

Solvent Accessibility Studies of β -Bends and Application to Cyclic Hexapeptides*

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Synopsis

The solvent-accessible surface areas (ASAs), of the atoms in tripeptides around the minimum-energy conformations of the β -bend types I, I', II, and II' have been computed as a first step in the systematic solvent accessibility study of secondary structures. The side chains chosen at the two middle positions of the bend are L-Ala, D-Ala, and Gly. The ASAs of the hydrogen atoms are reported here and are found useful in determining the type of β -bends in six examples of cyclic hexapeptides whose crystal structures are known. Comparison with observation showed that all the β -bends in these cyclic hexapeptides were correctly identified by the present method. This points to a possible use of the method in identifying β -bend types in solution.

INTRODUCTION

Information regarding the exposure of the different atoms, groups, and residues in a protein to the solvent surrounding it is useful in understanding its structure and function. The idea of solvent accessibility had been developed, and a quantity known as solvent-accessible surface area (ASA) had been computed for the first time by F. M. Richards and coworkers.¹ The concept of ASA has been applied by many workers to the study of protein stability and folding,²⁻⁵ as well as to smaller peptides.⁶⁻⁹ Recently, Snyder and coworkers¹⁰ have computed the exposure of NH hydrogen atoms in a cyclic hexadepsipeptide, roseotoxin B, by means of ASAs.

The work described here involves the computation of the ASAs of some β -bend conformations and forms a first step in a systematic study of the solvent accessibility of the secondary structures in proteins (helices, β -bends, extended strands, and β -sheets).

SYSTEMS AND METHODS

The systems used in the study are tripeptides in β -bend conformations of types I, II, I', and II',¹¹ one of which is shown in Fig. 1. The ideal values of the backbone torsional angles (ϕ_2, ψ_2), (ϕ_3, ψ_3) for these bend types are: type I ($-60^\circ, -30^\circ$), ($-90^\circ, 0^\circ$); type II ($-60^\circ, 120^\circ$), ($80^\circ, 0^\circ$);

* Part XII of the series, Stereochemical Studies on Cyclic Peptides. Part XI is Ramakrishnan, C. & Narasinga Rao, B. N. (1980) *Int. J. Pept. Protein Res.* 15, 81-85.

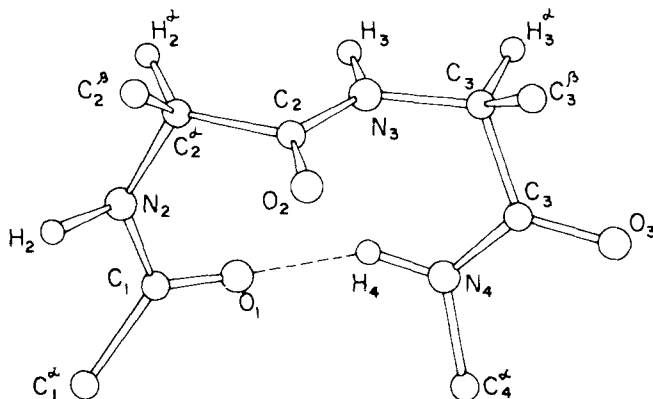


Fig. 1. Tripeptide in a β -bend conformation showing atom numbering and the 4 \rightarrow 1 hydrogen bond (- -).

type I' ($60^\circ, 30^\circ$), ($90^\circ, 0^\circ$); and type II' ($60^\circ, -120^\circ$), ($-80^\circ, 0^\circ$). The side chains L-Ala, D-Ala, and Gly (abbreviated to L, D, and G, respectively) are fixed at positions C_2^β and C_3^β leading to a total of nine tripeptides, namely, GL, GD, LG, DG, LL, DD, LD, DL, and GG. For each of these tripeptides, the potential energy, including hydrogen-bond energy, is calculated for hydrogen-bonded conformations in the regions of each of the bend types separately, varying the torsion angles (ϕ_2, ψ_2) , (ϕ_3, ψ_3) at 10° intervals. Standard potential functions and constants were used.¹² The conformations at the different grid points are then arranged in the increasing order of potential energy. In each case, 100 conformations, starting from the minimum upward, are used for ASA calculations. In fact, calculations were done only for types I and II, since types I' and II' are, respectively, their mirror images for enantiomeric sequences and have the same potential energy and ASA values.

The method used for computing ASAs is that of Lee and Richards¹ and employs the computer programs supplied by the latter. The method consists in rolling a solvent molecule down the surface of the tripeptide, making the maximum permissible contact with each atom. The van der Waals radii used are^{1,12,13}: C, 1.70 Å; C including attached hydrogens (used for C_1^α , C_4^α , and the C^β of Ala residue), 1.80 Å; O, 1.52 Å; N, 1.55 Å; H, 1.20 Å; and the solvent water molecule, 1.40 Å. In this study, attention has been confined to the ASAs of the hydrogen atoms alone because they stick out of the polypeptide backbone (as do the carbonyl oxygens) and, hence, their ASAs will be very sensitive to changes in conformation. Besides, the possibility of obtaining the solvent exposure of the hydrogens experimentally makes them the ideal choice in the efforts to distinguish conformations from ASA values. The ranges over which the ASAs of the hydrogen atoms vary for these conformations are given in Table I, each section of the table

TABLE I
 Ranges of ASAs (in \AA^2) of Hydrogen Atoms for the Different Types of β -Bends with
 Different Middle Residues

Atom	Type of β -Bend			
	I	I'	II	II'
Gly-L-Ala bend				
H ₂	18.1-20.2	17.5-20.9	20.1-21.6	20.3-21.6
H ₃	2.8-10.4	5.2-15.3	4.4-18.5	3.3-12.7
H ₄	0.0-5.2	0.0-5.1	0.0-5.6	0.0-4.3
H ₂ ¹	22.1-24.6	18.5-21.6	22.2-24.5	15.9-19.7
H ₂ ²	18.7-21.4	22.1-24.6	14.9-19.7	22.2-24.1
H ₃ ³	12.9-19.8	19.8-22.1	18.3-21.9	11.6-19.7
Gly-D-Ala bend				
H ₂	17.5-20.9	18.1-20.2	20.3-21.6	20.1-21.6
H ₃	5.2-15.3	2.8-10.4	3.3-12.7	4.4-18.5
H ₄	0.0-5.1	0.0-5.2	0.0-4.3	0.0-5.6
H ₂ ¹	22.1-24.6	18.7-21.4	22.2-24.1	14.9-19.7
H ₂ ²	18.5-21.6	22.1-24.6	15.9-19.7	22.2-24.5
H ₃ ³	19.8-22.1	12.9-19.8	11.6-19.7	18.3-21.9
L-Ala-Gly bend				
H ₂	13.0-14.7	18.5-20.7	14.8-15.7	20.7-21.4
H ₃	5.3-12.0	5.5-15.3	8.8-16.9	1.5-7.4
H ₄	0.0-5.2	0.0-5.2	0.0-5.6	0.0-5.7
H ₂ ²	16.3-18.6	19.4-21.9	12.7-16.1	18.9-20.1
H ₃ ¹	22.6-25.0	15.5-22.6	15.3-21.9	20.7-26.1
H ₃ ²	15.5-22.6	22.6-24.6	22.5-24.8	13.9-22.2
D-Ala-Gly bend				
H ₂	18.5-20.7	13.0-14.7	20.7-21.4	14.8-15.7
H ₃	5.5-15.3	5.3-12.0	1.5-7.4	8.8-16.9
H ₄	0.0-5.2	0.0-5.2	0.0-5.7	0.0-5.6
H ₂ ²	19.4-21.9	16.3-18.6	18.9-20.1	12.7-16.1
H ₃ ¹	22.6-24.6	15.5-22.6	13.9-22.2	22.5-24.8
H ₃ ²	15.5-22.6	22.6-25.0	20.7-26.1	15.3-21.9
L-Ala-L-Ala bend				
H ₂	13.0-14.7	18.1-20.7	14.7-15.7	20.7-21.4
H ₃	2.6-7.6	5.2-15.3	4.7-17.6	0.3-3.5
H ₄	0.0-5.2	0.0-5.1	0.0-5.6	0.0-4.4
H ₂ ²	16.3-18.6	19.4-21.9	12.5-16.9	18.6-20.0
H ₃ ³	12.8-19.7	19.8-22.1	18.3-21.9	11.3-19.4
D-Ala-D-Ala bend				
H ₂	18.1-20.7	13.0-14.7	20.7-21.4	14.7-15.7
H ₃	5.2-15.3	2.6-7.6	0.3-3.5	4.7-17.6
H ₄	0.0-5.1	0.0-5.2	0.0-4.4	0.0-5.6
H ₂ ²	19.4-21.9	16.3-18.6	18.6-20.0	12.5-16.9
H ₃ ³	19.8-22.1	12.8-19.7	11.3-19.4	18.3-21.9

(continued)

TABLE I (continued)

Atom	Type of β -Bend			
	I	I'	II	II'
L-Ala-D-Ala bend				
H ₂	12.7-15.3	18.5-20.6	14.8-15.7	20.7-21.4
H ₃	5.1-12.2	3.4-10.5	5.1-12.5	1.1-8.1
H ₄	0.0-5.1	0.0-5.2	0.0-4.3	0.0-5.7
H ₂ [‡]	16.1-18.9	19.4-21.9	12.7-16.1	18.6-20.6
H ₃ [‡]	19.9-22.1	12.8-19.8	12.6-19.5	18.3-22.0
D-Ala-L-Ala bend				
H ₂	18.5-20.6	12.7-15.3	20.7-21.4	14.8-15.7
H ₃	3.4-10.5	5.1-12.2	1.1-8.1	5.1-12.5
H ₄	0.0-5.2	0.0-5.1	0.0-5.7	0.0-4.3
H ₂ [‡]	19.4-21.9	16.1-18.9	18.6-20.6	12.7-16.1
H ₃ [‡]	12.8-19.8	19.9-22.1	18.3-22.0	12.6-19.5
Gly-Gly bend				
H ₂	18.1-20.2	18.1-20.2	20.3-21.6	20.3-21.6
H ₃	5.3-15.3	5.3-15.3	7.6-17.6	7.6-17.6
H ₄	0.0-5.2	0.0-5.2	0.0-5.6	0.0-5.6
H ₂ ^{‡1}	22.1-24.6	18.7-21.4	22.3-24.2	15.8-19.7
H ₂ ^{‡2}	18.7-21.4	22.1-24.6	15.8-19.7	22.3-24.2
H ₃ ^{‡1}	22.6-25.0	15.5-22.6	15.3-22.4	22.0-24.8
H ₃ ^{‡2}	15.5-22.6	22.6-25.0	22.0-24.8	15.3-22.4

giving the values corresponding to one of the nine tripeptides. The values in Table I reveal the following points relevant to distinguishing bend types.

1. The ASA of H₄ (see Fig. 1 for atom numbering) is uniformly low (5.7 Å² or lower) as this is directly involved in the 4 → 1 hydrogen bond. Thus, the ASA of H₄ cannot be expected to help in distinguishing bend types.

2. The H₂ atom is well exposed to the solvent, as may be seen from the table, although its ASA values vary within a narrow range of about 2 Å².

3. The ASAs of the H₃ atom vary a good deal. The ranges for the different bend types are different, although overlap is common. The wider spectrum of the ASA of H₃ is quite in accord with our expectations, because this atom forms part of the middle peptide unit of the β -bend, whose tilt accounts for the difference between the type I and type II bends.

4. The total number of H ^{α} atoms varies from 2 to 4, depending on the residues of C₂[‡] and C₃[‡] (Fig. 1). Their ASAs are generally high, vary from one bend type to another, and the ranges are different despite overlap.

5. Considering the standard bend varieties L-L type I, L-D type II,

D-D type I', and D-L type II' [LL(I), LD(II), DD(I'), DL(II')], the H₂ atom is more accessible in LD(II) than in LL(I), whereas for H₃ the reverse is the case. In the case of H₃, the ASA ranges for LL(I) and LD(II) overlap somewhat, with the latter being more accessible. DD(I') and DL(II') are mirror images of LL(I) and LD(II), respectively.

APPLICATION OF ASA CALCULATIONS TO CYCLIC HEXAPEPTIDES

Procedure

The indication that bend types can be distinguished using hydrogen ASA was checked by application to cyclic hexapeptides (CHP). From among the dozen or so CHPs whose x-ray crystal structures are available, the six listed in Table II were chosen for our study.

The usefulness of the procedure is based on the assumption that it will be possible in the not-too-distant future to obtain experimentally the ASA values of hydrogen atoms of peptides in solution from nmr spectroscopy. However, lacking experimental data, it is necessary at present to simulate the "observed" values of ASAs by computation from the x-ray structure coordinates. The following procedure is used to identify the bend type:

1. Where the positions of hydrogen atoms are not reported, they are fixed using standard geometry.
2. All non-Gly side chains are stripped of their side-chain atoms from C γ onward in order to equate them to L-Ala or D-Ala. The ASAs of the hydrogen atoms in these simplified molecules are calculated as in the case of β -bends, and the values so obtained serve as "observed" values.
3. The bend type is now determined by comparing the "observed" ASAs with the theoretical values for the bends given in Table I. If

TABLE II
Cyclic Hexapeptides Used in this Study

Name	Sequence
CHP1	cyclo(L-Ala-L-Ala-Gly-Gly-L-Ala-Gly) ^a
CHP2	cyclo(Gly-Gly-D-Ala-D-Ala-Gly-Gly) ^b
CHP3	cyclo(L-Ala-L-Ala-Gly-L-Ala-Gly-Gly) ^a
CHP4	cyclo(Gly-L-His-Gly-L-Ala-L-Tyr-Gly) ^c
CHP5	cyclo(Gly-D-Leu-L-Leu-Gly-D-Leu-L-Leu) ^d
CHP6	cyclo(L-Leu-L-Phe-Gly-D-Leu-D-Phe-Gly) ^e

^a From Ref. 14.

^b From Ref. 15.

^c From Ref. 16.

^d From Ref. 17.

^e From Ref. 18.

d_1^j, d_2^j, \dots are the differences between the "observed" ASA of the j th hydrogen atom (in each bend) and the mean of the lower and upper limits given in Table I for each bend type, then the sum of the differences is given by

$$D_t = \sum_{j=1}^n |d_t^j|$$

where $j = 1, 2, \dots, n$ corresponds to the n hydrogen atoms in the peptide and t stands for the bend type. The bend type for which the value of D is the lowest has been taken as the most possible bend.

RESULTS

The details of the steps in arriving at the bend types from the ASA values are first illustrated with one cyclic hexapeptide *cyclo(L-Ala-L-Ala-Gly-Gly-L-Ala-Gly)* (CHP1), and the results on the other molecules are presented in summary form.

The ASAs of the hydrogens in CHP1 are shown schematically in Fig. 2. The first step is to determine the location of the bends in the CHP. In the diagram, it can be seen that the atoms H_3 and H_6 have very low ASAs (0 and 1.4 Å²) clearly showing that these are involved in hydrogen bonding and that the two bends are composed of residues 6-1-2-3 and 3-4-5-6.

The next step lies in identifying the type of bend, the details of

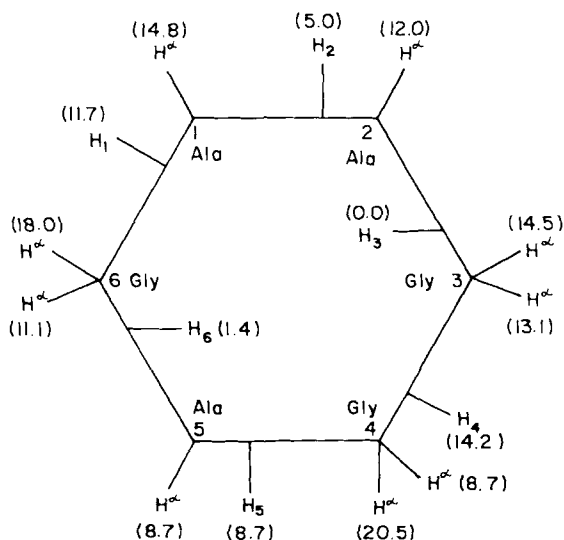


Fig. 2. Schematic diagram of the cyclic hexapeptide *cyclo(L-Ala-L-Ala-Gly-Gly-L-Ala-Gly)*. The values in parentheses are the ASAs of the hydrogen atoms in the molecule.

TABLE III
 ASA Values of the Hydrogen Atoms of cyclo(L-Ala-L-Ala-Gly-Gly-L-Ala-Gly) and
 Comparison with Corresponding Values for Typical Bends^a

Bend Type	ASA of Hydrogen Atoms (\AA^2)					Sum of Differences, D_i (\AA^2)
	H ₂	H ₃	H ₂ ¹	H ₂ ²	H ₃	
<u>Bend 1: L-Ala-L-Ala</u>						
"Observed"	11.70	5.00		14.80	12.00	
I	13.85 (2.15)	5.10 (0.10)		17.45 (2.65)	16.25 (4.25)	9.15 ^b
II	15.20 (3.50)	11.15 (6.15)		14.70 (0.10)	20.10 (8.10)	17.85
I'	19.40 (7.70)	10.25 (5.25)		20.65 (5.85)	20.95 (8.95)	27.75
II'	21.05 (9.35)	1.90 (3.10)		19.30 (4.50)	15.35 (3.35)	20.30
<u>Bend 2: Gly-L-Ala</u>						
"Observed"	14.20	8.70	8.70	20.50	8.70	
I	19.15 (4.95)	6.60 (2.10)	23.40 (14.70)	20.10 (0.40)	16.35 (7.65)	29.80
II	20.85 (6.65)	11.45 (2.75)	23.35 (14.65)	17.30 (3.20)	20.10 (11.40)	38.65
I'	19.20 (5.00)	10.25 (1.55)	20.05 (11.35)	23.35 (2.85)	20.95 (12.25)	33.00
II'	20.95 (6.75)	8.00 (0.70)	17.80 (9.10)	23.15 (2.65)	15.65 (6.95)	26.15 ^b

^a Differences between the two are given in parentheses.

^b Bend type with the lowest D_i value.

which are given in Table III. The first line gives the "observed" ASA values for each hydrogen atom, and below it are given the corresponding ideal values for the bend types obtained from the appropriate section of Table I. The difference (D_i) between the "observed" and the ideal values is given in parentheses. From the sum of the D values in the last column, it can be seen that the first bend belongs to type I and the second to type II', although in the latter case, the difference between the lowest and the next higher ASA values is not as pronounced as in the former. These deductions agree with those made from the (ϕ, ψ) values.

The complete results on the six CHPs are given in Table IV, where the "observed" (simulated) hydrogen ASA values, along with the assignment of the bend types based on them (corresponding to the lowest D value), are listed. These assignments are seen to agree, in all cases, with those based on the (ϕ, ψ) values given in the last four columns.

TABLE IV
ASAs of the Hydrogen Atoms in Six Cyclic Hexapeptides and Assignments of β -Bend Types

Residues in Bends		"Observed" ASA Values (\AA^2)						Bend Type			Observed (ϕ, ψ) Values (deg)			
No.	Name	H ₂	H ₃	H ₃ ¹	H ₃ ²	H ₃ ¹	H ₃ ²	H ₃ ¹	H ₃ ²	Bend Type	ϕ_2	ψ_2	ϕ_3	ψ_3
CHP1														
1-2	L-Ala-L-Ala	11.7	5.0	—	14.8	—	12.0	—	12.0	I	-53	-43	-84	0
4-5	Gly-L-Ala	14.2	8.7	8.7	20.5	—	8.7	—	8.7	II'	84	-113	-106	-9
CHP2														
6-1	Gly-Gly	17.6	9.0	19.77	15.30	16.24	14.62	—	14.62	I	-70	-15	-106	16
3-4	D-Ala-D-Ala	11.4	5.5	13.5	—	13.4	—	—	—	I'	66	15	131	-31
CHP3														
1-2	L-Ala-L-Ala	9.3	4.0	—	13.9	—	9.8	—	9.8	I	-62	-32	-95	13
4-5	L-Ala-Gly	15.7	10.0	—	19.0	15.6	24.4	—	24.4	I'	54	38	91	-6
CHP4														
4-5	L-Ala-L-Tyr	3.1	6.5	—	19.8	—	16.2	—	16.2	I	-56	-35	-95	-10
1-2	Gly-L-His	19.8	13.0	15.7	24.7	—	14.9	—	14.9	II'	61	-135	-77	-10
CHP5														
2-3	D-Leu-L-Leu	12.6	9.8	12.6	—	—	13.1	—	13.1	II'	77	-116	-87	-4
5-6	D-Leu-L-Leu	13.2	11.3	13.6	—	—	13.2	—	13.2	II'	66	-121	-85	-4
CHP6														
1-2	L-Leu-L-Phe	12.7	6.6	—	17.5	—	11.8	—	11.8	I	-59	-32	-115	36
4-5	D-Leu-D-Phe	12.7	6.6	17.5	—	11.8	—	—	—	I'	59	32	115	-36

DISCUSSION AND CONCLUSION

In the present paper, only the ASAs of the hydrogen atoms in bends have been studied. This limited study points to the usefulness of ASAs in understanding and identifying some conformational features in small peptides. The described method for distinguishing bend types in cyclic hexapeptides is an offshoot of the calculations on ideal bend types. The agreement between the simulated "observed" ASA values of hydrogens and those calculated for the ideal bend types shows that if the ASA values for the CHPs in solution could be obtained experimentally, it would be possible to predict the type of β -bend occurring in them using the procedure presented here.

In the present study, we have used simulated values of proton ASAs. However, there are indications that such values may soon become experimentally available. For example, nitrosyl-induced enhancement in T_1 relaxation rates of protons have been used as a probe of conformation in peptides.^{19,20} Since the phenomenon is correlated to the extent of exposure of the proton to the surrounding solvent, it must be possible to derive ASA values from the enhancement in T_1 values. These can then be used to distinguish bend types.

Side-chain atoms beyond C^β have not been included in the calculations of ASA values described here. However, it would be useful to have a qualitative idea as to how a larger side chain can shield the hydrogen atoms from the solvent. A side chain at C_2^β reduces the ASA of the H_2 atom considerably (by 5 \AA^2 or more, depending on the bulkiness of the group), except when the torsion angle, χ^1 , is around 180° , at which value the side chain points away from H_2 . The ASA of the H_2^β atom would be affected to a smaller extent, whereas H_3 and H_3^β ASAs would hardly be affected. Similarly, a longer side chain at C_3^β would cut off parts of H_3 and H_3^β from solvent, but this effect may be masked by the larger spectrum of variation of the ASAs of these two atoms. A side chain at C_3^β has hardly any effect on the ASAs of H_2 and H_2^β .

The calculations on bends described here form part of our ASA study of secondary structures. The success of the present application is an encouraging sign to calculate ASAs for a general bend, defined in our earlier paper,²¹ which include those that do not have $4 - 1$ hydrogen bonds. It is possible to extend the computation to the different types of helices, extended strands, and β -sheets and to compare them with known protein structures so that information regarding the tendencies, if any, of the different elements of secondary structures to bury or expose themselves during the process of protein folding can be derived. Some of these studies are under way.

We would like to thank Professor Kenneth D. Kopple for useful suggestions and discussions. The solvent-accessibility programs were supplied by Professor F. M. Richards.

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Received May 8, 1984

Accepted November 27, 1984