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Influence of nature of side chain on conformation of alternating L- and D-stereo oligopeptides

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Abstract. Peptides containing residues with alternating D- and L-stereochemistry in the backbone have been studied for their single-strand helix-forming capability by molecular dynamics (MD) simulations. The influence of the nature of the side chain such as steric, branching and polarity on helix forming ability has been probed by studying *t*-Boc-(L-Ala-D-Ala)$_4$-OMe (small hydrophobic side chain), *t*-Boc-(L-Phe-D-Phe)$_4$-OMe (bulky hydrophobic side chain), *t*-Boc-(L-Val-D-Val)$_4$-OMe (β-branch in side chain), *t*-Boc-(D-alloIle-L-Ile)$_3$-OMe (γ-branch in side chain), and *t*-Boc-(L-Ala-D-Ser)$_4$-OMe (hydrophobic and hydrophilic side chains). Besides this, the effect of unsymmetrical α,α-disubstitution with alternating D- and L-stereochemistry at Cα carbons on helix stability has also been investigated. The results show that such peptides, with the exception of those with α,α-disubstitution, have a unique ability to form β-helices.

Keywords. DL-peptides; conformation; molecular dynamics; β-helix.

1. Introduction

Various peptide antibiotics contain D-amino acids in addition to L-residues. The antibacterial peptides gramicidin A, enniantin B and valinomycin have regular alternating sequences of L- and D-residues. This has generated some interest in polypeptide chains with regular sequences of L- and D-amino acids. These polymers assume besides α-helical conformations [1–4], other structures [5] different from those characterising the poly(L-amino acid) chains. The β-helix is one such structure for these sequences. It is particularly fascinating, because it possesses a hollow core and hence an ability to form molecular channels, permeable to small ions or neutral molecules [6,7], and also forms inclusion complexes [8]. The functioning of gramicidin A has been explained by a transmembrane channel, spanning the lipid bilayer of biomembranes [9].

A β-helix differs from the commonly encountered α-helix in its H-bonding pattern. This is clearly seen in figure 1, which shows schematically the H-bonding patterns for right-handed and left-handed β-helices (figure 1a) and for right-handed

*For correspondence

and left-handed α-helices (figure 1b). The H-bonds in a β-helix point in both up and down directions along the helix axis but are in the same direction for an α-helix. The β-helical structures have a unique sequence of sign reversal of the ϕ and ψ angles for the LD-pair of amino acids i.e. the sign of the ϕ and ψ angles follows the pattern $-,+,+,-$. This is a distinct feature that further differentiates β- and α-helices, the latter having the same sign for all ϕ and ψ angles (negative for right-handed and positive for left-handed α-helices).

Some theoretical investigations of peptides with amino acids having alternating D- and L-configuration have appeared in the literature [3,5,7,10]. The earliest work[5] reported was by Ramachandran and Chandrasekharan in 1972, on the basis of conformational theory. Later papers use energy functions that range from simple in form, to types that are the forerunners of the modern forcefields [3,7,10]. Nevertheless, many of these studies do give some insight into the conformations possible for such peptides. A couple of experimental investigations by NMR and X-ray diffraction techniques on homopolymers with alternating D- and L-residues have been reported. X-ray [11] and NMR [12] in cyclohexane results indicate that *t*-Boc-(L-Val-D-Val)$_4$-OMe assumes an antiparallel double-stranded left-handed β-helical structure. The β-helix however, is markedly less stable in chloroform. The poly *t*-Boc-(L-Ile-D-Ile)$_n$-OMe ($n = 3$ or 4) exists as β-helix in cyclohexane [13], while a related peptide *t*-Boc-(D-alloIle-L-Ile)$_3$-OMe in the solid state [14] shows a contrasting U-shaped conformation, stabilised by intramolecular H-bonds along with α- and β-turns. To the best of our knowledge there has been no thorough study of such peptides by modern theoretical methods such as molecular dynamics (MD) simulations, Monte Carlo (MC) or systematic conformational search (SC) techniques.

We have investigated the role played by structural factors such as the nature of the side chain like bulkiness (steric), branching and hydrophilicity/hydrophobicity on peptide backbone with alternating D- and L-stereochemistry to adopt the β-helical motif. To this end, various model peptides were studied such as *t*-Boc-(L-Ala-D-Ala)$_4$-OMe (small hydrophobic side chain), *t*-Boc-(L-Phe-D-Phe)$_4$-OMe (bulky

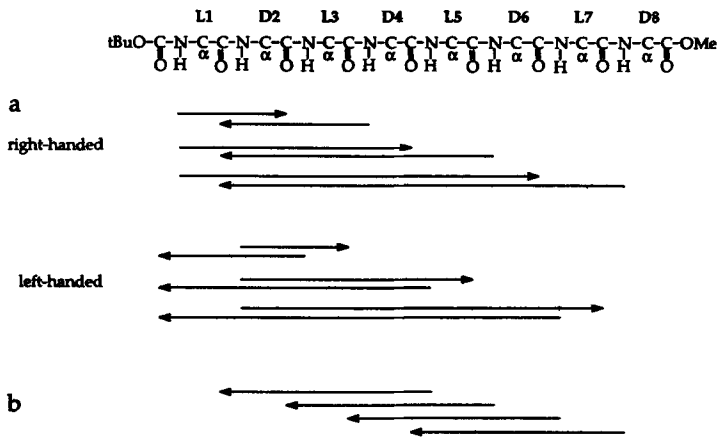

Figure 1. Hydrogen bonding patterns for right-handed and left-handed (**a**) β-helices, and (**b**) α-helices.

hydrophobic side chain), *t*-Boc-(L-Val-D-Val)$_4$-OMe (branching at β-position in side chain), *t*-Boc-(D-alloIle-L-Ile)$_4$-OMe (γ-branch in side chain, D-alloIle is diastereoisomeric with L-Ile in that only the configuration at the Cα has been inverted) and *t*-Boc-(L-Ala-D-Ser)$_4$-OMe (hydrophobic/hydrophilic side chains). Besides these, we have also investigated the effect of disubstitution on the α-carbon atom, with unsymmetrical alkyl groups and alternating L- and D-stereochemistry, by studying *t*-Boc-(L-Iva-D-Iva)$_4$-OMe (Iva stands for isovaline i.e. α-amino-α-methyl butyric acid). For all these peptides, a comparison has been made with the corresponding peptides composed solely of L-residues.

2. Methodology

The MD calculations were performed on a Silicon Graphics IRIS Indigo workstation with molecular modelling software from MSI, USA. *Insight II* (v. 2·3) provided the graphical interface. The molecules were built in an extended conformation ($\phi = \psi = 180°$) using the *Biopolymer* module in *Insight II*. MD simulations were done using *Discover* (v. 2·9). The energy was calculated using the CFF91 forcefield [15,16]. Bond stretching was expressed by an harmonic potential. The cross-terms expressing coupling between the internal degrees of freedom were not included in the energy expression. In the Coulomb term, the dielectric constant (ε) was set to 1·0. The starting structure was initially minimised with 100 steps of steepest descents, followed by 100 steps of conjugate gradients to remove any 'bad contacts' in the molecule.

To search the conformational space of these peptides, two different protocols were used for the MD simulations.

In the first simulation, the initial minimizations were followed by slow heating of the molecule to 1000 K in steps of 100 K. At each new temperature the structure was equilibrated for 2 ps. On reaching 1000 K, MD simulations were carried out for 100 ps, during which time structures were sampled every 1·0 ps giving rise to a trajectory of 100 structures. Temperature control during equilibration was achieved by velocity scaling, while during the data collection period (100 ps) weak coupling to a temperature bath with a time constant of 0·1 ps was used [17]. Newton's equations of motion were integrated every 1·0 fs using the Verlet algorithm [18]. Each sampled structure was cooled to 0 K in 15 steps by a short MD run (5 ps) where the temperature at each new step (i) is obtained from the following equation

$$T\text{cool}_i = T\text{start}\,[3(1-i/S)^2 - 2(1-i/S)^3], \quad \text{for } i \leq S,$$

where S is the total number of steps and Tstart is the starting temperature (in this case 1000 K). The rate of cooling is dependent only on the number of steps, S. We show in figure 2 the 'cooling curves' resulting from steps of $S = 5$, 10 and 15. We have used 15 cooling steps. At the end of the cooling cycle, the temperature of the system is 0 K. These '0 K structures' were then subjected to minimizations with 1000 steps of conjugate gradients, at which point most structures had a gradient of 0·001 kcal/mole/Å or lower.

The second simulation strategy is completely different in spirit from the above. The initial minimised structure was slowly heated to 1000 K in steps of 100 K,

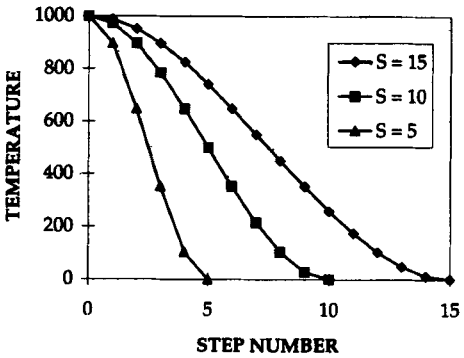

Figure 2. The cooling profiles resulting from the number of steps $S = 5$, 10 and 15.

taking care to equilibrate sufficiently (2 ps) at each new temperature. On reaching 1000 K, dynamics was run for 2 ps. This structure was then cooled slowly in steps of 100 K up to a temperature of 300 K, by a 2 ps dynamics run at each new temperature. The structure was then minimised by 100 steps of steepest descents, followed by 1000 steps of conjugate gradients or until the gradient fell below a threshold value of 0·001 kcal/mole/Å. This structure was stored and became a starting structure for a second 'heat-cool' cycle as described above. This was repeated 100 times to give a total of 100 structures in the second simulation.

Structures from both simulations were analysed using the *Analysis* and *Decipher* modules in *Insight II* for secondary structural patterns. The global minimum referred to in the next section refers to the lowest energy structure obtained from the two simulations.

Ideally, we would have liked to include explicit solvent molecules in these simulations, but such a study is prohibitively expensive in terms of computer time and usage for the size and the number of peptides and the simulation protocols considered here. Nevertheless, it is heartening to note that our predicted results for the peptide *t*-Boc-(L-Val-D-Val)$_4$-OMe is in line with the NMR finding of β-helices in solution [12].

3. Results and discussion

The LD-copolymer of Ala, {*t*-Boc-(L-Ala-D-Ala)$_4$-OMe} shows a high preference for a right-handed β-helix. The global minimum and 50% of all helical structures within 15 kcal/mole of the global minimum are right-handed β-helices. Another 25% of the structures are partly β-helical with slight distortions at the first two residues at the N-terminal end. A structure with some α-helicity for the central residues (residue numbers 4–6) of the peptide is seen but is 17 kcal/mole higher than the global minimum. The rest of the helical structures could not be assigned to any standard type. The global minimum of the L-homopolymer, *t*-Boc-(L-Ala)$_8$-OMe is a right-handed α-helix and many local minima within 10 kcal/mole have the α-helix motif.

Substitution at the α-position by an ethyl group – *t*-Boc-(L-Iva-D-Iva)$_4$-OMe – suppresses the helix-forming capacity of the LD-peptide. The trajectory is dominated by structures that are characterised by γ-bends and β-turns. The global minimum is characterised by two β-turns. The first one is associated with the tetrad spanning amino acids 2 to 5 in the sequence, the second β-turn commences at amino-acid 4 and terminates at amino acid 7. Every possible linear sequence of tetrad beginning from residue number 1 has been found in a β-turn with equal frequency. This suggests that every linear sequence of tetrad in this peptide has the propensity to fold in β-turns. All β-turns are of type III or its mirror image III′. On the other hand, for the corresponding L-copolymer *t*-Boc-(L-Iva)$_8$-OMe, the global minimum and several local minima within 2 kcal/mole are all π-helices. No other type of helix is seen. Thus, LD-peptides constructed from amino acids with disubstitution at the Cα position are incapable of assuming any helical structure. However, the corresponding L-homopolymers are unaffected in their helix-forming capability by such substitution.

Replacing D-Ala by a hydrophilic residue, D-Ser, in the LD-copolymer to form *t*-Boc-(L-Ala-D-Ser)$_4$-OMe, results in a very slight destabilisation of the β-helix. The global minimum in this case is not a β-helix, but a structure with γ-bends at Ala3 and Ala5. Nevertheless, β-helices are found within 1–8 kcal/mole above the global minimum. The population of β-helices among the various helical structures within 15 kcal/mole of the global minimum is $\approx 50\%$. The remaining structures show partial β-helix formations at the N- or the C-terminii. All the β-helices are right-handed. In case of the L-homopolymer, *t*-Boc-(L-Ala-L-Ser)$_4$-OMe, only α-helices are encountered and are 5–15 kcal/mole higher in energy than the global minimum which is characterised by γ-bends at Ala1, Ala3 and Ala5. Both right- and left-handed α-helices are encountered, but right-handed helices have lower energies than the left-handed ones. Thus, the introduction of a hydrophilic residue like D-ser neither greatly destabilises the β-helix nor alters the proportion of β-helical structures in the *ensemble*.

For *t*-Boc-(L-Val-D-Val)$_4$-OMe with a branch at β-position in the side chain, the global minimum is a left-handed β-helix. A single right-handed β-helix is observed but is 10 kcal/mole higher in energy than the left-handed helix. An α-helix is also seen and is about 19 kcal/mole higher in energy compared to the global minimum (β-helix). But for the L-homopolymer, *t*-Boc-(L-Val)$_8$-OMe, α-helices are found within 10 kcal/mole of the global minimum which has a U-shaped backbone with a loop around the central residues stabilised by three hydrogen bonds viz. Val1NH....Val8CO, Val3NH....Val6CO and Val8NH....Val1CO. Most α-helices have small distortions in the backbone torsion angles at the terminal residues. The α-helices are all right-handed as in the previous cases.

For a residue like Ile with a branch at the γ-position in the side chain, the DL-copolymer, *t*-Boc-(D-alloIle-L-Ile)$_3$-OMe, displays greater propensity than in the previous cases to form a β-helix. The global minimum is a right-handed β-helix and many left-handed ones are encountered within 10 kcal/mole of the global minimum. Thus, both left- and right-handed β-helices are favoured, but there is a slight predominance of left-handed helices with this peptide. The most stable structures for the L-homopolymer of Ile, *t*-Boc-(L-Ile)$_6$-OMe lie in shallow broad pools highly populated with either α- or 3_{10}-helices.

Table 1. Backbone torsion angles of some helices obtained for various peptides by MD simulations.

	Stereochemistry of amino acids															
	L1		D2		L3		D4		L5		D6		L7		Ḋ8	
LD-Peptides	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ
t-Boc-(L-Ala-D-Ala)$_4$-OMe	–83	78	83	–133	–86	74	86	–94	–126	81	81	–87	–139	88	85	–
t-Boc-(L-Ala-D-Ser)$_4$-OMe	–84	70	94	–131	–85	75	82	–69	–150	105	68	–116	–115	23	156	–
t-Boc-(L-Val-D-Val)$_4$-OMe	–104	146	86	–83	–84	112	113	–94	–79	91	143	–100	–84	105	126	–
t-Boc-(D-alloIle-L-Ile)$_3$-OMe*	–	–	84	–76	–77	135	87	–81	–81	80	142	–85	–86	–	–	–
t-Boc-(L-Phe-D-Phe)$_4$-OMe	–94	151	88	–85	–87	110	121	–97	–80	92	146	–98	–88	109	150	–
	Residue number															
	1		2		3		4		5		6		7		8	
L-Peptides	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ
t-Boc-(L-Ala)$_8$-OMe	–58	–33	–62	–46	–67	–43	–60	–47	–65	–42	–64	–42	–70	–39	–137	–
t-Boc-(L-Ala-L-ser)4-OMe	–61	–30	–61	–54	–66	–44	–60	–51	–69	–47	–59	–38	–85	10	–151	–
t-Boc-(L-Iva)8-OMe	–53	–34	–54	–20	–54	–31	–56	–26	–55	–27	–55	–32	–63	–20	+38	–
t-Boc-(L-Val)8-OMe	–58	–36	–61	–33	–80	–49	–67	–26	–68	–37	–82	–48	–87	70	–88	–
t-Boc-(L-Ile-)6-OMe	–61	–17	–63	–39	–65	–45	–62	–45	–64	–45	–139	–71	–	–	–	–
t-Boc-(L-Phe)8-OMe	74	–55	–57	–32	–59	–23	–63	–55	–70	–27	–68	–45	–79	–34	–87	–

*Stereochemistry of residues in the sequence is D1, L2, D3, L4,..... .

A bulky side chain (Phe) as present in *t*-Boc-(L-Phe-D-Phe)$_4$-OMe does not appear to hinder β-helix formation. The global minimum is a left-handed β-helix. Many other helices are found within 4·0 kcal/mole of the global minimum and are an assortment of both right- and left-handed β-helices. No α-helix was encountered for the LD-peptide. But in case of its L-homopolymer, *t*-Boc-(L-Phe)$_8$-OMe only α-helices are observed. Some deviations of the backbone torsion angles from the ideal values are noted for the residues, especially at the N-terminal.

The backbone torsion angles of representative helical structures for each of the above classes of peptide are given in table 1.

3.1 *Conformation of side chains in β-helices*

In *t*-Boc-(L-Phe-D-Phe)$_4$-OMe, the side chains show a strong preference for the tg^+ (χ_1, χ_2) arrangement which is followed by a tg^- structure. In *t*-Boc-(D-alloIle-L-Ile)$_3$-OMe a g^-t (χ_1, χ_2) conformation is the most favoured by the side chains. Both *tt* and tg^+ states are the next preferred ones. The lone side chain torsion in *t*-Boc-(L-Val-D-Val)$_4$-OMe takes on the *trans* arrangement.

4. Conclusions

It is evident that peptides containing residues with alternating stereochemistry in the backbone have a distinct ability to form β-helical structures which are not accessible to peptides having solely L-amino acids. The bulk, branching, hydrophilicity/hydrophobicity or stereochemistry of the side chain does not disturb the intrinsic propensity of such peptides to adopt β-helical structures. Also, disubstitution at the Cα of the residues destabilises the β-helix. However, the nature (right- or left-handed) of the β-helix is influenced by type of residues forming the peptide. A small side chain promotes right-handed β-helix formation as seen in the cases of *t*-Boc-(L-Ala-D-Ala)$_4$-OMe and the analog, *t*-Boc-(L-Ala-D-Ser)$_4$-OMe. Bulky side chains and side chains with β- or γ-branches favour both right- and left-handed β-helices.

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