The γ Turn, a Possible Folded Conformation of the Polypeptide Chain. Comparison with the β Turn

George Némethy* [1a] and Morton P. Printz[1b,c]

The Rockefeller University, New York, New York 10021. *Received July 11, 1972*

ABSTRACT: A new tripeptide conformation is proposed which allows complete reversal of the direction of a polypeptide chain. This conformation, which for identification we have termed the γ turn, can connect two strands of an antiparallel pleated-sheet structure like the well-recognized β turn; however, it requires one less residue for this purpose. The proposed conformation is stabilized by two hydrogen bonds. One of them, a strongly bent bond, forms a seven-membered ring structure which was proposed recently as a stable conformation. The other hydrogen bond is straight and is analogous to those in antiparallel pleated sheets. Conformational energy computations indicate that the γ turn is a stable structure and that it can occur in a sequence consisting of L-amino acids, in contrast to the reduced stability of the β turn for such a sequence. It is proposed that nmr coupling constants can distinguish in a unique manner between the two types of turns. The dihedral angles for the γ turn are (ϕ_1, ψ_1, ω_1; ϕ_2, ψ_2, ω_2; ϕ_3, ψ_3) 172, 128 −170; 68, −61, 172; −131, 162°.

Folded conformations in which the polypeptide chain reverses its direction over a few residues have been recognized recently as a frequent component of protein structure.[2] Several proteins contain such sequences, for example, lysozyme,[3] carboxypeptidase A,[4] and bovine trypsin inhibitor.[5] Similar backbone peptide conformations have been reported for several oligopeptide hormones, synthetic polypeptides, and antibiotics; *e.g.*, for gramicidin SA.[6] It is of interest to compare the energetics of various ways of folding a polypeptide chain leading to sharp reversals of chain direction. The present paper serves this purpose.

The most frequently proposed folding structure is the β turn or β bend, which was proposed to occur in many small peptides,[6,7] may be found in some proteins as well,[2,3,8] and has been postulated to play an important role during the process of the folding of the polypeptide chain.[9] It consists of four amino acid residues, two of which (the first and fourth) are connected by two NH · · · OC hydrogen bonds corresponding to an antiparallel β structure and are linked as well by means of the second and third residue.[10]

In a recent study,[11,12] leading to proposed models for angiotensin II, we found another structure allowing a similar folding back of the polypeptide chain and involving three instead of four residues. This structure was referred to in our preliminary communication[12] as the γ turn. While the β turn and the γ turn are not unique, they are both energetically favorable structures. The postulated γ turn may in some cases even be preferable to the β burn. Thus we propose it as a possible general feature of polypeptide and protein conformations. In this paper, intramolecular potential energy computations of the β turn and the γ turn for various amino acid sequences are compared.

In an alternate nomenclature, based on the hydrogen-bonding pattern,[10b,13] the β turn and the γ turn could be described also as a 1–4 bend and a 1–3 bend, respectively.

Description of the Computation

Conformations are defined in terms of the dihedral angles for rotations about covalent bonds (ϕ, ψ, and ω for the backbone, χ's for the side chains), following the recently proposed standard conventions.[14] A frequently used set of values for bond lengths and bond angles was taken,[15] no angle bending was permitted. The coordinates of each atom were generated by means of the usual matrix techniques for coordinate transformations.[13,15,16] Intramolecular potential energies were computed following the usual procedures (reviewed, for example, in ref 15) as the sum of torsional energies for internal rotation, nonbonded (dispersion) interactions, dipole interactions, and hydrogen bonds. The functional forms for the various contributions and the constants used in them were those given by Scheraga,[15] except as otherwise noted.

A Lennard-Jones 6–12 potential with constants given by Scheraga[15,17] was used. Except for terminal methyl groups (treated as "extended atoms"), hydrogen atoms were considered separately. Electrostatic interactions between bond dipoles were computed in terms of Coulomb interactions between partial charges on each atom ("monopole" approximation[15,18]), using an effective dielectric constant[15,19] of 3.5. The energy of the hydrogen bond was computed by the method described by Poland and Scheraga.[18] The constants

(1) (a) To whom communications should be addressed at Laboratoire d'Enzymologie Physico-chimique et Moléculaire, Université de Paris-Sud, 91405 Orsay, France. (b) Basic Sciences Building, U. C. S. D., La Jolla, Calif. 92037. (c) Supported in part by N. I. H. Grant AM 02493.

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TABLE I
DIHEDRAL ANGLES FOR THE STABLE CONFORMATIONS OF THE γ TURN[a]

Sequence	Residue	φ, deg	ψ, deg	ω, deg
-L-Ala$_3$-	1	172	128	−170
	2	68	−61	172
	3	−131	162	
-Gly$_3$-	1	171	125	−170
	2	70	−61	172
	3	−129	165	

[a] Defined according to the standard conventions.[14]

TABLE II
INTRAMOLECULAR POTENTIAL ENERGIES OF A γ-TURN TRIPEPTIDE AND OF A β-TURN TETRAPEPTIDE FOR AMINO ACID SEQUENCES WITH VARIOUS CONFIGURATIONS

Sequence[a]	Energy, kcal/mol	
	γ Turn	
G-G-G	−15.3	
L-L-L	−14.1	
D-D-D	+10.2	
L-D-L	−13.3	
	β Turns[b]	
	β-I	β-II
G-G-G-G	−20.7	−17.9
L-L-L-L	−12.5	−13.6
D-D-D-D	−1.1	+5.8
L-D-L-L	−19.6	
L-L-D-L		−18.3

[a] Abbreviations used: G = glycyl, L = L-alanyl, D = D-alanyl residue. [b] See text for the definitions of the β-I and the β-II forms.

were chosen to give a minimum of −5.5 kcal/mol for the total hydrogen bond energy of a linear N—H···O—C bond at an H···O distance of 1.85 Å. No account was taken of any interactions with the solvent.

The barriers of internal rotation about the N-C$^\alpha$, C$^\alpha$-C, and C$^\alpha$-C$^\beta$ bonds were taken as 0.6, 0.2, and 2.8 kcal/mol, respectively.[15] In contrast to most published computations on small peptides, limited internal rotation was allowed around the peptide C′-N bond, following suggestions[20,21] that out-of-plane distortions of the peptide amide group are easily possible. Instead of the more elaborate procedure described by Winkler and Dunitz,[21] we assumed a simple sinusoidal dependence of the torsional energy on the angle ω, in order to avoid a large increase in the number of variables. The torsional energy was computed as

$$U(\omega) = \frac{U_0}{2}(1 - \cos 2\omega) - \frac{U_1}{2}(1 - \cos \omega) \qquad (1)$$

with U_0 = 19.0 and U_1 = 2.0 kcal/mol. This corresponds to an energy difference of 2.0 kcal/mol between the cis and trans peptide and to an energy barrier of 20 kcal/mol, measured from the trans form.[13]

The most stable conformations were obtained by minimizing the total potential energy as a function of the dihedral angles, using a program based on Powell's function minimization technique.[22] Throughout the computations, it is assumed that the tri- or tetrapeptide considered is part of a longer polypeptide chain so that no free amino- or carboxy-terminal groups were considered. However, the energy values listed in the tables do not include any interactions with the preceding and following parts of the peptide chain. The computations were carried out on the PDP-15 computer of the Rockefeller University Computer Center.

(20) M. Levitt and S. Lifson, *J. Mol. Biol.*, **46**, 269 (1969).
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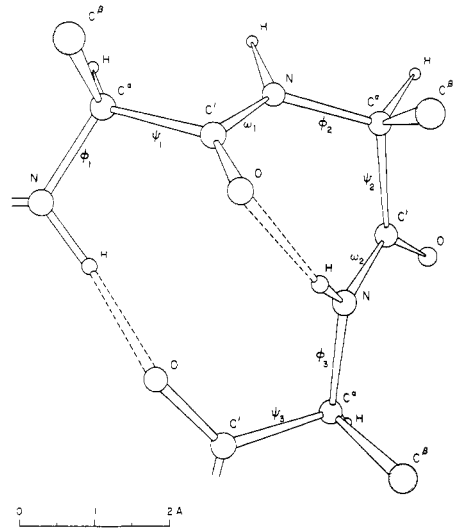

Figure 1. Perspective drawing of a tripeptide segment of a polypeptide chain, folded into a γ turn. The hydrogen bonds $N_1H_1\cdots O_3C_3$ and $N_3H_3\cdots O_1C_1$ are marked by dashed lines. The shaded circles represent the C$^\beta$ atoms of the side chains for L-amino acids. The dihedral angles φ, ψ, and ω, describing the rotation around each of the backbone bonds for each residue, are indicated next to each bond.

The Structure of the γ Turn

The dihedral angles describing the conformation and the total intramolecular energy are given in Tables I and II. The dihedral angles of the Gly$_3$ and Ala$_3$ sequences differ slightly, as a result of changing side-chain–backbone interactions, but the overall structure does not change significantly. Substitution of other amino acids or incorporation of the tripeptide into a longer sequence causes only very slight changes in the dihedral angles (*cf.* the angles listed for the proposed model of angiotensin II, an octapeptide.[12] The structure is characterized by two hydrogen bonds (Figure 1). One of them, between the N—H of residue 3 and the O=C of residue 1, results in a seven-membered ring. This ring structure, corresponding to ϕ_2, ψ_2 near 60, −60, has been proposed recently in several studies as a stable conformation.[23–25] While the hydrogen bond is very strongly bent, it has a normal H···O distance, and it still makes a sizable contribution to the stabilization of the folded seven-membered ring (Table III). The folding of residue 2 also permits the formation of a second hydrogen bond between the N—H of residue 1 and the O=C of residue 3. This bond is nearly straight and of optimal strength (Table III), corresponding to hydrogen bonds in the antiparallel β structure. Residues preceding and following a γ-turn tripeptide can take up several conformations, including that of the antiparallel β structure, without strain.

(23) V. F. Bystrov, S. L. Portnova, V. I. Tsetlin, V. T. Ivanov, and Y. A. Ovchinnikov, *Tetrahedron*, **25**, 493 (1969).
(24) G. M. Crippen and H. A. Scheraga, *Proc. Nat. Acad. Sci. U. S.*, **64**, 42 (1969).
(25) B. Maigret, B. Pullman, and D. Perahia, *J. Theor. Biol.*, **31**, 269 (1971).

Table III
Characteristic Features of the Hydrogen Bonds in the γ Turn

	$N_1H_1\cdots O_3C_3$	$N_3H_3\cdots O_1C_1$
H···O distance, Å	1.82	1.78
Total energy, kcal/mol	−5.5	−4.5
NHO angle, deg	166	138
HOC angle, deg	166	102

A comparison of glycyl and alanyl residues in various positions along the turn (Table IV) indicates that an L-alanyl side chain can be placed into position 1 or 3 without any strain on the γ turn (favorable nonbonded interactions with the $C^\beta H_3$ group in position 3 actually lower the energy as compared with glycine), and into position 2 without much difficulty. On the other hand, a D residue cannot be placed into position 1 or 3 without disrupting the β-turn structure, and a D-alanyl side chain is somewhat less favorable even in position 2. This is in marked contrast with D-amino acid substitutions in the β turn (see below). An important characteristic of the γ turn is that L side chains all extend on the same side of the backbone turn and are near each other. Thus, significant favorable nonbonded interactions can exist between larger side chains which would further stabilize the structure, as, *e.g.*, in angiotensin.[12] Table V summarizes results for longer side chains. The energy values listed are the differences between L-2-aminobutanoic acid and alanine, for side-chain rotations (χ_1) corresponding to local energy minima. Since two staggered low-energy conformations are available for one C^γ atom, all L-amino acid residues can occur in each position of the γ turn, including the C^β-branched amino acids.

The dihedral angles listed in Table I for the L-amino acid γ turn may appear to fall slightly outside the low-energy regions shown in most (ϕ, ψ) conformational energy maps (see, *e.g.*, ref 13, 15). However, this discrepancy is only apparent. Most published conformational energy maps were computed with the assumption of a completely rigid C′–N bond in the trans conformation (ω = 180°). The possibility of restricted rotation about this bond leads to the diminution of some repulsive nonbonded interactions and therefore to an expansion of the low-energy regions of the conformational maps.[26]

Actually, some rotation about the C′–N bonds, as indicated by the ω values in Table I, is necessary for the stabilization of the hydrogen bonds in the γ turn; otherwise, the structure could not be formed at all. However, the small energy of torsional rotation (a total of 0.9 kcal/mol for both bonds) is more than compensated for by the energy of the hydrogen bonds. This energy would be even lower had we used the more precise procedure described by Winkler and Dunitz[21] instead of eq 1. Permitting some bending of bond angles would distribute the energy of distortion value over several smaller values.

Comparison of the β Turn

Both structures can furnish the turning point for polypeptide chain folding into an antiparallel β structure. The backbone intramolecular potential energy *per residue* is about equal for the two turns (*cf.* the entries for Gly_3 and Gly_4 respectively, in Table II). However, the number of residues needed for achieving the turn is one less in the γ turn than in the β turn.[27] As a result, in a hairpinlike antiparallel structure with a β turn, there can be one more hydrogen bond *per residue* than in the corresponding structure with a β turn.

There is a marked difference between the two types of turns in the placing of side chains. As is well known,[7,10,28] the β turn can exist in two forms. In one of them (to be referred to here as β-I), it is preferred that residue (i + 1) be glycine or a D-amino acid, as reflected by the energy values in Table II. In the other form (β-II), residue (i + 2) should be preferably glycine or a D-amino acid. The presence of L-alanine in the positions indicated increases the energy by about 5 or 8 kcal/mol, respectively. Thus the stability of the perfect β turn is reduced in an all-L-amino acid sequence. In contrast, either an L- or a D-amino acid residue can be accomodated easily in position i + 1 of the γ turn: the respective energies are only 2.0 and 2.8 kcal/mol above that

Table IV
Intramolecular Potential Energy Changes for the Substitution of L-Alanyl or D-Alanyl Residues for Glycyl Residues in the γ Turn and the Two Forms of the β Turn[a]

	Energy, kcal/mol		
	Residue		
Substitution	i	i + 1	i + 2
	γ Turn		
Gly → L-Ala	0.0	2.0	−0.8
Gly → D-Ala	11.6	2.8	11.1
L-Ala → D-Ala	11.6	0.8	11.9
	β-I Turn		
Gly → L-Ala		8.5	0.6
Gly → D-Ala		1.5	8.7
L-Ala → D-Ala		−7.0	8.1
	β-II Turn		
Gly → L-Ala		1.1	5.0
Gly → D-Ala		12.1	0.2
L-Ala → D-Ala		11.1	−4.8

[a] See text for the definitions of the β-I and the β-II forms.

Table V
Energies of a $C^\beta H_2$–$C^\gamma H_3$ Side Chain in Various Residues of the γ Turn, Relative to the Energy of an Alanyl Side Chain in the Same Position, and for Values of the C^α–C^β Dihedral Angle χ^1 in the Stable Side-Chain Conformations

Residue	Conformation[a]	χ^1, deg	ΔE, kcal/mol
i	g^-	55	−1.9
	t	−177	−3.0
	g^+	(−63)	(−2.5)[b]
i + 1	g^-	No stable position	
	t	−167	−2.5
	g^+	−52	−2.3
i + 2	g^-	59	−1.2
	t	(−176)	(−1.3)[c]
	g^+	−62	−1.7

[a] Staggered conformations are indicated with the notation of Flory: t = trans (χ^1 near 180°), g^- = gauche (χ^1 near 60°), g^+ = gauche (χ^1 near −60°). [b] Stable only for an N-terminal residue, not inside a polypeptide chain. [c] Stable only for a C-terminal residue, not inside a polypeptide chain.

(26) K. D. Gibson and H. A. Scheraga, *Biopolymers*, **4**, 709 (1966).

(27) In the γ turn, the two hydrogen bonds are i to i + 2 and i + 2 to i, while in the β turn they are i to i + 3 and i + 3 to i. This designation follows the recommended notation.[14]

(28) C. M. Venkatachalam, *Biopolymers*, **6**, 1425 (1968).

of glycine (Table IV). Thus the γ turn may be equivalent to or even more favorable than the β turn in all-L sequences not containing glycine.

While the two hydrogen bonds in the β turn are linear and hence presumably of maximal strength, they are not strongly shielded from the solvent by the side chains in a hairpinlike structure. The same is true about the linear i to $i + 2$ hydrogen bond in the γ turn. While the $i + 2$ to i hydrogen bond of the latter is bent, and hence intrinsically weaker (Table III), it is considerably more shielded by the backbone and the side chains.[11,12] Thus, both structures can explain an observed reduction of amide–water proton exchange rates.

A criterion for distinguishing between the γ turn and the β turn can be furnished by nuclear magnetic resonance spectroscopy, *viz.*, the determination of the $^3J_{NC}$ coupling constant.[29] From Figure 1 (A and B) of ref 29, the following values of $^3J_{NC}$ can be estimated for L sequences in each of the turns:[30] for the γ turn, about 2.3, 7.9, and 9.7 Hz; for the β-I turn, 8.8, 7.9, 8.7, and 8.9 Hz; for the β-II turn, 8.9, 4.0, 6.6, and 8.9 Hz. If positions $i + 1$ or $i + 2$, respectively, of the two forms of the β turn were occupied by a D residue, corresponding to the most stable conformations, the sequences of coupling constants would become about 8.9, 2.6, 8.7, and 8.9 Hz for the β-I turn and 8.9, 4.0, 7.1, and 8.9 Hz for the β-II turn. In the γ turn, D substitution in position $i + 1$ would result in the sequence 2.3, 4.4, and 9.7 Hz. Thus, within the limitations of the method, the coupling constants can be used to distinguish uniquely between the various turns for a known amino acid sequence. Nmr studies are in progress in angiotensin II.

After submission of this manuscript, we obtained from Dr. B. W. Mathews details of the electron density map of thermolysin. These indicate the presence of a γ turn, with dihedral angles very similar to those proposed in this paper, occurring as residues 25–27 of thermolysin. A description of this structure, as well as a comparison with the present predictions, is being published.[32] Dr. Matthews also points out[32] the possibility of another form of the γ turn, based on the alternate form of the seven-membered hydrogen-bonded ring.[23]

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(30) Recently, Ramachandran, *et al.*, presented[31] a revised equation for $^3J_{NC}$ as a function of the N–C$^\alpha$ dihedral angle. With that equation, similar sequences of the coupling constants for the three postulated turns are obtained as shown in the text. Using the equation in ref 31, the differences between the high and low values of $^3J_{NC}$ are smaller than listed here; however, the sequences of values are still characteristically different for the various conformations.

(31) G. N. Ramachandran, R. Chandrasekharan, and K. D. Kopple, *Biopolymers*, **10**, 2113 (1971).

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Equation-of-State Parameters for Poly(dimethylsiloxane)

Hsiang Shih and P. J. Flory*

Department of Chemistry, Stanford University, Stanford, California 94305.
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ABSTRACT: The thermal expansivity $\alpha = V^{-1}(\partial V/\partial T)_p$ and thermal pressure coefficient $\gamma = (\partial p/\partial T)_V$ for poly(dimethylsiloxane) (PDMS), mol wt $\approx 10^5$, have been determined accurately over the temperature ranges 20–207 and 24–161°, respectively. Characteristic parameters v^*, T^*, and p^* calculated from these results are compared with those for other polymers and for low molecular weight liquids. For PDMS, α is much larger and γ much smaller than for any other polymer. The characteristic pressure p^* appears to be a more reliable index of the intermolecular energy than the cohesive energy density for this purpose. It is approximately the same for a polymer as for corresponding low molecular weight liquids. For PDMS and HMDS (hexamethyldisiloxane) p^* assumes exceptionally low values of 341 and 358 J cm^{-3}, respectively, which are duplicated only by the fluorocarbons ($p^* \approx 360$ J cm^{-3}).

The investigations reported here were carried out with the objective of determining the density ρ, the thermal expansivity $\alpha = V^{-1}(\partial V/\partial T)_p$, and the thermal pressure coefficient $\gamma = (\partial p/\partial T)_V$ for PDMS [poly(dimethylsiloxane)] over a wide range of temperature and with the accuracy required for evaluation of the reduction parameters needed for the treatment of solutions of this polymer. Results reported previously are limited in scope and in some instances are of uncertain accuracy. Comprehensive measurements of the equation-of-state parameters appear not to have been carried out heretofore on PDMS of high molecular weight.

Experimental Section

A sample of PDMS having a viscosity-average molecular weight of about 10^5 was obtained from the General Electric Co. Low molecular weight constituents were removed by fractional precipitation from a 0.5% solution in ethyl acetate using methanol as precipitant according to the procedure described previously.[1] Addition of methanol to the solution in the ratio of one to three parts by volume served to precipitate *ca.* 55% of the polymer at 30°. After addition of the precipitant, the temperature was raised to 50° to restore homogeneity, whereupon the solution was allowed to cool to 30° with gentle stirring. Separation of the precipitated phase and recovery of the polymer therein was carried out in the usual manner.[1]

Apparatus and procedures used for determination of the equation-of-state parameters have been described.[2,3] The cells used

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(2) R. A. Orwoll and P. J. Flory, *ibid.*, **89**, 6814 (1967).
(3) H. Höcker, G. J. Blake, and P. J. Flory, *Trans. Faraday Soc.*, **67**, 2251 (1971).