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The conformational properties of dehydrobutyrine and dehydrovaline: theoretical and solid-state conformational studies

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Dehydrobutyrine is the most naturally occurring dehydroamino acid. It is also the simplest dehydroamino acid having the geometrical isomers *E*/*Z*. To investigate its conformational properties, a theoretical analysis was performed on *N*-acetyl-α,β-dehydrobutyrine *N*′-methylamides, Ac-(*E*)-ΔAbu-NHMe and Ac-(*Z*)-ΔAbu-NHMe, as well as the dehydrovaline derivative Ac-ΔVal-NHMe. The ϕ, ψ potential energy surfaces and the localised conformers were calculated at the B3LYP/6-311++G(d,p) level of theory both *in vacuo* and with inclusion of the solvent (chloroform, water) effect (SCRF method). The X-ray crystal structures of Ac-(*Z*)-ΔAbu-NHMe and Ac-ΔVal-NHMe were determined at 85 and 100 K, respectively. The solid-state conformational preferences for the studied residues have been analysed and compared with the other related structures. Despite the limitations imposed by the $C^{\alpha} = C^{\beta}$ double bond on the topography of the side chains, the main chains of the studied dehydroamino acids are more flexible than in standard alanine. The studied dehydroamino acids differ in their conformational preferences, which depend on the polarity of the environment. This might be a reason why the nature quite precisely differentiates between ΔVal and each of the ΔAbu isomers, and why, particularly so with the latter, they are used as a conformational tool to influence the biological action of usually small, cyclic dehydropeptides. Copyright © 2010 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: nisin; stendomycin; syringopeptin; bogorols; laxaphycin; pahayokolides; antrimycins; lasiodine-A

Introduction

α,β-Didehydro-α-amino acids (in short: dehydroamino acids, ΔXaa) belong to non-standard amino acids occurring in natural peptides. The unsaturated character of the carbon–carbon double bond enables dehydroamino acids to play an effector role, for example, by covalently bonding a toxin molecule to an enzyme [1]. The $C^{\alpha} = C^{\beta}$ bond deprives the α-carbon atom of asymmetry. It prevents rotation of the side chain around the χ^1 torsion angle and the β-substituent in the side chain can adopt only two positions: *Z* or *E*. In results, the dehydroamino acids reveal different conformational features from standard analogues. The presence of the $C^{\alpha} = C^{\beta}$ bond is often important [2] or even indispensable [3–5] for the biological action of the dehydropeptides. Furthermore, the geometrical isomer (*Z* or *E*) can have a spectacular impact on the behaviour of the dehydropeptide [3,6]. This shows that the geometry of the side chain influences the conformational properties of the dehydroamino acid residue, and thus, the bioactive conformation of the whole dehydropeptide molecule.

Dehydrobutyrine, ΔAbu, is the simplest dehydroamino acid that may form the geometrical isomers. Both isomers – (*Z*)-ΔAbu and (*E*)-ΔAbu – occur in nature. *Z*-Dehydrobutyrine was found in nisins, one of the oldest known antibacterial agents, used extensively as a food preservative [7]. It is present in molecules of tiopeptide antibiotics (micrococcins, thiocillins, QN3323 and cyclothiazomycin) highly modified, sulfur-containing, macrocyclic peptides inhibiting protein synthesis in bacteria [8]. It is also found in stendomycin, an antifungal peptide antibiotic [9], kahalalides G and F, with the latter being used in clinical trials for treatment of prostate cancer [10,11], FK228, a histone deacetylase (HDAC) inhibitor [12,13] and the closely related cormycin A [14], syringopeptin [15] and pseudomycins [16,17] displaying phytotoxic antimicrobial activity. *E*-Dehydrobutyrine constitutes bogorols, a new cationic peptide antibiotic [18]; laxaphycin A, a cell growth inhibitory cyclic peptide [19]; lobocyclamide A, which exhibits moderate antifungal activity [20], as well as the related somamides and dolastatin 13 of still unknown biological activities [21,22]. Both isomers are found in largamide H, inhibiting chymotrypsin [23] and pahayokolides, inhibiting a number of cancer cell lines [24,25].

In nature dehydroamino acids which have the alkyl group in both *Z*- and *E*-positions also occur. Dehydrovaline (ΔVal) was found in a linear alkaloid, lasiodine-A, inhibitor of the uncoupled electron transport in chloroplasts [26]. Dehydrovaline and dehydroisoleucine (ΔIle) constitute antrimycins and cirratiomycins,

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tuberculostatic heptapeptide antibiotics [27]. They were found in FR225659, the novel gluconeogenesis inhibitors and potent microtubule depolymerisers [28]. Dehydroisoleucine is present in phomopsin A, a fungal metabolite that binds to microtubules [29].

A comprehensive general view of the conformational properties of dehydroamino acids, including ΔAbu and ΔVal, using theoretical methods was presented by Thormann and Hofmann [30]. A characteristic conformational pattern of the dehydroamino acids, determined by the possible conjugation between the $C^{\alpha} = C^{\beta}$ double bond and neighbouring amide groups was shown and the steric effects of the *Z* and *E* substituents were noticed [30]. The studies performed in solution revealed that in weakly polar environment (DCM, chloroform), ΔAbu (especially the isomer *E*) and ΔVal prefer extended conformations [31–33]. The conformational characteristic of peptides containing dehydroamino acids was reviewed by Jain and Chauhan [31]. However, it was clearly stated that in the case of ΔAbu and ΔVal containing peptides, there was a need for further studies due to an insufficient amount of data.

The aim of this work is to bring a deeper insight into the conformational properties of the ΔAbu and ΔVal residues as well as to evaluate some of the differences between those dehydroamino acid residues which have alkyl substituents in the β-position of the side chain.

Experimental Methods

Theoretical Calculation

The conformational properties of dehydropeptides were studied on the basis of the following molecules of *N*-acetyl-α,β-dehydroamino acid *N*′-methylamides: Ac-(*E*)-ΔAbu-NHMe, Ac-(*Z*)-ΔAbu-NHMe and Ac-ΔVal-NHMe (Figure 1) using the Gaussian 09 package [34]. Calculations were performed on the *trans*-amide bonds (ω_0, ω_1 ~180°). The ϕ, ψ potential energy surfaces of the studied molecules were created on the basis of 84 points calculated at the B3LYP/6-311++G** level of theory. In each of these structures, the geometrical parameters were fully relaxed, except for the constrained torsion angles ϕ and ψ. The 30° increment was applied for the ϕ, ψ main-chain dihedral angles. Within the range −180° to 150° for the torsion angles ϕ and within the range 0–180° for the torsion angles ψ. Due to the achiral α-carbon, inversion through the centre of symmetry (ϕ, ψ = 0°, 0°) yields equivalent structures (i.e. $(\phi, \psi) \rightarrow (-\phi, -\psi)$); therefore full ($\phi$, ψ) potential energy surface maps were obtained in this way. The energy surfaces were obtained using the Surfer 8 program with the radial basis function as a gridding method (Golden Software, Inc. 2002). To estimate the effects of environment (chloroform, water) on the shapes of the energy surfaces, the calculations were conducted in each grid point using a self-consistent reaction field (SCRF) model, with the geometrical parameters fully relaxed, except for the constrained torsion angles ϕ and ψ. The polarisable continuum model (PCM) was chosen [35,36]. The possible energy minima of every low-energy region of the potential energy surfaces were checked by full geometry optimisation of the selected structure at the B3LYP/6-311++G** level *in vacuo* as well as in the chloroform and water mimicking environment using the PCM model. Frequency analyses were carried out to verify the nature of the minimum state of all the stationary points obtained and to calculate the zero-point vibrational energies (ZPVEs).

The notations of the conformations applied in literature were invented for standard amino acids. No one fits to the dehydroamino acids, which usually adopt the values of the main-chain torsion angles ϕ and ψ close to borderline of the conformational regions of the standard analogues. Therefore, some conformers were not strictly labelled according to the nomenclature to avoid the confusion. Additionally, three common nomenclatures for diamide models were applied in tables [37–39] with intentions of better understanding and for convenience of the readers.

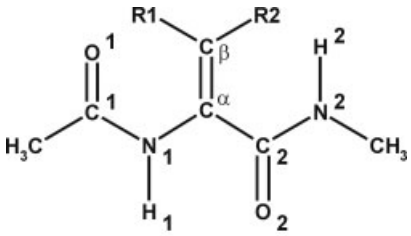

Figure 1. General formula and atom numbering of the model compounds containing the residues studied in this work. Ac-(*E*)-ΔAbu-NHMe (R_1 = H, R_2 = Me), Ac-(*Z*)-ΔAbu-NHMe (R_1 = Me, R_2 = H) and Ac-ΔVal-NHMe ($R_1 = R_2$ = Me).

X-Ray Crystallography

Ac-(*E*)-ΔAbu-NHMe, Ac-(*Z*)-ΔAbu-NHMe and Ac-ΔVal-NHMe were synthesised as described earlier [40]. Only Ac-(*Z*)-ΔAbu-NHMe and Ac-ΔVal-NHMe were obtained in the crystal form. Intensity data for Ac-(*Z*)-ΔAbu-NHMe (at 85 K) and Ac-ΔVal-NHMe (at 100 K) were collected on an Oxford Diffraction Xcalibur PX diffractometer, with the graphite-monochromated MoKα radiation (λ = 0.71073 Å), equipped with an Oxford Cryosystems cooler. Both structures were solved by direct methods and refined on F^2 by the full-matrix least-squares method. The crystal structure of Ac-ΔVal-NHMe is characterised by the presence of whole-molecule orientational disorder. The molecules are disordered in two sites, with equal occupancies (0.5), one rotated by 180° to the other about the pseudo twofold axis approximately through the double C4═C5 bond (Figure 2). All non-H atoms, in both structures, were refined with anisotropic thermal parameters. All of the H-atoms in the structure of Ac-ΔVal-NHMe and the H-atoms bound to C-atoms in the structure of Ac-(*Z*)-ΔAbu-NHMe were included in the geometrically calculated positions and refined using the riding model. The H-atoms bound to N-atoms in the structure of Ac-(*Z*)-ΔAbu-NHMe were located in subsequent difference Fourier maps and isotropically refined. The Oxford Diffraction software was used during the data collection, cell refinement and data-reduction processes (CrysAlis CCD and CrysAlis RED in KUMA KM4 and Xcalibur PX Software. Oxford Diffraction Ltd, Abingdon, England, 2009). SHELXS-97, SHELXL-97 [41] and Mercury [42] programmes were used for the structure solution, refinement and structure drawing.

Crystal data for Ac-(Z)-ΔAbu-NHMe

$C_7H_{12}N_2O_2$, M = 156.19, crystal size 0.6 × 0.11 × 0.08 mm, monoclinic, space group $P2_1/c$, a = 7.019(3), b = 16.013(7), c = 7.895(4) Å, β = 109.82(5)°, V = 834.8(7) Å^3, ρ_{calcd} = 1.243 g/cm^3, Z = 4, μ = 0.092 mm^{-1}, reflections collected 11393, R_{int} = 0.0558, data/parameters 3039/111, GOF on F^2 1.022, R_1 (all data) = 0.0760, wR_2 (all data) = 0.1445.

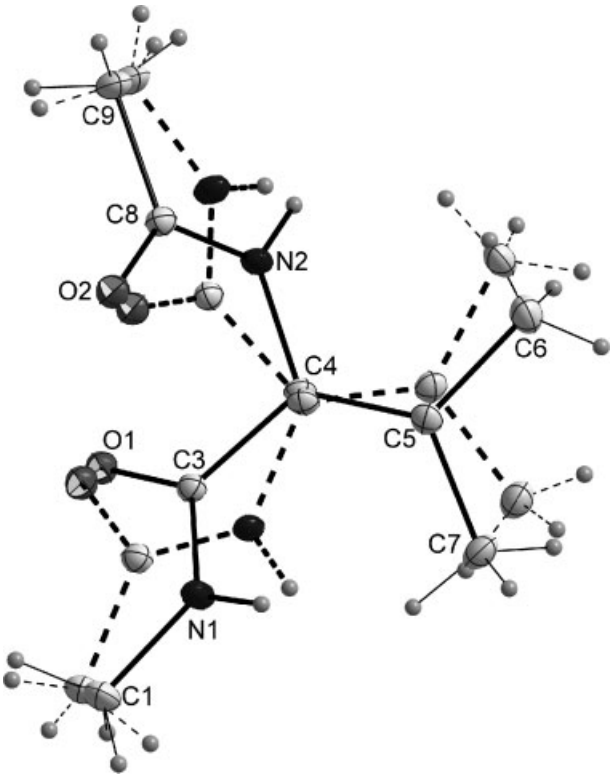

Figure 2. The disordered molecule of Ac-ΔVal-NHMe in the crystal structure at 100 K. The two orientations are distinguished by full and broken lines. Displacement ellipsoids are plotted at the 10% probability level.

Crystal data for Ac-ΔVal-NHMe

$C_8H_{14}N_2O_2$, $M = 170.21$, crystal size $0.36 \times 0.30 \times 0.25$ mm, monoclinic, space group $C2/c$, a = 16.572(6), b = 7.486(3), c = 8.576(3) Å, $\beta = 119.78(5)^\circ$, $V = 923.4(8)$ Å^3, $\rho_{calcd} = 1.224$ g/cm^3, $Z = 4$, $\mu = 0.089$ mm^{-1}, reflections collected 8740, $R_{int} = 0.0200$, data/parameters 2523/113, GOF on F^2 1.009, R_1 (all data) = 0.0675, wR_2 (all data) = 0.1087.

The crystallographic data for Ac-(*Z*)-ΔAbu-NHMe and Ac-ΔVal-NHMe have been deposited at the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC 770515 and CCDC 770516, respectively. The data can be obtained free of charge via ww.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223 336033; e-mail: deposit@ccdc.cam.uk).

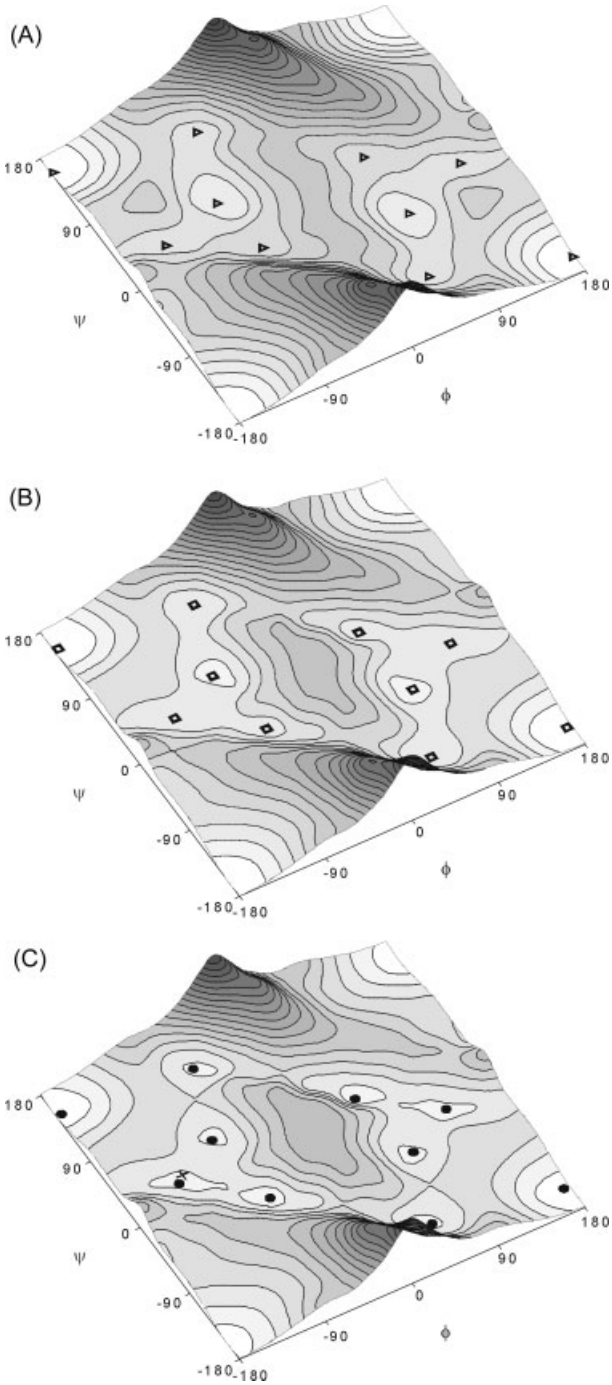

Figure 3. The φ, ψ potential energy surfaces and the conformers of the Ac-(*E*)-ΔAbu-NHMe molecule. Calculation performed: (A) *in vacuo*, (B) chloroform mimicking environment and (C) water mimicking environment. Crosses depict the conformations found in the solid state.

Results

Theoretical Conformational Analysis

Ac-(E)-ΔAbu-NHMe

Figure 3 and Table 1 present the conformational preferences of the Ac-(*E*)-ΔAbu-NHMe molecule. The calculation performed *in vacuo* reveals the following conformers presented according to their increasing energy: C5, C7, β, $\beta 2$ and α. Each of these conformers has its mirror counterpart, which has the opposite sign of all torsion angles but the same energy. Therefore, they are not shown in Table 1. The most stable is the extended conformer C5 and its energy is considerably lower than the remaining conformers. The second conformer, C7, is 1.4 kcal/mol higher in energy. The energy gap between the lowest conformer C5 and the highest conformer α is of the value 3.4 kcal/mol. Regardless of the environment simulated, neither the number nor the kind of conformer changes. A weakly polar environment, mimicking chloroform solvent, changes the energy order only between the two conformers highest in energy, $\beta 2$ and α. Again, the extended conformer C5 is much more stable than the others. However, the energy gap between the lowest conformer, C5, and the highest conformer, $\beta 2$,

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Table 1. Conformers of the Ac-(*E*)-ΔAbu-NHMe molecule together with the known solid-state conformation of the (*E*)-ΔAbu residue

Conformer code[a]						
[37]	[38]	[39]	ϕ (°)	ψ (°)	Energy (hartrees)	ΔE (kcal/mol)
In vacuo						
C5	E	β_L	−178.3	160.4	−533.913154	0.00
C7	C	γ_L	−72.0	48.5	−533.910959	1.38
β	F	ε_L	−48.0	126.0	−533.908726	2.78
$\beta 2$	B	δ_L	−139.2	24.3	−533.908659	2.82
α	A	α	−66.0	−23.5	−533.907770	3.38
Chloroform mimicking environment						
C5	E	β_L	−175.9	155.1	−533.923116	0.00
C7	C	γ_L	−74.4	52.0	−533.921437	1.06
β	F	ε_L	−47.9	131.4	−533.921261	1.17
α	A	α	−64.5	−28.5	−533.920887	1.40
$\beta 2$	B	δ_L	−130.1	24.0	−533.920698	1.52
Water mimicking environment						
α	A	α	−62.5	−31.5	−533.928004	0.00
C5	E	β_L	−174.6	151.7	−533.927947	0.03
β	F	ε_L	−47.9	133.2	−533.927536	0.29
$\beta 2$	B	δ_L	−126.5	22.3	−533.926692	0.82
C7	C	γ_L	−75.3	51.5	−533.926292	1.07
Solid-state conformation						
			−118.97	29.29	[43]	

[a] Each calculated conformer has its mirror counterpart.

decreases and does not exceed 1.5 kcal/mol. A much more polar environment, mimicking water, changes considerably the energy order of the conformers to the following: α, C5, β, $\beta 2$ and C7. The helical conformer α becomes the most stable conformer, but the stability of the conformer C5 of almost the same energy should also be noted. The third is the collagen-like conformer β. The last is the conformer C7. Nevertheless, the conformers differ in energy barely by 1.1 kcal/mol.

Ac-(Z)-ΔAbu-NHMe

Figure 4 shows the conformational maps of the Ac-(*Z*)-ΔAbu-NHMe calculated *in vacuo* and in two solvents. The energies of all its conformers are compared in Table 2. The calculations performed *in vacuo* revealed eight conformers: C5, C7, $\beta 2$, β and their mirror counterparts. The energy gap between the conformers does not exceed 2.4 kcal/mol. Similar to the (*E*)-ΔAbu isomer, the most stable is the extended conformer C5 and the second is the conformer C7, 1.3 kcal/mol higher in energy. In contrast to (*E*)-ΔAbu, the considerable stability of the conformer $\beta 2$ should be noted. The conformer α is also missing and this part of the map is enveloped in the broad region of the conformer C7. The conformer α appears in the weakly polar environment, mimicking chloroform solvent and becomes the second in energy order. The extended conformer, C5, is still the most stable, but interestingly, the conformers differ in energy only within 0.8 kcal/mol. In a more polar environment, mimicking water solvent, as in the case of (*E*)-ΔAbu, the most stable is the helical conformer α. However, the second is the conformer $\beta 2$, not the conformer C5. The third is the collagen-like conformer β. The difference in energy between the three lowest conformers, α, $\beta 2$ and β, does not exceed 0.8 kcal/mol. The conformers C5 and C7 are much less stable. In polar environment, the substituent in the position (*Z*) of the side

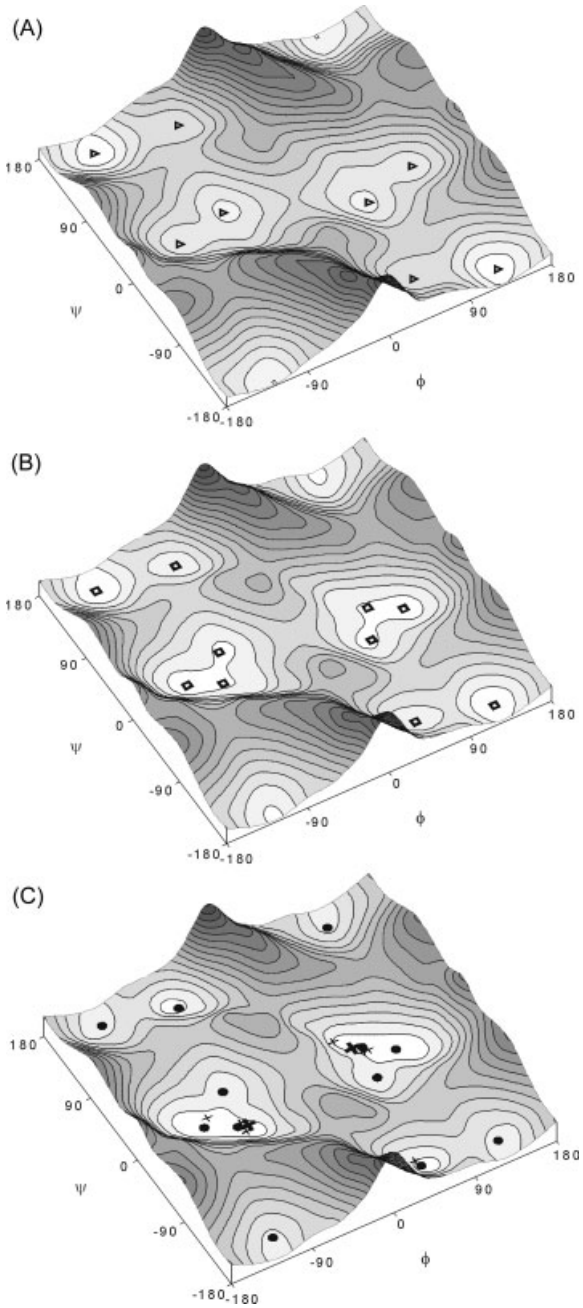

Figure 4. The α, β potential energy surfaces and the conformers of the Ac-(*Z*)-ΔAbu-NHMe molecule. Calculation performed: (A) *in vacuo*, (B) chloroform mimicking environment and (C) water mimicking environment. Crosses depict the conformations found in the solid state. Bold crosses present the conformations of the compounds studied in this work.

chain imposes a steric hindrance and the area of the conformer C5 is divided. The additional extended conformer appears, labelled as C5′, but of the highest energy. In comparison to the chloroform mimicking environment, the energy gap between the conformers increases twofold, up to 1.6 kcal/mol.

Ac-ΔVal-NHMe

Figure 5 and Table 3 present the conformational preferences of the Ac-ΔVal-NHMe molecule. The calculations performed *in vacuo* reveal 12 conformers: C7, C5, $\beta 2$, β, α, C5′ and their mirror

Table 2. Conformers of the Ac-(*Z*)-ΔAbu-NHMe molecule together with the known solid-state conformations of the (*Z*)-ΔAbu and (*Z*)-ΔLeu residues

Conformer code[a]						
[37]	[38]	[39]	ϕ (°)	ψ (°)	Energy (hartrees)	ΔE (kcal/mol)
In vacuo						
C5	E	β_L	−131.5	159.6	−533.914923	0.00
C7	C	γ_L	−64.0	29.3	−533.912800	1.34
β2	B	δ_L	−121.0	13.3	−533.912041	1.81
β	F	ε_L	−49.3	139.9	−533.911047	2.44
Chloroform mimicking environment						
C5	E	β_L	−129.6	157.8	−533.925300	0.00
α	A	α	−83.8	−6.5	−533.924883	0.26
β2	B	δ_L	−114.0	10.2	−533.924789	0.32
β	F	ε_L	−50.7	142.5	−533.924174	0.71
C7	C	γ_L	−67.2	30.1	−533.924105	0.75
Water mimicking environment						
α	A	α	−76.8	−13.5	−533.932012	0.00
β2	B	δ_L	−104.2	3.3	−533.931542	0.30
β	F	ε_L	−51.3	143.8	−533.930784	0.77
C5	E	β_L	−128.5	157.2	−533.930148	1.17
C7	C	γ_L	−68.7	29.3	−533.929437	1.62
C5′	E*	β_L	−120.0	−155.6	−533.929393	1.64
Solid-state conformation						
Z-ΔAbu			−67.38	−16.74	This work	
Z-ΔAbu			67.38	16.74	This work	
Z-ΔAbu			−72.34	−11.21	[44]	
Z-ΔAbu			−72.02	−22.37	[45]	
Z-ΔAbu			−96.52	11.32	[43]	
Z-ΔLeu			54.43	31.15	[46]	
Z-ΔLeu			80.36	7.98	[47]	
Z-ΔLeu			73.82	7.87	[48]	
Z-ΔLeu			47.91	−137.18	[49]	

[a] Each calculated conformer has its mirror counterpart.

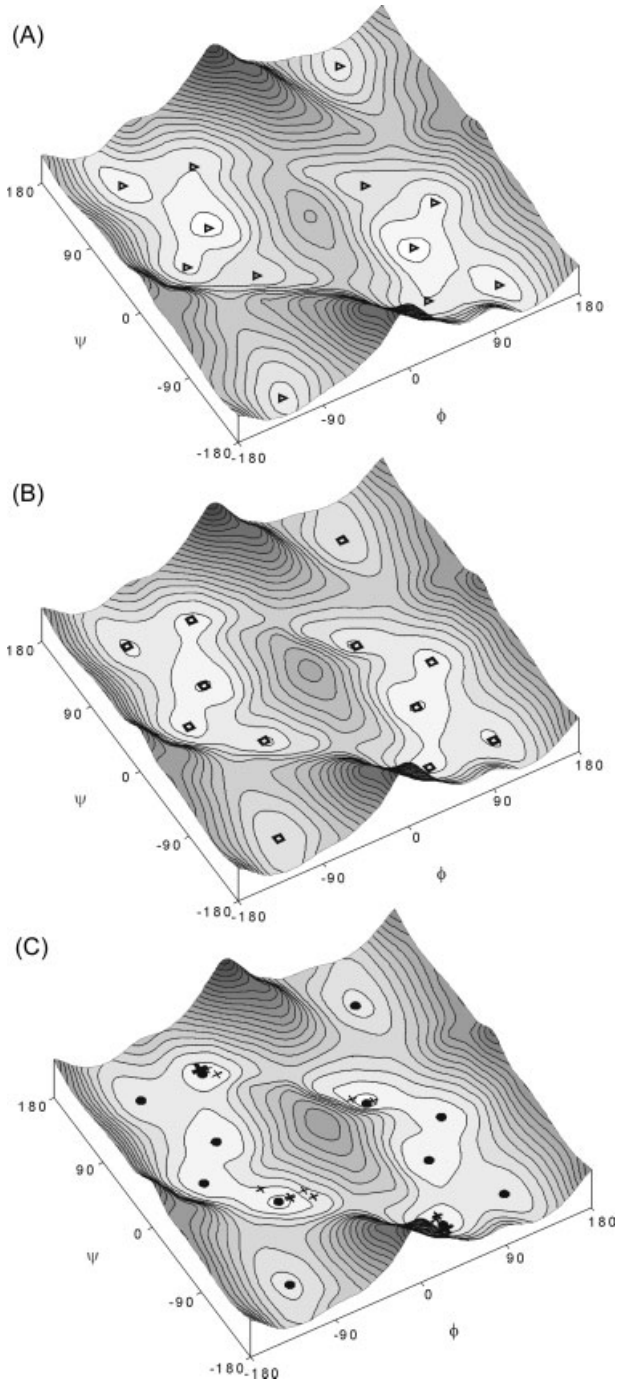

Figure 5. The α, β potential energy surfaces and the conformers of the Ac-ΔVal-NHMe molecule. Calculation performed: (A) *in vacuo*, (B) chloroform mimicking environment and (C) water mimicking environment. Crosses depict the conformations found in the solid state. Bold crosses present the conformations of the compounds studied in this work.

counterparts. The presence of the second extended conformer C5′ should be noted, which was seen only for (*Z*)-ΔAbu in a water environment. Another considerable difference, in comparison to the ΔAbu analogues, is the lowest in energy conformer C7. The energy gap between the lowest conformer C7 and the highest conformer C5′ equals 2.6 kcal/mol. The number and the kind of the conformers do not change, regardless of the environment simulated. In a chloroform mimicking environment, the conformer C7 is still the most stable. The energy order goes as follows: C7, β, β2, α, C5 and C5′. Except for the conformer C5′, the energy difference between the conformers is small, about 1.1 kcal/mol. In a water mimicking polar environment, similarly to ΔAbu, the helical conformer α becomes the most stable. However, the second is the conformer β. The least stable are the extended conformers C5 and C5′. The energy of the four remaining conformers, α, β, C7 and β2, is placed within 1.0 kcal/mol.

X-ray crystal structures – molecular conformations and interactions

Figure 6 depicts the molecular conformations and arrangements of the Ac-(*Z*)-ΔAbu-NHMe and Ac-ΔVal-NHMe molecules in the solid state. The determined bond distances and valence angles in both studied compounds are similar to those found in other related structures [50–55]. The torsion ϕ and ψ angles adopted for Ac-(*Z*)ΔAbu-NHMe and Ac-ΔVal-NHMe molecules are −67.4°, −16.7° and 50.5°, −139.2°, respectively, together with the opposite values (67.4°, 16.7° and −50.5°, 139.2°) characteristic for each two molecules related by inversion centres presented in the crystal structures. All secondary amides are *trans*. The common feature found in both crystal structures is a linear, catemeric molecular arrangement. The main driving forces responsible for the molecular associations are the N–H···O hydrogen bonds.

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Table 3. Conformers of the Ac-ΔVal-NHMe molecule together with the known solid-state conformations of the ΔVal and related residues

Conformer code[a]						
[37]	[38]	[39]	ϕ (°)	ψ (°)	Energy (hartrees)	ΔE (kcal/mol)
In vacuo						
C7	C	γ_L	−75.6	57.3	−573.211026	0.00
C5	E	β_L	−119.7	137.7	−573.209193	1.15
β2	B	δ_L	−117.7	23.1	−573.208805	1.39
β	F	ε_L	−52.7	123.3	−573.208693	1.46
α	A	α	−69.9	−21.7	−573.207166	2.42
C5′	E*	β_L	−122.2	−159.4	−573.206805	2.64
Chloroform mimicking environment						
C7	C	γ_L	−77.7	59.7	−573.222189	0.00
β	F	ε_L	−50.4	130.9	−573.221158	0.65
β2	B	δ_L	−115.6	22.0	−573.220867	0.83
α	A	α	−65.2	−28.2	−573.220806	0.87
C5	E	β_L	−117.2	132.9	−573.220390	1.13
C5′	E*	ε_D	−114.6	−140.4	−573.218681	2.20
Water mimicking environment						
α	A	α	−64.3	−30.9	−573.228376	0.00
β	F	ε_L	−50.1	133.3	−573.227500	0.55
C7	C	γ_L	−78.6	58.3	−573.227239	0.71
β2	B	δ_L	−113.9	19.9	−573.226860	0.95
C5′	E*	ε_D	−109.6	−129.9	−573.225522	1.79
C5	E	β_L	−115.9	131.6	−573.225356	1.89
Solid-state conformation						
ΔVal			−50.51	139.17	This work	
ΔVal			50.51	−139.17	This work	
ΔVal			−37.41	−48.10	[50]	
ΔVal			−53.92	−32.93	[51]	
ΔVal			−73.54	−14.11	[52]	
ΔVal			−41.60	−37.85	[53]	
ΔVal			−44.17	135.89	[54]	
ΔVal			−38.75	126.07	[55]	
(*E*)-ΔIle			−53.68	−34.77	[50]	
(*E*)-ΔIle			53.30	39.77	[56]	
(*E*)-ΔIle			70.31	29.67	[56]	
(*Z*)-ΔIle			36.72	−123.52	[55]	
ΔVal[b]			50.54	−121.27	[57]	
ΔVal[b]			47.83	−125.01	[57]	
ΔVal[b]			38.78	−126.13	[58]	
ΔVal[b]			51.16	−122.44	[58]	
ΔVal[b]			48.02	−124.76	[58]	

[a] Each calculated conformer has its mirror counterpart.
[b] (4-alkylcyclohexylidene)glycine.

The hydrophilic parts of the molecules direct to the 'inside' of the chains, whereas the hydrophobic ones are involved in C–H···O interactions. In both crystal structures each molecule is involved in four N–H···O hydrogen bonds (eight, in total, in the case of the disordered in two sites Ac-ΔVal-NHMe molecules), two with the preceding and two with the following molecule forming a chain. Thus, the mean planes of molecules are placed nearly perpendicularly towards the chain direction. The Ac-(*Z*)-ΔAbu-NHMe molecules (Figure 6A), linked through the N–H···O hydrogen bonds, are arranged alternately (the *N*-terminus is facing the *C*-terminus). The side chains are positioned on one side of the molecular chain. Each molecule in the chain directs its N–H groups of both amides towards one neighbouring molecule while the carbonyl groups towards the other molecule. Thus, each molecule is the donor of the hydrogen bonds for one molecule and at the same time the acceptor for another molecule. In the structure of Ac-ΔVal-NHMe the situation is very similar, but complicated by the disorder. The each site molecules (Figure 6B), connected by the N–H···O hydrogen bonds, are arranged parallel (the *N*-terminus is facing the *N*-terminus). The side chains show an alternate arrangement: up and down, along the molecular chain. Each Ac-ΔVal-NHMe molecule simultaneously plays the role of a hydrogen-bond donor and acceptor for the neighbouring molecules in the chain.

Discussion

The $C^{\alpha} = C^{\beta}$ double bond is the main structural feature of α,β-dehydroamino acids. The first result of its presence is the lack of asymmetry of the α-carbon atom. This influences the shape of the ϕ, ψ potential energy surface of the dehydroamino residue, which is symmetrical through the point (ϕ, $\psi = 0^{\circ}, 0^{\circ}$). In consequence, the conformers of the dehydroamino acids are paired. Each conformer has its mirror counterpart, equal in energy, but for which the signs of all torsion angles have the opposite values (Figures 3–5). Therefore, the number of the conformers of the studied molecules, presented in Tables 1–3 should be doubled (not shown for clarity).

To compare the conformational properties of the studied dehydroamino acid to the standard chiral amino acid, the calculated conformers of the alanine diamide are presented in Table 4. Considering all conformers, including the mirror counterparts, it can be seen that the studied dehydroamino acids' residues possess a greater number of the conformers than the alanine residue. Due to the lack of asymmetry of the α-carbon atom, the dehydroamino acids do not have the conformers C7eq and C7ax, but instead, they have the conformer C7 and conformer −C7, both equal in energy. Again, instead of the right- and left-handed helical conformers, α_R and α_L, there are the conformers α and its counterpart $-\alpha$, equally accessible. Similarly, there are not only the conformers C5, β, β2 and C5′, but also the conformers −C5, $-\beta$, $-\beta$2 and −C5′. Therefore, the dehydroamino acid residues can simultaneously adopt a conformation accessible for both L- and D-amino acids. Additionally, the energy gaps between the lowest and highest conformers of the studied ΔAbu and ΔVal residues are smaller than for those of the standard alanine residue, regardless of the environment simulated (Tables 1–4). Furthermore, for each of the simulated polar environments, the studied dehydroamino acids' residues have a greater number of low-energy conformers ($\Delta E < 2$ kcal/mol) than the standard alanine residue. On the basis of the results presented, it can be concluded that the $C^{\alpha} = C^{\beta}$ double bond in the side chain increases the conformational freedom of the studied dehydroamino acids residues in comparison to standard analogue.

On the other hand, the studied dehydroamino acids reveal considerable conformational stability. All the conformers revealed by the calculation performed *in vacuo* remain in the polar environment. In the case of the (*E*)-ΔAbu and ΔVal residues both the number and the kind of the conformers presented *in vacuo* exist also in polar environment. Only for the *Z*-dehydrobutyrine, the conformers α and C5′ appear with the increases of the polarity of the environment. However, the energy order of the conformers depends on the position of the side chain. This shows

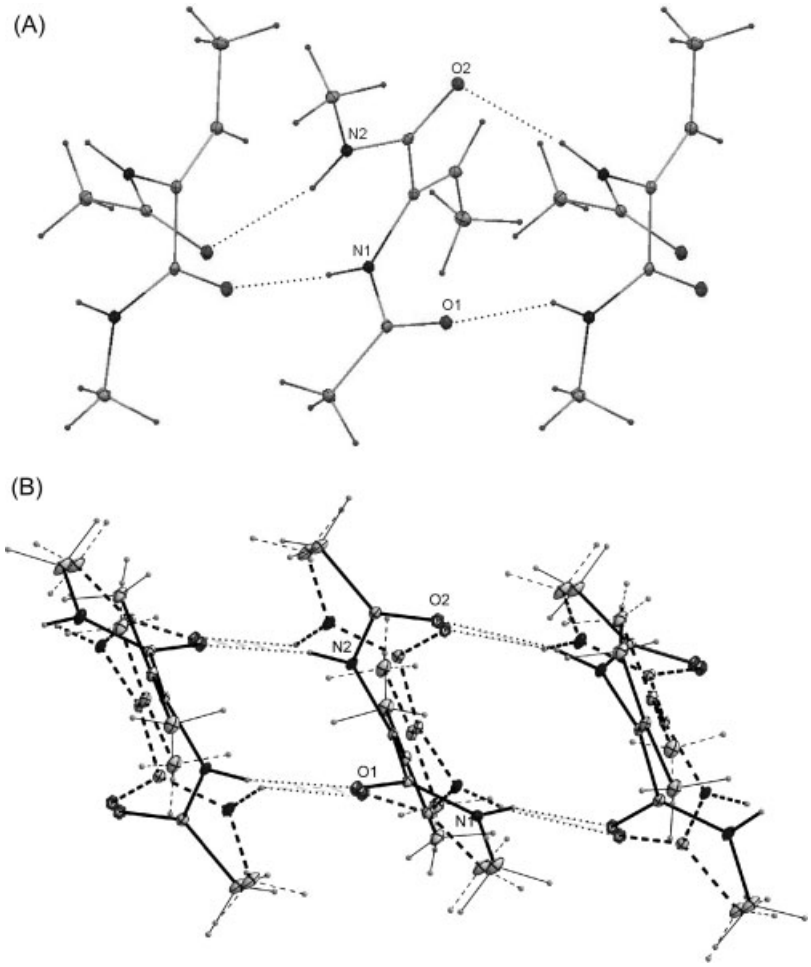

Figure 6. Association of molecules in the crystal structures of: (A) Ac-(*Z*)-ΔAbu-NHMe and (B) Ac-ΔVal-NHMe. The dotted lines indicate N–H···O hydrogen bonds. Displacement ellipsoids are plotted at the 10% probability level.

the differences in the conformational preferences of the studied residues.

In the (*E*)-ΔAbu residue, the β-methyl group does not limit the torsion angle ϕ, which can adopt the value close to $\sim 180^{\circ}$. It enables the π-electron conjugation, characteristic for dehydroamino acids, which envelops the nearly planar arrangement of the $C^{\alpha} = C^{\beta}$ double bond and the flanking amide groups. The concomitant existence of such a π-electron conjugation and the hydrogen C5-type N–H···O bond gives the extended conformation a considerable stability. Therefore, the (*E*)-ΔAbu residue strongly prefers the extended conformation C5, particularly in a weakly polar environment. This is supported by the IR study performed in DCM where the conformation C5 was determined [33]. Simulation of a more polar environment shows the small difference in energy, within 1.1 kcal/mol, between the conformers, which indicates that all of them should be accessible. So far only one crystal structure containing the (*E*)-ΔAbu residue is known, Ac-Pro-(*E*)-ΔAbu-NHMe, where the proline residue and the internal N-H···O hydrogen bond stabilises the adopted conformation [43]. The conformation of the (*E*)-ΔAbu residue (ϕ, $\psi = -119^{\circ}$, 29°) corresponds to the calculated conformer $\beta 2$ (Figure 3C, Table 1). All this together shows that the (*E*)-ΔAbu residue can reveal a bimodal behaviour. In a weakly polar environment it acts as a conformational stiffener adopting primarily the extended conformation C5. In a more polar environment it acts as a conformational releaser with almost all conformers potentially adopted (and doubled), and thus, it is much more flexible than standard amino acid residue.

In the (*Z*)-ΔAbu residue, the β-methyl group in the *Z*-position limits the torsion angle ϕ, which cannot adopt a value close to $\sim 180^{\circ}$. The planarity of the arrangement of the $C^{\alpha} = C^{\beta}$ double bond and the flanking amide groups in the conformer C5 is much more difficult to obtain. As in the case of the isomer *E*, the conformer C5 is still the lowest in energy, also in the weakly polar solvent, which is confirmed by the IR study [31,32]. However, the value of the torsion angle ϕ is about 50° less than for the conformer C5 of (*E*)-ΔAbu. It influences both the π-electron conjugation and the N–H···O hydrogen bond. In consequence, the energy gap between the lowest conformer C5 and the other conformers decreases. It can particularly be seen when a chloroform solvent is simulated. The relative energy amongst conformers does not exceed 0.8 kcal/mol, which is two times smaller than in the case of the isomer *E*. All conformers of (*Z*)-ΔAbu presented in Table 2 (and their mirror counterparts) are within the energy of the three low-energy conformers of the standard alanine (Table 4).

In contrast to the *E*-analogue, an increase in the polarity of the environment increases the energy differences amongst the conformers. Two conformers, α and $\beta 2$, have visibly lower energy than the others. This is supported by the scarce crystallographic data (Figure 4C, Table 2). In the hereunto presented crystal

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Table 4. Conformers of the Ac-L-Ala-NHMe molecule obtained on the basis of the maps earlier published [59]

Conformer code						
[37]	[38]	[39]	ϕ (°)	ψ (°)	Energy (hartrees)	ΔE (kcal/mol)
In vacuo						
C7eq	C	γ_L	−83.5	76.0	−495.826207	0.00
C5	E	β_L	−155.0	159.2	−495.825134	0.68
$\beta 2$	B	δ_L	−115.1	13.6	−495.822498	2.33
C7ax	C*	γ_D	73.1	−56.1	−495.822136	2.56
α_L	A*	α_D	72.0	19.7	−495.818051	5.12
α'	G	δ_D	−164.6	−44.7	−495.816409	6.15
Chloroform mimicking environment						
C5	E	β_L	−151.4	154.9	−495.836526	0.00
C7eq	C	γ_L	−84.6	74.3	−495.836384	0.09
$\beta 2$	B	δ_L	−102.6	0.2	−495.835056	0.92
C7ax	C*	γ_D	73.3	−54.6	−495.832757	2.36
α_L	A*	α_D	69.2	25.3	−495.830733	3.63
α_D	F*	ε_D	58.9	−134.3	−495.829481	4.42
Water mimicking environment						
C5	E	β_L	−152.0	154.9	−495.841460	0.00
α_R	A	α_L	−89.9	−12.0	−495.841354	0.07
β	F	ε_L	−74.4	142.7	−495.840671	0.50
α_L	A*	α_D	64.5	33.4	−495.837589	2.43
C7ax	C*	γ_D	73.4	−53.1	−495.837415	2.54
α_D	F*	ε_D	58.5	−139.2	−495.835771	3.57

structure, the (*Z*)-ΔAbu residue adopts the conformations (ϕ, $\psi = -67^\circ, -17^\circ$ and $67^\circ, 17^\circ$), which corresponds to the calculated conformer α and $-\alpha$. Also, two crystal structure conformations, as known from the literature, correspond to the calculated conformer α [44,45] and one to the conformer $\beta 2$ [43]. Comparison of the conformers α and $\beta 2$ of both geometrical isomers shows the greater stability of those (*Z*)-ΔAbu (Tables 1 and 2). The β-methyl group in the *Z*-position does not limit the torsion angle ψ, which can adopt a value close to $\sim 0^\circ$. The arrangement of the $C^\alpha = C^\beta$ double bond and the *C*-terminal amide group is more planar in these conformers and, possibly, they gain additional stabilisation from the arousing π-electron conjugation. All this together indicates that the (*Z*)-ΔAbu residue can reveal more diverse conformational properties than its geometrical isomer *E*. In a weakly polar environment it possesses considerable conformational freedom and acts as a conformational releaser. In a more polar environment its conformation is more restricted. However, it is still much more flexible than standard amino acid residue.

It should be noted that in the solid state, (*Z*)-dehydroleucine, (*Z*)-ΔLeu, having a longer alkyl substituent in the *Z*-position, also reveals a tendency to adopt the conformations α [46], $\beta 2$ [47,48] and β [49] (Table 2). This shows that the conformational pattern of the (*Z*)-ΔAbu residue can be valid for all dehydroamino acids having an alkyl group in the position *Z* in the main chain.

In the dehydrovaline residue, the β-methyl groups are both in the *E*- and *Z*-positions. Such rigid arrangement of the bulky side chains influences primarily the extended conformer C5 which is no longer the lowest in energy. The presented calculations performed *in vacuo* and in a chloroform mimicking environment show a tendency towards the conformer C7. Similar results were obtained earlier [30]. Nevertheless, the IR study clearly indicates that the extended conformation dominates in solution [32,60].

The calculations performed in the water mimicking environment show that in the more polar environment the dehydrovaline residue adopts primarily the conformation α and β. It is confirmed by the conformations in the solid state (Table 3, Figure 5C). The literature data shows that the conformation α [50–53] and β [54,55] is adopted in the crystals. In the hereunto presented solid structure Ac-ΔVal-NHMe, the residue adopts the conformations of the torsion angles ϕ, $\psi = -51^\circ$, 139° and 51°, -139°, which correspond to the calculated conformer β and $-\beta$. It should be noted that dehydroizoleucine also reveals a similar tendency [50,55,56]. Furthermore, synthesised novel axially chiral dehydroamino acids (4-alkylcyclohexylidene)glycines, adopt the conformation β [57,58]. This indicates that the conformational properties presented by dehydrovaline can be generalised to entire family of dehydroamino acids having the β-alkyl substituents in both the *Z*- and *E*-positions of the side chain.

Apart from the conformational properties of the studied dehydroamino acids, their associational features should be mentioned. Both the Ac-ΔVal-NHMe and Ac-(*Z*)-ΔAbu-NHMe molecules adopt in the crystal structures a linear, catemeric molecular arrangement, where the molecules are connected with each other by means of two N–H···O hydrogen bonds (Figure 6). Analogous Ac-ΔAla-NHMe and Ac-(*Z*)-ΔPhe-NHMe reveal different molecular interactions. The Ac-(*Z*)-ΔPhe-NHMe molecules form three-dimensional network, where each molecule is connected by single N–H···O hydrogen bond to four other molecules [61]. In the case of Ac-ΔAla-NHMe, there is a two-dimensional layered network. The molecules form dimers joined together by two N–H···O hydrogen bonds. Additionally, each molecule of the dimer forms two N–H···O hydrogen bonds with two neighbouring dimers. As a result each Ac-ΔAla-NHMe dimer is surrounded by four other dimers [62].

Conclusion

Dehydrobutyrine is the simplest dehydroamino acid that may form the geometrical isomers *Z* and *E*. Both isomers occur in natural peptides which indicate that the position of the alkyl group in the side chain has considerable and various influence on the biological activity of the dehydropeptide. The properties of the ΔAbu residues differ from those of the ΔVal residue, which represents the simplest dehydroamino acid with the alkyl groups in both *Z*- and *E*-positions. It was found that in a highly polar environment the studied dehydroamino acids reveal a tendency to adopt primarily the helical conformation α. Additionally, the (*Z*)-ΔAbu residue prefers a semi-extended conformation $\beta 2$, whereas the ΔVal residue tends to adopt the conformation β. The (*E*)-ΔAbu residue seems to be very flexible, with no clear conformational preferences. The dehydroamino acids with longer alkyl chain, such as (*Z*)-ΔLeu as well as ΔIle or artificial (4-alkylcyclohexylidene)glycines reveal similar conformational properties to ΔAbu and ΔVal, respectively. This enables an assumption that, at least in a polar environment, ΔAbu and ΔVal can constitute a conformational pattern of analogous structures. As the polarity of the environment decreases, both dehydrobutyrine isomers prefer the extended conformation C5. This particular tendency towards the extended conformation shows the (*E*)-ΔAbu residue. In contrast, the (*Z*)-ΔAbu residue reveals considerable conformational flexibility. The lack of the asymmetry of the α-carbon atom, the greater number of conformers and the smaller differences in their relative energy reveal that despite the limitation imposed on the side

chain, the studied dehydroamino acids should be perceived as conformationally more flexible than standard amino acids. This is a possible reason why nature often applies them in small, cyclic peptides and why amongst naturally occurring dehydroamino acids, dehydrobutyrine is the most prevalent.

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