Peptide Models 6. New β-Turn Conformations from ab Initio Calculations Confirmed by X-ray Data of Proteins[⊥]

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Abstract: In an attempt to determine intrinsically stable hairpin geometries, a number of triamide conformations of For-Ala-Ala-NH_2 were investigated using ab initio calculations (HF/3-21G). Previous ab initio calculations of selected diamides of single amino acid residues (e.g., For-Ala-NH_2) suggested that the α_L-type backbone conformation ($\phi \approx -54°$, $\psi \approx -45°$) is not a minimal energy structure, although in globular proteins the $(\alpha_L)_n$ units (referred to as α-helices) are the most frequently found conformations. The lack of the α_L conformation made the application of ab initio calculations in peptide geometry analyses questionable. In contrast, for triamides (e.g., For-Ala-Ala-NH_2) the appearance of the α_L backbone subconformation is confirmed in the $\alpha_L\delta_L$ conformation (usually referred to as type I β-turn). This intrinsically stable conformation is the most frequently found hairpin structure in proteins. The existence of the ϵ_L conformation ($\phi \approx -60°$, $\psi \approx 120°$) in chiral diamides (such as For-Ala-NH_2, For-Ser-NH_2, or For-Val-NH_2) has never been confirmed by ab initio studies, although X-ray analyses of proteins revealed the existence of the polyproline II conformation $[(\epsilon_L)_n]$ a long time ago. The herein presented stable $\gamma_D\epsilon_L$ and $\delta_D\epsilon_L$ hairpin conformations, calculated by ab initio methods, legitimize the "missing" ϵ_L backbone geometry. The fact that some legitimate backbone conformations (α_L and ϵ_L) appear only in triamides and not in diamide systems assigns a specific role to triamide models in understanding protein conformations. The importance of some triamide conformations, especially type I and type II β-turns, is emphasized. This study summarizes all the possible [18 (30) conformations depending on the d or τ "selection rule"] hairpin geometries determined for For-Ala-Ala-NH_2 using ab initio computations. We were able to identify all 30 ab initio yielded conformations as backbone substructures of globular proteins, determined by X-ray crystallography. The 30 optimized triamide structures present a unique opportunity to understand the conformational behavior of β-turns (β-bends or hairpins). This may have far-reaching consequences in understanding the β-turn-mediated protein folding.

Introduction

Besides the two major secondary structural elements[1–3] [the α-helix $(\phi_i = -60° \pm 30°, \psi_i = -60° \pm 30°)_n$, and the β-pleated sheet $(\phi_i = -150° \pm 30°, \psi_i = 150° \pm 30°)_n$], the third most frequently found[4,5] structural unit in globular proteins is the β-turn conformation.[6–8] Smith and Pease[8] reviewed in detail the reverse turns in peptides and proteins. Reverse turns, hairpins, β-bends, or β-turns are structural elements consisting of four successive amino acid residues (labeled 1, 2, 3, and 4) at positions i, $i + 1$, $i + 2$, and $i + 3$ in proteins. The variety of definitions suggested in the past quarter of a century clearly illustrates the evolution of the β-turn concept. Adhering to the original definitions of Venkatachalam,[6] β-turns are classified into conformational types by their values of ϕ_{i+1}, ψ_{i+1}, ϕ_{i+2}, and ψ_{i+2} torsional angles. On the basis of the four backbone torsional angle values of the second and third residues, there are three major types of folded conformations: I, II, and III β-turns (Chart I). About a decade later, in the analysis of the β-turn content of globular proteins, a distance criteria was also introduced.[9a,b] Accordingly, the C_1^α - C_4^α distance must be shorter than 7 Å. Often an intramolecular H-bond can be found in β-turns, where the NH of the $i + 3$ residue points toward the carbonyl oxygen of the ith residue (1 ← 4-type H-bond) as shown in Figure 1. Although this 1 ← 4 hydrogen bond has never been proved to be a necessary condition for β-turns, it is frequently found in peptides and proteins on the basis of X-ray[9a,c] and NMR structure determinations,[10] and therefore a misconception has developed over the years that such a hydrogen bond is an essential structural feature of β-turns. This H-bond pattern was also used as a criterion for β-turn assignment in proteins.[9a,c]

The Vankatachalam[6]-predicted $\psi_{i+2} = 0°$ value for several types of β-turns was not satisfied in several structure assignments in globular proteins. Consequently, Chou and Fasman[9b] suggested a larger tolerance for the ψ_{i+2} torsional angle ($-50° \leq \psi_{i+2} \leq 50°$). More recently, Wilmot and Thorton[11] demonstrated that the $\psi_{i+2} = 0°$ criterion is one of the reasons that numberous turns are identified as "distorted." The experimental value of ψ_{i+2} is often around 45° or −45°. This finding is in perfect agreement with our previous analysis[12,13] of ab initio Ramachandran maps,

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[⊥] Dedicated to the memory of Professor M. Kajtár, the late virtuoso of stereochemistry.

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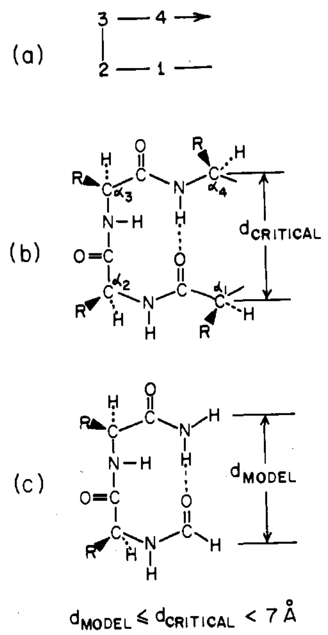
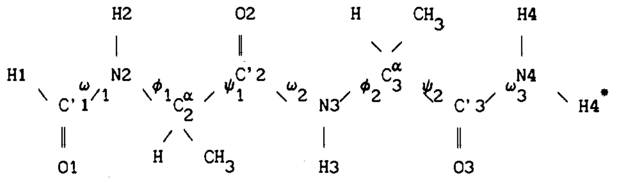


Figure 1. (a) Sequence of four successive amino acid residues forming a β-turn backbone conformation. (b) A schematic representation of the 1 ← 4 hydrogen bond in a β-turn structure and the 7-Å upper limit of the critical distance ($d_{\mathrm{crit}} = d$) which assigns the secondary structural elements according to the "classical" definition. In model compounds (e.g., For-Ala-Ala-NH_2) the model distance (d_{model}) is always shorter than the critical distance for a peptide with real C_1^α and C_4^α atoms (c).

Chart I

TYPE	ϕ_{i+1}	ψ_{i+1}	ϕ_{i+2}	ψ_{i+2}	topological code
I	-60	-30	-90	0	$\alpha_L\alpha_L$ $\alpha_L\gamma_L$ $\alpha_L\delta_L$
II	-60	120	80	0	$\varepsilon_L\alpha_D$ $\varepsilon_L\gamma_D$ $\varepsilon_L\delta_D$
III	-60	-30	-60	-30	$\alpha_L\alpha_L$

Chart II



where none of the nine conformational minima had ψ values around 0° while values around −45° (α_L, δ_D, or γ_D) and around 45° (α_D, δ_L, or γ_L conformations) were found. Therefore, we propose to distinguish[13] the three different forms of type I and type II β-turns on the basis of ψ_{i+2}. These conformations are identified by the topological codes given in Chart I. The type III β-turn is determined as a single turn of a 3_{10} helix and has therefore a single topological code only.

The smallest N- and C-terminal protected β-turn model, incorporating two chiral amino acids, has the structure shown in Chart II.

In an *N*-formyl dipeptidamide model, the two amino acids represent the second and the third residues of a β-turn (cf. Figure 1). In this model the H1–C'1 bond of the N-terminal formyl group is shorter by ≈0.5 Å and the N4–H4* bond length of the C-terminal amide group is shorter by 0.6 Å, as compared to the corresponding C_i^α – C'1 and the N4 – C_{i+4}^α bonds. Due to this shortening, the distances d_{model} and d_{crit} may differ from each other by no more than 0.6 Å + 0.5 Å = 1.1 Å, depending on the orientation of the two bonds (Figure 1).

Although the classification of β-turns is traditionally made on the basis of the backbone torsional angle values (ϕ_i, ψ_i), the degree of folding or unfolding of a β-turn can be defined in a simpler way, on the basis of the twisting of the hairpin conformation. In agreement with the pioneering work of Levitt,[9a] we have recently introduced[14] the C_i^α - C_{i+1}^α - C_{i+2}^α - C_{i+3}^α torsional angle labeled as τ (see Figure 2) that describes the overall angularity of the backbone conformation with values $-180° \leq \tau \leq 180°$. The global minimum (the $\gamma_L\gamma_L$ conformation), for example, has $\tau_{\gamma_L\gamma_L} = 168.4°$, which can quantitatively describe the degree of unfolding (Figure 3). Considering the criterion that the C_1^α – C_4^α distance must be shorter than 7 Å, only a fraction of the β-turn conformations ($-90° \leq \tau \leq 90°$) can be assigned as such.

For the first three of the eight different types (I–VIII) of β-turns (Table I), their mirror image conformations (types I', II', and III' β-turns) have also been suggested previously.[6] Although both the type I β-turn and the type I' β-turn conformations incorporate only L-amino acids, they have *conformationally*

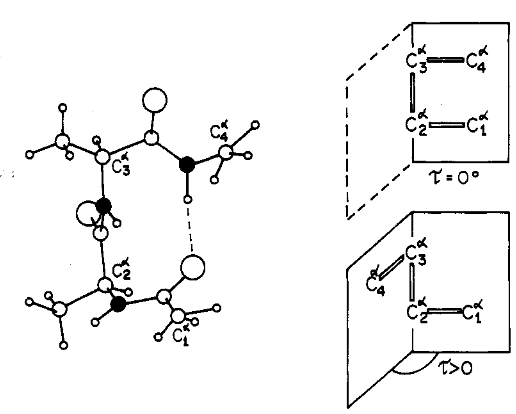


Figure 2. (Left) Hairpin conformation of a polypeptide chain. A schematic illustration of an untwisted β-turn and a backbone twisted by τ degrees is shown on the right.

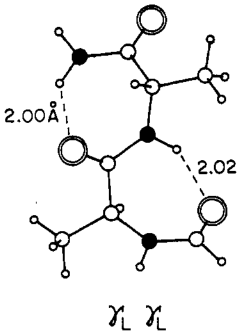


Figure 3. $\gamma_L\gamma_L$ conformation of For-L-Ala-L-Ala-NH_2 corresponding to a rather twisted backbone geometry ($\tau_{H_1-C_2^\alpha-C_3^\alpha-H_4} = 167.8°$).

Table I. Different Types of β-Turns[a] in Peptides Identified on the Basis of Experimental Studies[6,8,11]

type	ϕ_1	ψ_1	ϕ_2	ψ_2	new code(s)[b]
I	−60	−30	−90	0	$\alpha_L\alpha_L$, $\alpha_L\gamma_L$, $\alpha_L\delta_L$
I'	60	30	90	0	$\alpha_D\alpha_D$, $\alpha_D\gamma_D$, $\alpha_D\delta_D$
II	−60	120	80	0	$\epsilon_L\alpha_D$, $\epsilon_L\gamma_D$, $\epsilon_L\delta_D$
II'	60	−120	−80	0	$\epsilon_D\alpha_L$, $\epsilon_D\gamma_L$, $\epsilon_D\delta_L$
III	−60	−30	−60	−30	$\alpha_L\alpha_L$
III'	60	30	60	30	$\alpha_D\alpha_D$
IV	ambiguously defined				
V	ambiguously defined				
VIa	−60	120	−90	0	$\epsilon_L\alpha_L$, $\epsilon_L\gamma_L$, $\epsilon_L\delta_L$
VIb	−120	120	−60	0	$\beta_L\alpha_L$, $\beta_L\gamma_L$, $\beta_L\delta_L$
VII	ambiguously defined				
VIII	−60	−30	−120	120	$\alpha_L\beta_L$

[a] Only types I, II, and III have more than sporadic occurrence in globular proteins according to X-ray studies. [b] In degrees.

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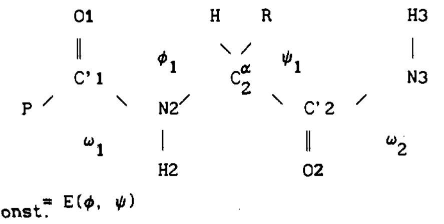
Chart III



enantiomeric peptide backbones.[12] Therefore, the conformational enantiomer of the $\alpha_L\alpha_L$ conformation (type I [or III] β-turn) is the $\alpha_D\alpha_D$ conformation labeled traditionally as the type I′ [or III′] β-turn. (Duplications reported in Table I originate from the historic evolution of the concept; both the type I and the type III β-turns have an $\alpha_L\alpha_L$-like backbone geometry, but these were derived from different sources and therefore are labeled differently.) The extraction of the remaining β-turn conformations, fulfilling the angularity ($-90° \leq \tau \leq 90°$) or distance ($d \leq 7$ Å) criteria, is hardly possible on the basis of earlier approaches. Even if more β-turn conformations exist than can be detected by analyzing a 4D-Ramachandran[13]-type potential energy hypersurface (PEHS), these structures, due to the rate occurrence of these conformations in globular proteins, cannot be extracted from the X-ray data analyses of these proteins.

Multidimensional conformational analysis (MDCA)[15] predicted[12] nine minima on the full Ramachandran map,[16] $E = E(\phi,\psi)$, where the torsional angles are defined according to IUPAC-IUB[17] convention as shown in Chart III. (Let us assume that ω_1 and ω_2 are constants; usually ω_1 and $\omega_2 = 180°$ or sometimes 0°). Each of these nine minima represents the only energy minimum in a given catchment region.[18]

Figure 4 shows the occurrence of the nine minima (α_L, α_D, β_L, γ_L, γ_D, δ_L, δ_D, ϵ_L, ϵ_D) in an idealized fashion. It should be noted that according to the IUPAC-IUB convention[17] the ϕ and the ψ values vary between −180° and 180°. This domain is indicated by the dashed square in the center of Figure 4. For various reasons,[12,13] during conformational analysis it is convenient to use a different cut of the same potential energy surface (PES), namely any of the four identical quadrants encircled by the solid lines in Figure 4. With this choice of representation, the spatial arrangement of the nine minima may be specified as shown in Chart IV. Torsional angles ϕ_{TOP} and ψ_{TOP} span the range from 0° to 360° in accordance with the earlier suggestion,[12] while in the IUPAC-IUB convention[17] these ϕ and ψ variables vary from −180° to 180°.

During the conformational analysis of PCONH-CHR-CONHQ using ab initio calculations,[19-24] the absence of the α_L and ϵ_L peptide conformations was noted. By the calculation of several dozens of relaxed grid points, the shape of the PES for For-Ala-NH$_2$ was recently analyzed in the vicinity of the α_L conformation, but no minimum was found.[24] Nevertheless, the shape of the PES, in the area where the α_L conformation is expected to be, suggests that even a minor stabilizing force (such as a hydrogen bond) could result in a local minimum, giving legitimacy to the α_L backbone structure. To the best of our knowledge, various experimental methods, including X-ray crystallography, have never identified the α_L backbone conformation for any single amino acid diamides, which agrees perfectly with the ab initio results. Nevertheless, each of the nine minima specified in Chart IV are legitimate both in terms of multidimensional conformational analysis and on the basis of X-ray-analyzed structures in larger peptides and proteins.[25,26] (The conformational oligomers $(\alpha_L)_n$ and $(\epsilon_L)_n$, the so-called α-helix and poly-L-proline II secondary structural units, occur frequently in globular proteins.) However, the absence of the mentioned minima (α_L and ϵ_L) in the

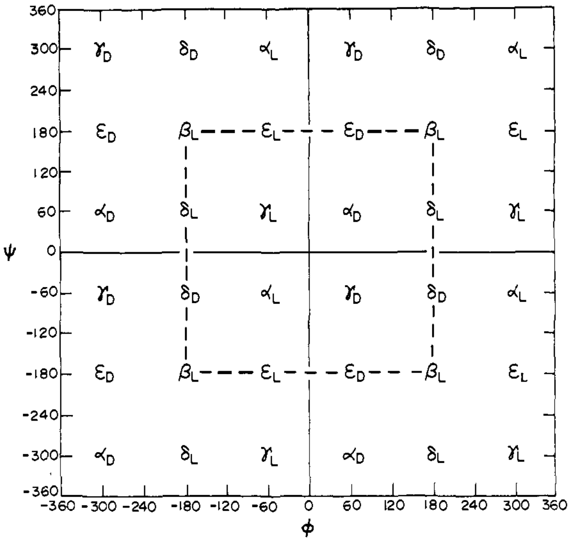


Figure 4. Idealized PES topology for a single amino acid residue involving two complete cycles of rotation in both ϕ and ψ (the location of the minima are specified by their names in terms of subscripted Greek letters).

Chart IV

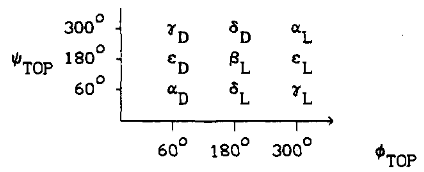


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Table II. Experimental Conformation of Selected Single Amino Acid Diamides Obtained from X-ray Crystallography

amino acid	N-terminal	C-terminal	ϕ^a	ψ	conformation	ref
Gly	MeCO-	-NH^{i}Pr	−78	160	ϵ_L	25a
Gly	^{t}BuCO-	-NH^{i}Pr	−112	142	ϵ_L	25b
Gly	^{i}PrCO-	-NH^{i}Pr	−169	175	β_L	25c
L-Met	MeCO-	-NMe$_2$	−126	162	β_L	25d
DL-Val^b	MeCO-	-NMe$_2$	−92(−90)	123(122)	ϵ_L	25e
L-Val	MeCO-	-NMe$_2$	−132	77	δ_L	25f
DL-Leu	MeCO-	-NHEt	−91	144	ϵ_L	25g
DL-Leu	MeCO-	-NHEt	−91	144	ϵ_L	25h
L-Gln	^{t}BuCO-	-NHMe	−72	165	ϵ_L	25i
L-Ser^c	^{t}BuCO-	-NHMe	−92	151	ϵ_L	25j

[a] In degrees. [b] There were two molecules of different geometries in the unit cell. [c] Side chain torsional angles are $\chi_1 = 70°$, $\chi_2 = 88°$.

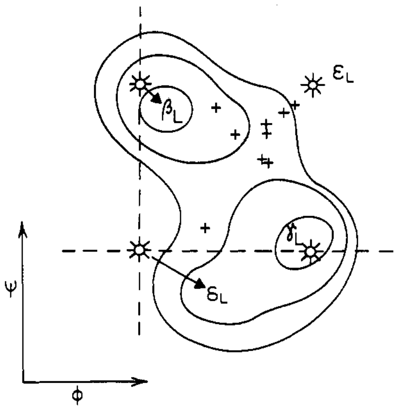

Figure 5. Illustration of the "Grand Canyon" region of a 2D-Ramachandran map that includes the β_L, γ_L, ϵ_L, and δ_L conformations. The idealized conformations are denoted by open stars, while the arrows indicate the approximate shifts of the ideal conformation to the actual ones.

simplest peptide model (PCONH-CHR-CONHQ) could have far-reaching consequences, resulting in the conclusion that polypeptide backbone conformations cannot be modeled using PCONH-CHR-CONHQ-type models. This would mean that, from a conformational point of view, proteins cannot be simply regarded as the polymers of -CONH-CHR-CONH-systems but must be considered as built from larger substructures. According to selected crystallographic data, amino acid diamides adopt minimal energy conformations (Table II) close to ϵ_L, γ_L, and δ_L on the $E = E(\phi,\psi)$ PES. These three minima are also close to each other geometrically. These crystallographically determined minima are located in a common region of a "Grand Canyon" (as shown in Figure 5) that has been constructed[23] using Pople's ab initio energy contour diagram.[21] Type I and II β-turns incorporate the α_L and ϵ_L conformations at the second [or $(i + 1)$th] position of the hairpin conformation (see Table I). These backbone geometries were not obtained from ab initio studies of chiral diamide models[19-24] (e.g., For-Ala-NH$_2$ or For-Val-NH$_2$). The conformational enantiomers of these β-turns, the type I′ and the type II′ structures, incorporate not the "unstable" α_L and ϵ_L subconformations but the stable α_D and ϵ_D backbone orientations.

Several papers have been published[9,11] detailing results obtained by using theoretical predictions and statistical analyses of β-turn distribution frequencies in globular proteins. However, the question of whether β-turns may be considered not only as a recognizable secondary structural element in proteins[9] but also as intrinsically stable conformational elements of simple triamides *has still not been answered.* Although experimental evidence accumulated from studies of model compounds (e.g., -Pro-Xxx-) in apolar solvents,[26o-r] such as CCl$_4$, suggests that β-turn conformations are the most enthalpically favored patterns for several small peptides, the lack of gas-phase evidence may suggest the following alternatives. It is quite possible that β-turn conformations exist only in an environment created by solvation, long-range interactions, intermolecular H-bonds, etc. On the other hand, β-turns may be intrinsically stable structures and they may exist even in a vacuum without the stabilizing interaction of any side chain or environmental effect.

The X-ray crystallography of dipeptide derivatives (e.g., PCO-Xxx-Yyy-NHQ) having a hairpin geometry (see Table III) shows that the folded conformation is always stabilized by intermolecular and/or environmental interactions.[26a-n] In solution the stabilizing effect of solvents can never be excluded. Since gas-phase data on tripeptide structures are currently not available, there are no experimental data which prove or disprove the intrinsic stability of β-turns. In the 1970s, Scheraga and others investigated β-turn conformations using molecular mechanics calculations.[27] The structural analysis of Ac-Xxx-Yyy-NHMe-type peptides resulted in useful geometrical data, although these force-field calculations cannot answer the question of "existence" or "nonexistence" due to the experimental origin of the parameters used. For example, force-field calculations (e.g., ECEPP/2) for Ac-L-Ala-NHMe resulted in a stable α_L structure that must be regarded as an "artifact" in view of recent ab initio calculations.[12,19-24] Reliable answers can only be expected from high-level ab initio calculations. Although some attempts have been made[28] to include correlation energy in the case of diamides of single amino acids, the triamide systems presently remain much too large for such sophisticated calculations. Even at a lower level of theory, only a limited number of computations have been published[13,14,23,29,30] on selected triamide conformations. The *more than two dozen* optimized dipeptide diamide geometries reported here should provide a unique opportunity for analysis of the conformational behavior of β-turns, which hopefully will lead to a more accurate understanding of protein 3D-structures.

Computational Methods

The fully relaxed minimal energy conformations were calculated using full geometry optimizations by gradient methods with the GAUSSIAN 90 program[31] using the 3-21G basis set[32] on a Cray X-MP/28 supercomputer. This work is exploratory in its nature, and calculations using a larger basis set at the HF or MP level of theory may be performed

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Table III. Selected Experimental Conformations of Dipeptide Derivatives Determined by X-ray Crystallography

amino acids	N-terminal	C-terminal	ϕ_1[a]	ψ_1	ϕ_2	ψ_2	β-turn type	conformation	ref 26
Pro-Gly	^{t}BuCO-	-NHMe	−71	157	−76	175		$\epsilon_L\epsilon_L$	a
Pro-Ala	^{i}PrCO-	-NH^{i}Pr	−59	136	66	14	II	$\epsilon_L\alpha_D$	b
Pro-D-Ala	^{i}PrCO-	-NH^{i}Pr	−62	137	96	3	II	$\epsilon_L\alpha_D$	b
Pro-Asp	^{t}BuCO-	-NHMe	−57	134	59	26	II	$\epsilon_L\alpha_D$	c
Pro-Asn	^{t}BuCO-	-NHMe	−59	138	66	11	II	$\epsilon_L\alpha_D$	c
Pro-Asp(OMe)	^{t}BuCO-	-NH^{i}Pr	−66	−20	−91	6	I	$\alpha_L\delta_L$	c
Pro-His	^{t}BuCO-	-NHMe	−63	−22	−70	−20	I	$\alpha_L\delta_L$	d
D-Ala-Pro	^{t}BuCO-	-NH^{i}Pr	60	−140	−89	9	II′	$\epsilon_D\gamma_L$	e
D-Ala-D-Pro	^{t}BuCO-	-NH^{i}Pr	64	−152	83	−156		$\epsilon_D\epsilon_D$	f
Gly-Pro	^{t}BuCO-	-NHMe	−79	174	−85	−22	VIa	$\epsilon_L\alpha_L$	g
Pro-Gly	^{t}BuCO-	-NH^{i}Pr	−64	137	84	−3	II	$\epsilon_L\gamma_D$	h
Ala-Pro	^{i}PrCO-	-NH^{i}Pr	−129	76	−67	−22	VIb	$\delta_L\alpha_L$	i
Gly-Gly	^{t}BuCO-	-NH^{i}Pr	−69	−25	−89	3	I	$\alpha_L\alpha_L$	j
Ala-Gly	^{t}BuCO-	-NH^{i}Pr	−68	132	−83	2	VIa	$\epsilon_L\alpha_L$	j
Pro-Thr	^{t}BuCO-	-NHMe	−66	−22	−103	7	I	$\alpha_L\gamma_L$	k
Pro-Pro	^{t}BuCO-	-NHMe	−60	138	−95	−7	VIa	$\epsilon_L\alpha_L$	l
Pro-D-Pro	^{t}BuCO-	-NHMe	−58	134	83	−7	II	$\epsilon_L\gamma_D$	l
Pro-Pro	^{t}BuCO-	-NHMe	−60	138	−95	−7	VIa	$\epsilon_L\alpha_L$	m
Pro-Cys(Me)	^{t}BuCO-	-NHMe	−61	132	62	17	II	$\epsilon_L\alpha_D$	m
Pro-Phe	^{t}BuCO-	-NHMe	−64	139	62	23	II	$\epsilon_L\alpha_D$	m
Pro-Tyr	^{t}BuCO-	-NHMe	−59	137	73	9	II	$\epsilon_L\alpha_D$	m
Pro-D-Tyr	^{t}BuCO-	-NHMe	−64	137	73	9	II	$\epsilon_L\alpha_D$	m
Pro-Ser	^{t}BuCO-	-NHMe	−60	−30	−75	−11	I	$\alpha_L\alpha_L$	n
Pro-D-Ser	^{t}BuCO-	-NHMe	−59	133	76	8	II	$\epsilon_L\alpha_D$	n
Pro-D-Ser	^{t}BuCO-	-OMe	−64	−29	76	17		$\alpha_L\alpha_D$	n

[a] In degrees.

Table IV. Comparison of the ϕ and ψ Torsional Angles Obtained in ab Initio Calculations under "Normal" and "Tight" Optimizations on Selected Conformations of For-L-Ala-NH$_2$ and For-L-Ala-L-Ala-NH$_2$

		torsional angle[a]			
compd	conformation	type	normal[b]	tight[c]	change
For-Ala-NH$_2$	α_D	ϕ	63.8	63.8	0.0
		ψ	32.7	32.7	0.0
	β_L	ϕ	−168.4	−168.3	0.1
		ψ	170.9	170.5	0.4
	γ_L	ϕ	−84.4	−84.5	0.1
		ψ	67.7	67.3	0.4
For-Ala-Ala-NH$_2$	$\gamma_L\gamma_L$	ϕ	−84.2	−84.2	0.0
		ψ	67.0	67.0	0.0
		ϕ	−84.9	−85.0	0.1
		ψ	66.4	66.4	0.0

[a] In degrees. [b] Max force $\leq 3 \times 10^{-4}$ au. [c] Max force $\leq 1 \times 10^{-5}$ au.

Table V. Optimized ab Initio SCF (3-21G) Geometries[a] for Type I′ β-Turn Conformations of For-L-Ala-L-Ala-NH$_2$

init[b]	$\alpha_D\alpha_D$	$\alpha_D\gamma_D$	$\alpha_D\delta_D$	$\alpha_D\beta_L$
conv[c]	$\alpha_D\alpha_D$	$\alpha_D\gamma_D$	$\alpha_D\delta_D$	$\alpha_D\beta_L$
ω_1	171.2	173.5	171.0	176.2
ϕ_1	60.4	62.3	64.1	60.2
ψ_1	28.3	37.0	16.8	33.0
ω_2	179.0	−172.9	−175.6	−177.5
ϕ_2	62.3	74.1	150.8	−173.6
ψ_2	24.9	−58.0	−40.0	169.8
ω_3	179.1	174.9	−174.2	177.5
d_{model}[d]	5.33	6.06	4.30	7.03
τ_{model}	−66.9	−79.3	−11.0	61.6
d_{crit}[e]	5.97	6.64	4.64	7.42
τ_{crit}	−70.8	−80.4	−12.7	57.8
O1···HN4	2.03	3.76	2.16	6.42
O1···N4	3.03	4.00	3.11	6.06
O1–NH4–N4	172.4	97.1	160.3	−64.5
O1···HN3	3.03	3.20	2.87	3.18
O1···N3	3.10	3.27	3.05	3.19
O1–HN3–N3	−84.5	−85.3	90.7	−81.5
O2···HN4	3.06	1.93	4.10	5.11
O2···N4	3.23	2.83	4.61	5.16
O2–NH4–N4	91.1	147.1	114.9	−87.1
max force	1.8×10^{-5}	1.1×10^{-4}	8.4×10^{-5}	3.6×10^{-5}
rms force	6.5×10^{-6}	3.6×10^{-5}	2.7×10^{-5}	1.1×10^{-5}
E	0.956 139	0.950 869	0.949 915	0.954 609
ΔE	4.73	8.04	8.64	5.69
SDB[f]	28	3	1	26
LDB[g]	77	10	4	72

[a] Torsion angles (ω, ϕ, ψ) in degrees, distances in angstroms, forces in au, energy (E) in hartrees, and energy differences (ΔE) in kcal/mol relative to $E(\gamma_L\gamma_L)$[−656.963 681 hartree)]. [b] Initial backbone conformation (calculated by ECEPP/2). [c] Converged backbone conformation. [d] C_i^α and C_{i+3}^α in accordance with classical β-turn definition must be shorter than 7 Å. In For-Ala-Ala-NH$_2$ the two C$^\alpha$ atoms are replaced by hydrogens (H1 and H4*) (cf. Chart II); therefore, the model distance (d_{model}) is shorter than $C_i^\alpha - C_{i+3}^\alpha$ (Figure 1) by no more than 1.1 Å. In such a case, τ is H1–C2$^\alpha$–C3$^\alpha$–H4. [e] Critical distances for β-turn assignment ($C_i^\alpha - C_{i+3}^\alpha$) were extrapolated using ab initio calculated bond lengths and bond angles on the basis of the determined N–H and C′–H distances. In such a case, τ is C1$^\alpha$–C2$^\alpha$–C3$^\alpha$–C4$^\alpha$. [f] Small Data Base (for the list of nonidentical proteins, see ref 13). [g] Large Data Base (for the list of proteins, see ref 13).

in the future on selected minima. The authors are fully aware of the limitations of the 3-21G basis set, although work by Pople and co-workers[21a,b] indicates that in the case of peptides even the 3-21G basis set is sufficient. The initial geometries of the selected triamides were generated on the basis of the previously optimized For-L-Ala-NH$_2$ structures.[12] The For-L-Ala-L-Ala-NH$_2$ geometries were then subsequently optimized. The final forces along the internal coordinates in the relaxed structures ranged from 2.2×10^{-6} to 2.1×10^{-4} au, while the value of the root mean square of the forces (rms) was between 5.4×10^{-7} and 5.7×10^{-5} au. One may be concerned about the convergence of geometry optimization under these normal conditions. The ultimate test of accuracy, in this particular case, is not so much the magnitude of the residual force but the "self-consistency" of the ϕ and ψ values as these torsional angles characterize peptide conformations. In order to check this point, "normal" and "tight" optimizations have been carried out on selected conformations. As may be seen from Table IV, the absolute value of torsional angle changes, i.e., $|\Delta\phi|$ and $|\Delta\psi|$, are very small. Since the geometry-optimized ϕ and ψ values are needed in the present paper mainly to determine which catchment region a computed conformation belongs to (these characteristic conformations are separated from each other by several tens of degrees), the achieved accuracy is more than adequate (Table IV).

The $\gamma_L\gamma_L$ conformation was found as the global minimum (E[RHF] = −656.963 681 hartrees) and was used as reference point for ΔE calculations.

Scope

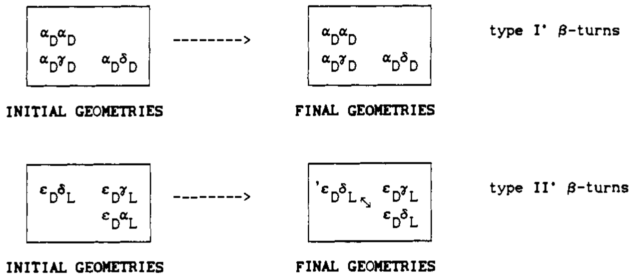
Several different types of criteria (such as distance, torsional angle, or the 1 ← 4 hydrogen bonding) have been used to identify twisted hairpin conformations since Vankatachalam.[6] We now use an objective measure, recently defined[14] and based on the angularity (or twisting) parameter τ, in agreement with the work

Table VI. Optimized ab Initio SCF (3-21G) Geometries for Type I and Type II′ β-Turn Conformations of For-L-Ala-L-Ala-NH$_2$[a]

init	$\delta_L\delta_L$, $\alpha_L\alpha_L$, $\alpha_L\delta_L$	$\delta_L\beta_L$, $\alpha_L\epsilon_L$, $\alpha_L\beta_L$	$\delta_L\delta_D$, $\alpha_L\delta_D$	$\epsilon_D\delta_L$, $\epsilon_D\alpha_L$	$\epsilon_D\gamma_L$	$\epsilon_D\gamma_D$	$\epsilon_D\epsilon_D$
conv	$\alpha_L\delta_L$	$\delta_L\beta_L$	$\delta_L\delta_D$	$\epsilon_D\delta_L$	$\epsilon_D\gamma_L$	$\epsilon_D\gamma_D$	$\epsilon_D\epsilon_D$
ω_1	−171.7	−175.9	−172.8	−177.4	−171.9	−171.3	−169.6
ϕ_1	−68.6	−121.3	−126.8	56.1	64.0	66.9	67.7
ψ_1	−17.5	17.6	23.8	−129.7	−172.8	−178.1	−178.2
ω_2	177.8	176.4	171.1	177.2	−175.3	174.9	−166.1
ϕ_2	−113.1	−169.4	−173.7	−112.4	−86.0	75.8	63.7
ψ_2	21.3	169.6	−45.6	26.6	66.2	−86.0	−170.6
ω_3	176.0	178.1	−176.5	176.5	−176.2	−177.7	−179.0
d_{model}	4.83	7.25	5.66	5.29	5.99	6.97	8.00
τ_{model}	41.1	19.5	−9.9	−35.0	−36.7	71.3	77.8
d_{crit}	5.32	7.56	5.95	5.82	6.52	7.52	8.56
τ_{crit}	43.7	20.8	−5.6	−36.9	−41.5	69.5	75.4
O1···HN4	2.11	7.55	5.49	1.99	3.58	5.04	7.79
O1···N4	3.07	6.98	5.74	2.98	4.19	5.91	7.14
O1–HN4–N4	162.5	−51.9	99.4	169.9	121.6	148.6	−71.7
O1···HN3	3.01	3.63	3.55	3.33	4.46	4.64	4.72
O1···N3	3.15	4.12	4.15	3.21	4.09	4.23	4.28
O1–HN3–N3	−89.0	112.7	121.2	−74.6	−62.1	−59.9	−58.2
O2···HN4	3.50	5.10	4.55	3.34	2.05	1.91	4.61
O2···N4	3.98	5.15	4.85	3.89	2.90	2.80	4.02
O2–HN4–N4	111.9	−87.31	101.8	115.9	141.9	146.5	−62.1
max force	1.5×10^{-5}	5.6×10^{-5}	8.7×10^{-5}	1.4×10^{-4}	1.3×10^{-4}	1.8×10^{-4}	8.0×10^{-6}
rms force	5.8×10^{-6}	1.6×10^{-5}	2.7×10^{-5}	4.9×10^{-5}	4.6×10^{-5}	3.9×10^{-5}	2.8×10^{-6}
E	0.958 677	0.956 57	0.945 712	0.955 958	0.952 062	0.947 257	0.940 468
ΔE	3.13	4.46	11.28	4.85	7.29	10.31	14.57
SDB	129	14	4	1	1	1	1
LDB	435	37	9	1	8	8	1

[a] Units, abbreviations, and parameters are the same as those used in Table V.

Scheme I



Scheme II

of Levitt,[9a] such that for a reverse β-turn the value of this τ (torsional angle involving C_i^α, C_{i+1}^α, C_{i+2}^α, C_{i+3}^α peptide backbone atoms) must be in the $-90° \le \tau \le 90°$ range. The following problems were studied: (1) How many of the 81 legitimate[13] backbone conformations of a triamide (e.g., For-Ala-Ala-NH$_2$) would qualify as β-turns? (2) Can ab initio molecular orbital (MO) computations indicate whether a β-turn has intrinsic stability? If yes, which of the total of 81 legitimate minima have such an "intrinsic" stability? (3) Is a $1 \leftarrow 4$-type intramolecular H-bond a necessary structural condition for β-turns? If not, which types of β-turns have a $1 \leftarrow 4$ hydrogen bond and which do not have such a bond? (4) Can any of the "intrinsically unstable" α_L and/or ϵ_L diamide conformations be stabilized and therefore included in a β-turn?

Results and Discussions

In the first part of this study type I′ and type II′ β-turn conformations were analyzed ($\alpha_D\alpha_D$, $\alpha_D\gamma_D$, $\alpha_D\delta_D$, $\epsilon_D\alpha_L$, $\epsilon_D\gamma_L$, $\epsilon_D\delta_L$). These conformations were expected to be minimal energy geometries on the basis of previous conformational analyses of ab initio surfaces.[12] The computed results are given in Tables V and VI (cf. Scheme I).

All three relaxed conformations containing an α_D-type geometry ($\alpha_D\alpha_D$, $\alpha_D\gamma_D$, $\alpha_D\delta_D$) at the first position are typical type I′ β-turns, although only the $\alpha_D\alpha_D$ structure incorporates a $1 \leftarrow 4$-type H-bond (Table V and Figures 6 and 7). The conformational parameters of $\epsilon_D\gamma_L$ did not change qualitatively during the optimization, and the structure remained a β-turn. Only the optimization of the $\epsilon_D\alpha_L$-type initial geometry resulted in a qualitatively new backbone conformation ($\epsilon_D\delta_L$) which is a more "open" conformation (Scheme I). The original $\epsilon_D\alpha_L$ conformation with $d_{\epsilon D\alpha L} = 6.47$ Å and $\tau_{\epsilon D\alpha L} = -55.3°$ values fulfills both the distance ($d \le 7$ Å) and the angularity ($-90° \le \tau \le 90°$) criteria of a β-turn, as does the $\epsilon_D\delta_L$ geometry ($d_{\epsilon D\delta L} = 5.82$ Å and $\tau_{\epsilon D\delta L} = -36.9°$). On the basis of an earlier molecular mechanics (MM) study on dipeptide diamides,[13] two more conformations ($\epsilon_D\gamma_D$ and $\epsilon_D\epsilon_D$) among the type I′ and type II′ β-turns were also incorporated in the analysis. Although the d values of the $\epsilon_D\gamma_D$ and $\epsilon_D\epsilon_D$ conformations are longer than expected for a conventional β-turn ($d_{\epsilon D\gamma D} = 7.52$ Å and $d_{\epsilon D\epsilon D} = 8.56$ Å), on the basis of the angularity of the backbone conformation (τ value) both of these are hairpin conformations, while the $\tau_{\epsilon D\gamma D} = 69.5°$ and $\tau_{\epsilon D\epsilon D} = 75.4°$ values are smaller than 90° but larger than −90°, respectively.

In contrast to the above β-turns, the type I β-turns ($\alpha_L\alpha_L$, $\alpha_L\gamma_L$, $\alpha_L\delta_L$) as well as the type II β-turn conformations ($\epsilon_L\alpha_D$, $\epsilon_L\gamma_D$, $\epsilon_L\delta_D$) have an intrinsically unstable α_L or ϵ_L conformation as their first residue. Starting with the ab initio geometry optimization for the type I β-turn conformation, using geometries obtained from molecular mechanics, neither the $\alpha_L\alpha_L$ nor the $\alpha_L\gamma_L$ conformations were found to be minimum energy structures (Scheme II and Table VI).

The $\alpha_L\delta_L$ structure incorporates a stabilizing intramolecular H-bond, which may suggest that all α_L conformations must be stabilized with a hydrogen bond as found in the $\alpha_L\delta_L$ conformation. On the basis of the topological analysis of an idealized PES[12,13] or a MM calculation associated with a diamide system, three different type I β-turn structures ($\alpha_L\alpha_L$, $\alpha_L\gamma_L$, $\alpha_L\delta_L$) are expected. These three different backbone conformations were also confirmed by the backbone analyses of X-ray determined protein structures (Table VII); 1113 occurrences for the $\alpha_L\alpha_L$, 14 occurrences for the $\alpha_L\gamma_L$, and 129 occurrences for the $\alpha_L\delta_L$ substructures were found in the Small Data Base.[13] The present finding that only the $\alpha_L\delta_L$ conformation is a minimum energy structure is congruent with previous ab initio calculations, as this is the only $\alpha_L x_L$ or $\alpha_L x_D$

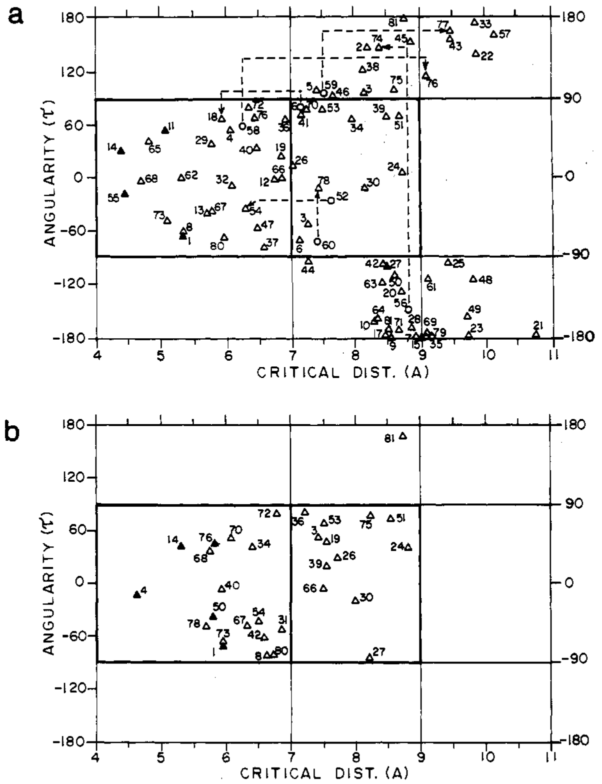


Figure 6. (a) Critical distance (d_{crit}) vs τ (the angularity of the conformation) of the 75(81) Ac-Ala-Ala-NHCH$_3$ conformations, calculated by the ECEPP/2 method. (The six conformations marked by *, shown among the 81 symbols in this figure caption below, are the annihilated conformations [for details, see refs 13 and 14]) represented by ○ on the top portion of this figure. Conformations incorporating 1 ← 4-type H-bonds are plotted as ▲. The numbers and the conformations they represent are as follows: 1, $\alpha_D\alpha_D$, 2, $\alpha_D\alpha_L$; 3, $\alpha_D\beta_L$; 4, $\alpha_D\delta_D$; 5, $\alpha_D\delta_L$; 6, $\alpha_D\epsilon_D$; 7, $\alpha_D\epsilon_L$; 8, $\alpha_D\gamma_D$; 9, $\alpha_D\gamma_L$; 10, $\alpha_L\alpha_D$; 11, $\alpha_L\alpha_L$; 12, $\alpha_L\beta_L$; 13, $\alpha_L\delta_D$; 14, $\alpha_L\delta_L$; 15, $\alpha_L\epsilon_D$; 16, $\alpha_L\gamma_L$; 17, $\alpha_L\epsilon_L$*; 18, $\alpha_L\gamma_D$; 19, $\beta_L\alpha_D$; 20, $\beta_L\alpha_L$; 21, $\beta_L\beta_L$; 22, $\beta_L\delta_D$; 23, $\beta_L\delta_L$; 24, $\beta_L\epsilon_D$; 25, $\beta_L\epsilon_L$; 26, $\beta_L\gamma_D$; 27, $\beta_L\gamma_L$; 28, $\delta_D\alpha_D$; 29, $\delta_D\alpha_L$; 30, $\delta_D\beta_L$; 31, $\delta_D\delta_D$; 32, $\delta_D\delta_L$; 33, $\delta_D\epsilon_D$; 34, $\delta_D\epsilon_L$; 35, $\delta_D\gamma_D$; 36, $\delta_D\gamma_L$; 37, $\delta_L\alpha_D$; 38, $\delta_L\alpha_L$; 39, $\delta_L\beta_L$; 40, $\delta_L\delta_D$; 41, $\delta_L\delta_L$; 42, $\delta_L\epsilon_D$, 43, $\delta_L\epsilon_L$, 44, $\delta_L\gamma_D$; 45, $\delta_L\gamma_L$; 46, $\epsilon_D\alpha_D$; 47, $\epsilon_D\alpha_L$; 48, $\epsilon_D\beta_L$; 49, $\epsilon_D\delta_D$; 50, $\epsilon_D\delta_L$; 51, $\epsilon_D\epsilon_D$; 52, $\epsilon_D\epsilon_L$*; 53, $\epsilon_D\gamma_D$; 54, $\epsilon_D\gamma_L$; 55, $\epsilon_L\alpha_D$; 56, $\epsilon_L\alpha_L$*; 57, $\gamma_L\alpha_L$; 58, $\epsilon_L\beta_L$; 59, $\epsilon_L\delta_D$*; 60, $\epsilon_L\delta_L$*; 61, $\epsilon_L\epsilon_D$*; 62 $\epsilon_L\epsilon_L$; 63, $\epsilon_L\gamma_D$; 64, $\epsilon_L\gamma_L$; 65, $\gamma_D\alpha_D$; 66, $\gamma_D\alpha_L$; 67, $\gamma_D\beta_L$; 68, $\gamma_D\delta_D$; 69, $\gamma_D\delta_L$; 70, $\gamma_D\epsilon_D$; 71, $\gamma_D\epsilon_L$; 72, $\gamma_D\gamma_D$; 73, $\gamma_D\gamma_L$; 74, $\gamma_L\alpha_D$; 75, $\gamma_L\beta_L$; 76, $\gamma_L\delta_D$; 77, $\gamma_L\delta_L$; 78, $\gamma_L\epsilon_D$, 79, $\gamma_L\epsilon_L$; 80, $\gamma_L\gamma_D$; 81, $\gamma_L\gamma_L$. (b) d_{crit} vs τ distribution of the 30 hairpin conformations of For-Ala-Ala-NH$_2$, calculated by the ab initio method. Note that according to the original definition, based on the critical distance[9] only 18 of the 30 conformations are β-turns and only 5 ($\alpha_D\alpha_D$ [type III′ β-turn], $\alpha_D\delta_D$ [type I′ β-turn], $\alpha_L\delta_L$ [type I β-turn], $\epsilon_D\delta_L$ [type II′ β-turn], and $\gamma_L\delta_D$) have the 1 ← 4-type H-bond (tus).

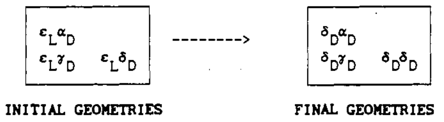
conformation of For-Ala-Ala-NH$_2$ which has an intramolecular H-bond (x_L stands for α_L, β_L, γ_L, δ_L, and ϵ_L; x_D stands for α_D, γ_D, δ_D, and ϵ_D). The present ab initio calculation resulted in the $\alpha_L\delta_L$ (type I β-turn) conformation ($\phi_{i+1} = -69°$, $\psi_{i+1} = -18°$, $\phi_{i+2} = -113°$, $\psi_{i+2} = 21°$), which is remarkably close to that predicted by Vankatachalam ($\phi_{i+1} = -60°$, $\psi_{i+1} = -30°$, $\phi_{i+2} = -90°$, $\psi_{i+2} = 0°$).[6] On the basis of molecular mechanics calculations,[13,14] three additional β-turn conformations ($\alpha_L\beta_L$, $\alpha_L\delta_D$, and $\alpha_L\epsilon_L$), each containing the α_L subconformation, may also be minimal energy structures.[13,14] The $\alpha_L\beta_L$ conformation was recently assigned to be a β-turn by Wilmot and Thorton[11] on the basis of protein X-ray data analyses, where the torsional angles are close to $\phi_{i+1} = -60°$, $\psi_{i+1} = -30°$, $\phi_{i+2} = -120°$, $\psi_{i+2} = 120°$. The present ab initio calculations do not confirm the existence of such an $\alpha_L\beta_L$ or $\alpha_L\epsilon_L$ conformation for For-Ala-Ala-NH$_2$. These two initial conformers were shifted to $\delta_L\beta_L$, (Table VI). Although this is a qualitatively new backbone conformation ($\phi_{i+1}^{\delta L\beta L} = -121°$, $\psi_{i+1}^{\delta L\beta L} = 18°$, $\phi_{i+2}^{\delta L\beta L} = -169°$, $\psi_{i+2}^{\delta L\beta L} = 170°$), it has never been assigned previously as a β-turn. It fulfills the angularity criteria of the hairpin conformation with $\tau = 20.8°$. A similar shift of the $\alpha_L\delta_D$ was also observed to be a $\delta_L\delta_D$ β-turn conformation. This second structure seems to be a "perfect" β-turn on the basis both criteria, with a critical distance significantly shorter than 7 Å ($d = 5.95$ Å) and a backbone angularity rather close to 0 ($\tau = -5°$). Due to its relatively high energy compared to the $\gamma_L\gamma_L$ conformation ($\Delta E = 11.28$ kcal/mol), only sporadic occurrence is expected, as confirmed by X-ray analyses (Table VI). The appearance of 4 (9) representatives of $\delta_L\delta_D$ in the Small Data Base (SDB) and Large Data Base (LDB)[13] confirms the instability of the calculated conformation. Since it is unreasonable to expect that any type of statistical analysis based on protein X-ray data will reveal the existence of such a rare β-turn, the present theoretical results are of unique value.

Table VII. Frequency of Type I and I′ as Well as Type II and II′ β-Turn Structures in Selected Proteins[a]

Type I		
$\alpha_L\alpha_L$[b]	$\alpha_L\gamma_L$	$\alpha_L\delta_L$
1113 (8103)	14 (62)	129 (435)
Type I′		
$\alpha_D\alpha_D$	$\alpha_D\gamma_D$	$\alpha_D\delta_D$
28 (77)	3 (10)	1 (4)
Type II		
$\epsilon_L\alpha_D$	$\epsilon_L\gamma_D$	$\epsilon_L\delta_D$
29 (90)	3 (8)	2 (16)
Type II′		
$\epsilon_D\alpha_L$	$\epsilon_D\gamma_L$	$\epsilon_D\delta_L$
15 (43)	1 (8)	1 (1)

[a] Frequency values are for the Small Data Base (SDB) and in parentheses for the Large Data Base (LDB) (see ref 13). [b] $\alpha_L\alpha_L$ triamides may participate in the α-helix, the 3_{10} helix, or the type I β-turn.

Scheme III



According to Table I, all three forms of the type II β-turn backbone conformation incorporate an ϵ_L substructures in their i+1 positions. In all optimizations the ϵ_L conformation of the first residue has been shifted to the more stable δ_D conformation (Scheme III). In contrast to preliminary molecular mechanics investigations,[13,14] none of these three $\epsilon_L x_D$ conformations were found to be minima for For-Ala-Ala-NH$_2$ according to our ab initio calculations.

All three type II β-turn ($\epsilon_L x_D$) structures were shifted to the corresponding $\delta_D x_D$ minima on the 4D-Ramachandran type map (Scheme III). Two of the three new backbone conformations ($\delta_D\alpha_D$ and $\delta_D\gamma_D$) are distorted and become extended conformations. Such a backbone angularity change is authentically monitored[14] by the shift $\Delta\tau(\epsilon_L\alpha_D \rightarrow \delta_D\alpha_D) = -152° \rightarrow -18° = 134°$ and $\Delta\tau(\epsilon_L\gamma_D \rightarrow \delta_D\gamma_D) = -163° \rightarrow -66° = +97°$. Although the third conformational switch ($\epsilon_L\delta_D \rightarrow \delta_D\delta_D$) resulted in a new backbone category, it still may be regarded as a β-turn conformation ($d_{\delta D\delta D} = 6.87$ Å and $\tau_{\delta D\delta D} = -51.4°$). Of the three most important β-turns, type I ($\alpha_L\alpha_L$, $\alpha_L\gamma_L$, $\alpha_L\delta_L$), type II ($\epsilon_L\alpha_D$, $\epsilon_L\gamma_D$, $\epsilon_L\delta_D$), and type III ($\alpha_L\alpha_L$), only one ($\alpha_L\delta_L$) was found to be a minimal energy conformation in the ab initio calculations. Using only the X-ray determined β-turn geometries as input conformations for ab initio studies, even with their mirror images [type I′($\alpha_D\alpha_D$, $\alpha_D\gamma_D$, $\alpha_D\delta_D$), type II′ ($\epsilon_D\alpha_L$, $\epsilon_D\gamma_L$, $\epsilon_D\delta_L$), and type III′ ($\alpha_D\alpha_D$) β-turns], no more than seven β-turn-like backbone conformations can be calculated for For-Ala-Ala-NH$_2$ ($\alpha_D\alpha_D$, $\alpha_D\gamma_D$, $\alpha_D\delta_D$, $\epsilon_D\gamma_L$, $\epsilon_D\gamma_D$, $\epsilon_D\epsilon_D$, and $\alpha_L\delta_L$).

Table VIII. Ab Initio SCF (3-21G) β-Turn Conformations of For-L-Ala-L-Ala-NH$_2$ Predicted by Multidimensional Conformational Analyses[a]

init	$\beta_{L}\alpha_{D}$	$\beta_{L}\epsilon_{D}$	$\beta_{L}\gamma_{D}$	$\beta_{L}\gamma_{L}$	$\delta_{D}\beta_{L}$	$\epsilon_{L}\delta_{D}$, $\delta_{D}\delta_{D}$	$\delta_{D}\epsilon_{L}$[b]	$\delta_{D}\delta_{L}$, $\delta_{D}\gamma_{L}$	$\delta_{L}\epsilon_{D}$[c]	$\gamma_{D}\beta_{L}$	$\gamma_{D}\delta_{D}$	$\gamma_{D}\alpha_{L}$, $\gamma_{D}\delta_{L}$	$\gamma_{D}\epsilon_{L}$	$\gamma_{D}\gamma_{L}$	$\delta_{L}\alpha_{D}$, $\gamma_{L}\alpha_{D}$	$\gamma_{L}\delta_{D}$	$\gamma_{L}\epsilon_{D}$	$\gamma_{L}\beta_{L}$	$\gamma_{L}\gamma_{D}$	$\gamma_{L}\gamma_{L}$
conv	$\beta_{L}\alpha_{D}$	$\beta_{L}\epsilon_{D}$	$\beta_{L}\gamma_{D}$	$\beta_{L}\gamma_{L}$	$\delta_{D}\beta_{L}$	$\delta_{D}\delta_{D}$	$\delta_{D}\epsilon_{L}$	$\delta_{D}\gamma_{L}$	$\delta_{L}\epsilon_{D}$	$\gamma_{D}\beta_{L}$	$\delta_{D}\delta_{D}$	$\gamma_{D}\delta_{L}$	$\gamma_{D}\epsilon_{L}$	$\gamma_{D}\gamma_{L}$	$\gamma_{L}\alpha_{D}$	$\gamma_{L}\delta_{D}$	$\gamma_{L}\epsilon_{D}$	$\gamma_{L}\beta_{L}$	$\gamma_{L}\gamma_{D}$	$\gamma_{L}\gamma_{L}$
ω_1	179.2	178.0	179.2	178.9	176.6	174.2	175.2	174.7	–177.0	175.0	174.1	172.0	–177.2	174.3	–172.4	–177.5	176.5	–176.1	–174.3	–174.4
ϕ_1	–167.6	–169.0	–167.2	–167.7	–176.6	178.0	–174.2	178.9	–161.8	76.1	73.8	75.5	72.6	73.8	–85.8	–79.3	–80.9	–86.8	–84.5	–84.2
ψ_1	168.4	172.4	168.4	169.3	–43.6	–45.6	–55.0	–44.3	55.7	–51.2	–57.6	–52.7	–66.7	–58.3	64.0	75.8	75.8	71.4	68.6	67.0
ω_2	172.1	–171.5	172.1	–177.5	–178.5	178.7	154.1	–173.2	–152.1	–178.7	178.9	–171.9	160.6	–173.7	176.6	–173.6	–156.8	–179.7	176.6	–174.1
ϕ_2	62.1	65.7	75.6	–85.1	–167.4	–172.9	–79.0	–85.5	62.6	–161.6	–170.5	–122.1	–73.7	–83.6	62.4	176.7	64.3	–164.0	72.7	–84.9
ψ_2	35.3	–175.5	–57.2	68.2	170.2	–49.7	171.7	68.8	–173.8	169.2	–45.4	22.9	168.8	67.1	33.0	–35.2	–176.5	168.6	–57.3	66.4
ω_3	179.1	–179.2	–178.2	–178.8	177.7	–173.0	179.7	–179.3	–179.1	177.6	–179.3	176.9	179.0	–179.4	178.9	–178.2	–178.9	177.8	–177.6	–178.9
d_{model}	7.13	8.43	7.27	7.67	7.74	6.54	6.36	6.89	6.47	7.06	5.81	5.34	5.87	6.33	5.50	5.28	5.54	7.66	6.27	7.92
τ_{model}	49.2	40.2	28.0	–83.0	–16.2	–51.5	45.2	85.2	–62.3	–0.5	–46.7	40.4	57.0	84.4	–65.5	43.7	–51.2	77.7	–83.5	167.8
d_{crit}	7.56	8.83	7.73	8.22	8.00	6.87	6.42	7.23	6.60	7.51	6.34	5.77	6.09	6.80	5.97	5.84	5.70	8.25	6.74	8.76
τ_{crit}	49.0	42.8	31.1	–83.0	–18.4	–51.4	43.3	82.2	–59.8	–4.7	–47.3	38.3	53.2	80.6	–64.9	47.0	–47.9	78.4	–79.9	168.4
O1–HN4	5.96	8.81	6.29	6.53	8.72	6.23	8.11	6.63	8.05	6.23	3.19	3.61	5.99	5.26	3.76	2.20	5.90	6.40	5.22	5.57
O1–N4	6.69	8.17	6.96	7.16	8.20	6.65	7.37	7.01	7.33	5.54	3.62	4.20	5.34	5.28	4.64	3.14	5.27	5.74	5.33	5.71
O1–HN4–N4	134.8	–47.3	128.8	125.5	–55.7	110.7	–39.0	108.8	–40.7	–42.5	107.7	120.1	–45.9	–85.3	148.5	–157.4	–47.1	–45.0	90.7	93.1
O1–NH3	5.06	5.05	4.99	5.08	4.57	4.41	4.46	4.45	4.01	2.01	1.88	1.91	1.90	1.90	2.01	2.07	1.99	2.16	1.98	2.02
O1–N3	5.15	5.18	5.13	5.15	4.87	4.84	4.85	4.85	4.57	2.88	2.81	2.83	2.80	2.81	2.88	2.82	2.85	2.96	2.87	2.88
O1–NH3–N3	–89.7	91.6	92.7	–88.8	102.1	109.8	107.7	108.0	118.8	144.3	151.8	149.5	147.8	149.1	143.6	130.4	142.8	135.4	146.6	142.4
O2–NH4	3.20	4.55	1.93	2.04	5.09	4.61	4.53	2.04	4.56	4.95	4.62	3.54	4.35	1.99	3.21	4.58	4.62	4.99	1.90	2.00
O2–N4	3.24	4.14	2.82	2.89	5.14	4.87	4.31	2.88	4.18	5.04	4.89	4.06	4.12	2.85	3.27	4.89	4.22	5.07	2.79	2.86
O2–NH4–N4	–83.0	–59.8	146.7	140.7	–87.2	99.7	–71.0	140.3	–61.7	–89.2	99.7	114.5	–70.1	142.3	–84.7	102.0	–60.2	–88.9	146.9	142.6
max force	1.5×10^{-4}	2.1×10^{-4}	1.7×10^{-4}	1.0×10^{-4}	4.7×10^{-5}	3.1×10^{-6}	1.3×10^{-4}	1.2×10^{-4}	1.1×10^{-4}	1.1×10^{-4}	1.6×10^{-4}	6.7×10^{-5}	1.6×10^{-4}	6.0×10^{-5}	1.2×10^{-4}	1.2×10^{-4}	1.7×10^{-4}	3.3×10^{-5}	1.6×10^{-4}	6.4×10^{-5}
rms force	3.6×10^{-5}	4.5×10^{-5}	4.4×10^{-5}	2.7×10^{-5}	1.5×10^{-5}	9.7×10^{-7}	3.8×10^{-5}	3.3×10^{-5}	2.9×10^{-5}	3.0×10^{-5}	3.6×10^{-5}	2.6×10^{-5}	4.7×10^{-5}	1.9×10^{-5}	4.0×10^{-5}	3.1×10^{-5}	5.1×10^{-5}	1.0×10^{-5}	4.0×10^{-5}	2.1×10^{-5}
E	0.952 663	0.949 539	0.956 752	0.961 052	0.951 769	0.940 844	0.954 315	0.952 531	0.954 077	0.952 732	0.949 771	0.953 949	0.953 218	0.960 326	0.955 569	0.956 465	0.955 072	0.957 037	0.959 946	0.963 681
ΔE	6.91	8.87	4.34	1.65	7.47	14.33	5.88	7.00	6.11	6.87	8.73	6.11	6.57	2.11	5.09	4.53	5.40	4.17	2.34	0.00
SDB	12	9	1	20	3	4	9	2	1	1	1	1	2	0	9	2	1	38	9	8
LDB	35	31	3	83	11	29	12	5	3	5	3	6	13	5	19	16	5	113	19	41

[a] Units, abbreviations, and parameters are the same as those used in Table V. [b] The $\delta_{D}\epsilon_{L}$ conformation contains an 8-membered intramolecular H-bond, where O3···H2 = 2.07 Å, O3—N2 = 3.05 Å with an H-bond angle (O3–H2–N2) = 163.1°. [c] The $\delta_{L}\epsilon_{D}$ conformation contains an 8-membered intramolecular H-bond, where O3···H2 = 2.07 Å and O3···N2 = 2.99 Å with an H-bond angle (O3–H2–N2) = 151.2°.

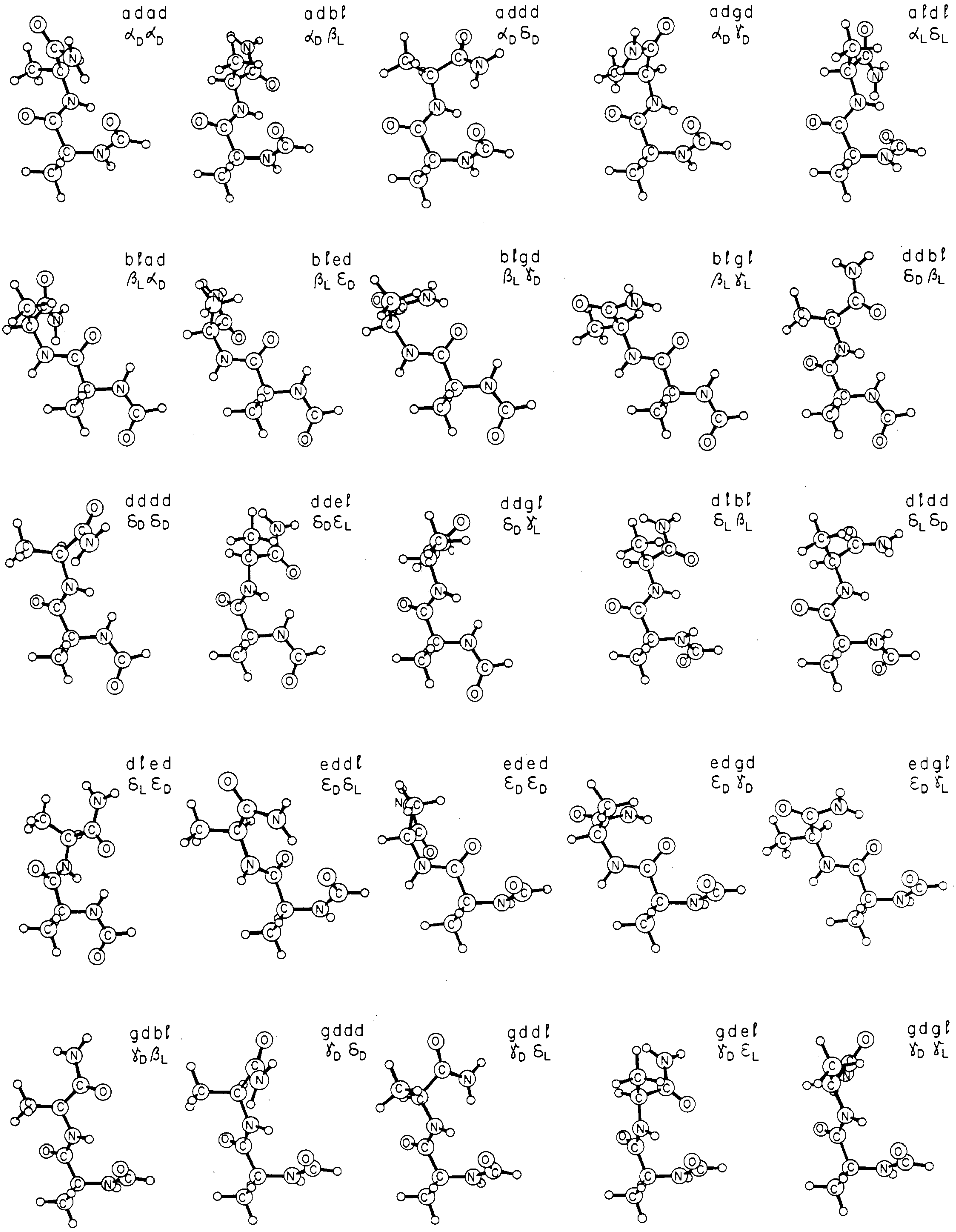


Figure 7. Continued

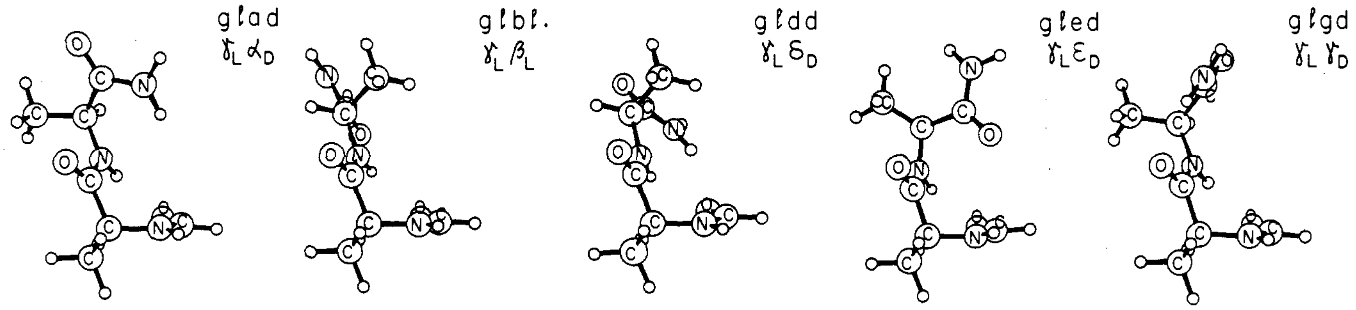


Figure 7. All 30 β-turn conformations resulting from ab initio calculations.

By contrast, MDCA considerations predict a significantly larger number of β-turn geometries. Using molecular mechanics (ECEPP/2), 26 β-turn conformations are expected if the stricter $d = 7$ Å criterion is accepted, and a total of 36 different backbone orientations are predicted if the $-90° \leq \tau \leq 90°$ threshold value is applied. These preliminary considerations were extremely useful, while ab initio calculations resulted in 18 β-turns according to the stronger distance criteria and an additional 12 (total of 30) (Figure 6b) according to the backbone angularity criteria ($-90° < \tau < 90°$). Selected conformational parameters for the 30 β-turn backbone conformations are listed in Tables V, VI, and VIII.

The $\gamma_L\alpha_D$ conformation has a folding pattern similar to that of $\alpha_L\delta_L$, but the middle amide plane is twisted by $\sim$150° (Figure 7). The critical distance is somewhat larger in $\gamma_L\alpha_D$ (d = 5.97 Å) than that found in $\alpha_L\delta_L$ (d = 5.32 Å), but the similar absolute value of τ in both conformations ($\tau_{\alpha L\delta L} = 44°$, $\tau_{\gamma L\alpha D} = -65°$) reflects a highly similar degree of folding. This conformation is relatively close to a type II β-turn. If β-turns are evaluated on the basis of their degree of refolding ($\tau \approx 0$), then the $\gamma_D\beta_L$ conformation will be the most perfect one ($\tau_{\gamma D\beta L} = -4.7°$) (Figure 6b). Such a folding pattern ($\phi_{i+1} \approx 80°$, $\psi_{i+1} \approx -60°$, $\phi_{i+2} \approx -160°$, $\psi_{i+2} \approx 160°$), assigned and labeled here as a β-turn for the first time, was also found in globular proteins (see Table VIII), despite its small probability.

The critical distances in the $\delta_L\delta_D$, $\gamma_L\epsilon_D$, $\gamma_L\alpha_D$, $\gamma_D\delta_L$, and $\gamma_D\delta_D$ conformations (Table VIII) are at least 1 Å shorter than the previously defined 7 Å as an upper limit for β-turns. On the basis of the backbone angularity value (τ), these conformations are perfect hairpin geometries (Figure 7). By contrast, the $1 \leftarrow 4$ intramolecular H-bond is missing in all of these conformations, strongly suggesting that for β-turn-like geometries such an interaction ($1 \leftarrow 4$ H-bond) is not a necessary condition. It seems that such an intramolecular H-bond is necessary where the β-turn structure incorporates the α_L conformational subunit. It is also interesting to note that two different β-turn conformations, $\delta_D\gamma_L$ and $\gamma_D\gamma_L$ (Table VIII), result in very similar d_{crit} values as well as almost identical τ values, $d_{\delta D\gamma L} = 7.23$ Å, $d_{\gamma D\gamma L} = 6.80$ Å and $\tau_{\delta D\gamma L} = 82°$, $\tau_{\gamma D\gamma L} = 81°$, respectively. This suggests that more than a single combination of the subconformations may result in the same degree of hairpin twisting.

Up to the present, ab initio calculations have shown that, due to the unfavorable eclipsed interaction of the amide proton and the β carbon atoms (H–N–C^α–C^β torsional angle is approximately $-20°$), the ϵ_L backbone conformation is unstable. (As published previously,[12,19-24] the ϵ_L backbone conformation was annihilated in For-Gly-NH_2, For-Ala-NH_2, and For-Val-NH_2.) The $\delta_D\epsilon_L$ and $\gamma_D\epsilon_L$ relaxed conformations (rms forces 3.8×10^{-5} and 4.7×10^{-5} au, respectively) reported herein are unique exceptions (see Table VIII and Figure 7). There is no direct interaction (like an intramolecular hydrogen bond) between the third and the second amide groups of the molecule oriented in the ϵ_L conformation. However, it is presumable that indirect effects may influence the $\delta-$ polarity of the carbonyl oxygen in the central amide. The increased $\delta-$ charge on the oxygen can stabilize the ϵ_L conformation of the "second half" of the molecule. This speculation, however, must be investigated by ab initio calculations using larger basis sets and taking correlation effects into account.

Conclusion

For the first time, ab initio-type calculations on the multidimensional conformational problem of dipeptide diamides resulted in a complete set of relaxed β-turn conformations of For-Ala-Ala-NH_2. The geometries are intrinsically stable hairpin conformations. It was shown that if an α_L substructure is incorporated in a β-turn conformation (e.g., $\alpha_L\delta_L$ in type I β-turn), a favorable H-bond interaction is required to stabilize such a β-turn. By contrast, for β-turns not containing an α_L conformational subunit, the existence of such a $1 \leftarrow 4$ H-bond is not required. Thus, while the $\alpha_D\alpha_D$ conformation contains a $1 \leftarrow 4$-type H-bond, the $\delta_L\delta_D$ folding pattern contains no H-bonds at all. Therefore, ab initio calculations confirmed that, while the $1 \leftarrow 4$ H-bond may be present in β-turns, it is not a necessary condition for the stabilization of such structures. These calculations lead to the conclusion that the $1 \leftarrow 4$-type intramolecular H-bond is more related to the α_L-type substructure than to the true nature of a β-turn backbone conformation. The ab initio calculations reported in this paper resulted in 18 β-turns according to the stronger distance criteria ($d \leq 7$ Å) and an additional 12 structures (a total of 30 β-turns) according to the backbone angularity criteria ($-90° < \tau < 90°$).

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