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Predicted conformation of poly(dehydroalanine): A preference for turns ☆

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Abstract

Repetitive conformations of poly(dehydroalanine) were studied using molecular mechanics. An exhaustive search of the conformational space was carried out on a ΔAla octapeptide model, using the AMBER force field and the ΔAla parameters of Alagona et al [26], under three dielectric conditions, $\varepsilon = 1$ (vacuum), $\varepsilon = r$ and $\varepsilon = 4r$ (solvent). In all cases, two major groups of low-energy conformers were found, one corresponding to a regular 3/10 helix or type III turn, the other to an irregular conformation, $\Phi = -157$ to $-170°$, $\Psi = -1$ to $15°$ which however can be found in the i + 2 position of gamma-turns. The data confirm that ΔAla may induce turn-like structures in peptides and also indicate that it may confer flexibility to the peptide chain.

1. Introduction

α,β-Dehydroamino acids are found in several naturally occurring peptides of bacterial origin, and also form an integral part of polycyclic peptide antibiotics like risin, epidermin and subtilin [1–4]. Incorporation of these residues in peptides confers increased resistance to enzymatic degradation and for this reason dehydro residues have been used to design peptide analogues with highly altered bioactivities [5–8].

The inherent structural features of dehydropeptides, such as the planarity of the double bond between C_α and C_β atoms and the geometric restrictions of the side chain, offer a means of introducing local constraints on the peptide backbone. Conformational studies carried out both in solution and in solid state (mostly on dehydrophenylalanine containing peptides) have suggested that β-turn structures are favoured in short sequences [9–12], whereas helical conformations are stabilised in longer sequences [13–18]. However, crystal structure studies of short peptides containing a dehydroalanine (ΔAla) residue have shown that in all cases this residue exhibits an extended conformation [19–21]. Solution conformation studies using NMR and CD spectroscopy supported these finding and, in addition, have pointed out the unusual ability of this residue to induce an inverse γ-turn conformation in the preceding residue of the peptide chain [21,22]. Theoretical investigations and molecular mechanics simulation studies, on the other hand, have suggested that despite the side-chain rigidity due to the presence of the α-β double bond, considerable backbone flexibility may be expected in ΔAla and ΔPhe containing peptides [23,24].

These findings make it worthwhile to assess the conformational preferences of the corresponding homopolymers, which remain an open problem. A better understanding of these structures will be useful in peptide design and in enzyme mimetics. Here we descibe the conformational preferences of poly(dehydroalanine) determined by molecular mechanics calculation on an octapeptide model and provide evidence that ΔAla has a propensity to assess conformations characteristic of type III turns (3/10 helices) and γ-turns.

2. Materials and methods

The minimum energy conformations of poly(dehydroalanine) were determined using a poly-ΔAla octapeptide model, acylated at the N-terminus and methylamidated on

☆ This paper is dedicated to the memory of George Némethy, who recently deceased, in recognition of his contributions to this field, without which this work could not have been accomplished.

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Table 1
The lowest energy structures obtained on constrained minimisation

	$\varepsilon = 1$			$\varepsilon = r$			$\varepsilon = 4r$		
	Φ (°)	Ψ (°)	Energy (kcal/mol)	Φ (°)	Ψ (°)	Energy (kcal/mol)	Φ (°)	Ψ (°)	Energy (kcal/mol)
1	−50	−20	284.8	−50	−30	157.8	−130	−20	117
2	−50	−30	285	−50	−20	159	−140	−10	118.3
3	−60	−20	289	−60	−20	162.3	−140	0	121.5
4	−60	−10	290	−60	−10	164.6	−50	−20	122.6
5	−40	−40	293.5	−40	−40	166	−140	10	123
6	−40	−30	293.6	−40	−30	167.2	−50	−30	123.3
7	−70	−10	299.5	−60	−30	172.7	−60	−10	123.4
8	−60	−30	299.9	−50	−40	173	−60	−20	123.5

the C-terminus. The molecular mechanics calculations were performed with the SYBYL package version 6.01 (Tripos Associates, St. Louis, MO, USA). The AMBER force field [25] was used in al energy minimisations with the ΔAla force-field parameters of Alagona et al. [26]. A cut-off of 50 Å was used in all cases in order to include all possible non bonded interactions. As a first approximation, a grid-search like calculation was performed within the range of −180° to 180°, in which the backbone angles (Φ and Ψ) were set uniformly for all residues in the octapeptide molecule, and were varied with a 10° increment. Each conformer was then energy minimised with fixed Φ and Ψ angles until the RMS of the gradient fell below 0.1 kcal/mol Å. Low-energy conformers were then subjected to unconstrained minimisation (without fixing the backbone dihedral angles) until the RMS of the gradient fell

Table 2
The lowest energy (kcal/mol) structures obtained on unconstrained minimisation

	Starting angles (°)		Minimised average angles (°)		Total energy	E_{VdW}	E_{ele}	E_{Hbond}	E_{tor}	E_{bond}	E_{ang}
	Φ	Ψ	Φ	Ψ							
$\varepsilon = 1$											
1	−50	−20	−33.3	−29.9	244.9	4.4	159.2	−3.6	60.3	6.5	18.2
2	−50	−30	−32.7	−30.9	245.8	2.7	160.1	−3.6	66	6	14.6
3	−60	−10	−33.3	−30	244.9	4.4	159.2	−3.6	60.1	6.5	18.3
4	−60	−20	−32.9	−30.8	246.1	3	161	−3.3	64.8	6	14.8
5	−40	−30	−33.3	−29.9	244.9	4.4	159.2	−3.6	60	6.5	18.3
6	−40	−40	−32.6	−30.8	245.8	3.7	160	−3.7	66.2	6	14.6
7	−130	−20	−160.0	2.0	258.7	0.5	210.5	−2.2	18	10.7	20.0
8	−140	−10	−157.4	5.3	258.1	−0.5	200	−2.1	19.8	8.4	16.3
9	−140	0	−156.8	3.5	261.1	0.6	208.7	−1.9	22.5	9.86	21.1
$\varepsilon = r$											
1	−50	−20	−32.9	−31.3	119.3	3.3	34.1	−3.5	68.6	4.4	12.3
2	−50	−30	−32.6	−31.1	119.3	3.5	34.1	−3.5	68.4	4.4	12.4
3	−60	−10	−32.8	−31.3	119.3	3.4	34.2	−3.5	68.5	4.4	12.3
4	−60	−20	−32.9	−30.8	119.3	3.4	34.2	−3.5	68.5	4.4	12.3
5	−40	−30	−32.9	−31.4	119.3	3.3	34.2	−3.5	68.6	4.4	12.3
6	−40	−40	−32.8	−31.3	119.3	3.4	34.1	−3.4	65.6	4.4	12.3
7	−130	−20	−159.7	1.7	120.3	1.7	74.8	−2.1	16.8	8.8	20.0
8	−140	−10	−160.9	11.4	144.2	0.9	97.6	−0.1	21	8.4	16.3
9	−140	0	−162.6	6.6	144.2	0.8	96	−0.1	23	8.2	16.3
$\varepsilon = 4r$											
1	−50	−20	−32.4	−28.6	86.8	9.8	14	−1	43.2	3.5	17.1
2	−50	−30	−32	−27.8	86.8	10.4	14.1	−1	41.8	3.6	17.9
3	−60	−10	−32	−28.1	86.8	10.1	14.1	−1	42.4	3.6	17.6
4	−60	−20	−32	−28	86.8	10.4	14.1	−1	41.9	3.6	17.8
5	−40	−30	−32.1	−28.3	86.8	10.1	14	−1	42.6	3.6	17.5
6	−40	−40	−32.4	−28.6	86.8	9.8	14	−1	43.5	3.5	17.0
7	−130	−20	−169.4	8.3	61.3	−0.37	30.2	0	5.3	5.8	20.5
8	−140	−10	−169.4	8.2	61.3	−0.31	30.1	0	5.4	5.8	20.4
9	−140	0	−169.2	8.4	61.3	−0.6	30.2	0	5.4	5.8	20.6

below 0.05 kcal/mol Å. We repeated these calculations with all 3 different dielectric conditions: $\varepsilon = 1$, $\varepsilon = r$ and $\varepsilon = 4r$.

A high-resolution subset of the Brookhaven data bank (provided with the SYBYL package) was used to locate 73 residues in proteins that have dihedral angles in the range $\Phi = -140$ to $-170°$ and $\Psi = 0$ to 15°.

3. Results and discussion

Our strategy to find the lowest energy conformations of polydehydroalanine octapeptide consisted in two steps: (i) Exhaustive sampling the Φ,Ψ space in steps of 10° and minimising the resulting 1296 conformers with fix backbone dihedrals ('constrained minimisation' Table 1) and

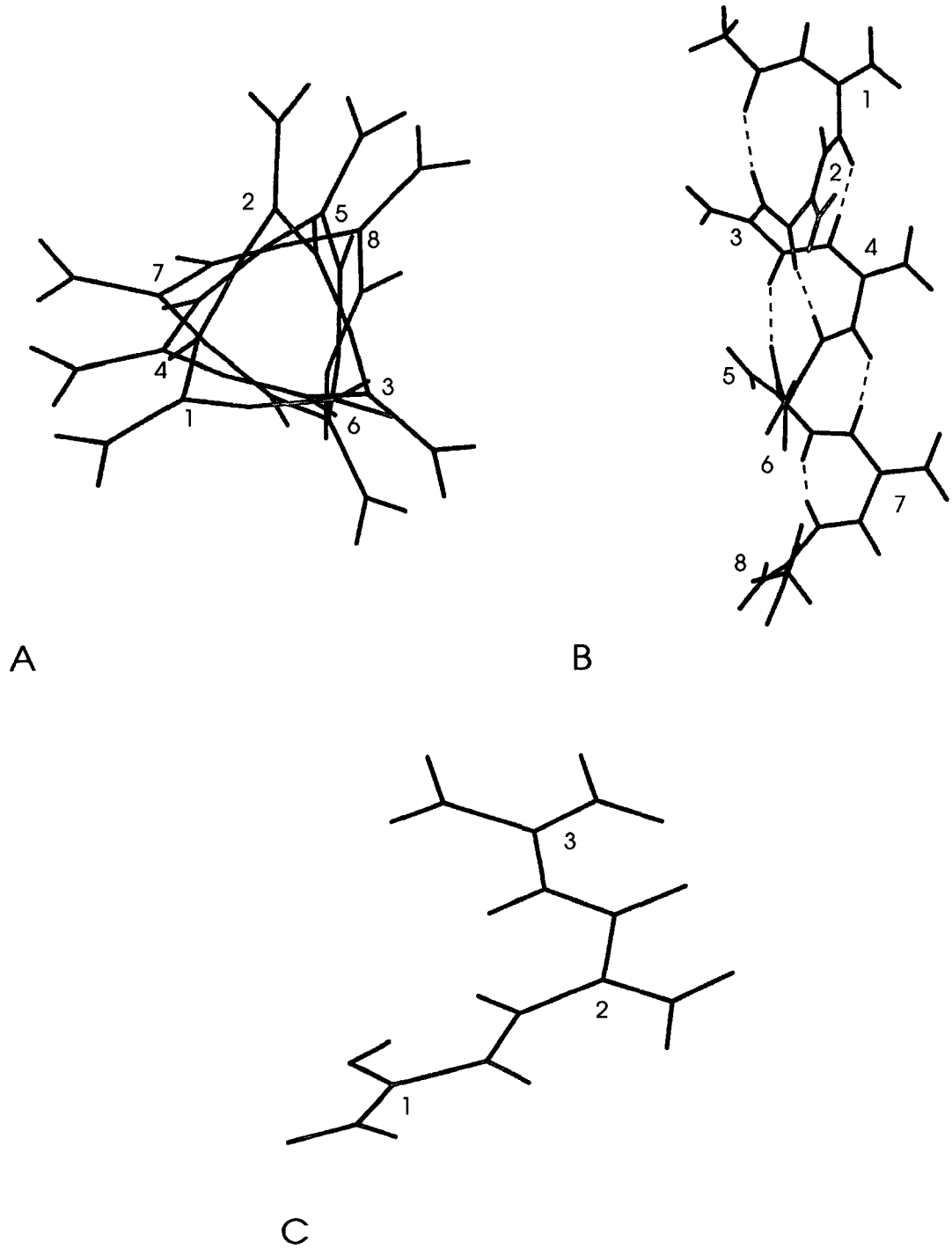

Fig. 1. Schematic representation of the lowest energy structures obtained for the poly-ΔAla octapeptides. (A) and (B) 3/10 helix (structure 8 in Table 2C), and (C) turn-like conformation (tripeptide detail).

(ii) subjecting the 300 lowest energy conformers to unconstrained minimisation in order to find the final energy minima (Table 2). This metod is apparently quite robust when tested with other amino acid homopolymers; for example it gives regular α-helices for a poly-alanine octapeptide in all dielectric conditions tested ($\varepsilon = 1$, $\varepsilon = r$ and $\varepsilon = 4r$, data not shown).

According to Table 2 the low energy conformers obtained under the three dielectric conditions fall in two categories. The first type is 3/10 helix ($\Phi = -32$ to $-33.3°$), $\Psi = -30.0°$ (-28.3 to $-31.3°$) with a characteristic $(i,i+3)$ H-bond pattern and regular chain topology (Fig. 1A and B). The second category is an unusual structure without H-bonds, characterised by average dihedral angles $\Phi = -157°$ to $-170°$ (min. $-170°$, max. $-146°$) $\Psi = 1$–$10°$ (min. $-1°$, max. $12°$) (Fig. 1C). The structure corresponding to the second category is an unusual helix with approx. 4.5 residues/turn and a pitch of approx. 3.2 Å/turn. The homopolymer structure (partially shown in Fig. 1C) is unlikely to occur in proteins or peptides, however, since there is apparently no possibility to form H-bonds that could stabilise the helix (not shown). In contrast to the 3/10 helix, the average Φ,Ψ values of this structure somewhat vary with the dielectric conditions (Table 2). Both types of structures are regular in the sense that the dihedral angles have relatively low standard deviation values within the octapeptide (not shown), those in the 3/10 helix are typically lower than 2° (Table 2). In order to see if structures similar to the second type of ΔAla conformers occur in proteins, we performed a search of the protein structure database for residues with similar dihedral angles ($\Phi = -140$ to $-170°$, $\Psi = 0$ to $15°$). 73 such residues were found, all of them in regions in which the main chain changes direction, such as turns, hairpins, loops and kinks between regular structures etc. (not shown). So, for brevity, we use the name 'turn-like' to designate this conformation. An analysis of the H-bond patterns revealed that the largest group of the retrieved regions is a γ-turn [27,28] (16 cases) and the residue in question invariably occupies the $i+2$ position which immediately follows the centre of the turn. In fact, 3 classical and 13 inverse γ-turns have such a conformation in the $i+2$ position (Table 3). This is in excellent agreement with the experimental finding ΔAla induces a γ-turn in the preceding residue of the peptide chain [21,22].

As expected, the energy of the final conformers depends on the method used for calculating the dielectric constant (Table 2). At $\varepsilon = 1$ (vacuum), 3/10 helix is more stable than the turn-like structure by about 1.9 kcal/residue. At $\varepsilon = 4r$ (solution), the turn-like conformers seem to be more stable, their total energies are about 3 kcal/residue lower than those of the 3/10 helices. The reason of this variance is that the dominating energy factors, i.e. the electrostatic and the torsional terms, change differentially

Table 3
Location of residues with dihedral angles $\Phi = -140°$ to $-170°$ and $\Psi = 1$ to $15°$ in γ-turns

Name of protein	PDB code	Sequence position	Amino acids loop numbering			Main chain dihedral angles					
			i	$i+1$	$i+2$	Φ_i	Ψ_i	Φ_{i+1}	Ψ_{i+1}	Φ_{i+2}	Ψ_{i+2}
IgG Kol Fab' [c]	2FB4	L49–L51	R	D	A	48	57	70	−54	−143	1
IgG B-J 1 dimer [c]	2RHE	A51–53	Y	N	D	55	45	71	−46	−144	12
Catabolic activator pr.	3GAP	A153–155	Q	P	D	−44	168	−72	45	−142	14
IgG * G1 Fab fragment	2HFL	L123–125	L	T	S	−55	−54	−54	4	−166	3
IgG Fab fragment	1MCP	L131–133	L	T	S	−50	−54	−56	39	−168	10
IgG Fab fragment	1MCP	H136–138	P	P	A	−60	128	−54	63	−159	8
IgG Fab'	1F19	H87–89	T	S	E	51	144	−65	31	−164	9
Carboxypeptidease	4CPA	I28–30	W	N	S	−102	114	−83	46	−169	13
Tomato bushy stunt virus	2TBV	A169–171	G	T	T	−130	153	−52	31	−146	0
GAP dehydrogenase	4GPD	I197–199	G	A	A	−59	76	−53	20	−148	11
GAP dehydrogenase [c]	4GPD	I300–302	S	K	T	−159	89	53	−40	−163	14
Cellobiohydrolase	1CBH	A29–31	N	P	Y	−140	176	−69	25	−152	12
Ferredoxin reductase	1FNR	122–124	N	D	A	−91	−174	−66	21	−152	9
Horse deoxy-haemoglobin [b]	2DHB	B40–42	R	F	F	−70	−50	−84	16	−144	4
Xylose isomerase [b]	3XIA	100–102	D	G	G	−174	168	−95	58	−144	14
Leucin-binding protein [b]	2LBP	66–68	V	N	D	−62	−72	−65	27	−141	9
	Poly-ΔAla 'turn-like' conformers [a]								$\varepsilon = 1$	-158	4
									$\varepsilon = r$	-161	7
									$\varepsilon = 4r$	-169	8
	Ideal γ-turn [d]					−172	128	68	−61	−131	162
	classical inverse					−172	128	−78	65	−131	162

[a] Average values calculated from Table 2.
[b] End of α-helix.
[c] Classical γ-turns. All other entries are inverse γ-turns.
[d] After Némethy and Printz [27].

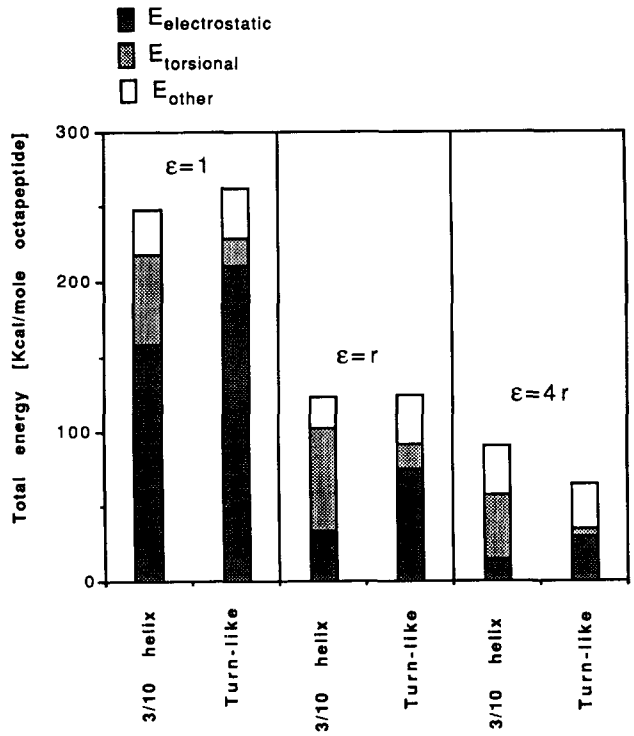

Fig. 2. Distribution of energy terms under different dielectric conditions. The energy components of the lowest energy structures in Table 2 are displayed for each dielectric condition.

when passing from vacuum ($\varepsilon = 1$) to solution ($\varepsilon = 4r$) (Fig. 2).

Our data thus suggest that ΔAla may have two low energy conformations in peptides. One of them, the 3/10 helix or type III turn, is characterised by $(i,i+3)$ H-bonds. The other one seems to be a preferred conformation in γ-turns of proteins which are characterised by $(i,i+2)$ H bonds. In other terms, both of the low energy conformers of ΔAla are related to turns, so ΔAla appears to be a good candidate for inducing turns in synthetic peptides. Furthermore, the two conformations have quite similar energies in solvent-like conditions so it can be supposed that ΔAla residues may confer flexibility to polypeptide chains, as previously suggested [23,24]. It is interesting to note that a similar flexibility was predicted for homopeptides of isoaminobutyric acid (Aib), another achiral amino acid lacking the C_α hydrogen. For shorter poly-Aib peptides, the 3/10 and extended conformations were equally probable [28], for longer peptides however helical conformations (3/10 or a) seemed to be more favoured [29,30].

The scope of our conclusions is naturally limited by the fact that a relatively short peptide was used in the calculations and the stability of helices may vary with the peptide length. Molecular dynamics simulations with explicit solvent molecules are nderway in order to further study the conformations of ΔAla residues, and these may give additional insights into the problem of flexibility around these residues.

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