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Cyclic peptides—Small and big and their conformational aspects

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Abstract. Cyclic peptides form an interesting class of compounds for study by conformational analysis, by virtue of their unique conformational features and biological properties. The small cyclic peptides having 3–6 peptide units in their ring, show a variety of conformational characteristics such as occurrence of *cis* peptide units, flexibility of peptide dimension and variety in hydrogen bonding. The different possible conformations of cyclic tri- and hexa-peptides are given and certain specific conformational features are discussed for cyclic tetra and pentapeptides. For higher cyclic peptides, the hydrogen bonding requirement for stability of the backbone of the ring, is seen to be kept to a minimum. These various features and their significance are examined and discussed in the light of energy minimization studies and analysis of available experimental data.

Keywords. Cyclic peptides; hydrogen bonding; symmetry; cyclic hexapeptides; energy minimization; peptide conformation.

Introduction

Cyclic peptides offer an interesting class of compounds for study using conformational methods. The condition of ring closure brings about restrictions on the possible conformations compared to a linear peptide. Still it is possible to have a considerable variety of conformations. In the last few years there has been a steady increase in the number of cyclic peptides studied both in solution and solid state as well as by conformational means. In this paper an attempt has been made to review and analyse the available information, with special emphasis on symmetry and hydrogen bonding. A number of review articles dealing with various aspects of cyclic peptides are available in literature and particular mention can be made of Karle (1981a), Kessler (1982), Ovchinnikov and Ivanov (1982).

Cyclic tripeptides

Cyclic tripeptides which contain nine membered rings are in general strained systems Not many cyclic tripeptides are known. Those on which crystal structure information is available are cyclo trisarcosyl (Groth, 1976a) and others containing Prolyl and N-BzlGlycyl residues. One common feature in all these is the occurrence of *cis* peptide units which is geometrically a necessary factor for the formation of cyclic tripeptides. Earlier conformational studies on cyclic tripeptides have been done by Venkatachalam (1968), who has considered a three-fold symmetric conformation. Recent extensive work in our laboratory (Manjula, 1976; Ramnarayan, K. and Ramakrishnan, C., unpublished results) indicates three distinct conformations that are possible for a cyclic

tripeptide. These are shown in figure 1, in which the principal torsional angles are also marked. Figure la shows the symmetric form and lb an asymmetric form. These two are composed of three *cis* peptide units. The conformation shown in figure 1c has a rare feature of a *trans* peptide unit in combination with two *cis* peptide units.

The symmetric conformation is energetically more favourable than the other two. An

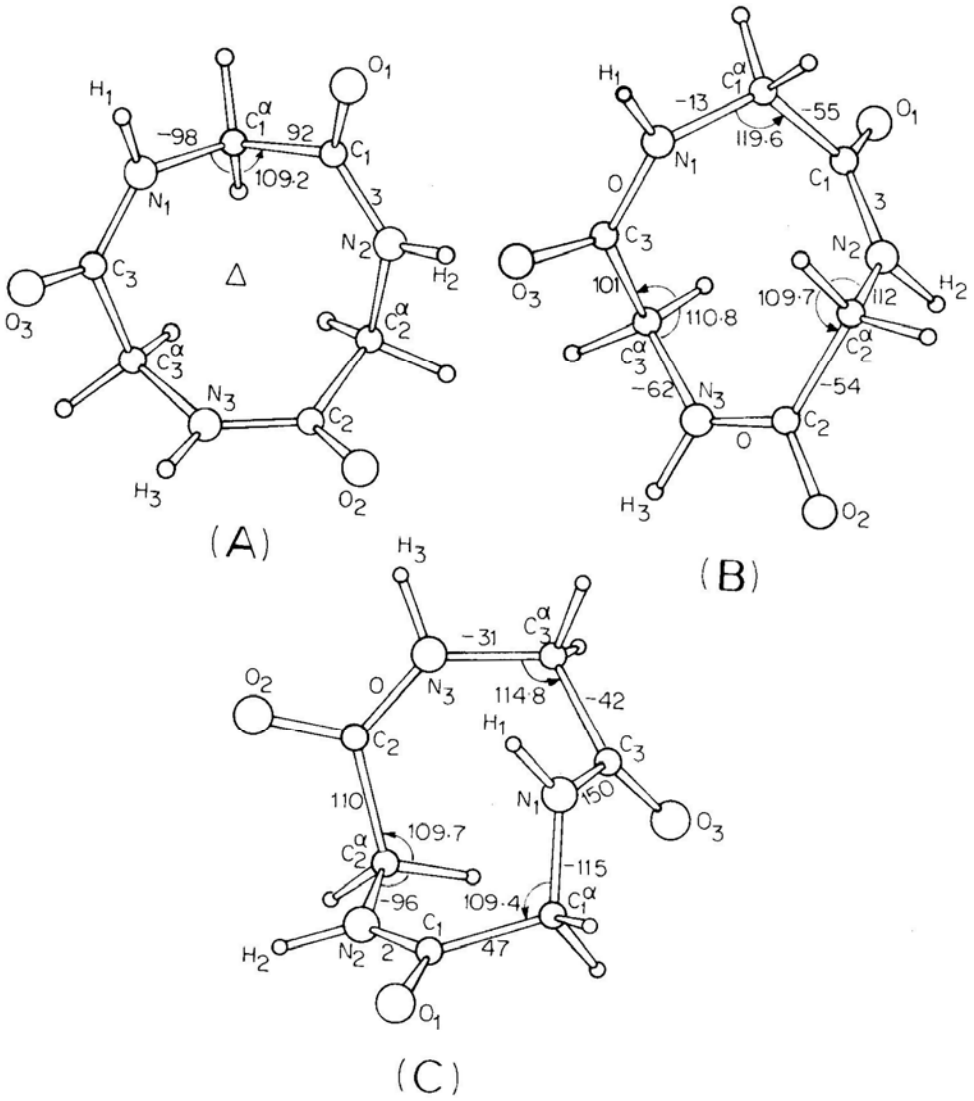


Figure 1. Projection of the minimum energy conformations of cyclic tripeptides.

A. Three fold symmetric conformation with all three *cis* peptide units

B. Nonsymmetric conformation with all three *cis* peptide units c–c–c

C. Nonsymmetric conformation with two *cis* and one *trans* peptide units. c–c–t

The bond angles (°) $NC^{\alpha}C$ and the torsion angles (°) about the bonds are also given.

interesting aspect of both symmetric and asymmetric conformations is that the ϕ value at all the α-carbon atoms are such that they are automatically suitable for the accommodation of a Prolyl residue which is very well known to take up *cis* form with equal ease as *trans* form. While the symmetric form can accommodate only homo-isomeric form of Prolyl residue (all L or all D), the asymmetric form can accommodate only a heterogeneous sequence (LLD or DDL). This is well supported by observation in that the conformations of the compounds cyclo-tri-L-Prolyl (Druyan *et al*., 1976) and cyclo(L-Pro-L-Pro-L-Hypro) (Kartha and Ambady, 1975) take up the symmetric (or near symmetric) form and that of cyclo (L-Pro-L-Pro-D-Pro) (Bats and Fuess, 1980) and cyclo (L-Pro-BzlGly-D-Pro) (Kessler *et al*., 1983a) take up the asymmetric form.

Kessler and his group have done extensive studios both in solution and solid state, on cyclic tripeptides containing NBzlGly and Prolyl residues. While cyclo (BzlGly-L-Pro-L-Pro) (Bats and Fuess, 1982) and cyclo($BzlGly_2$-L-Pro) (Bats and Fuess, 1980) take up symmetric conformations as would be expected, cyclo (Nitro BzlGly-L-Pro_2) assumes the asymmetric conformation in the solid state (Kessler *et al*., 1983b), though this can easily be accommodated in a symmetric form. The reason for this is still not clear and needs further study.

Another noteworthy feature of both the 'all-*cis*' conformations is that none of the N-H groups points inside the ring and hence there will not be steric hindrance if the hydrogen is substituted by other groups such as methyl, benzyl, etc. Such N-substituted groups are also known for their occurrences in *cis* peptide units.

It has been shown by Venkatachalam (1968) that the rather strong steric hindrance that exists between the H^{α}s in the symmetric conformation can be relieved by tilting the C^{α}-H^{α} vectors away from the centre of the ring. Though to some extent the short contacts are relieved by this process, there results undue distortion of the bond angles at C^{α} atoms. On the other hand, Manjula (1976) has shown that a large nonplanarity of the order of 20–30° can relieve this short contact without much distortion at the α-carbon atom. There is a third possibility, namely, the peptide dimensions can be suitably changed in order to provide more space in the centre of the ring. Recent energy minimization studies in our group (to be published), do indicate that an increase in the internal peptide bond angles $C^{\alpha}CN$ and CNC^{α} will be preferred to either large nonplanarity or large distortion of the bond angles at C^{α}. The average values of some of the bond lengths and bond angles of the *cis* peptide unit that occurs in different molecules are given in table 1, in which the standard dimensions are those given by Ramachandran and Venkatachalam (1968) and Ramachandran and Sasisekharan (1968). It can be seen that the bond lengths C^{α}–C and C–N are marginally higher and the bond angles $C^{\alpha}CN$ and CNC^{α} are definitely higher than the standard value for cyclic tripeptides, while this is not so in the case of *cis* peptide units occurring in other (non tri) cyclic peptides (a more detailed analysis will be published elsewhere). The values obtained in the energy minimization studies for these angles are 121° and 129° respectively, which are on the higher side too. From these it can be concluded that the *cis* peptide unit has undergone slight expansion of the angles in order to provide space for the H^{α} atoms to be accommodated without steric hindrance. Thus the *cis* peptide unit, while remaining reasonably rigid in other cyclic peptides, has shown tendency for need based flexibility in cyclic tripeptides. In fact, in a recent crystal structure

Table 1. Average dimensions of the *cis* peptide unit under different conditions.

	$C^{\alpha}C$	C-N	N-C^{α}	$C^{\alpha}CN$	CNC^{α}
Standard	1·53	1·32	1·47	118	126
Cyclic di	1·50	1·33	1·46	118·1	125·4
Cyclic non-tri	1·52	1·33	1·46	118	125·1
Cyclic tri	1·54	1·34	1·47	120	128
Minimized symmetric conformation	1·53	1·32	1·47	120	128
Minimized non-symmetric conformation	1·54	1·32	1·47	121	129

determination of a cyclic tripeptide (Kessler, 1983b) one of the values of the angles $C^{\alpha}CN$ is as high as 135° and that of CNC^{α} is 131°.

Cyclic tetrapeptides

Cyclic tetrapeptide consisting of a ring system of 12 atoms, is less strained than a cyclic tripeptide. This is the smallest cyclic peptide which can be formed with all *trans* peptide units. The crystal structure information on cyclic tetrapeptides (Groth, 1970; Declercq *et al*., 1975; Flippen and Karle, 1976; Ueno and Shimizu, 1983; Swepston *et al*., 1981; Chiang and Karle, 1982) indicates a preponderance of Sarcosyl and Prolyl residues, which have nearly equal preference for *cis* and *trans* peptide units. Conformations with all *trans* peptide units (Ramakrishnan and Sarathy, 1968) and conformations with alternating *cis* and *trans* peptide units (Manjula and Ramakrishnan, 1979) can be formed with planar units and having different symmetry elements in the molecule.

Among the crystal structure studies, cyclo(L-Pro-Sar)$_2$ (Ueno and Shimizu, 1983) prefers an all *cis* conformation, while dihydrochlamydocin (Flippen and Karle, 1976) favours an all *trans* structure, inspite of its having a Prolyl residue. A study of the Sarcosyl containing cyclic peptides clearly shows that the backbone rings of the cyclic peptides contain atleast one *cis* peptide unit each.

Four of the cyclic tetrapeptides contain Prolyl residues: dihydrochlamydocin (Flippen and Karle, 1976), cyclo (L-Pro-Sar)$_2$ (Ueno and Shimizu, 1983) and cyclo (L-Ala-L-Pro-L-Phe-L-Pro) (Chiang and Karle, 1982). Among these dihydrochlamydocin alone does not contain a *cis* peptide unit. The reason for this is the presence of an Aib residue in the sequence. Had this compound taken up a *cis-trans-cis-trans* structure, then Aib must occupy either a *trans-cis* junction or a *cis-trans* junction. Recent studies indicate that Aib residues cannot be easily accommodated in such a heterogeneous junction without serious steric hindrance. Even with the *trans-trans* junction the sterically allowed regions for an Aib residue in the (ϕ, ψ) plane is very restricted and centred around the α -helical regions. Recently Kawai *et al* (1983) have proposed a *trans-trans-trans-cis* conformation for Ala^4 chlamydocin and it is satisfying to note that Aib occurs at a *trans-trans* junction.

Earlier studies on cyclic tetrapeptides with four-fold and two-fold symmetric conformations have not yielded any hydrogen bonding within the ring, whereas the only observed all *trans* conformation of dihydrochlamydocin contains two γ-turns in the ring having two 3→1 hydrogen bonds. The reason for this can be sought in the sequence of this compound cyclo(Iabu-L-Phe-D-Pro-LX) which has got a Pro and an Aib occurring diagonally across in the sequence. Both of these are capable of supporting a γ-turn in a dipeptide. The energy map of a pair of two linked peptide units with Aib at the junction α-carbon atom has been worked out by Narasinga Rao (1982), who has taken into account the hydrogen bond energy too. This map shows that the minimum at the γ-turn region is only about 2 kcal mol^{-1} higher than the global energy minimum occurring at the α-helical regions of the map. Hence Aib can take up this conformation without much difficulty if this is going to help in the formation of the cyclic ring.

Cyclic pentapeptides

The stability of a cyclic pentapeptide ring can be achieved by different schemes of hydrogen bonding. The two types of well known hydrogen bonding namely 4→1 (β-turn) and 3→1 (γ-turn) are easily possible in cyclic pentapeptides. One can have a wide variety of conformations that can be worked out from energy considerations. Ramakrishnan and Narasinga Rao (1982) and Narasinga Rao (1982) have given the details of the different conformations that one can have in cyclic pentapeptide.

Observationally, crystal structures of five compounds are known. They are: cyclo (Gly-L-Pro-Gly-D-Ala-L-Pro) (Karle, 1978), cyclo (Gly-L-Pro-L-Ser-D-Ala-L-Pro) (Karle, 1979), cyclo(D-Phe-L-Pro-Gly-D-Ala-L-Pro) (Karle, 1981b), cyclo(L-Thr-D-Val-L-Pro-Sar-Me-Ala) (Mauger *et al*., 1982) and cyclo (D-Phe-L-Pro-Gly-D-Ala-L-Pro) (Karle, 1984). In all these cases there is a β-turn with a 4→1 hydrogen bond in the cyclic pentapeptide ring. Four of the five compounds have got two Prolyl residues in their sequence. Since proline is well known to bring about β-turns, it is not surprising that all these compounds have favoured a 4→1 hydrogen bond in their ring. Conformational studies on cyclic pentapeptides with β-turns (Manjula and Ramakrishnan, 1979) show that when three units of a cyclic pentapeptide are in β-turn orientation, the remaining two peptide units have a near γ-turn conformation. However, an actual $\beta\gamma$ arrangement can be brought about only with significant nonplanarity in atleast one of the peptide units involved in the γ-turn (Narasinga Rao, 1982). It is interesting to note that the residue at the centre of the γ-turn is proline both in the case of cyclo(Gly-L-Pro-Gly-D-Ala-L-Pro) and cyclo(D-Phe-L-Pro-Gly-D-Ala-Pro).

Hence it can be concluded that a combination of nonplanarity, ring closure condition and occurrence of Prolyl residue at the proper place in the sequence has resulted in an additional hydrogen bond of the type 3→1, thereby increasing the stability. It will be interesting to watch for any cyclic pentapeptide that will take up $\beta\gamma$ conformation in solid state without a Prolyl residue at the γ-turn centre. Such a structure can throw more light on any change in peptide dimensions and nonplanarity requirements for a cyclic pentapeptide.

Cyclic hexapeptides

Amongst all cyclic peptides, small or big, cyclic hexapeptides have been studied most extensively by experimental methods. This is mainly owing to the ease of synthesis of cyclic hexapeptides and the ability of the cyclic hexapeptide backbone to take up well defined and stable structures, with all the peptide bonds in *trans* configuration, relatively easily. A recent review on cyclic peptides (Ovchinnikov and Ivanov, 1982) gives a list of more than 100 synthetic cyclic hexapeptides. An examination of the observed crystal structures shows that cyclic hexapeptides with no sequence symmetry and those of the type $(A_1\text{–}A_2\text{–}A_3)_2$, where A_1 , A_2 and A_3 are various amino acid residues, take up conformations featured by two β-turns, many of which are stabilized by their associated hydrogen bonds. Table 2 gives a list of β-turns observed in cyclic hexapeptide structures as obtained from crystal structure studies. It can be seen that the

Table 2. β-turns in cyclic hexapeptides*.

Name	Types of β-turns	Reference
β-turns of the same type		
c(Gly-L-Pro-D-Phe)$_2$	II II	Brown and Yang, 1979
c(L-Ala-L-Pro-D-Phe)$_2$	II II	Brown and Teller, 1976
c(Gly-L-Pro-D-Ala)$_2$	II II	Kostansek *et al.*, 1979b
c(Gly-D-Leu-L-Leu)$_2$-A	II′ II′	Varughese *et al.*, 1981
c(Gly-D-Leu-L-Leu)$_2$-B	II′ II′	Varughese *et al.*, 1981
c(L-Val-D-Phe-L-Pro)$_2$	II′ II′	Flippen-Anderson, 1979
β-turns and its inverse turn		
c(Gly)$_6$-A	I I′	Karle and Karle, 1963
c(Gly)$_6$-B	I I′	Karle and Karle, 1963
c(L-Leu-L-Phe-Gly-D-Leu-D-Phe-Gly)	I I′	Hossain *et al.*, 1979
c(Gly-Gly-Gly-Gly-D-Ala-D-Ala)	I I′	Karle *et al.*, 1970
c(L-Ala-L-Ala-Gly-L-Ala-Gly-Gly)	I I′	Hossain and van der Helm, 1978
c(Gly-L-Leu-Gly)$_2$	I I′	Brown and Rosen, 1981
c(Gly-L-Tyr-Gly)$_2$	I I′	Shamala, N. 1977
c(L-Phe-D-Leu-Gly-D-Phe-D-Leu-Gly)	II II′	Barnes and van der Helm, 1982
β-turns of different types		
Ferrichrome-A	I II	van der Helm *et al.*, 1981; Zalkin *et al.*, 1966
Ferrichrome	I II	van der Helm *et al.*, 1980
Ferrichrysin	I II	Norrestam *et al.*, 1975
Alumichrome A	I II	van der Helm *et al.*, 1981
c(Gly-L-Pro-Gly)$_2$	I II	Kostansek *et al.*, 1979a
c(L-Phe-D-Leu-Gly-L-Phe-L-Leu-Gly)	I II	Barnes and van der Helm, 1982
c(Gly-L-Ala-L-Ala-Gly-Gly-L-Ala)	I II′	Hossain and van der Helm, 1978
c(Gly-L-His-Gly-L-Ala-L-Tyr-Gly)	I II′	Yang *et al.*, 1981
c(Gly-L-Pro-L-Val-L-Phe-L-Phe-L-Ala)	I II′	Chiang *et al.*, 1982; Karle and Chiang, 1984

* Not all the β-turns have a 4→1 hydrogen bond.

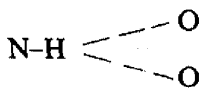
cyclic hexapeptide backbones are made up of different combinations of type I and II β-turns or their inverses and these can be classified into different categories as follows:

(a) Cyclic hexapeptides made up of tripeptide fragments having the same type of β-turns, giving rise to near or exact two-fold symmetric cyclic hexapeptides. The first six cyclic hexapeptides given in table 2 are of this category.
(b) Cyclic hexapeptides made up of dissimilar β-turn tripeptide fragments: These can be further divided into two types in which:
  (i) The two tripeptides fragments have β-turns, one being inverse to the other (I, I′, II, II′) . Examples of such cyclic hexapeptides are given in entries 7–14 of table 2.
  (ii) The two β-turns are completely dissimilar. The entries 15–24 of the table 2 can be seen to fall under this category, some of which are combinations of one type I β-turn and one type II β-turn, while the others (21–23 of table 2) have a type I β-turn and a type II′ β-turn.

From the NMR studies of cyclic hexapeptides in solution, it has been found that one of the major conformations of cyclic hexapeptides especially of the type $c(A_1–A_2–A_3)_2$ is one which has an average two-fold molecular symmetry and is stabilized by hydrogen bonded β-turns (see for example Gierasch *et al*., 1981; Kopple *et al*., 1978; Blaha and Budesinsky, 1973; Bovey *et al*., 1972; Torchia *et al*., 1972a,b).

The various possible conformations of cyclic hexapeptides can be obtained by using stereochemical criteria and energy calculations. The earliest work in this group using energy calculations had been on six-fold symmetric cyclic hexapeptides and on two-fold symmetric cyclic hexapeptides having types I and II β-turns (Ramakrishnan and Sarathy, 1969; Sarathy and Ramakrishnan, 1971). Recently using energy minimization techniques, the accommodation of different hydrogen bonded secondary structural features like β-turns and γ-turns in the cyclic hexapeptide backbone were studied, and a summary of the results is given below:

(a) Different combinations of type I, II, I′ and II′ β-turn tripeptide fragments can be linked to form geometrically and energetically feasible cyclic hexapeptides. Different subtypes are possible for each of these combinations and these depend mainly on:
  (i) The conformation at the C^{α}s where the two tripeptides link and (ii) in the hydrogen bonding schemes of the β-turn tripeptide itself. The conformations at the linking C^{α}s has been found to be either in the extended region or in the 3→1 hydrogen bonded (γ or inverse γ) regions of the (ϕ, ψ) map. The different hydrogen bonding schemes found present in the component β-turns of these theoretically worked out cyclic hexapeptides are:
    (i) A β-turn with only a 4→1 hydrogen bond
    (ii) A type I or I′ β-turn having a 4→1 and a 3→ 1 hydrogen bonds occurring in a bifurcated form of the type



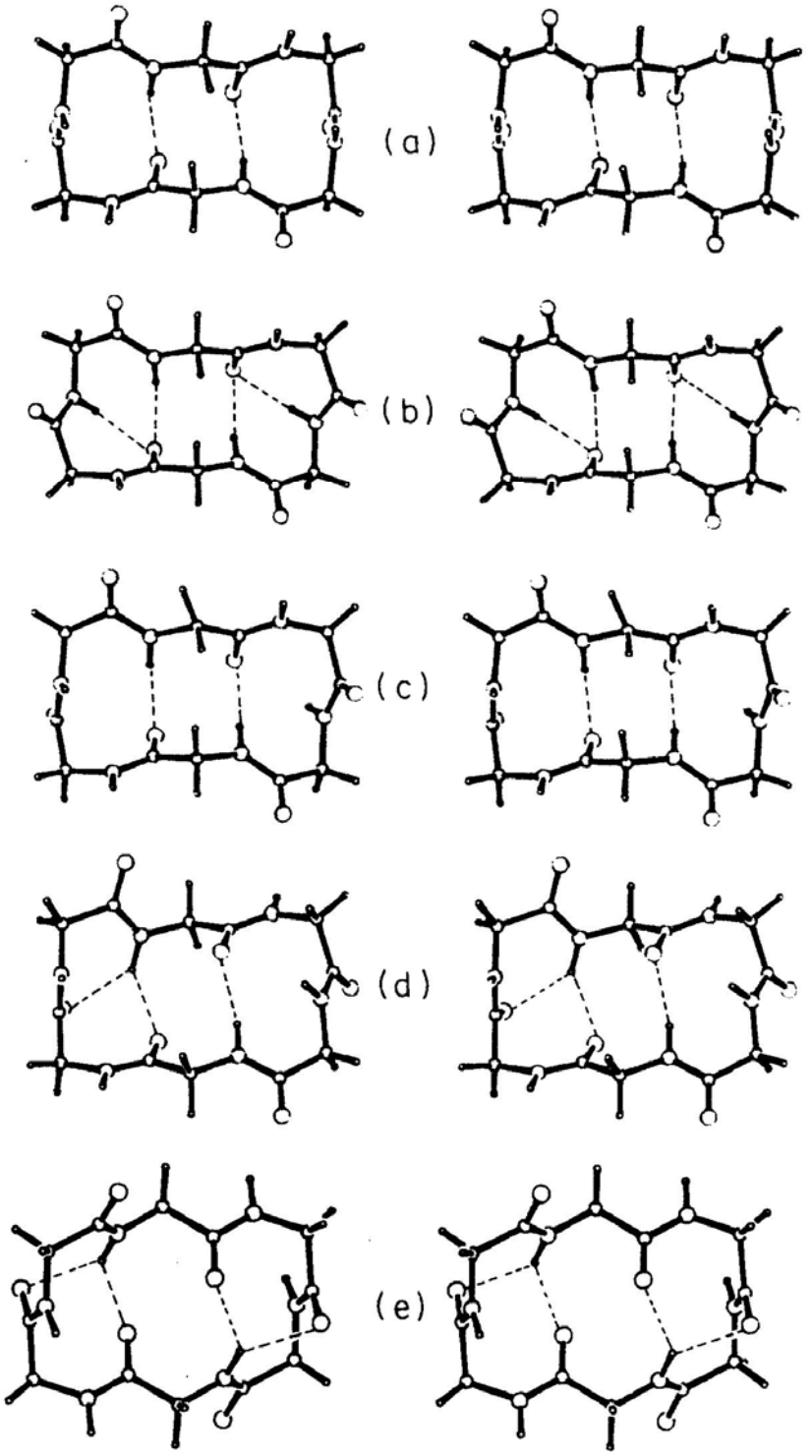


Figure 2. (a–e)

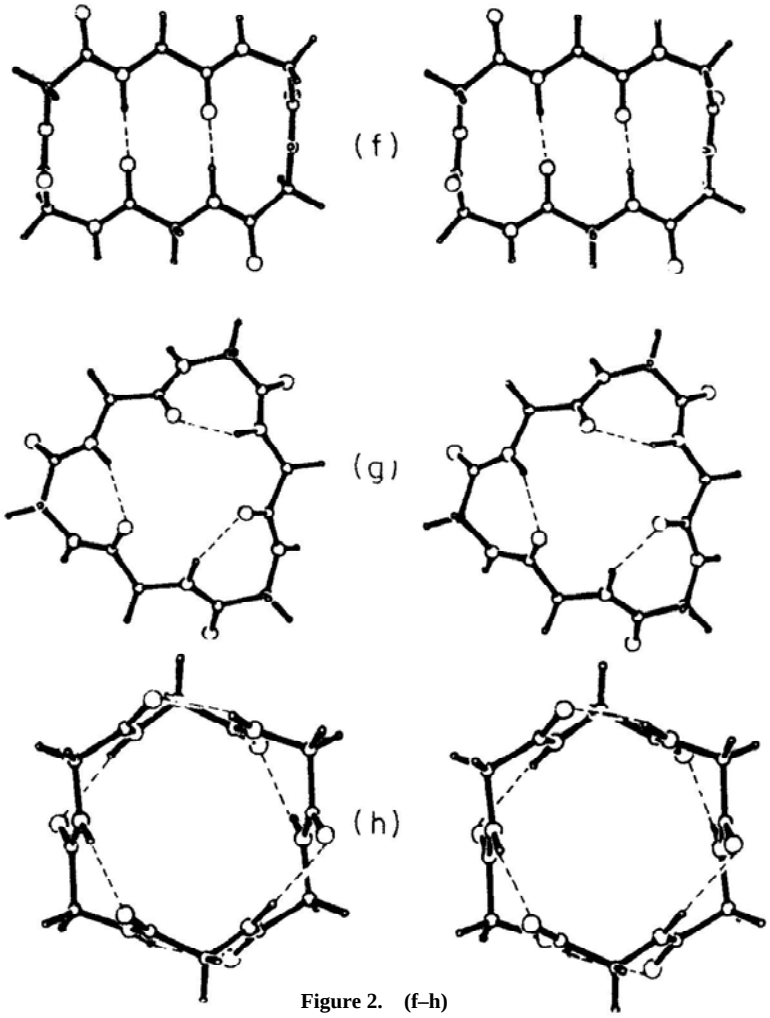


Figure 2. (f–h)

Figure 2. Stereodiagrams of cyclic hexapeptides with different hydrogen bonding schemes, worked out using energy minimization studies: a to f has a pair of β turns.
(a), Type I--I (b), Type II-II (c),Type I–II; (d),Type I–II′, (e),Type I-I′; (f),Type II-II′; (g), Three fold symmetrical having three γ turns; (h), S_6 symmetrical having three γ turns and three inverse γ turns.
The hydrogen bonds are shown as broken lines.

(iii) A type II.or II′ β-turn having both the 4→1 and 3→1 hydrogen bonds formed with the same acceptor oxygen atom *i. e.*,

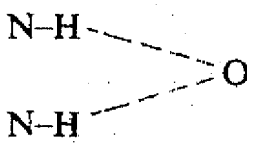


Figure 2a–f shows examples of cyclic hexapeptides made up of different combination of b –turns alongs with their various hydrogen bonding schemes.

Another class of cyclic peptides of the sequence type $c(A_1–A_2)_3$ in the heterodetic (depsipeptide) and homodetic (all peptides) forms are known to take up three-fold symmetric structures. Some of the examples of such types like the Enniatin B–KI complex (Dobler *et al*., 1969), Beauvericin hydrated (Geddes and Akrigg, 1976) in the solid state and a solution conformer of $c(Pro\text{-}Gly)_3$ and its cation complexes (Madison *et al*., 1974) show a three-fold symmetric structure. Energy calculations have been done by Mitra (1978) in this group of cyclic hexadepsipeptides having a three-fold symmetry using grid search procedures. Recently energy minimization studies of cyclic hexapeptides having a three-fold symmetry were done. The basic unit for which only one hydrogen bonding scheme is possible *viz.,* a 3→ 1 hydrogen bond across the dipeptide. Therefore starting with such a stable dipeptide conformation, *viz.,* one in which there is a 3→1 hydrogen bond, the possible conformational types of cyclic hexapeptides with the three-fold symmetry were worked out. The energy minimization studies show that two types of C_3-symmetric structures with three 3→1 hydrogen bonds are possible.

(a) One in which three extended conformations are interleaved with the three 3→1 hydrogen bonded γ-turn or inverse γ conformations.
(b) The other which has alternate y or inverse γ-turn with six 3→1 hydrogen bonds and an overall S_6 molecular symmetry.

Figure 2g,h show the two types of conformations with C_3 and S_6 symmetry and having three and six 3→1 hydrogen bonds respectively.

In the case of $c(Pro\text{-}Gly)_3$, the possibility of a two β-turn structure is ruled out owing to the presence of the three alternate $N–C^{\delta}$ groups. Therefore the next stable hydrogen bonding scheme i.e., the 3→1 hydrogen bond is preferred. Further, since the Pro residue is known to induce a 3→1 hydrogen bonded turn, a three 3→1 hydrogen bonded structure is expected in $c(Pro\text{-}Gly)_3$, one of the solution conformers of $c(Pro\text{-}Gly)_3$ in nonpolar solvents is known to take up this structure.

Many of the cyclic hexapeptides studied experimentally contain an L-Pro residue (Gierasch *et al*., 1981; Kopple *et al*., 1973, 1974, 1981, 1983). Since a Pro residue can occur as a *cis* Pro or as a *trans* Pro form with equal ease, some of the solid state and solution structures of cyclic hexapeptides are known to have *cis* Pro peptide bonds. The crystal structures of cyclo (L-Pro-L-Pro-Gly-L-Pro-L-Leu-Gly) (Nakashima *et al*., 1984), cyclo $(Gly\text{-}L\text{-}Pro\text{-}L\text{-}Pro)_2$ (Czugler *et al.,* 1982) its Mg^{2+} complex (Karle and Karle, 1981) and cyclo $(L\text{-}Phe\text{-}L\text{-}Pro\text{-}D\text{-}Ala)_2$ (Kartha *et al*., 1984) have two *cis* peptides in the cyclic hexapeptide backbone. The peptide conformation sequence in the former two have *cis* peptide units occurring consecutively, while those of the latter two are separated by two *trans* units. In addition, the crystal structure of cyclo $(L\text{-}Pro\text{-}Gly)_3$ (Kartha *et al*., 1982) shows one *cis* peptide unit in the cyclic hexapeptide backbone. The occurrence of the *cis* peptide units as a major conformation in solution has been thought of to be dependent on the solvent and the bulkiness of the residue preceding Pro (Sarkar *et al*., 1984; Kopple *et al*., 1981,1983), for the $c(L\text{-}A_1\text{-}L\text{-}Pro\text{-}D\text{-}A_3)_2$ types of cyclic hexapeptides.

Cyclic peptides having more than 6 residues

It is quite obvious that as one moves towards big cyclic peptides the conformational possibilities and the number of hydrogen bonding schemes increase considerably. Any

minimization studies in a multidimensional space is naturally bound to give a large number of minima and the selection from among these minima becomes difficult, in the absence of established procedures. In the present section, the various observed structures of these higher cyclic peptides are looked into to get any useful information on the general conformational aspects.

There are three examples of Sarcosyl containing higher cyclic peptides. They are cyclo hepta Sarcosyl (Groth, 1975), cyclo(L-Pro-Sar)$_4$ (Shimizu *et al*., 1983) and cyclo deca Sarcosyl (Groth, 1976b). As is to be expected there are a large number of *cis* peptide units in the ring. The ratio of *cis: trans* in these cases are 4:3, 4:4 and 6:4 respectively. There are two examples of higher cyclic peptides containing N-methylated amino acids. They are Ilamycin B_1 (Iitaka *et al*., 1974), which contains two N-methyl-Leu residues and cyclosporin A (Petcher *et al*., 1976), containing seven N-methylated amino acids including one Sarcosyl. The former contains two *cis* involving the N-methylated residues and it is surprising that the latter contains only one *cis* peptide unit inspite of the abundance of N-methyl residues.

From the above it is clear that it is virtually impossible at this stage to predict the number of *cis* peptide units or the sequence of *cis* and *trans* residues that occur in these cases.

Regarding the hydrogen bonding in these higher cyclic peptides, Karle (1981a) has given detailed description of the types of hydrogen bonds occurring in the crystal structures of cyclic peptides. As can be seen from these, the number of hydrogen bonds does not increase in proportion to the size of the cyclic peptide. A close examination of these structures reveals that, two or three good hydrogen bonds positioned suitably in the ring can bring in the necessary folding of the polypeptide chain to form a cyclic peptide and also stability to the conformation.

In the cyclic heptapeptide Ilamycin B (Iitaka *et al*., 1974) six of the seven peptide units are formed by two β-turns and the seventh one is involved in a weak 5→1 hydrogen bond. The cyclic octapeptide β-amanitin (Kostansek *et al*., 1978) has got a pair of 5→1 hydrogen bonds and each one of these can support four peptide units (in the same way as a 4→1 hydrogen bond stabilizes three peptide units). The complexed and uncomplexed forms of antamanide and its analogues (Karle *et al*., 1973, 1979; Karle 1974a, b, 1977; Karle and Duesler, 1977) brings out the observation that the hydrogen bonding is of 4→1 type in complexed and 5→1 type in uncomplexed forms. The reason for this can be sought for in the occurrence of the other coordination bonds in the complex. Lastly the cyclic dodecapeptide prolinomycin, cyclo(D-Val-L-Pro-L-Val-D-Pro)$_3$ (Hamilton *et al*., 1980) and the pentadecapeptide cyclo (L-Val-L-Pro-Gly-L-Val-Gly)$_3$ (Cook *et al*., 1980) have six and three 4→1 hydrogen bonds respectively.

Conclusion

Thus the present analysis based upon the energy minimization studies carried out in our group on small cyclic peptides and the available data on crystal structures of small and big cyclic peptides indicate the following:

(i) The cyclic tri and tetrapeptides are good model compounds to give us the nature of the peptide units including their flexibility.

(ii) In the case of cyclic tetra to hexapeptides the ring gets its stability through hydrogen bonding, and in this 4→1 hydrogen bonding is strongly preferred to 3→1 type.

(iii) As the number of units increases, the effect of hydrogen bonding is such that the ring gets its stability from a minimum number, two or three only. Hence the remaining oxygen atoms can easily take part in coordination bonds with metal atoms and thereby form complexes.

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