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Stereochemical studies on cyclic peptides—VIII. Conformational analysis of hydrogen bonded cyclohexaglycyl molecule with a centre of inversion symmetry*†

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Abstract. A study on the conformational aspects of cyclo-hexaglycyl having inversion symmetry has been made. The cyclic backbone has been assumed to have two internal 4→1 types of NH...O hydrogen bonds. This molecule has been found to take up two types of conformations designated as A^* and B^* having nearly the same energy values. The theoretical conformations have been compared with the conformations of cyclohexaglycyl hemihydrate observed in the crystal structure. Two molecules with an approximate inversion symmetry are close to the conformation of the type B^* and two other molecules with exact inversion symmetry correspond nearly to the types B^* and A^*. Comparison with the theoretically possible conformations of cyclohexaglycyl molecule with 2-fold symmetry has been made. The preference of inversion symmetry and preferred ranges of ψ for glycyl molecules is discussed.

Keywords. Conformation; cyclic peptides; cyclohexaglycyl; stereochemistry; symmetry—two-fold, inversion; hexapeptides, cyclic.

1. Introduction

The results of stereochemical analysis on cyclic hexapeptides with intracyclic 4→1 type of hydrogen bonds having two-fold axis of symmetry for the backbone was reported in the earlier parts of this series (Ramakrishnan and Sarathy 1969; Sarathy and Ramakrishnan 1972). This paper deals with the results of similar analysis on cyclic hexapeptide having the same 4→1 type of hydrogen bonds, but with a centre of inversion in the backbone of the molecule. So far, the crystal structures of three cyclic hexapeptides, namely, ferrichrome A tetrahydrate (Zalkin *et al* 1966), cyclohexaglycyl hemihydrate (Karle and Karle 1963) and cyclo-di-D-alanyl-tetraglycyl trihydrate (Karle *et al* 1970) are known. Of these, the structure of ferrichrome A tetrahydrate (Zalkin *et al* 1966) does not possess an inversion symmetry in the molecule. In the cyclohexaglycyl hemihydrate (Karle and Karle 1963), some of the molecules have an exact centre of inversion for the backbone ring. In some other molecules, the parameters indicate a near inversion symmetry between the two halves of the molecule. In the case of the compound, cyclo-di-D-alanyl-tetraglycyl trihydrate (Karle *et al* 1970), the molecule has an approximate centre of inversion as

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†Part VII of the series by Sarathy and Ramakrishnan (1972).

far as the backbone is concerned. Furthermore, some of the molecules in the structure of cyclohexaglycyl hemihydrate and also the molecule of cyclo-di-D-alanyltetraglycyl trihydrate have a pair of 4→1 type of intracyclic hydrogen bonds in the ring.

2. Generation of cyclic hexapeptide with inversion symmetry

A system of three peptide units having 4→1 type of hydrogen bond is first generated (NH group of third peptide unit being hydrogen bonded to the C=O of the first unit.) As the cyclohexapeptide molecule is assumed to have inversion symmetry, the midpoint of the line joining C_1^α and C_4^α atoms of the three peptides system is taken as the centre of inversion thereby generating another system of three peptide units. The introduction of the centre of inversion at the midpoint of the virtual bond $C_1^\alpha \ldots C_4^\alpha$ will lead to the formation of a closed cyclic hexapeptide ring with an inversion symmetry. This is shown schematically in figure 1. However, the value of the angle $\tau(NC^\alpha C)$ at the junctions C_1^α and C_4^α may not always turn out to be geometrically permissible. Hence, out of the various hydrogen bonded three peptide systems, only those which, when forming a cyclic hexapeptide molecule by the inversion symmetric operation, yield a value in the range 108° to 112° for the angle $\tau(N_1C_1^\alpha C_1)$ are selected and considered for further analysis.

The possible combinations of the parameters ϕ_2, ψ_2, ϕ_3, ψ_3 of the tripeptide fragment which yield a 4→1 type of hydrogen bond have been worked out and listed by Venkatachalam (1968a) corresponding to 110° of $\tau(N_2C_2^\alpha C_2)$ and $\tau(N_3C_3^\alpha C_3)$.* In fact, the total number of such combinations, when the torsional angles ϕ and ψ are varied at 10° intervals, runs up to a little more than 1000. But, when the inversion symmetry condition is applied, the number of possible cyclohexapeptide combinations works out to be about 150. Hence, in the present study, the bond angles τ at C_2^α and C_3^α

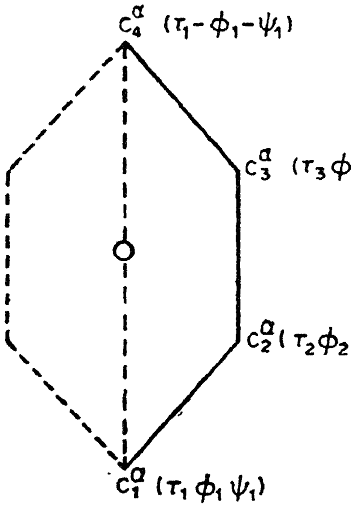

Figure 1. Schematic diagram showing an inversion symmetrical cyclic hexapeptide structure.

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*The nomenclature and conventions adopted are those given by IUPAC-IUB Commission, *Biochemistry* 9, 3471 (1970).

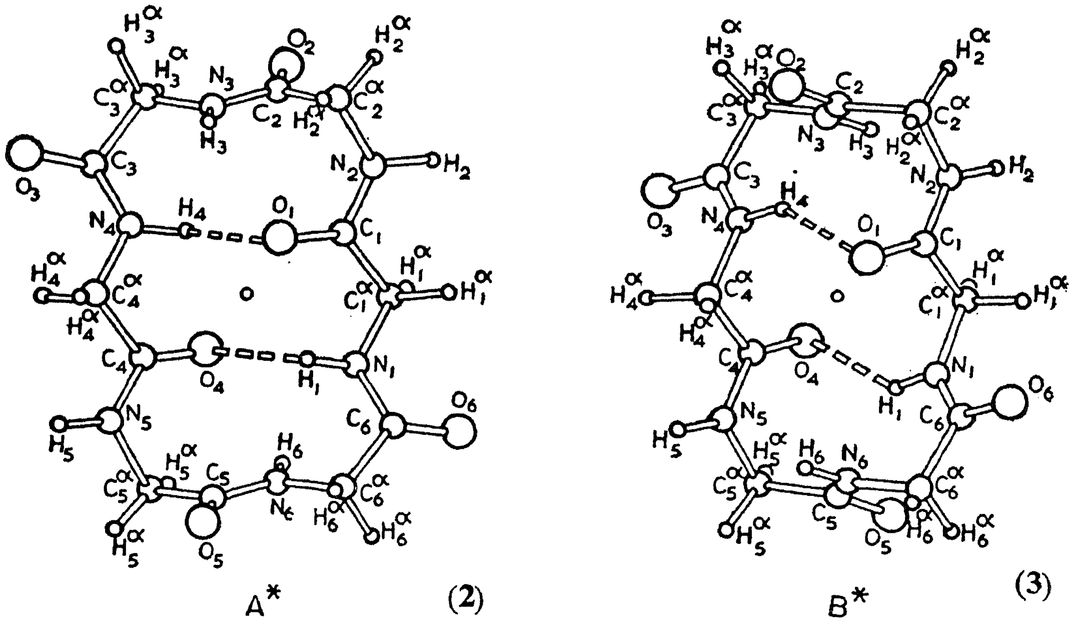
have also been varied from 108° to 112° at 2° interval and after this flexibility in the bond angles, the number of permissible cyclohexapeptide conformations increases to about 1500.

In general, any cyclic hexapeptide molecule with planar peptide units, gets fully specified, when the three parameters, ϕ, ψ and τ are specified at each of the six α-carbon atoms. But in the present case, by virtue of the inversion symmetry, if the parameters at the three consecutive α-carbon atoms are specified, the parameters at the other three α-carbon atoms get automatically defined by the relations given in table 1.

Earlier studies (Venkatachalam 1968b, Chandrasekaran *et al* 1973) have shown that two types of 4→1 hydrogen bonded tripeptide conformations are possible. In this paper, they are referred to as tripeptide fragments *A* and *B*. It is found that when inversion symmetry operation is made on these two tripeptide systems, each of these can lead to a geometrically favourable cyclohexapeptide structure. Thus, two distinct cyclohexapeptide conformations with inversion symmetry are possible and these are designated as *A** and *B**. The typical values of the parameters ϕ and ψ corresponding to these two conformations are given in table 2 and their projections on the plane containing the centre of inversion are shown in figures 2 and 3. The

Table 1. Relationship for the conformational parameters of cyclohexapeptide structure with inversion symmetry

C^α	Parameters			C^α	Parameters		
C_1^α	τ_1	ϕ_1	ψ_1	C_4^α	τ_1	$-\phi_1$	$-\psi_1$
C_2^α	τ_2	ϕ_2	ψ_2	C_5^α	τ_2	$-\phi_2$	$-\psi_2$
C_3^α	τ_3	ϕ_3	ψ_3	C_6^α	τ_3	$-\phi_3$	$-\psi_3$



A* (2) B* (3)

Figures 2 and 3. The two types of conformations (*A** and *B**) of cyclohexaglycyl with an inversion symmetry and 4→1 type of hydrogen bonds. The structure is shown as projected on to a plane containing the atoms C_1^α, C_2^α and centre of inversion.

Table 2. Conformational parameters (in degrees) of the two types of hydrogen bonded cyclohexapeptide structures with inversion symmetry

Type	ϕ_1	ψ_1	ϕ_2	ψ_2	ϕ_3	ψ_3
A^*	−160	179	−70	100	140	−40
B^*	−97	179	−60	−40	−90	20

two conformations A^* and B^* do differ quite a bit in the overall envelope and the disposition of the hydrogen bonds with respect to the hexapeptide ring.

3. Energy functions

The potential energy of the cyclic hexapeptide is evaluated taking into account the following contributing factors: (a) The energy (V_{nb}) due to Van der Waals type of interaction which is calculated using the Buckingham "6-Exp" type of function with constants as used by Brant and Flory (1965); (b) The energy (V_τ) due to the bond angle (τ) distortion from the tetrahedral value, (c) the energy (V_{tor}) due to the torsional distortion and (d) the electrostatic energy (V_{es}). The forms and the constants of the functions used for (b), (c) and (d) are those given by Ramachandran and Sasisekharan (1968) and are the same as used in the earlier study (Sarathy and Ramakrishnan 1972); (e) the hydrogen bond energy (V_{hb}) is calculated using the relation

$$V_{\text{hb}}\,(\text{kcal/mole}) = V_{\text{min}} + p_1\Delta^2 + q\exp(p_2\Delta)\theta^2$$

where $\Delta = R - R_{\text{min}}$, R is the hydrogen bond length (N...O) and θ is the hydrogen bond angle ($\text{H}-\hat{\text{N}}\ldots\text{O}$). The values of the constants used are the same as those given in Ramachandran *et al* (1971) namely $V_{\text{min}} = -4{\cdot}5$ kcal/mole, $R_{\text{min}} = 2{\cdot}95$Å, $p_1 = 25$, $p_2 = 2$ and $q = 10^{-3}$. The total energy of the system is given by V_{tot} where

$$V_{\text{tot}} = V_{\text{nb}} + V_\tau + V_{\text{tor}} + V_{\text{es}} + V_{\text{hb}}$$

As is known from the previous study (Sarathy and Ramakrishnan 1972), contributions due to torsional and bond angle distortions do not appreciably affect the relative stabilities of the various conformations. So, in the tables and discussion that follow the sum ($V_{\text{nb}} + V_\tau + V_{\text{tor}}$) denoted as V_{NB} and V_{TOT} only are considered separately.

4. Results and discussions

The energy calculations have been carried out with the system having glycyl residues at all the six α-carbon atoms (thus corresponding to cyclohexaglycyl). Tables 3a and 3b list the conformations of types A^* and B^* in the increasing order of the energy values V_{TOT} up to 0·6 kcal/mole per residue from their respective minimum energies. From the usual probabilistic considerations (employing the idea of the Boltzmann

Table 3a. Conformational parameters and energy values of hydrogen bonded conformations of cyclohexaglycyl of type A^* arranged according to the increasing order of V_{TOT}.

Conformational parameters in degrees									Energy in kcal/mole per residue	
τ_1	ϕ_1	ψ_1	τ_2	ϕ_2	ψ_2	τ_3	ϕ_3	ψ_3	V_{NB}	V_{TOT}
111	−160	179	108	−70	100	110	140	−40	−7·30	−1·14
110	−162	172	108	−60	100	112	140	−40	−7·04	−1·05
111	−162	172	110	−60	100	110	140	−40	−7·15	−1·03
111	−162	170	108	−60	100	110	140	−40	−6·93	−0·99
112	−164	169	110	−60	100	112	140	−40	−7·05	−0·98
111	−150	178	108	−70	100	110	140	−30	−7·01	−0·94
109	−166	174	108	−60	110	110	130	−40	−6·88	−0·94
110	−166	172	112	−60	100	110	140	−40	−6·89	−0·89
110	−164	173	108	−60	110	112	130	−40	−7·18	−0·89
110	−161	−178	108	−70	100	112	140	−40	−6·72	−0·88
110	−150	179	108	−70	100	112	140	−30	−7·18	−0·88
111	−162	−179	108	−70	110	110	130	−40	−7·06	−0·84
112	−164	169	110	−60	100	112	140	−40	−6·52	−0·82
111	−166	170	112	−60	100	108	140	−40	−6·04	−0·79
110	−165	172	110	−60	110	110	130	−40	−6·79	−0·79
110	−149	179	108	−70	90	112	150	−30	−6·64	−0·78
111	−165	171	112	−60	90	110	150	−40	−6·68	−0·77
110	−165	173	112	−60	90	112	150	−40	−6·96	−0·75
110	−163	172	110	−60	90	112	150	−40	−6·88	−0·74
110	−161	179	108	−70	110	110	130	−30	−6·83	−0·73
110	−152	179	110	−70	100	110	140	−30	−6·71	−0·73
112	−149	176	108	−70	100	108	140	−30	−6·97	−0·73
110	−152	170	108	−60	100	112	140	−30	−6·80	−0·72
109	−166	174	112	−60	100	112	140	−40	6·71	−0·72
112	−163	169	108	−60	110	108	130	−40	−6·29	−0·71
112	−178	164	112	−50	100	110	140	−50	−6·76	−0·71
109	−154	171	110	−60	100	112	140	−30	−6·79	−0·69
111	−151	177	108	−70	110	108	130	−30	−6·70	−0·67
111	−162	170	110	−60	90	110	150	−40	−6·75	−0·66
110	−151	179	110	−70	90	112	150	−30	−6·80	−0·66
109	−178	166	112	−50	100	112	140	−50	−6·61	−0·65
111	−150	178	110	−70	90	110	150	−30	−6·68	−0·65
109	−166	174	110	−60	110	112	130	−40	−6·76	−0·64
111	−151	177	110	−70	100	108	140	−30	−6·90	−0·63
109	−152	−179	110	−70	100	112	140	−30	−6·75	−0·62
112	−164	169	112	−60	90	108	150	−40	−6·61	−0·62
109	−153	171	108	−60	110	112	130	−30	−6·64	−0·57
110	−154	170	110	−60	100	110	140	−30	−6·68	−0·55
110	−165	172	108	−60	120	110	120	−40	−6·64	−0·54

Table 3b. Conformational parameters and energy values of hydrogen bonded conformations of cyclohexaglycyl of type B^* arranged according to the increasing order of V_{TOT}.

Conformational parameters in degrees									Enery in kcal/mole per residue	
τ_1	ϕ_1	ψ_1	τ_2	ϕ_2	ψ_2	τ_3	ϕ_3	ψ_3	V_{NB}	V_{TOT}
110	−97	179	112	−60	−40	112	−90	20	−7·73	−1·10
110	−96	179	112	−60	−30	112	−100	20	−7·72	−1·09
111	−95	180	110	−60	−40	112	−90	20	−7·72	−1·07
112	−93	179	108	−60	−40	112	−90	20	−7·59	−0·84
111	−94	178	110	−60	−30	112	−100	20	−7·56	−0·81
111	−107	−179	112	−60	−40	112	−90	10	−7·54	−0·80
111	−95	177	112	−60	−30	110	−100	20	−7·52	−0·74
111	−96	179	112	−60	−40	110	−90	20	−7·38	−0·73
111	−94	178	110	−60	−40	110	−90	20	−7·36	−0·58
112	−106	180	112	−60	−30	112	−90	10	−7·82	−0·58
110	−98	172	112	−50	−40	112	−90	20	−7·18	−0·57
111	−96	172	112	−60	−20	112	−110	20	−6·69	−0·50

distribution of states for a system in equilibrium), the range for each parameter in the list of low energy conformations represents the " most probable range " for each parameter. As can be seen from the tables, the minimum energy values corresponding to the types A^* and B^* conformations are nearly the same (the differences being only 0·04 kcal/mole per residue). Thus, from energy considerations, it can be said that both types of conformations can occur equally well. In fact, as will be mentioned in the next section, both types are found to occur in the crystal structure of cyclohexaglycyl hemihydrate.

4.1. *Comparison with the observed conformations in the crystal structure*

The theoretical deductions can be compared with the observed conformations in the crystal structure of cyclohexaglycyl hemihydrate (Karle and Karle 1963). The structure has 8 molecules in the unit cell (space group $P\bar{1}$ and there are four molecules in the asymmetric unit). Conformation-wise, there are five different conformers of the cyclohexaglycyl ring, in the structure. Table 4 lists information about the type, the hydrogen bond and the inversion symmetry of these five conformers. The conformational parameters of the four conformers are compared with those conformations listed in the tables 3a or 3b according to their types (A^* or B^*). The molecules I and II, which have a pair of intracyclic 4→1 hydrogen bonds and only an approximate centre of inversion, belong to the type B^*. The comparison between theory and the observation for these two molecules is given in table 5, in which the theoretical conformation which fits best with the observed conformation has been chosen (the conformation in the list, which gives the ' least sum of the squares of the deviations of all the parameters' from the observed conformer is taken as the one closest to the observed conformer). The maximum deviation is only about 13°, which indicates a good agreement especially in view of the fact that the parameters ϕ_2, ψ_2, ϕ_3, ψ_3 have been varied only at 10° intervals.

The other two molecules (III and IV) have exact inversion symmetry, but do not have 4→1 type of intracyclic hydrogen bond. The molecule III belongs to B^* type, whereas the molecule IV belongs to A^* type. However, it is found that these molecules are distorted very much from the minimum energy conformations.

On examining the various cyclohexaglycyl molecules in the observed crystal structure, especially from the point of view of hydrogen bonding, an interesting observavation emerges out. In the case of the two molecules I and II of cyclohexaglycyl structure while all the (NH) groups are involved in hydrogen bonding, only two C=O groups take part in hydrogen bonding. What is more, the same oxygen atom takes

Table 4. Hydrogen bond and symmetry details of the different conformers observed in the crystal structure of cyclo-hexaglycyl hemihydrate (Karle and Karle 1963)

Molecule	Type	Occurrence of 4 → 1 type of hydrogen bond	Inversion Symmetry
I	B^*	Yes	Approximate
II	B^*	Yes	Approximate
III	B^*	No	Exact
IV	A^*	No	Exact
V	A and B	No	—

Table 5. Comparison of the parameters[a] obtained from theory with observation in the case of molecules I and II[b]

Parameters	Theory Type B*	Observation Molecule I	Deviation	Observation Molecule II	Deviation
τ_1	111	108	3	108	3
ϕ_1	−107	−120	13	−121	14
ψ_1	−179	−166	13	−168	11
τ_2	112	111	1	113	1
ϕ_2	−60	−69	9	−69	9
ψ_2	−40	−30	10	−30	10
τ_3	112	113	1	112	0
ϕ_3	−90	−94	4	−95	5
ψ_3	10	11	1	9	1
τ_4	111	110	1	110	1
ϕ_4	107	114	7	115	8
ψ_4	179	165	14	168	11
τ_5	112	112	0	113	1
ϕ_5	60	69	9	68	8
ψ_5	40	33	7	33	7
τ_6	112	112	0	111	1
ϕ_6	90	92	2	93	3
ψ_6	−10	−7	3	−5	5
V_{TOT}	−0·8	−0·6	0·2	−0·5	0·3
$N_4\ldots\ldots O_1$(Å)	2·9	3·0	0·1	3·1	0·2
$H_4\hat{N}_4O_1$(°)	20	14	6	9	11
$N_1\ldots\ldots O_4$(A)	2·9	3·0	0·1	3·0	0·1
$H_1\hat{N}_1O_4$(°)	20	17	3	10	10

[a] The conformational parameters are in degrees and the energy values are in kcal/mole per residue

[b] These molecules have an approximate centre of inversion

part as acceptor in both intracyclic and intermolecular hydrogen bonding. In the crystal structure, these molecules occur at a level crystallographically designated as $b = \frac{1}{2}$, where there are no water molecules. On the other hand, in the molecules III and IV, four out of six (NH) groups and *all the oxygen atoms* take part in intermolecular hydrogen bonds. Interestingly, if $N\ldots O$ distance and $H—\hat{N}\ldots O$ angle (which correspond to a possible 4→1 type of hydrogen bond in the ring) are calculated, they turn out to be 4·5 Å and approximately 20°.* This can be taken to indicate that due to the large number of intermolecular hydrogen bonds (some of them involving water molecules which are present at the level $b = 0$), the intracyclic $N\ldots O$ distance has increased and hence the hydrogen bond broken.

For a visual comparison, the projections of the observed and the calculated conformations are shown superimposed in figure 4. Figures 4a and 4b indicate a good agreement for the two molecules I and II. Figures 4c and 4d indicate only a gross agreement. The peptide units which are not involved in the intracyclic hydrogen bond do show some difference (the difference in tilt about the virtual $C^\alpha\ldots C^\alpha$ bond

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*The hydrogen bond angle is calculated by fixing hydrogen atom in the peptide plane.

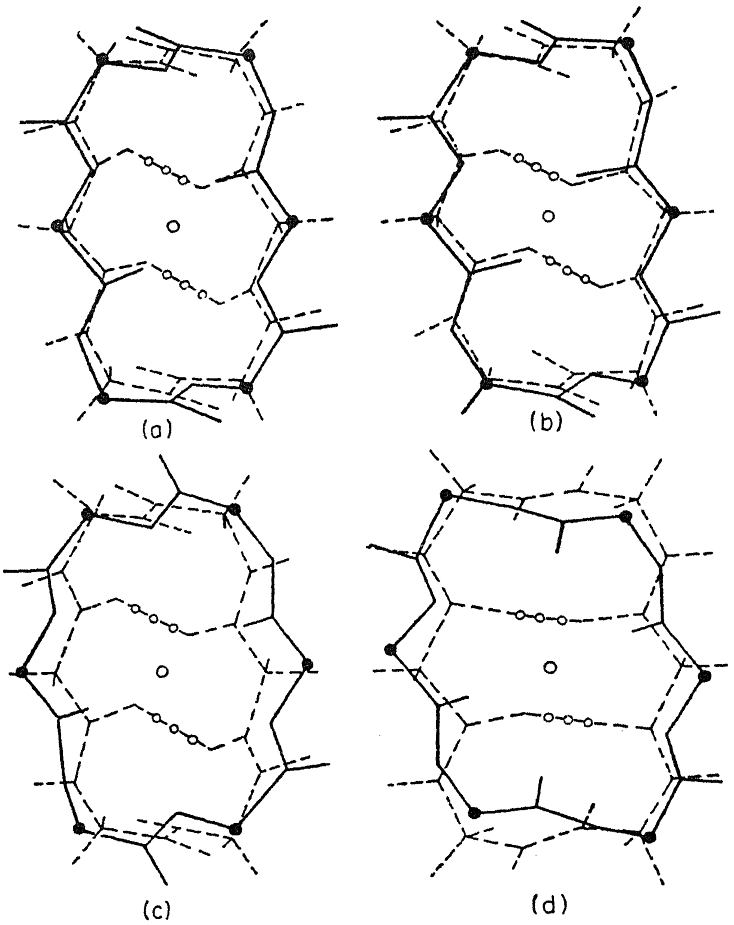

Figure 4. Projections of the observed (——) and theoretical (- - - -) conformations of cyclohexaglycyl. 4→1 type of hydrogen bonds are indicated by (-o-o-o-).

being about 45°). This can be understood if we take into account the fact that both the nitrogen and the oxygen of these units are subjected to external pull by an extensive intermolecular hydrogen bond network, which is not present for the molecules I and II.

The fifth conformer listed in table 4 has neither an inversion symmetry nor an intracyclic hydrogen bond. It is made up of tripeptide fragments *A* and *B* (The conformational parameters of the fragment of type *A* are close to that of molecule IV and parametres of type *B* are close to that of molecule III). As in the case of molecules III and IV, all the oxygen atoms are involved in external hydrogen bonding and also the N...O distance and $H—\hat{N}...O$ angle corresponding to a possible 4→1 hydrogen bonding are of 4·5 Å and 20°, respectively. This lends support to the proposition assumed for molecules III and IV that the external hydrogen bonds do have an effect of increasing the N...O distance and thereby weakening or breaking the internal hydrogen bond.

Summarizing, it can be said that occurrence of both *A** and *B** types of conformations are possible according to theory and do occur in crystal structure. The cooperative effect of the various hydrogen bonds present in a crystal packing puts a limit to the extent of comparison that could be made between theory and observation and subject to this limitation the comparison can be said to be satisfactory.

4.2. *Comparison with two-fold symmetrical ring conformation of cyclohexaglycyl*

In the earlier parts of this series (Ramakrishnan and Sarathy 1969; Sarathy and Ramakrishnan 1972) a study of the conformations of cyclohexaglycyl with two-fold symmetry for the backbone ring (which is possible for any cyclohexapeptide) has been made. Since the cyclohexaglycyl molecule can take up either of these symmetry features, it would be interesting to compare the results of the inversion symmetry with those of the two-fold symmetrical ring conformation (low energy conformations of the latter being designated as *A′* and *B* as in Sarathy and Ramakrishnan 1972). Type *A′* has the minimum energy for cyclohexaglycyl with two-fold rotation symmetry and the value of the minimum energy is equal to —3·9 kcal/mole per residue. It can be noticed that the total energy value of the minimum energy conformation with a two-fold symmetry is lower than that of the minimum energy conformation with inversion symmetry by about 3·0 kcal/mole per residue. As has already been mentioned, some of the molecules in the crystal structure of cyclohexaglycyl hemihydrate (Karle and Karle 1963) have at least an approximate centre of inversion within the ring. Thus, an intriguing and interesting question arises as to why the molecule prefers to have an inversion symmetry rather than a two-fold symmetry.

A possible place to look for an answer to this question is the conformation of tripeptide fragments of the types *A* and *B*. A study of the tripeptide conformations has been made for alanyl residue (both L and D configurations) by Chandrasekharan *et al* (1973). Study of these conformations with glycyl residues, which is applicable to cyclohexaglycyl molecule, has now been made. The regions around the minima of tripeptide fragments A and B enclosed by ϕ, ψ values at C_2^α and C_3^α are marked separately in figures 5a and 5b (Combination of a pair of points—one each from figures 5a and 5b defines a tripeptide fragment). The shaded regions in the figures correspond to conformations of tripeptide fragments capable of forming either two-fold or inversion symmetrical structures. Especially in figure 5b, for the *A* type of conformations, the regions suitable for these two symmetries are distinct. However,

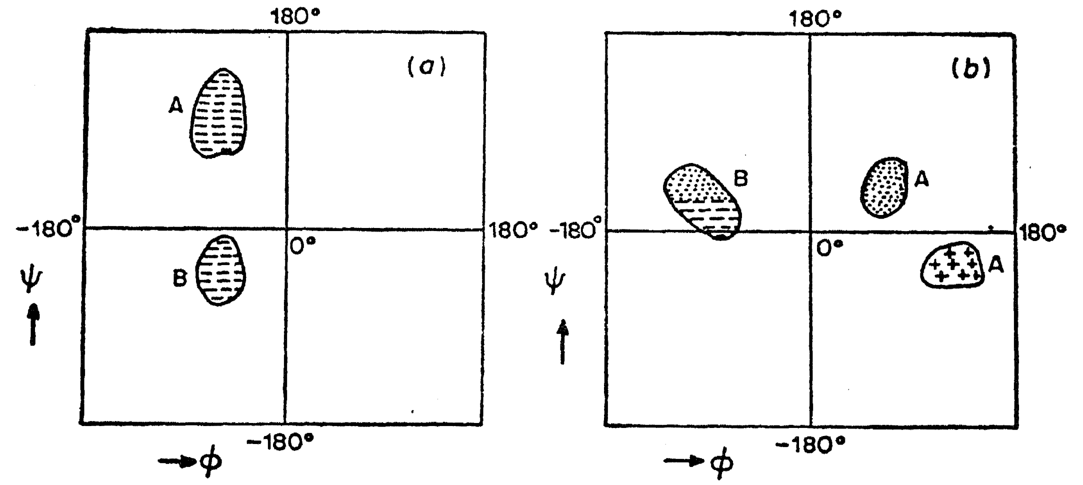

Figure 5. The (ϕ, ψ) plot at (a) C_2^α and (b) at C_3^α of tripeptide conformations around energy minima, which can form either two-fold symmetrical or inversion symmetrical hydrogen bonded cyclic hexapeptides. ::: conformations that can form two fold symmetrical structures only. ‡‡‡ conformations that can form inversion symmetrical structures only. ⁼⁼⁼ conformations that can form both types of symmetrical structures.

from the minimum energy considerations of tripeptides, it is not possible to choose either one of these as being more probable than the other. Thus, this approach does not enable to explain the preference of inversion symmetry over the two-fold symmetrical case.

Since the molecule has only glycyl residues, it was thought appropriate to analyse the values of the conformational parameters ϕ, ψ as observed in various amino acid and peptide crystal structures containing glycyl residues. Attention has been concentrated especially on the parameter ψ. The distribution of the parameter ψ is shown in figure 6, which shows that there are maxima around 0° or ± 180°. The observed ϕ and ψ values for glycyl residues in proteins and in small peptides as determined by x-ray crystallographic methods has been earlier plotted in a (ϕ, ψ) map (Sasisekharan 1973). It can also be seen in that map that the observed values of ψ are concentrated around 0° or ± 180°. This preference of ψ around 0° or ± 180° can be used as a criterion to test the theoretical conformations. The values of ψ of the two-fold and inversion symmetrical minima of the two types of conformations are listed in table 6. The value of ψ_2 is nearly the same for both two-fold and inversion symmetrical conformations of the types *A* and *B*. The value of ψ_3 is nearly the same for both kinds of symmetrical conformations of B-type. However, for *A*-type, they differ by 80°. But, the actual values, namely + 40° and − 40° are equally distributed around 0° and hence does not lend to a choice between them. The value of ψ_1 is −69° for the two-fold symmetrical case and 179° for the inversion case (and it is the same for both *A* and *B* types). Of these, naturally, the ψ for the inversion symmetrical case is at the maximum of the ψ distribution, while for the two-fold symmetrical case it is nearly at the minimum. Thus, the "preferred ψ value" criterion favours the inversion symmetrical structure over the two-fold symmetrical structure.

A modified torsional potential function for the rotation ψ with a two-fold nature has been proposed (Kolaskar *et al* 1975). Using this function, energy calculation has been repeated for cyclohexaglycyl. It is then found that the energy difference between inversion and two-fold symmetrical conformations of cyclohexaglycyl is brought down to about 1·7 kcal/mole per residue. But, between the two symmetrical conformations, the two-fold symmetrical conformation still has a lower energy.

The solution conformation of cyclohexaglycyl molecule is not yet known. NMR study on cyclo-(Gly-Gly-Gly-Gly-D-Ala-D-Ala) (Tonelli and Brewster 1972) shows that the cyclic hexapeptide is a flexible molecule in solution rapidly interconverting between a few low energy conformations, none of which resembles, even approximately,

Table 6. Value of ψ parameter (in degrees) at three α-carbon atoms in the theoretical minimum energy conformations with 2-fold and inversion symmetry†

Parameter	A-Type 2-fold (A′)[a]	A-Type Inversion (A*)	B-Type 2-fold (B)[a]	B-Type Inversion (B*)
ψ_1	−69	179	−69	179
ψ_2	110	100	−40	−40
ψ_3	40	−40	30	20

[a] For the definition of the conformations *A′* and *B*, see table 1 in Sarathy and Ramakrishnan (1972).

† The values of ψ at the remaining three α-carbon atoms can be obtained using the information given in table 1.

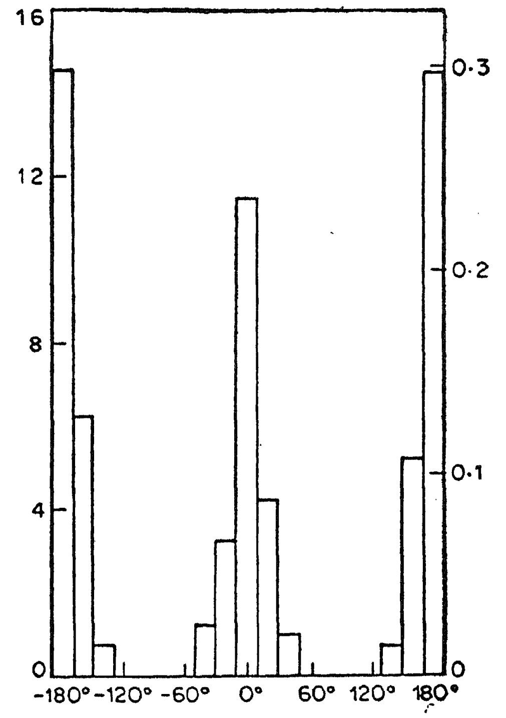

Figure 6. Histogram showing the distribution of the parameter ψ as observed in the glycine residues of the crystal structures of amino acides and peptides.

the crystalline conformation. The solution conformation of cyclo-(Gly-L-Tyr-Gly)$_2$ (Kopple *et al* 1972) is found to have a c_2-symmetry for the hexapeptide ring. However, the recent crystal structure study using packing method on cyclo-(Gly-L-Tyr-Gly)$_2$ indicates an approximate centre of inversion for the ring (Ramachandran and Shamala, private communication). On the other hand, a very recent crystal structure report (Brown and Teller 1976) on another cyclic hexapeptide, namely, cyclo (L-AlA-L-Pro-D-Phe)$_2$ (consisting of all non-glycyl residues) shows a c_2-symmetry for the backbone ring. Thus, it appears that cyclic hexapeptides rich in glycyl residues prefer inversion symmetrical conformation in crystalline state.

4.3. *Other conformational studies on cyclohexaglycyl*

The conformation of cyclohexaglycyl molecule using energy minimisation method has been given by Go and Scheraga (1973). These authors have used the method for cyclohexaglycyl molecule with different types of symmetry elements present in the ring. They have obtained eight minima for conformations with a centre of inversion. It should be mentioned that our emphasis in this paper is mostly on hydrogen bonded cyclic hexapeptide units which can have a centre of inversion in the ring. Of the eight minima that they have obtained, two of them, namely, HG_{11} and HG_{13} can be taken to approximately correspond to our B^* and A^* conformations, respectively. None of the minima from HG_8 to HG_{11} does have the type C (see figure 6 in Go and Scheraga 1973) hydrogen bonds, which is found in molecules I and II of the cyclohexaglycyl crystal structure. Type B hydrogen bond (3→1 type) of Go and Scheraga (1973) is not formed in the hexapeptide fragments used for the formation of cyclohexaglycyl structure in our study, as the value of ψ_3 at C_3^α should be considerably different for such a hydrogen bond. We would like to mention that the basic tripeptide unit is more likely to have a 4→1 type of hydrogen bond in it. In fact, as mentioned by Kopple (1972) this type of hydrogen bond forms a common feature in cyclic peptides with five or more residues. This hydrogen bond can subsequently be broken due to the packing effect when the molecules form a crystal. Further support for the occurrence of such hydrogen bonded peptide sections in a cyclic hexapeptide is available from crystal structures (Zalkin *et al* 1966; Karle *et al* 1970) and NMR studies (Torchia *et al* 1972; Kopple *et al* 1969).

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References

Brant D A and Flory P J 1965 *J. Am. Chem. Soc.* **87** 2791

Brown J N and Teller R G 1976 *J. Am. Chem. Soc.* **98** 7565

Chandrasekaran R, Lakshminarayanan A V, Pandya U V and Ramachandran G N 1973 *Biochem. Biophys. Acta.* **303** 14

Go N and Scheraga H A 1973 *Macromolecules* **6** 525

IUPAC-IUB Commission 1970 *Biochemistry* **9** 3471

Karle I L and Karle J 1963 *Acta. Cryst.* **16** 969

Karle I L, Gibson J W and Karle J 1970 *J. Am. Chem. Soc.* **92** 3755

Kolaskar A S, Sarathy K P and Sasisekharan V 1975 *Curr. Sci.* **44** 35

Kopple K D 1972 *J. Pharm. Sci.* **61** 1345

Kopple K D, Ohnishi M and Go A 1969 *J. Am. Chem. Soc.* **91** 4264

Kopple K D, Go A, Logan R H Jr and Savrda J 1972 *J. Am. Chem. Soc.* **94** 973

Ramachandran G N and Sasisekharan V 1968 *Adv. Protein Chem.* **23** 283

Ramachandran G N, Chandrasekaran R and Chidambaram R 1971 *Proc. Indian Acad. Sci.* **A74** 270

Ramachandran G N and Shamala N Private communication

Ramakrishnan C and Sarathy K P 1969 *Int. J. Peptide Protein Res.* **1** 103

Sarathy K P and Ramakrishnan C 1972 *Int. J. Peptide Protein Res.* **4** 1

Sasisekharan V 1973 in *The Jerusalem Symposium on quantum Chemistry and Biochemistry* ed. E D Bergmann and B Pullman **5** 36

Tonelli A E and Brewster A I 1972 *J. Am. Chem. Soc.* **94** 2851

Torchia D A, Wong S C K, Deber C M and Blout E R 1972 *J. Am. Chem. Soc.* **94** 616

Venkatachalam C M 1968a Thesis University of Madras

Venkatachalam C M 1968b *Biopolymers* **6** 1425

Zalkin A, Forrester J D and Templeton D H 1966 *J. Am. Chem. Soc.* **88** 1810