $\phi$ ,  $\psi$ , and  $\omega$ , and two positions of C $\gamma$  atom for a given set of the variables are necessary to calculate the energy. In addition to the calculation of energies on polyproline, it may be also meaningful to estimate energies for a proline residue in a polypeptide chain since such a situation is usually found to occur in actual proteins. Therefore, ( $\phi$ ,  $\psi$ ) energy maps of di-peptide Pro-Gly are made and compared with those of polyproline.

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## METHOD

## 1. Geometry of Prolyl Units

All the bond lengths in a prolyl residue were assumed to have fixed values, and some of the bond angles were also fixed as shown in Fig. 1. These values were determined mainly by the Ramachandran's recommended values<sup>4)</sup> for the prolyl residue as described below. The configuration of the pyrrolidine ring, as treated in the previous paper, was determined in relation to the rotational angle about N-C<sup>α</sup> bond ( $\phi$ ). The C<sup>γ</sup> atom was assumed to occupy one of the two positions opposite each other, *i. e.* one of them being on the plane C<sup>α</sup>-N-C<sup>β</sup> ( $\gamma$ 1-Position), which coincides with the planar-peptide plane in trans or cis configuration, and the other on C<sup>β</sup>-C<sup>α</sup>-N plane ( $\gamma$ 2-position). When a value of  $\phi$  and a position of C<sup>γ</sup> atom are given, the coordinates of C<sup>γ</sup> atom can be calculated under the condition of the constant lengths of C<sup>β</sup>-C<sup>γ</sup> and C<sup>γ</sup>-C<sup>δ</sup> bonds. Consequently,  $\tau$ (C<sup>α</sup>-C<sup>β</sup>-C<sup>γ</sup>),  $\tau$ (C<sup>β</sup>-C<sup>γ</sup>-C<sup>δ</sup>) and  $\tau$ (C<sup>γ</sup>-C<sup>δ</sup>-N) (and positions of H atoms attached to C<sup>γ</sup>, C<sup>β</sup> and C<sup>δ</sup>) can also be determined. The changes in these bond angles are plotted against  $\phi$  in Fig. 2. It is clearly shown that the deviation of  $\phi$  from the value of  $-60^\circ$  leads to a large distortion of the bond angles, giving rise to a high value of bending energy. Therefore the allowable range for  $\phi$  may be estimated from the energy calculation as shown in previous paper,<sup>1)</sup> where we obtained,  $-80^\circ < \phi < -40^\circ$  for an approximate limit of the range.

In the peptide part, it is necessary to exchange the angle  $\tau$ (C<sup>α</sup>NC') to  $\tau$ (C<sup>β</sup>NC') according to the conversion from trans configuration to cis because of the symmetrical relationship of the pyrrolidine ring against the peptide bond.<sup>4)</sup> The values in parentheses in Fig. 1 are those for cis configuration. When the rotation about the peptide bond ( $\omega$ ) was at an intermediate value, the following relations were assumed for  $\tau$ (C<sup>α</sup>NC') and  $\tau$ (C<sup>β</sup>NC') respectively in order to combine the two values continuously.

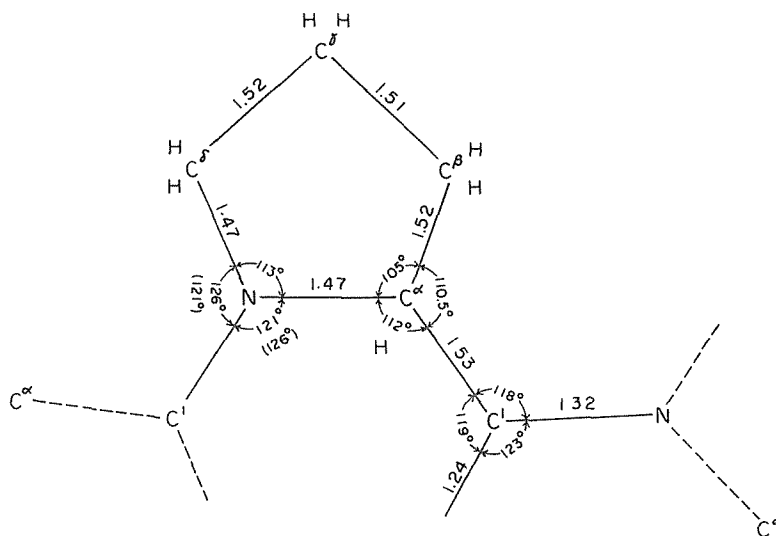


Fig. 1. The geometry of a prolyl residue used in the calculation is shown.

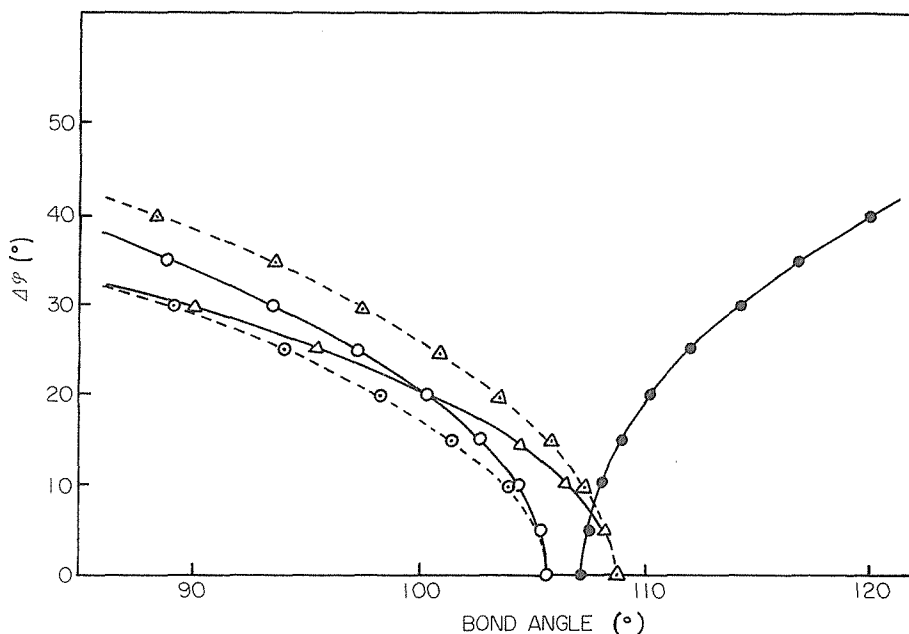


Fig. 2. The extent of distortion in the ring is expressed by bond angles of  $\tau(C^\beta C^\gamma C^\delta)$  (●),  $(C^\alpha C^\beta C^\gamma)$  ( $\Delta$ ,  $\triangle$ ), and  $\tau(C^\gamma C^\delta N)$  ( $\circ$ ,  $\odot$ ) as a function of  $\Delta\phi (= -60^\circ - \phi)$ . The full line is for  $\gamma 1$  position and the dashed line for  $\gamma 2$  position.

$$\tau(C^\alpha N C') = 126^\circ - 5^\circ (\sin \omega/2)^2.$$

$$\tau(C^\beta N C') = 121^\circ + 5^\circ (\sin \omega/2)^2.$$

There have been some arguments<sup>5)</sup> about a value for the bond angle  $\tau(NC^\alpha C')$ . Since the tetrahedral angle  $109.5^\circ$  for the angle is thought to be too small in most cases, we have selected the value of  $112^\circ$ . The choice for this angle is important because it has an effect on the helical dimensions of polyproline.

## 2. Energy Functions and Parameters

The total conformational energy consists of four different kinds of contributions, *i. e.*, bending energy, torsional energy, non-bonded interaction and electrostatic interaction. Hydrogen bonding is not necessary to take into account for an isolated polyproline. Bending energies were taken in consideration for the three variable bond angles in pyrrolidine ring, *i. e.*,  $\tau(C^\alpha C^\beta C^\gamma)$ ,  $\tau(C^\beta C^\gamma C^\delta)$  and  $\tau(C^\gamma C^\delta N)$ . The potential function was assumed to be proportional to the square of the degree of the distortion from the standard angle of the normal tetrahedral angle ( $=109.5^\circ$ ), as described in previous paper.

Torsional energies for all the C-C or C-N bonds in the ring were included in addition to the usual torsional energies along the main chain, *i. e.*,  $\Delta E_\phi(N-C^\alpha$  bonds),  $\Delta E_\phi(C^\alpha-C'$  bond), and  $\Delta E_\omega(C'-N$  bond). The functions and the parameters for the latter three along the main chain are the same as those usually used.<sup>6)</sup> The three fold potential function was employed about the bonds in the ring (*i. e.*,  $C^\alpha-C^\beta$ ,  $C^\beta-C^\gamma$ ,  $C^\gamma-C^\delta$ , and  $C^\delta-N$  bonds) for each rotational angle  $\chi_i$ , and the barrier height of 3 Kcal/mol was assumed to be common among these rotational potentials. The Lennard-Jones 6-12 potential was

employed for the non-bonded interaction and energy parameters used were the same as those of Ooi *et al*<sup>6)</sup> (a hydrogen atom radius of 1.275 Å was used in the present paper).

The dipole moment of the C'=O bond in the peptide part was necessary to take into account for electrostatic interactions. The monopole approximation was used by putting partial charges at the C' and O atom so as to reproduce a moment of 2.4 Debye. As a dielectric constant the value of D=4 was used.

### 3. Definition of Dipeptide Pro-Gly

The dipeptide Pro-Gly is defined as in Fig. 3 in accord with that of Go and Scheraga.<sup>7)</sup> The dimension for each bond length and bond angle is the same as that mentioned before. The two peptide bond parts in it are fixed in trans-planar configuration. Therefore, only  $\phi$  and  $\psi$  in the prolyl residue are the independent variables for each position of C' atom.

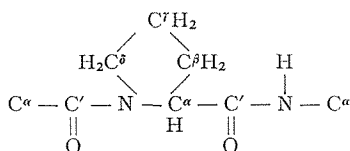


Fig. 3. Atomic arrangement of dipeptide Pro-Gly is shown.

## RESULTS AND DISCUSSION

The  $(\phi, \psi)$  energy maps with trans-planar peptide ( $\omega=180^\circ$ ) of Pro-Gly and poly-L-proline are shown in Figs. 4 and 5 respectively. The  $\phi$  axis of abscissa in each energy map is shown only in the neighborhood around  $\phi \approx -60^\circ$ , since out of this region, as described before, is completely prohibited with resulting large deformations or unclosure of the pyrrolidine ring. The energy values shown are relative ones against the lowest energy minimum for Pro-Gly and polyproline, respectively. The regions below 10 Kcal (per residue for polyproline) are contoured in the maps. All the maps hereafter are shown as follows; a contour of 10 Kcal/mol is represented by the outermost full line, 5 Kcal/mol by the dashed line, and smaller energy contours are indicated by the energy (Kcal/mol) on the contour lines. The lowest minimum for Pro-Gly is located around at  $(\phi=\psi=-50^\circ)$  with  $\gamma_1$  position (Fig. 4a), but the energy surface is so broad that the energy differences with other minima are small, *i. e.*, the order of 100~200 cal/mol. Symmetrical split against  $\phi$  of 1 Kcal/mol contours with respect to  $\phi \doteq -60^\circ$  is due to the energy contribution from the ring distortion. It is seen that the outlines of lower energies are almost similar to a normal dipeptide map, *e. g.*, the dialanine energy map,<sup>8)</sup> although within the limited region of  $\phi$ . It is especially interesting that the right-handed " $\alpha$ -helical region" (around  $\phi=-80^\circ \sim -40^\circ$  and  $\psi=-70^\circ \sim -30^\circ$ ) is fully included in the lower energy area of Pro-Gly irrespective to C' atom position. Taking into consideration that this region becomes disallowed in polyproline as seen in Fig. 5, it may be probable that a prolyl residue, if located among other kinds of residues in a polypeptide chain, could be folded into the  $\alpha$ -helical conformation. In actual proteins, however, prolyl residues were only found to be located at terminal ends of  $\alpha$ -helices or other parts of non-helical regions.<sup>9)</sup> If this is the general situation, the reason might be due not to the

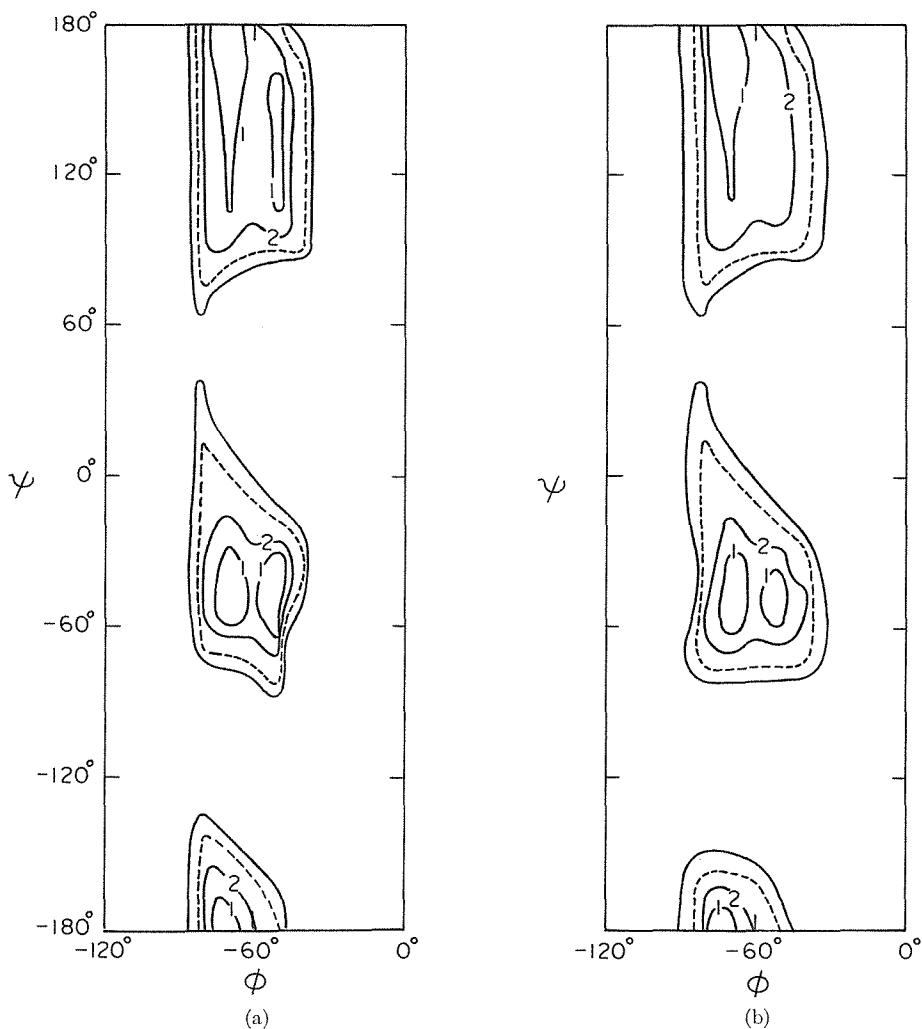


Fig. 4. Energy contour maps of Pro-Gly as a function of  $\phi$  and  $\psi$ . (a)  $\gamma_1$  position, (b)  $\gamma_2$  position. In these calculations,  $\omega$  was fixed as  $\pm 180^\circ$ . Outermost full lines indicate energy contour of 10 Kcal/mol, and dashed lines 5 Kcal/mol. The energy (Kcal/mol) of other contour lines is shown on the lines.

geometrical difficulty, *e. g.*, interatomic collisions of the prolyl residue but to incapability in formation of one of hydrogen bonds owing to the imino group, which would make a resulting  $\alpha$ -helix irregular and less stable.

The lowest energy minimum of polyproline was obtained by the energy minimization with respect to three variables,  $\phi$ ,  $\psi$ , and  $\omega$ . The location and the absolute total energy,  $\phi = -75^\circ$ ,  $\psi = 151^\circ$ ,  $\omega = -2^\circ$ , and  $E_t = 3.7$  Kcal/mol, are shown in Table 1 together with other energy minima. The contour areas with a lower energy in Fig. 5 are restricted to a smaller extent compared with those of Pro-Gly. The corresponding ranges for  $\psi$  show the same tendency to be restricted in the range ca.  $\psi = 100^\circ \sim 180^\circ$  and  $-180^\circ \sim -160^\circ$ , as had shown by other authors under the condition of the angle  $\phi$  fixed.<sup>2)</sup> The  $\alpha$ -helical area, however, is not completely excluded in our calculations,

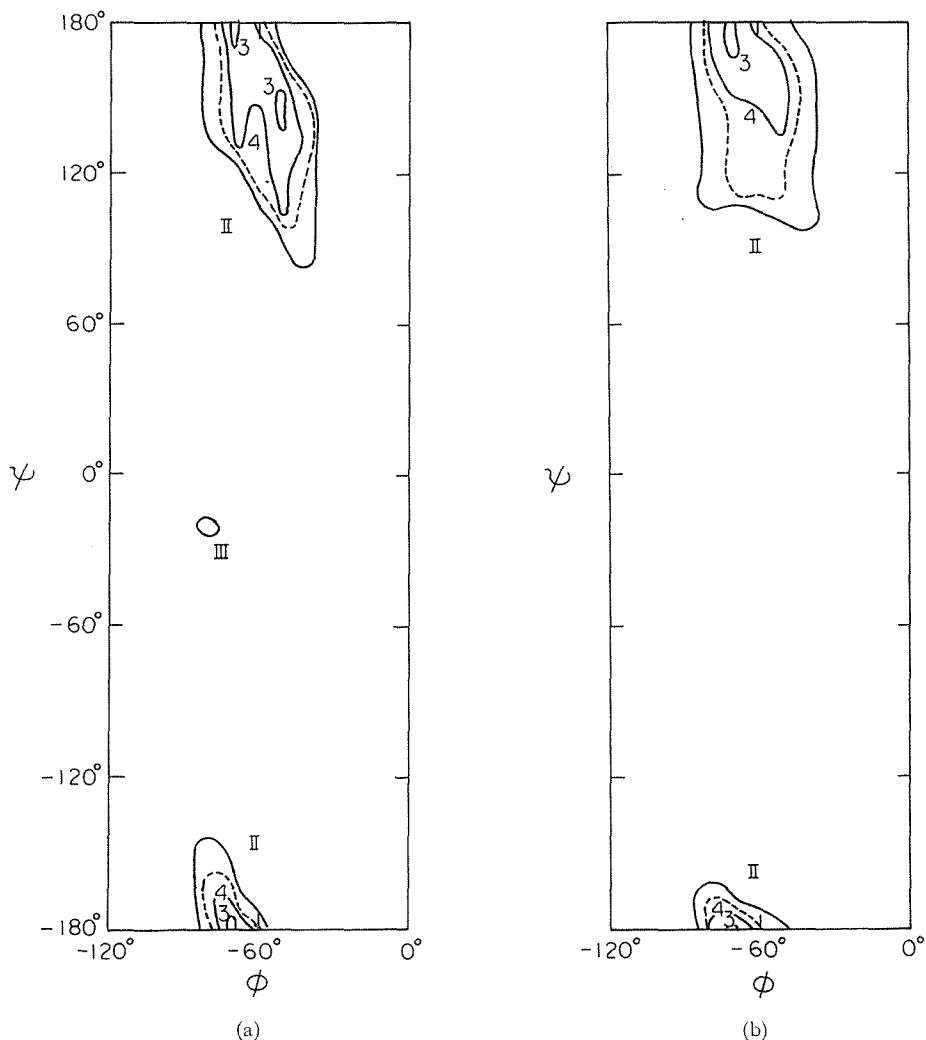


Fig 5. Energy contour maps of polyproline as a function of  $\phi$  and  $\psi$ .  
 (a)  $\gamma_1$  position, (b)  $\gamma_2$  position.  $\omega=180^\circ$ .

but is shown to have lower energies in the small limited  $(\phi, \psi)$  region only with the  $\gamma_1$  position of the  $C^\gamma$  atom. This area designated "area III" appears also in  $(\omega, \psi)$  maps shown later, where details will be referred to.

While the shape of area II is somewhat different depending on the choice of  $C^\gamma$  position as shown in Figs. 5a and 5b, there is a common feature that the range in  $\phi$  is not symmetrical about both sides of the line  $\phi=-60^\circ$ , and inclines toward the left hand side, *i.e.*, restricted in  $-80^\circ < \phi < -50^\circ$ . There are two minima in this area separated by a rather low energy barrier of several hundreds calories per mole. One of them lies around  $\phi=-75^\circ$  and the other  $\phi=-50^\circ$ . The experimental  $(\phi, \psi)$  value for the conformation of polyproline II ( $\phi=-76.6^\circ$ ,  $\psi=148.5^\circ$ ,  $\omega=180^\circ$ ) is located on one of the line described above, but shifts downwards from the minimum by ca.  $30^\circ$ . The energy for the conformation is higher than that of the minimum by about 2 Kcal/mol,

Table 1. Conformational Parameters and Absolute Energies at Minima, and those of PPI and PPII.

	Area	C <sup>r</sup> position	Dihedral angle (degree)			Helical parameter*		Absolute energy** (Kcal/mole)					
			$\phi$	$\psi$	$\omega$	$\theta$ (degree)	$d$ (Å)	Total	$E_{nb}$	$E_{es}$	$E_{rt}$	$E_{bd}$	$E_{tr}$
1	I	$\gamma 1$	-75.2	151.1	-2.0	114.4	1.733	3.67	-8.56	0.06	0.61	2.12	9.43
2		$\gamma 2$	-75.7	157.4	-3.5	117.8	1.836	4.01	-8.52	0.03	0.62	2.30	9.58
3	II	$\gamma 1$	-73.2	-176.4	174.7	-96.5	2.966	6.21	-5.86	-0.14	0.69	1.55	9.97
4		$\gamma 1$	-47.1	143.9	179.5	-101.0	2.671	6.30	-5.66	-0.22	0.65	1.48	10.05
5		$\gamma 2$	-74.2	177.3	178.5	-98.4	3.013	6.15	-6.01	-0.16	0.52	1.85	9.95
6		$\gamma 2$	-49.9	155.3	178.4	-96.1	2.690	7.06	-5.25	-0.16	0.63	0.99	10.95
7***	III	$\gamma 1$	-65.1	-49.8	-164.2	86.9	1.821	7.62	-6.77	0.07	2.07	0.58	11.67
8		$\gamma 2$	-59.4	-57.4	-163.2	84.9	1.813	8.84	-6.01	0.09	2.26	0.50	12.00
9	IV	$\gamma 1$	-77.1	-36.7	-30.1	135.7	2.396	10.20	-7.49	0.33	5.58	2.92	8.86
	PPI	$\gamma 2$	-82.3	160.4	0.0	108.0	1.900	5.92	-8.39	0.06	0.45	6.07	7.73
	PPII	$\gamma 1$	-76.6	148.5	180.0	-120.0	3.120	8.19	-3.87	-0.22	0.59	2.68	9.01

\*  $\theta$ : angular twist about the helical axis per unit,  $d$ : translation height along the helical axis per unit.

\*\* Abbreviations used;  $E_{nb}$ : non-bonded energy,  $E_{es}$ : electrostatic interaction,  $E_{rt}$ : sum of the rotational energies about  $\phi$ ,  $\psi$  and  $\omega$ ,  $E_{bd}$ : bending energies in the pyrrolidine ring,  $E_{tr}$ : torsional energies in the ring.

\*\*\* The conformation is shown in Fig. 10.

as shown in Table 1. The energy maps for the cis-configuration of the peptide group are shown in Figs. 6a and b. The low energy contours in the both maps (designated "area I") are similar with each other, and the global energy minimum in the whole ( $\phi$ ,  $\psi$ ,  $\omega$ ) space is located in this area as shown in Table 1. The energy is lower than the minimum in "area II" by about 2.5 Kcal/mol, suggesting that area I, when compared with area II, is energetically stable though entropically less stable.

The experimental values of  $\phi$  and  $\psi$  ( $\omega=0$ ) for polyproline I (PPI) is located near the minimum in the lower energy region. The energy at the point, however, is higher than the minimum value by 2.2 Kcal/mol (Table 1). Values of  $\phi$  and  $\psi$  which satisfy given values of the helical height and the number of residues per turn, are dependent on the geometry of the residue (some examples of other ( $\phi$ ,  $\psi$ ) values for PPI and PPII are found in Table V in Reference 5). It might be one of the reasons why the experimental

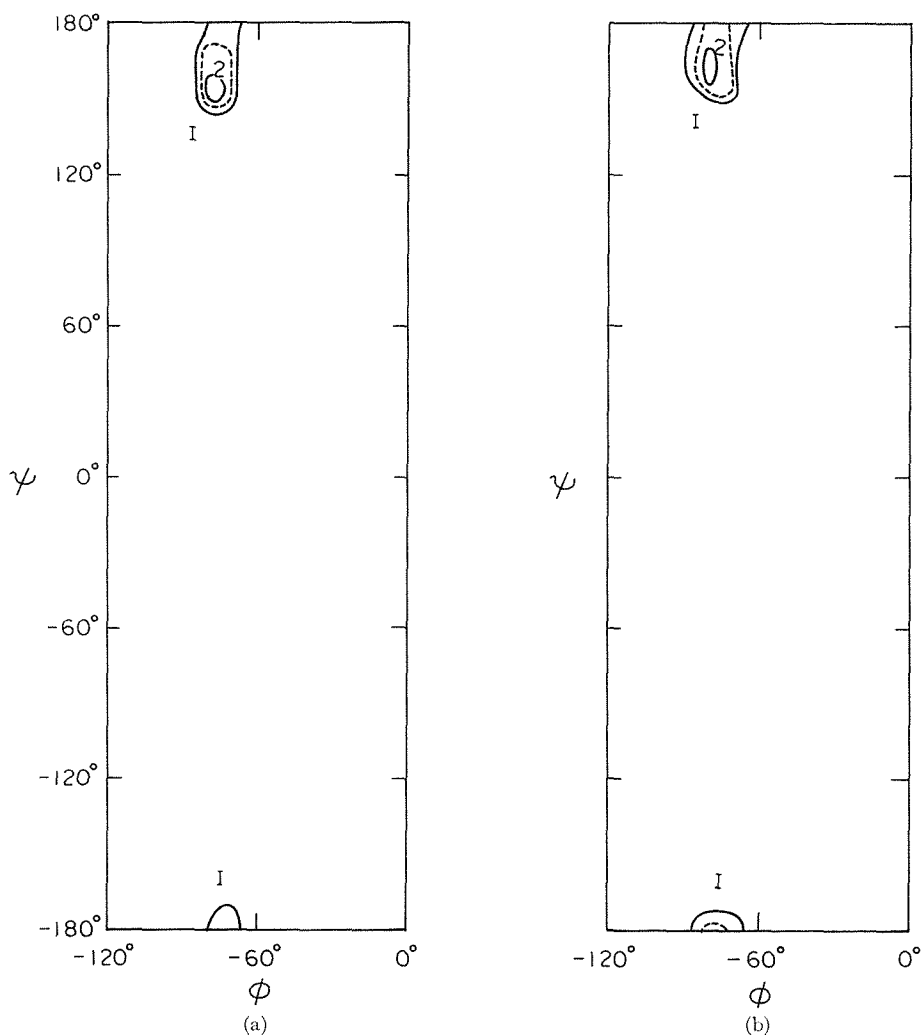


Fig. 6. Energy contour maps of polyproline with cis configuration of peptide ( $\omega=0$ ) as a function of  $\phi$  and  $\psi$ . (a)  $\gamma_1$  position, (b)  $\gamma_2$  position.



values of  $\phi$  and  $\psi$  do not coincide with the energy minimum for PPI and PPII. Another reason of the discrepancy might lie in the neglect of the intermolecular interactions in the present calculation; especially the conformation of PPII would be stabilized by the intermolecular interaction, since area II in Fig. 5 has a wider area of lower energies.

An energy map of  $(\omega, \psi)$  is a perpendicular section of  $(\phi, \psi)$  maps with a constant value of  $\phi$ , and we have chosen three representative values of  $\phi$ ,  $-60^\circ$ ,  $-80^\circ$ , and  $-50^\circ$ , so that the map of  $(\omega, \psi)$  may be made. The maps are shown in Figs. 7, 8, and 9, respectively. For  $\phi = -60^\circ$ , it is unnecessary to subdivide the energy map according to the  $C^r$  position, because of the planar structure of the pyrrolidine ring in this case. Energy values in general, however, are rather high owing to the torsional energies in the ring,<sup>1)</sup> and therefore, the areas of I and II seen in the map do not have low energy contours, resulting in the shift of the minimum point of area I to about  $-20^\circ$  from  $\omega = 0$ . The  $(\omega, \psi)$  maps of  $\phi = -80^\circ$  (Figs. 8a and b) involve the lowest energy regions of both areas I and II (see Figs. 5 and 6). The lowest energy region of area I (inside of the 2 Kcal/mol contour line) exists almost along the line  $\omega = 0$ , and the width in the direction is very narrow irrespective to the  $C^r$  position, while the region below 4 Kcal/mol in area-II has a wider width around  $\omega = \pm 180^\circ$ . Area-II appeared in Figs. 9a and b for  $\phi = -50^\circ$  has also lower energies than 3 Kcal/mol corresponding to the minimum in area-II of the  $(\phi,$

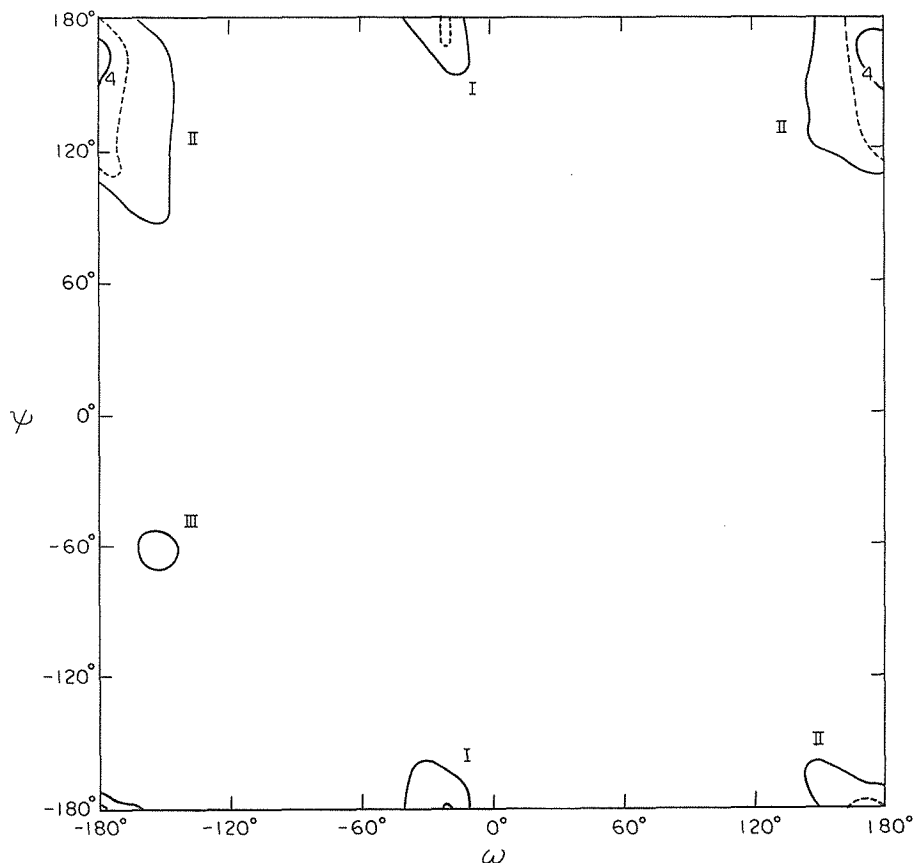


Fig. 7. Energy contour map of polyproline as a function of  $\omega$  and  $\psi$ .  $\phi = -60^\circ$ .

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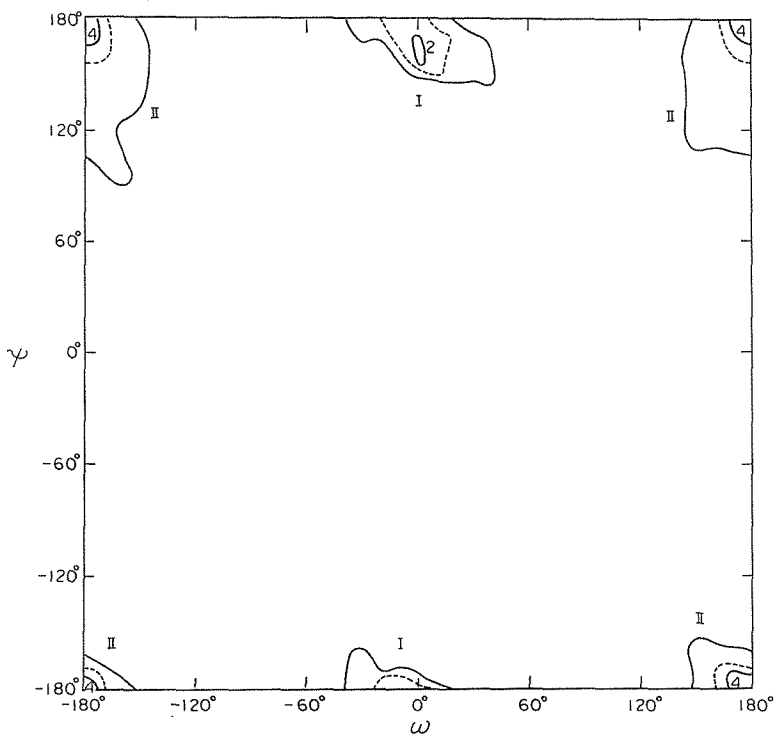
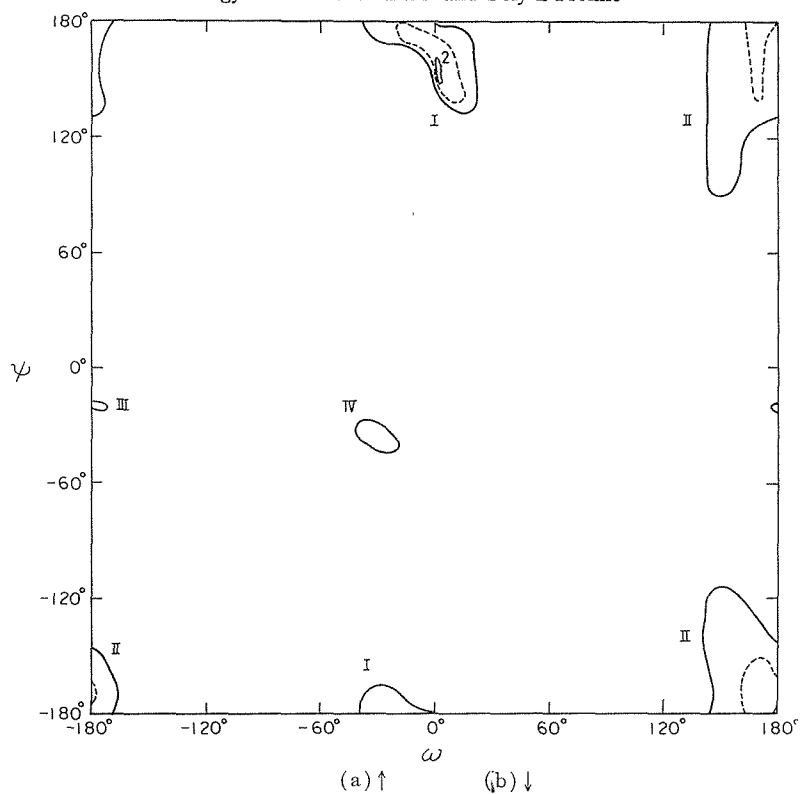


Fig. 8. Energy contour maps of polyproline as a function of  $\omega$  and  $\psi$ .  $\phi = -80^\circ$ .  
 (a)  $\gamma_1$  position, (b)  $\gamma_2$  position.

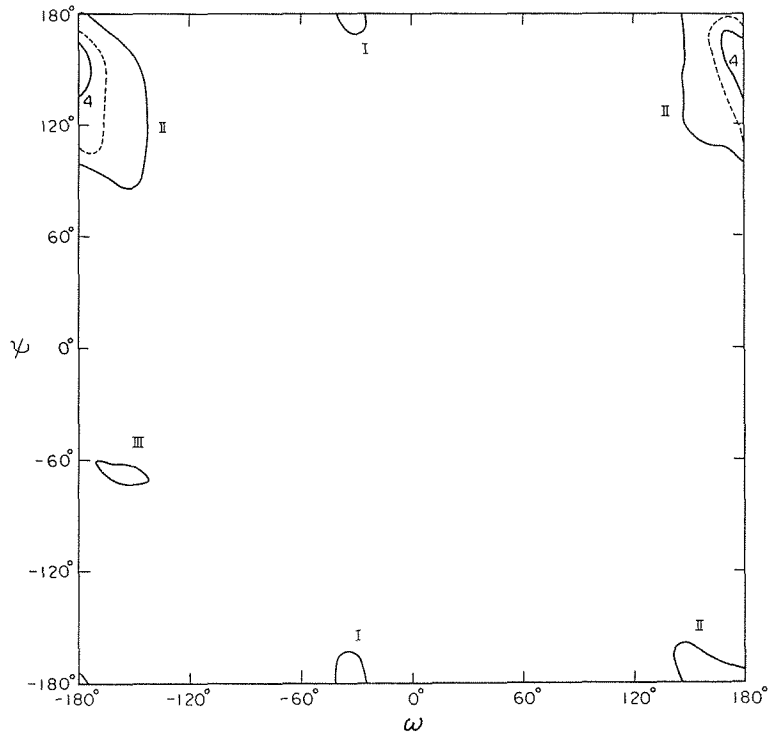
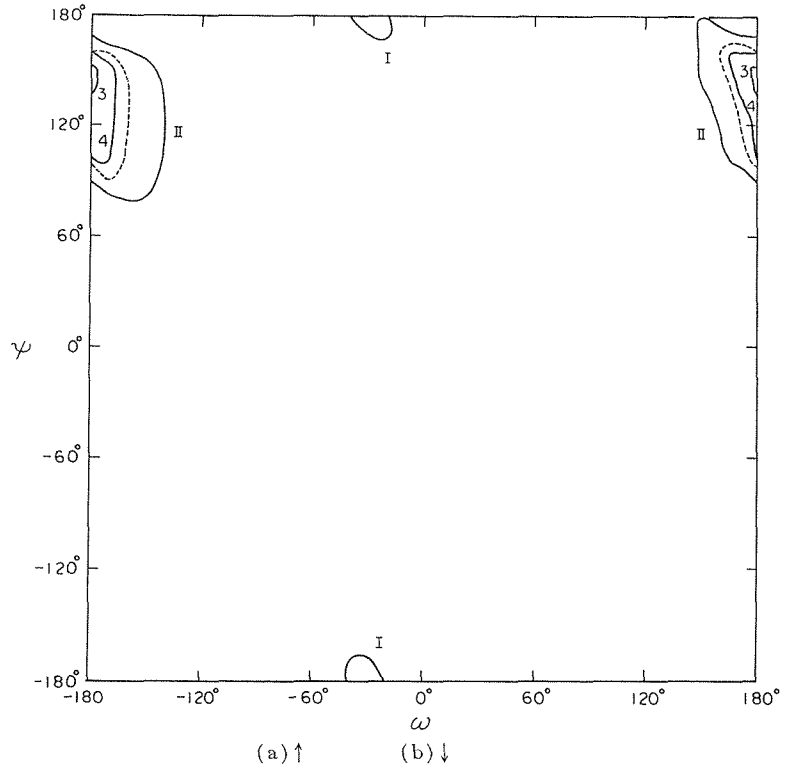


Fig. 9. Energy contour maps of polyproline as a function of  $\omega$  and  $\psi$ .  $\phi = -50^\circ$ .  
 (a)  $\gamma_1$  position, (b)  $\gamma_2$  position.

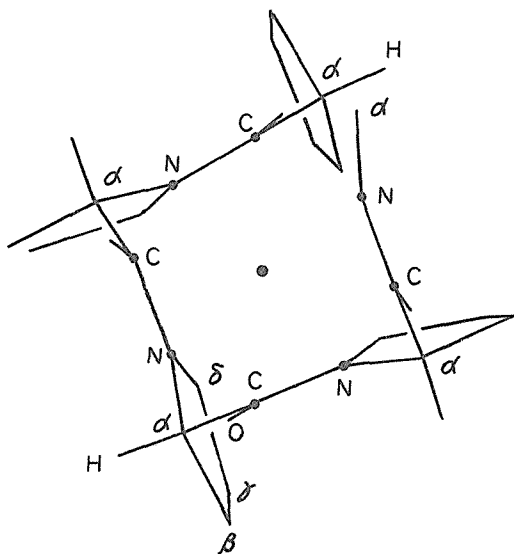


Fig. 10. The helical conformation of polyproline expected by the present calculation. Looking down the helical axis.  $\phi = -65.1^\circ$ ,  $\psi = -49.8^\circ$ ,  $\omega = -164.2^\circ$ .

$\psi$ ) map (Fig. 5). Area-I in Fig. 9, however, has no longer lower energy region and the location shift to the left hand side.

In addition to the areas of I and II, other energy contours, areas III and IV appear in Figs. 7, 8a and 9b. While area-III is found in the  $\alpha$ -helical region in Fig. 5a, the area of contour below 10 Kcal/mol runs obliquely through the  $(\phi, \psi, \omega)$  space where the lowest energy point in the area is located at about  $\omega = -165^\circ$  (see Table 1). The energy values at the minimum point is about 4 Kcal/mol higher than that of area-I. The helical conformation calculated at the point is shown in Fig. 10. The helix is stabilized mainly with non-bonded energy and distabilized with the rotational energy arised from the non-planar configuration of the peptide part (see No. 7 in Table 1). Area-IV seen in Fig. 8a also becomes below 10 Kcal/mol in relative energy. The helical conformations in this area, however, are not so stable as in area III (No. 9 in Table 1), because the extent of the deviation of the peptide from the planar structure is so large ( $\omega = -30^\circ$ ). It is not clear yet whether or not these regular helices of poly-L-proline other than PPI and PPII will be found experimentally. A helix corresponding to area-IV seems to be difficult to exist, but the helix of area-III might have a possibility to exist under a special condition of temperature and solvent.

In this article, our purpose was to search for the energetical aspects on polyproline, and further advance may be to have to introduce the entropic calculations including solvent effects, which are essential for the accurate estimation of their helical stability. The present results suggest that polyproline has more freedom than usually assumed.<sup>10)</sup>

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