Indian Journal of Biochemistry & Biophysics
Vol. 44, April 2007, pp. 106-113

Construction and design of single stranded collagen-like structure

Fateh Singh Nandel* and Avneet Saini

Department of Biophysics, Panjab University, Chandigarh160014, India

Received 06 October 2006; revised 10 February 2007

Polytheonamide B, a 48 residue long highly cytotoxic polypeptide extracted from marine sponges contains amino acids of alternate chirality and the N-terminal region is rich in t-Leu residues. The aim of this study is to analyze the effect of these alternate chiralities and conformational behavior of various model peptides containing t-Leu, in order to explore their role in designing bioactive peptides that shall offer advantages comparable to polytheonamide B, while circumventing its limitations. The conformational behavior of various peptides constructed from t-Leu of the form Ac-(L/D-X-L/D-Y)$_n$-NHMe, where X = Gly/Ala/Leu and Y = t-Leu has been studied and compared with the corresponding peptides containing Leu residue. The results show that the helix driving capacity of L and D forms of t-Leu is less than that of Leu residue. In poly t-Leu peptides, the population of collagen/inverse collagen-type structures or right/left handed-helical structures for L and D forms respectively is found to be chain length-dependent. The stability of the helical structures is increased by ~2 kcal per residue over the collagen-type structure in poly t-Leu peptides with chain length greater than five residues. Molecular view of peptides in collagen-type structure shows that the bulky side chains of t-Leu residues mask the NH moieties of the peptide bond, while the carbonyl groups lying along the helical groove are accessible to the small solvent molecules. Molecular model building suggests that one ethylene glycol molecule interacts by forming hydrogen bonds with carbonyl groups of two adjacent t-Leu residues. To the best of our knowledge, this is the first study of its own kind on the construction of a single-strand collagen/inverse collagen-type structure using unusual amino acid residues. Such synthetic collagen mimetic peptides shall exhibit specific affinity to natural collagen under controlled thermal conditions (heat or laser treatment) and hence can be explored as a new targeting method to attach therapeutic drugs to collagens in the living tissues and to biomaterials that incorporate natural collagens.

Keywords: L/D t-Leu helical tendency, Polytheonamide B, Peptide designing, Helix without hydrogen bonds, Single-strand collagen

Non-ribosomal peptides (NRPs)[1] form a large family of natural products including vancomycin, cyclosporin and bleomycin etc and are produced by poly-functional megasynthases- known as non-ribosomal peptide synthases (NRPs)[2]. NRPs are well documented in various microorganisms like bacteria, cyanobacteria and fungi[3-7]. Marine invertebrates, i.e., sponges and tunicates often contain NRPs that exert various biological activities[8]. Sponges of the order Lithistida are a rich source of bioactive peptides containing unusual amino acids[9] and polytheonamide B, a 48 residue long highly cytotoxic polypeptide extracted from these marine sponges contains D and L forms of unusual amino acids of various types[10]. It is a membrane-active peptide[9,10] and forms ion-conducting pores in the membrane. Sequence analysis of polytheonamide B reveals that: i) N-terminal part is rich in hydrophobic residues like t-Leu and βMeIle, ii) also contains dipeptide stretches in which the preceding residue to t-Leu is either Gly or Ala, iii) the stretch from 17 to 26 residues is rich in amino acids with smaller side chains like Gly and Ala, and iv) the C-terminal contains amino acid residues with side chains capable of hydrogen bonding by either acting as hydrogen-acceptor or hydrogen-donor viz., Asn-OH/Asm/OHAsm/OHVal/Ser, followed by L-amino acid residues with hydrophobic side chains.

As the N-terminal sequence of polytheonamide B is rich in t-Leu, it is worthwhile to study the conformational behavior of various model peptides containing t-Leu and explore the role of this residue in designing bioactive/biocompatible peptides. Hence, in the present study, the conformational behavior of the model peptides of the form Ac-(t-Leu)$_n$-NHMe, where n = 1-10 and Ac-(X-Y)$_n$-NHMe, where X = Gly/Ala/Leu (in L and D forms) and Y = t-Leu (in L and D forms) with n = 1, 3, 4 has been studied and results have been compared with the corresponding peptides containing Leu residues[11,12] to explore whether the helical driving capacity of t-Leu is similar to that of

Author for correspondence
Tel: 0172-2534125
E-mail: fatehhar@pu.ac.in; fateh_nandel@yahoo.com

Leu. The conformational results on these model peptides containing t-Leu may also provide a basis to explain the conformational behavior for the N-terminal decapeptide with the sequence Ac-Gly-β Meile-Gly-t-Leu-t-Leu-t-Leu-Ala-t-Leu-t-Leu-Ala-NHMe.

Methodology

The Φ, Ψ maps and χ potential energy curves for t-Leu in both L and D forms were constructed in model di- and tripeptides to have the knowledge of global, local and low energy minima by using standard bond lengths and bond angles[13,14]. The minima values for other amino acid residues used in the study were taken from the previous work[15,16]. Energy calculations were carried out using the quantum mechanical method PCILO[17] (pertubative configuration interaction using localized molecular orbital) on Sun W, Ultra 5-10; sparc. It may be pointed out that the minima obtained by PCILO calculations were also the minima at the *ab initio* level for the usual amino acids[18] and for dehydroamino acids[19-21]. In addition, the PCILO results[22,23] for the peptides containing usual and unusual amino acids were in conformity with *ab initio* results obtained in previous studies[24,25] and knowledge-based crystallographic data[26-30]. Conformational states were generated from the global, local and low energy minima in the Φ, Ψ maps and χ_i, χ_j curves/maps and their energies computed. Minimization was further refined by varying Φ, Ψ and χ values in the neighborhood of the minima so obtained in steps of 5 and then 2 degrees.

Results and Discussion

Peptides containing t-Leu

From the conformational results summarized in Table 1, it is obvious that the peptide Ac-(Gly-L-t-Leu)$_3$-NHMe can be realized in the right-handed helical structure along with the helical structure without hydrogen bonds having Φ, Ψ values in the neighborhood of 0^o, 90^o. Likewise, for the peptide Ac-(Gly-D-t-Leu)$_3$-NHMe, the 3_{10} left-handed helical structure and helix structure without hydrogen bonds with Φ, Ψ values in the neighborhood of 0º, -90º (Fig. 1) are found to be degenerate. At first sight, the structures with these Φ, Ψ values appear to be somewhat unusual, but PCILO and INDO level calculations on amino acids[21] have predicted minima for $\Phi = 0^o$ and $\Psi = \pm 90^o$. Also, *Ab initio* calculations at the HF/3.21G and HF/6.31 + G levels for the dipeptides of Gly and Ala[31] have predicted stationary point near $\Phi = 0^o$ and $\Psi = 90^o$. This helical structure without hydrogen bonds is found to be stabilized by carbonyl-carbonyl interactions between carbonyl oxygen of the i^{th} residue and carbonyl carbon of $i^{th}+1$ residue (Fig. 1) and hence shall be populated in the solvents of low dielectric constant, incapable of hydrogen bonding with the carbonyl moieties.

It is worth mentioning here that in the Ramachandran plots, based on the NMR-derived structure for 113 proteins, 84719 total residues plotted (Pro and Gly excluded), appreciable data points have been shown between left-handed helical and collagen-type structural regions[15,32], and between the right-handed helical and inverse collagen-type structural regions[31,33,34]. This supports that the minima corresponding to the Φ, Ψ values in neighborhood of 0^o, $\pm 90^o$ are not an overestimation. This structure with average Φ, Ψ values of –11º, 100º (Table 1) is characterized by rise per residue of -2.07 Å, rotation per residue of 116º and 3.1 residues per turn. These characteristics are analogous to the characteristics of usual 3_{10} helical structures.

In peptides constructed from amino acids of different chirality i.e. Ac-(L-Ala-D-t-Leu)$_3$-NHMe, both left-handed and distorted right-handed helical structures are found to be degenerate. The degeneracy of two states has been analyzed in terms of the hydrogen bonds and carbonyl-carbonyl interactions. In the 3_{10} left-handed helical structure, five intramolecular hydrogen bonds are formed, whereas in the distorted right-handed helical structure three ten-membered hydrogen bonded rings are formed. A molecular view of this peptide in the distorted helical structure (Fig. 2) clearly shows that the first and last ten-membered hydrogen bonded rings are formed due to the adoption of Φ, Ψ values in the inverse collagen-type structural region by D-t-Leu residue at position 2 and 6 and to the Φ, Ψ values of the 1st and 5th L-Ala residue in the right-handed helical region. This type of ten-membered hydrogen bonded rings has been reported in a number of cases[11,22]. On the other hand, the carbonyl-carbonyl interactions on the basis of distance are found to be stronger in the distorted helical structure as compared to the 3_{10} left-handed helical structure. Interestingly, the peptide Ac-(D-Ala-L-t-Leu)$_3$-NHMe can be realized only in the 3_{10} right-handed helical structure and its conformational

Table 1 — Conformational results for the t-leu peptides with ϕ, Ψ and χ (italics) values in degrees

Residue number								
1	2	3	4	5	6	7	8	ΔE (kcal/mol)
Ac-(Gly-L-t-Leu)$_3$-NHMe								
-50, -31	-52, -25	-50, -30	-50, -25	-50, -25	-50, -25			
	65		*60*		*60*			0
-16, 105	-30, 120	15, 75	-25, 115	10, 75	-25, 115			
	50		55		60			+1.78
Ac-(Gly-D-t-Leu)$_3$-NHMe								
49, 34	49, 26	51, 38	53, 22	53, 22	59, 16			
	61		*55*		*55*			0
5, -95	20, -110	-15, -70	25, -115	-15, -70	25, -115			
	65		65		65			+0.22
Ac-(L-Ala-L-t-Leu)$_3$-NHMe								
-42, -32	-50, -25	-50, -30	-50, -25	-50, -30	-54, -24			
	62		*60*		*62*			0
-20, 110	-30, 120	-20, 110	-25, 115	-20, 115	-30, 120			
	55		50		55			+9.22
Ac-(D-Ala-D-t-Leu)$_3$-NHMe								
52, 24	50, 25	50, 30	50, 25	52, 26	55, 20			
	61		*61*		*58*			0
20, -110	25, -115	25, -115	25, -115	20, -110	30, -120			
	70		65		65			+10.5
Ac-(L-Ala-D-t-Leu)$_3$-NHMe								
-25, -60	48, -118	-50, -30	-40, -35	-35, -45	30, -120			
	67		*55*		*65*			0
49, 31	49, 21	49, 26	49, 26	49, 31	49, 26			
	59		*63*		*62*			0
Ac-(D-Ala-L-t-Leu)$_3$-NHMe								
50, -120	-50, -25	-50, -30	-50, -25	-52, -32	-55, -17			
	60		*60*		*65*			+2.03
-52, -32	-52, -23	-52, -30	-50, -25	-50, -30	-55, -18			
	60		*60*		*65*			0
Ac-(L-Leu-L-t-Leu)$_3$-NHMe								
60, -30	-148, -20	-70, 23	10, 68	15, 59	15, 60			0
-18, -73	*73*	*-62, -73*	*55*	*60, -75*	*60*			
-49, -11	-49, -26	-49, -26	-49, -26	-49, -31	-24, -51			
85, 150	*70*	*65, 85*	*65*	*65, 80*	*45*			+9.97
-10, 145	-50, 155	-15, 105	-20, 110	-15, 105	-10, 95			
155, 80	*55*	*175, 80*	*50*	*165, 75*	*45*			+15.03
Ac-(D-Leu-D-t-Leu)$_3$-NHMe								
-65, 35	151, -170	-15, -68	74, -158	-15, -70	118, 170			0
53, 160	*35*	*63, 74*	*45*	*174, 162*	*45*			
54, 1	49, 16	49, 26	49, 26	49, 31	24, 51			+9.12
-55, 170	*55*	*-65, 155*	*55*	*55, 160*	*75*			
10, -150	50, -150	0, -85	10, -100	0, -95	-5, -75			+13.15
-165, 155	*70*	*180, 160*	*65*	*180, 160*	*65*			
Ac-(L-Leu-L-t-Leu)$_4$-NHMe								
-25, -115	-30, 120	-15, 105	-25, 115	-20, 110	-20, 115	-15, 105	-25, 115	
175, 70	*50*	*175, 70*	*55*	*180, 70*	*30*	*180, 70*	*50*	+12.12
-49, -31	-49, -21	-49, -31	-49, -26	-49, -31	-49, -26	-49, -31	-54, -16	
175, 50	*60*	*-175, 80*	*60*	*180, 75*	*60*	*-170, 80*	*60*	0
Ac-(D-Leu-D-t-Leu)$_4$-NHMe								
25, -115	30, -120	20, -110	19, -113	15, -105	23, -115	15, -110	20, -115	+14.03
-175, 165	*70*	*65, 70*	*70*	*180, 160*	*60*	*15, -110*	*70*	

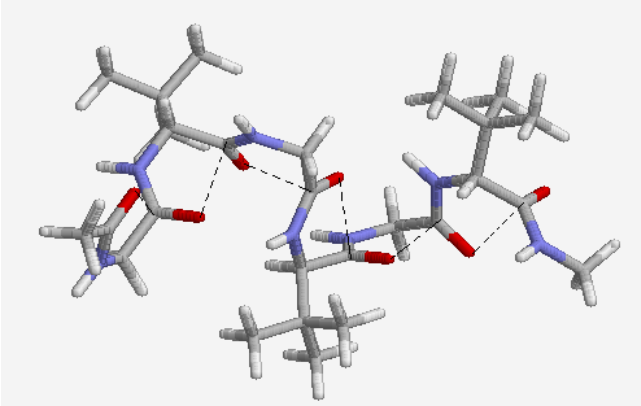

Fig. 1 — A view of the peptide Ac-(Gly-D-t-Leu)$_3$-NHMe in the helical structure without hydrogen bonds with Φ, Ψ values of 5º, -95º; 20º, -110º; -15º, -70º; 25º, -115º; -15º, -70º; 25º, -115º stabilized by the carbonyl carbonyl interactions [Oxygen atoms are shown in red]

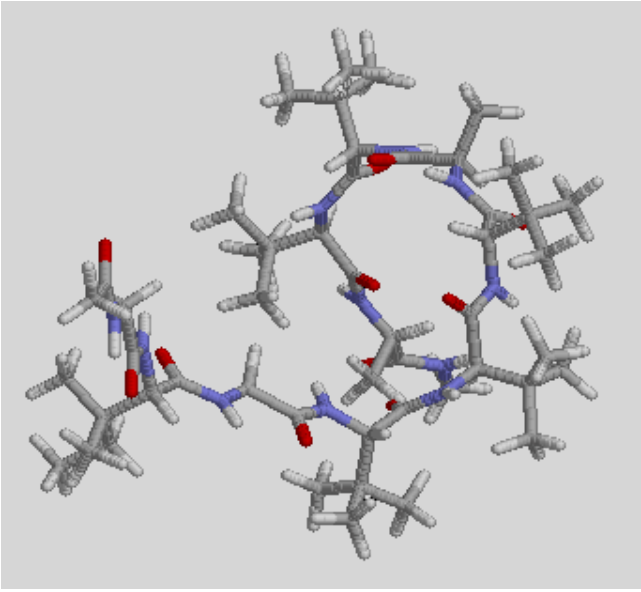

Fig. 3 — A molecular view of the N-terminal decapeptide Ac-Gly-βMeile-Gly-t-Leu-t-Leu-t-Leu-Ala-t-Leu-t-Leu-Ala-NHMe of polytheonamide B in the most stable conformational state shows the adoption of some kind of circular structure without hydrogen bonds in which the hydrophobic side chains point outwards

behavior is similar to the corresponding peptide containing Leu residue i.e. Ac-(D-Ala-L-Leu)$_3$-NHMe.

It may be mentioned that in the conformational study of the peptides Ac-(X-L/D-Leu)-NHMe with X = Gly, Aib, Abu, Val, Ile and Leu, the 3_{10} right/left-handed helical structure is the most stable in those cases, where the preceding residue to L/D-Leu is an

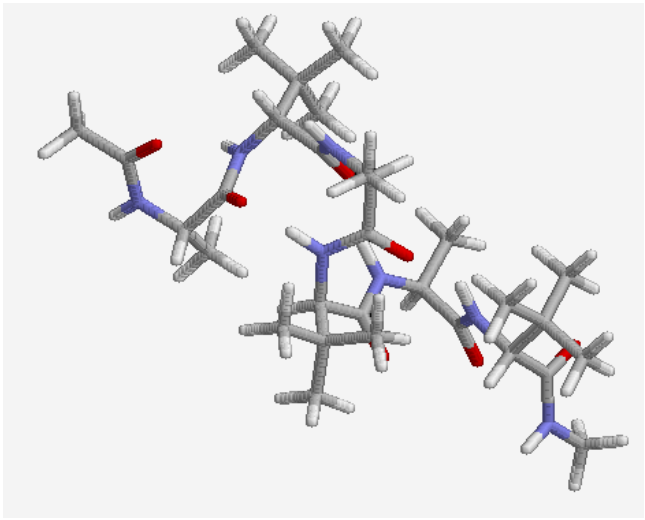

Fig. 2 — A molecular display of the peptide Ac-(L-Ala-D-t-Leu)$_3$-NHMe in the distorted right-handed helical structure showing formation of different type of 10-membered hydrogen bonded rings [The first and third 10-membered rings are formed due to the adoption of Φ, Ψ values by the D-t-Leu residue at 2nd and 6th positions in the inverse collagen-type structural region and due to the adoption of Φ, Ψ values by the 1st and 5th residues in the right-handed helical region]

amino acid residue with either L/D chirality and with a smaller aliphatic side chain i.e. Gly/Ala[11,12]. Thus, the conformational results for the peptides Ac-(Gly-L/D-t-Leu)$_3$-NHMe and Ac-(Ala-t-Leu)$_3$-NHMe of pure L/D and mixed chiralities are at somewhat variance with the corresponding results for the peptides containing Leu residue. This implies that the helical driving tendency of t-Leu is less than that of Leu residue, which is consistent with the experimental observation that t-Leu is predicted to be a poor helix-former[35]. Further, the conformational results for the peptides containing Leu and t-Leu reveal, that the behaviour of t-Leu is both chain length and environment-dependent i.e., L-t-Leu/L-Leu and D-t-Leu/D-Leu residues can adopt Φ, Ψ values in the left- or right-handed helical regions respectively. This observation is further supported by the conformational results on the N-terminal decapeptide Ac-Gly-βMeile-Gly-t-Leu-t-Leu-t-Leu-Ala-t-Leu-t-Leu-Ala-NHMe of polytheonamide B (Φ, Ψ values of 180˚, 180˚; -160˚, 120˚; 180˚, 180˚; -160˚, 120˚; 100˚, -170˚; -150˚, 130˚; 140˚, -160˚; -145˚, 130˚; 85˚, -175˚; -175˚, 130˚), which adopts a circular structure as shown in Fig. 3, in which the hydrophobic side chains point outwards.

Table 2a — Conformational results for poly t-Leu peptides [ϕ, Ψ and χ values (in parenthesis) are in degrees]

Ac-L-t-Leu-NHMe	
i) -30, 120 *(55)* 0	ii) -65, 0 *(65)* +16.17
Ac-D-t-Leu-NHMe	
i) –30, -120 *(65)* 0	ii) 65, 0 *(55)* +1.31
Ac-(L-t-Leu)$_2$-NHMe	
i) -30, 120 *(50);* -30, 120 *(55)* 0	ii) -49, -26 *(60);* -49, -26 *(60)* +1.48
iii) -65, 0 *(65);* -65, 0 *(65)* +2.09	
Ac-(D-t-Leu)$_2$-NHMe	
i) 26, -118 *(70)*; 26, -118 *(65)* 0	ii) 49, 26 *(60);* 54, 21 *(60)* +2.29
iii) 65, 0 *(55)*; 65, 0 *(55)* +2.34	
Ac-(L-t-Leu)$_3$-NHMe	
i) -30, 120 *(50);* -25, 120 *(50);* -30, 120 *(50)* +0.39	ii) -54, -21 *(60);* -54, -26 *(60);* -60, -15 *(65)* 0
Ac-(D-t-Leu)$_3$-NHMe	
i) 25, -120 *(70);* 30, -120 *(70);* 30, -120 *(70)* 0	ii) 49, 21 *(60);* 49, 26 *(60)*; 54, 16 *(60)* +1.34
Ac-(L-t-Leu)$_4$-NHMe	
i) -30, 120 *(50)*; -25, 120 *(50);* -30, 125 *(50);* -30, 120 *(50)* +0.50	ii) -49, -21 *(65);* -49, -21 *(60);* -49, -26 *(60);* -54, -26 *(60)* 0
Ac-(D-t-Leu)$_4$-NHMe	
i) 30, -125 *(70);* 30, -120 *(70)*; 30, -120 *(70);* 30, -120 *(65)* +1.36	ii) 49, 21 *(55);* 49, 21 *(60)*; 49, 26 *(60);* 54, 26 *(60)* 0

*The underlined values are ΔE/kcal mol^{-1} with respect to the most stable conformational state

Table 2b — Conformational results for poly t-Leu peptides [ϕ, Ψ and χ values (italics) are in degrees]

Residue number										ΔE (Kcal mol^{-1})
1	2	3	4	5	6	7	8	9	10	
Ac-(L-t-Leu)$_5$-NHMe										
-30, 120	-30, 120	-30, 120	-25, -115	-30, 120						+1.56
50	*55*	*55*	55	*55*						
-49, -21	-54, -26	-49, -26	-54, -31	-54, -16						0
60	*60*	*60*	*60*	*65*						
Ac-(D-t-Leu)$_5$-NHMe										
30, -125	25, -120	25, -115	30, -125	25, -115						+2.06
70	*70*	*65*	*70*	*65*						
49, 26	49, 26	49, 26	49, 31	49, 26						0
60	*60*	*60*	*65*	*60*						
Ac-(L-t-Leu)$_6$-NHMe										
-1, 85	14, 65	-18, 115	-23, 116	-27, 120	-19, 114					+1.41
50	*60*	*50*	*50*	*55*	*55*					
-44, -26	-49, -26	-49, -26	-49, -26	-54, -31	-64, -6					0
70	*65*	*60*	*65*	*55*	*60*					
Ac-(D-t-Leu)$_6$-NHMe										
20, -110	10, -95	25, -120	25, -115	26, -117	26, -120					+2.53
70	*70*	*70*	*70*	*65*	*65*					
44, 26	49, 26	49, 26	49, 31	49, 26	49, 21					0
55	*50*	*60*	*65*	*60*	*55*					
Ac-(L-t-Leu)$_8$-NHMe										
-25, 118	-29, 124	-31, 122	-23, 114	-23, 118	-27, 118	-23, 112	-31, 122			+4.42
50	*54*	*54*	*50*	*50*	*50*	*52*	*54*			
-49, -26	-49, -26	-49, -26	-49, -26	-49, -26	-49, -26	-49, -31	-54, -16			0
60	*60*	*55*	*55*	*55*	*55*	*55*	*60*			

(*Contd*)

Table 2b — Conformational results for poly t-Leu peptides [ϕ, Ψ and χ values (italics) are in degrees] — *Contd.*

Residue number										ΔE (Kcal mol^{-1})
1	2	3	4	5	6	7	8	9	10	
Ac-(D-t-Leu)$_8$-NHMe										
28, -122	30, -122	24, -114	28, -120	28, -122	32, -124	28, -116	28, -118			+5.25
68	*68*	*66*	*66*	*66*	*60*	*68*	*60*			
49, 26	49, 26	49, 26	49, 26	49, 26	49, 26	49, 31	49, 26			0
60	*60*	*65*	*65*	*65*	*65*	*65*	*60*			
Ac-(L-t-Leu)$_{10}$-NHMe										
-30, 125	-30, 120	-30, 120	-30, 120	-30, 120	-30, 120	-30, 125	-30, 120	-30, 120	-30, 120	+7.94
55	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*	
-45, -26	-49, -26	-49, -26	-49, -26	-49, -26	-49, -26	-49, -26	-49, -26	-49, -26	-56, -20	0
55	*60*	*60*	*55*	*55*	*55*	*55*	*60*	*55*	*60*	
Ac-(D-t-Leu)$_{10}$-NHMe										
35, -125	30, -115	25, -120	30, -125	25, -120	30, -120	30, -120	30, -120	30, -120	30, -120	+8.00
65	*70*	*65*	*65*	*70*	*70*	*70*	*70*	*70*	*70*	
52, 26	49, 26	49, 26	49, 26	49, 26	49, 26	49, 26	52, 26	49, 26	55, 20	0
60	*65*	*65*	*65*	*65*	*60*	*65*	*60*	*65*	*60*	

Poly t-Leu peptides

The conformational results for the various poly t-Leu peptides, summarized in Table 2 reveal that the most stable conformations are either collagen-type with Φ, Ψ values of ~ -30˚, 120˚/inverse collagen-type with Φ, Ψ values of 30˚, -120˚ or the 3_{10} helical structures with the handedness dictated by the chirality of t-Leu i.e. right-handed for L-form and left-handed for D-form. The stability of these structures is also found to be chain-length dependent, the collagen or inverse collagen-type structures being more stable than the helical structures up to a chain length of three residues, which is consistent with the crystallographic data analysis of proteins[28] and conformational studies on homopolypeptides[15,23]. With increase in chain length from trimers to the tetramers, the two forms i.e. collagen-type and usual helices are almost equally stable and in penta and hexa peptides i.e., Ac-(t-Leu)$_5$-NHMe and Ac-(t-Leu)$_6$-NHMe, the helical structures become slightly more stable than the collagen-type structures. A molecular view of Ac-(L-t-Leu)$_6$-NHMe in the collagen-type structure shown in Fig. 4 shows that only carbonyl groups are free (NH moieties are masked) for interactions, i.e. not involved in intra-hydrogen bonding and the structure is stabilized by strong carbonyl-carbonyl interactions between the carbonyl oxygen of the ith residue and the carbonyl carbon of the ith + 1 residue ($do_i..c_{i+1}$ = 2.15-2.34 Å, $dc_i..o_{i+1}$ = 2.97-3.21 Å, $dc_i..c_{i+1}$ = 2.63-2.70 Å and $do_i..o_{i+1}$ = 2.55-2.65 Å).

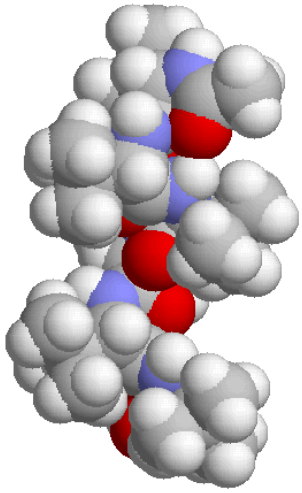

Fig. 4 — A molecular view of peptide Ac-(L-t-Leu)$_6$-NHMe in collagen-type structure with Φ, Ψ values of ~-30˚, 120˚ clearly shows that the peptide NH moiety is masked by the bulky and heavy side chains of t-Leu and only peptide carbonyls are accessible to small solvent molecules (N atoms and carbonyl oxygens are shown in blue and red respectively)

The importance of the carbonyl-carbonyl interactions[36,37] has been interpreted in terms of three main types of interaction motifs – i) a sheared antiparallel motif with two short carbon-oxygen interactions, ii) a perpendicular motif with only one short carbon-oxygen interaction, and iii) a highly

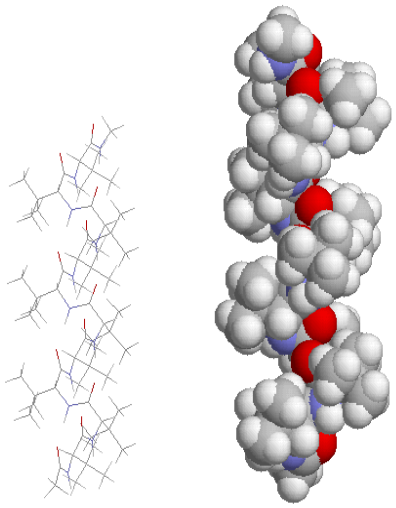

Fig. 5 — A graphical view of the molecule Ac-(L-t-Leu)$_{10}$-NHMe in the right-handed helical structure with Φ, Ψ values -49°, -26° and Ac-(D-t-Leu)$_{10}$-NHMe in the inverse collagen-type structure with Φ, Ψ values 30°, -120°

sheared parallel motif with only one short carbon-oxygen interaction by carrying out a systematic study between ketonic groups in the Cambridge structure database. The importance of coloumbic interactions between backbone carbonyls in proteins as a stabilizing factor in α-helices, β-sheets and right-handed twist often observed in β-strands is well-documented[38,39]. Carbonyl-carbonyl interactions also stabilize the partially allowed the Ramachandran conformations of aspartic acid and asparagines[40]. Recently, we have also shown that the helical structure without hydrogen bonds with Φ, Ψ values of 0°, ± 90° are stabilized by carbonyl-carbonyl interactions for the peptides constructed from achiral and unusual amino acids like Aib and Δ^zPhe[21,29,41]. The magnitude of these interactions is 4-10 kcal mol^{-1}, depending on the approach of carbonyl groups and is competitive with the hydrogen bond, in terms of their stabilization energy[21,29].

As expected, with further increase in chain length, the helical structure becomes more stable over the collagen-type structures in octa and decapeptides by 4 and 8 kcal mol^{-1}, respectively. In other words, stability of the helical structure increases by ~2 kcal per residue in going from the hexa to deca peptide. The origin of chain length dependence of these structures has been traced in terms of non-covalent interactions. With similar Φ, Ψ values, interactions in left and right-handed helix are identical and the same is found to be true for collagen and inverse collagen-type structures. The number of hydrophobic contacts is increased due to the additional helical turn as chain length increases and hence the magnitude of hydrophobic interactions increases. Thus, the conformational behavior of the peptides constructed from t-Leu is found to be only chain length-dependent. In non-polar solvents, the poly t-Leu peptides with more than six residues can be realized in helical structure, whereas in polar solvents capable of hydrogen bonding with these, the peptides can be realized into the collagen-type structures as the difference in stabilization energy per residue is only ~ 2 kcal.

A view of the molecule Ac-(L-t-Leu)$_{10}$-NHMe in the right-handed helical structure with Φ, Ψ values -49°, -26° and of Ac-(D-t-Leu)$_{10}$-NHMe in the inverse collagen-type structure with Φ, Ψ values 30°, -120° are shown in Fig. 5. In collagen-type structure, backbone is surrounded by t-Leu side chains that completely mask the NH moieties of peptide backbone i.e. making NH inaccessible to solvent molecules, whereas carbonyl groups positioned along the helical groove are exposed. Molecular model building suggests that small molecules like ethylene glycol through their hydroxyl groups interact with the carbonyl groups of collagen-type structure to further stabilize it. Modeling studies also suggest that one ethylene glycol molecule interacts with two carbonyl groups of adjacent t-Leu residues. As the Φ, Ψ values are very close to the Φ, Ψ values for the native collagen strand[42] and overall the molecule appears to be hydrophobic, this structure may be called single-stranded collagen structure. Thus, poly-t-Leu peptides and peptides containing Leu and t-Leu, i.e. Ac-(Leu-t-Leu)$_4$-NHMe of pure chiralities, in the presence of ethylene glycol may be exploited for their use as collagen mimetic peptides. The diameter of this single-stranded collagen (~ 15 Å) is comparable with the natural collagen[42] i.e., 14 Å. To the best of our knowledge, this is the first conformational study on peptides containing t-Leu and poly t-Leu peptides.

Conclusion

The peptides containing t-Leu of both L or D chirality i.e. Ac-(Gly-t-Leu)$_3$-NHMe are predicted to be populated in 3_{10} right/left handed helical structure respectively, along with the helical structures without hydrogen bonds. In the peptides constructed from Ala and t-Leu of single chirality, only 3_{10} right/left-handed helical structures are found to be the most stable for L and D forms respectively. For the peptide Ac-(D-Ala-L-t-Leu)$_3$-NHMe containing amino acid residues of mixed chiralities, the 3_{10} right-handed helical

structure is predicted to be more stable, whereas for the peptide Ac-(L-Ala-D-t-Leu)$_3$-NHMe the distorted right-handed and left-handed helical structures are found to degenerate. No regular structure in terms of Φ, Ψ values is predicted for the peptides Ac-(Leu-t-Leu)$_3$-NHMe constructed from amino acids of single or mixed chiralities. Comparison of conformational structure adopted by t-Leu peptides with the corresponding peptides containing Leu residue shows that the helical driving capacity of t-Leu residue is less.

In short chain poly t-Leu peptides, the collagen-type structure with L chirality or inverse collagen-type structure with D chirality are found to be the most stable. But, with chain length greater than six residues, the right or left-handed helical structures are found to be more stable than the collagen/inverse collagen-type structures by ~ 2 kcal per residue. The NH moiety of peptide backbone are masked by the symmetrical, bulky and hydrophobic side chain of t-Leu residues and only the carbonyl groups are accessible to small solvent molecules like ethylene glycol in the collagen/inverse collagen-type structure. One ethylene glycol molecule interacts with carbonyl groups of two adjacent t-Leu residues, leading to the stability of single-strand collagen/inverse collagen structure.

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