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Design of peptides with α,β-dehydro-residues: synthesis, crystal structure and molecular conformation of a tetrapeptide *Z*-ΔVal-Val-ΔPhe-Ile-Ome

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Abstract

This is the first designed peptide with a combination of a branched β-carbon ΔVal and a ΔPhe residues. The peptide *Z*-ΔVal-Val-ΔPhe-Ile-Ome was synthesized in solution phase. Single crystals were grown by slow evaporation from its solution in acetone–water mixture at 25 °C. The crystals belong to an orthorhombic space group $P2_12_12_1$ with $a = 12.513(2)$ Å, $b = 15.904(5)$ Å, $c = 17.686(2)$ Å and $Z = 4$. The structure was determined by direct methods and refined by least-squares procedure to an *R* factor of 0.082. The peptide adopts a 3_{10}-helical conformation with two intramolecular hydrogen bonds ($i + 3 \rightarrow i$) involving carbonyl oxygen atoms of carbobenzoxy group and ΔVal and NH groups of ΔPhe and Ile with distances of 2.764(6) and 3.047(7) Å, respectively. The structure determination has revealed that a tetrapeptide with ΔVal at ($i + 1$) and ΔPhe at ($i + 3$) positions adopts a folded conformation despite the presence of unfavorable branched β-carbon residues Val and Ile at ($i + 2$) and ($i + 4$) positions, respectively. The packing of the molecules in the unit cell is stabilized by two intermolecular hydrogen bonds involving NH groups of ΔVal and Val residues with carbonyl oxygen atoms of Val and ΔPhe residues belonging to a symmetry related molecule. The side chains of Val, ΔPhe and Ile form infinite chains of van der Waals interactions.

Keywords: Peptide design; X-ray diffraction; ΔVal residue; ΔPhe residue; Conformation; Crystal structure

1. Introduction

Dehydro-amino acid residues are strong inducers of folded conformations [1]. The branched β-carbon dehydro-residues such as ΔVal and ΔIle introduce steric constraints in peptides that differ significantly from those of ΔPhe and other non-branched β-carbon residues. In order to determine the combined effect of ΔVal and ΔPhe on the resulting conformation of a peptide, we have designed and synthesized a peptide with both ΔVal and ΔPhe at ($i + 1$) and ($i + 3$) positions, respectively. We report here the synthesis, crystal structure and molecular conformation of *Z*-ΔVal-Val-ΔPhe-Ile-Ome.

2. Experimental

The peptide *Z*-ΔVal-Val-ΔPhe-Ile-Ome was synthesized using the following six steps.

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2.1. Synthesis of Z-ΔVal-OH (1)

The compound (1) was synthesized by the condensation of 2-oxo-3-methyl-butanoic acid (0.9 g, 7.8 mmol) with benzyl carbamate (1.4 g, 9.4 mmol) and *p*-toluene sulfonic acid (0.27 g, 9.4 mmol) in dry benzene. The reaction mixture was refluxed at 100 °C using Dean and Stark water remover for 8 h. Then the solution was extracted with saturated sodium bicarbonate. The extracts were neutralized by adding concentrated hydrochloric acid drop wise to yield a white solid, which was filtered and recrystallized from benzene. The solid product of *Z*-ΔVal-OH was obtained with a yield of 67%.

2.2. Synthesis of Boc-Val-(β-OH)-Phe-OH (2)

To a precooled solution of (1) (1 g, 4.6 mmol) in tetrahydrofuran (THF), *N*-methylmorpholine (NMM) (0.5 ml, 4.6 mmol) and isobutylchloroformate (IBCF) (0.61 ml, 4.6 mmol) were added and stirred for 20 min at − 10 °C. To this, a precooled solution of Phe-(β-OH) (1 g, 5.5 mmol) in 1N NaOH (5.5 ml) was added and the mixture was stirred for 3 h at 0 °C and then at room temperature overnight. The organic solvent was removed in vacuo and the aqueous phase was acidified with citric acid to pH 3 and extracted with ethyl acetate. The organic layer was washed with water and dried over anhydrous sodium sulphate and evaporated to yield 80% of compound (2).

2.3. Synthesis of Boc-Val-ΔPhe-azlactone (3)

Compound (2) (1.78 g, 4.7 mmol) was reacted with anhydrous sodium acetate (0.46 g, 5.6 mmol) and freshly distilled acetic anhydride (10 ml) for 24 h at room temperature. The reaction mixture was then poured over crushed ice, the resultant product was washed with 5% sodium bicarbonate and water and finally recrystallized from acetone–water mixture to yield 82% of compound (3).

2.4. Synthesis of Boc-Val-ΔPhe-Ile-Ome (4)

To a solution of compound (3) (1 g, 2.9 mmol) in dichloromethane (DCM) (10 ml), Ile-Ome·HCl (0.63 g, 3.4 mmol) was added followed by triethylamine (TEA) (0.47 ml, 3.4 mmol). This mixture was stirred for 80 h. The solvent was evaporated and the residue was dissolved in ethyl acetate. It was washed with 10% sodium bicarbonate, 5% citric acid and water, respectively, and dried over anhydrous sodium sulphate. The solvent was removed under reduced pressure and the solid product (4) was obtained at a yield of 83%.

2.5. Removal of Boc from compound (4) to get TFA-Val-ΔPhe-Ile-Ome (5)

Compound (4) (2.16 g, 4.7 mmol) was dissolved in 0.5 ml of trifluoroacetic acid (TFA) and 0.5 ml of DCM and stirred for 1 h. The solvent was removed in vacuo to yield compound (5) on addition of dry ether with a yield of 78%.

2.6. Synthesis of Z-ΔVal-Val-ΔPhe-Ile-Ome (6)

To a solution of the compound (1) (0.18 g, 0.68 mmol) in DCM (10 ml), compound (5) was added along with TEA (0.4 ml, 2.71 mmol). The coupling was carried out as mentioned for (4) to obtain 74% of the final compound.

2.7. ^1H NMR of Z-ΔVal-Val-ΔPhe-Ile-Ome

In order to confirm the correctness of the final synthesis of the peptide, ^1H NMR spectra were recorded in $CDCl_3$ with 400 MHz Bruker DRX 400 instrument and the following results were obtained: δ 0.88–0.95 (m, 6H, $C^{\gamma 2}$, C^{δ} Ile); δ 1.04–1.06 (m, 2H, $C^{\gamma 1}$ Ile); δ 1.52 (m, 12H, $C^{\gamma 1}$, $C^{\gamma 2}$, ΔVal, Val); δ 1.77 (m, 3H, $C^{\gamma 1}$, C^{β} Ile); δ 1.98 (m, 1H, C^{β} Val); δ 3.74 (s, 3H, Ome); δ 4.46 (m, 1H, C^{α} Ile); δ 4.63–4.67 (m, 2H, –CH2, *Z*); δ 4.82–4.85 (d, 1H, C^{α} Val); δ 6.12 (bs, 1H, NH ΔVal); δ 6.31–6.33 (bd, 1H, NH Val); δ 7.26–7.28 (m, 11H, Ar, *Z*, ΔPhe, C^{β} ΔPhe); δ 7.52 (s, 1H, NH ΔPhe), δ 8.71 (s, 1H, NH Ile). The observed ^1H NMR spectra clearly indicated the synthesis of the correct peptide.

2.8. Structure determination

The peptide was crystallized from its solution in acetone–water mixture at room temperature

Table 1
The details of intensity data collection and refinement for *Z*-ΔVal-Val-ΔPhe-Ile-Ome

Molecular formula	$C_{34}H_{44}N_4O_7$
Molecular weight	620.73
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
a (Å)	12.513(2)
b (Å)	15.904(5)
c (Å)	17.686(2)
Vn (Å^3)	3519.6(4)
Z (molecules/unit cell)	4
dc (g cm^{-3})	1.17
F (000)	1328
Total no. of independent reflections	3710
No. of observed reflections ($I \geq 2\sigma(I)$)	2371
Radiation (λ, Cu K_α/Å)	1.5418
μr	0.67
Instrument used	Enraf-Nonius CAD4
Mode of data collection	$\omega-2\theta$
Crystal dimension (mm^3)	0.6 × 0.3 × 0.2
R	0.082
R_w	0.153
S (Goodness of fit)	1.05
Temperature (K)	293

(298 K) by slow evaporation method. The crystal data are given in Table 1. The unit cell parameters were refined by a least-squares fit of 25 high angle ($25 \leq \theta \leq 40°$) reflections. These reflections were centered individually on the diffractometer. Lorentz and polarization corrections were applied. The absorption corrections were not applied. The structure was determined by direct methods using the program SHELXS 97 [2]. The coordinates of non-hydrogen atoms were refined anisotropically using program SHELXL 97 [3]. The coordinates of hydrogen atoms were obtained from difference Fourier map and were included in the final cycles of refinement using isotropic temperature factors of non-hydrogen atoms to which they were attached. The final *R*-factor for 2371 observed reflections ($I \geq 2\sigma(I)$) was 0.082. The details of intensity data collection and refinement are given in Table 1. The atomic scattering factors used in these calculations were those of Cromer and Mann [4] for non-hydrogen atoms and Steward et al. [5] for hydrogen atoms. The final positional and equivalent isotropic thermal parameters of non-hydrogen atoms are given in Table 2.

Table 2
Atomic coordinates ($\times 10^4$) and equivalent isotropic thermal parameters ($\times 10^3$) of non-hydrogen atoms in *Z*-ΔVal-Val-ΔPhe-Ile-Ome (estimated standard deviations are given in parentheses)

Atoms	*x*	*y*	z	U_{eq} (Å^2)[a]
C_{01}	1384(12)	7901(7)	10855(6)	135(4)
C_{02}	1131(15)	7813(9)	11630(6)	143(6)
C_{03}	144(18)	7526(10)	11752(7)	163(7)
C_{04}	−543(14)	7274(8)	11205(9)	149(5)
C_{05}	−284(14)	7356(7)	10488(7)	133(4)
C_{06}	607(8)	7668(5)	10285(4)	79(2)
C_{07}	956(10)	7719(7)	9486(6)	120(3)
O_0	167(8)	8241(4)	9079(3)	147(3)
C'_0	−455(9)	7897(6)	8553(4)	97(3)
O'_0	−633(8)	7142(4)	8500(3)	129(3)
N_1	−919(5)	8467(3)	8093(3)	67(1)
C_1^α	−1497(5)	8248(4)	7450(3)	60(1)
C_1^β	−2429(6)	8647(4)	7286(4)	75(2)
$C_1^{\gamma 1}$	−2897(6)	9282(5)	7800(5)	91(2)
$C_1^{\gamma 2}$	−3073(6)	8491(5)	6589(5)	89(2)
C'_1	−1088(5)	7533(4)	6979(3)	63(1)
O'_1	−1692(4)	7005(3)	6709(3)	82(1)
N_2	−38(5)	7503(3)	6875(3)	59(1)
C_2^α	435(6)	6820(4)	6388(4)	66(2)
C_2^β	1513(8)	7043(5)	6094(6)	105(3)
$C_2^{\gamma 1}$	2356(6)	7139(6)	6727(8)	124(4)
$C_2^{\gamma 2}$	1502(12)	7780(6)	5576(7)	148(5)
C'_2	404(5)	5970(4)	6790(4)	61(2)
O'_2	728(4)	5339(3)	6438(3)	77(1)
N_3	52(4)	5931(3)	7489(3)	56(1)
C_3^α	−239(5)	5146(3)	7839(3)	56(1)
C_3^β	161(6)	4867(4)	8478(3)	67(2)
C_3^γ	1067(6)	5162(4)	8928(4)	75(2)
$C_3^{\delta 1}$	1216(10)	4854(6)	9649(5)	109(3)
$C_3^{\delta 2}$	1865(7)	5672(5)	8673(5)	95(2)
$C_3^{\varepsilon 1}$	2079(10)	5067(8)	10094(6)	136(4)
$C_3^{\varepsilon 2}$	2731(8)	5893(6)	9110(7)	118(4)
C_3^η	2807(10)	5591(7)	9822(7)	120(4)
C'_3	−1149(5)	4670(4)	7477(4)	65(2)
O'_3	−1313(4)	3926(3)	7628(3)	82(1)
N_4	−1752(4)	5115(3)	6990(3)	69(1)
C_4^α	−2578(5)	4692(5)	6528(4)	71(2)
C_4^β	−3700(7)	4973(6)	6729(5)	94(3)
$C_4^{\gamma 1}$	−3932(7)	4800(8)	7561(7)	126(4)
$C_4^{\gamma 2}$	−3946(7)	5866(6)	6509(6)	107(3)
C_4^δ	−5045(9)	6148(9)	6548(8)	141(4)
C'_4	−2363(7)	4794(6)	5697(5)	92(2)
O'_4	−2881(6)	4438(6)	5233(4)	149(3)
O_5^T	−1568(7)	5265(5)	5534(3)	120(2)
C_5^T	−1250(14)	5294(9)	4756(8)	158(5)

[a] $U_{eq} = (1/3)\sum_i \sum_j U_{ij} a_i a_j (\mathbf{a}_i \cdot \mathbf{a}_j)$.

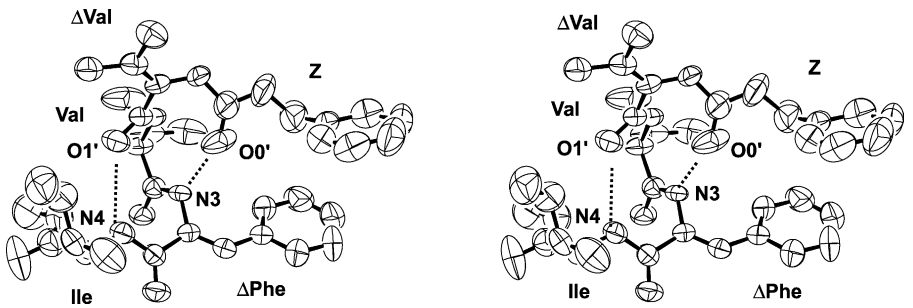

Fig. 1. Stereoview of the molecule *Z*-ΔVal-Val-ΔPhe-Ile-Ome. The residues and important atoms are labeled. The hydrogen bonds are shown by dotted lines.

3. Results and discussion

The stereoview of the peptide *Z*-ΔVal-Val-ΔPhe-Ile-Ome is shown in Fig. 1. The peptide molecule is characterized by 3_{10}-helical conformation which is stabilized by two intramolecular $(i+3) \rightarrow (i)$ hydrogen bonds.

3.1. Molecular dimensions

The C=C double bonds in the two dehydro-residues ΔVal and ΔPhe have bond lengths of 1.36(1) and 1.31(1) Å, respectively, and compare well with a classical C=C double bond distance of 1.337 Å [6].

3.2. Conformation of the peptide

The peptide *Z*-ΔVal-Val-ΔPhe-Ile-Ome adopts a 3_{10}-helical conformation with torsion angles $\phi 1 = -38.7(10)°$, $\psi_1 = -41.1(8)°$, $\phi_{2=} - 73.0(8)°$, $\psi_2 = -3.8(9)°$, $\phi_3 = -62.0(7)°$, $\psi_3 = -15.5(8)°$, $\phi_4 = -119.7(7)°$ and $\psi_4^{\mathrm{T}} = -4.6(10)deg$;. The structure is further characterized by the presence of two intramolecular $(i+3 \rightarrow i)$ hydrogen bonds involving NH groups of ΔPhe and Ile as donors and carbonyl oxygen atoms of *Z* group and ΔVal. The ΔVal is located at $(i+1)$ position of the first β-turn while ΔPhe is positioned at $(i+2)$ position of the second β-turn. Both dehydro-residues at their respective positions in isolated sequences generally induce type II β-turn conformation [7,8] except when surrounded by branched β-carbon dehydro-residues [9]. As observed in the present case, the mixing of these two dehydro-residues results in the formation of a right handed 3_{10}-helical structure. Although, the backbone torsion angles are significantly deviated from the ideal values of $-60°$, $-30°$, the characteristic hydrogen bonds are formed properly. The selected torsion angles in the peptide are given in Table 3. As indicated by the torsion angles of ΔVal ($\chi_1^{1,1} = -3.9(10)°, \chi_1^{1,2} = 176.4(7)°$) and ΔPhe ($\chi_3^1 = -10.8(11)°, \chi_3^{2,1} = 168.5(8)°, \chi_3^{2,2} = 18.0(12)°$), the side chains of both dehydro-residues are essentially planar. Therefore, both ΔVal at $(i+1)$ and ΔPhe at

Table 3
Selected torsion angles (°) involving non-hydrogen atoms in *Z*-ΔVal-Val-ΔPhe-Ile-Ome (estimated standard deviations are given in parentheses)

θ_0	$C_{07}-O_0-C_0'-N_1$	−163.1(8)
ω_0	$O_0-C_0'-N_1-C_1^{\alpha}$	172.1(7)
ϕ_1	$C_0'-N_1-C_1^{\alpha}-C_1'$	−38.7(10)
$\chi_1^{1,1}$	$N_1-C_1^{\alpha}-C_1^{\beta}-C_1^{\gamma 1}$	−3.9(10)
$\chi_1^{1,2}$	$N_1-C_1^{\alpha}-C_1^{\beta}-C_1^{\gamma 2}$	176.4(7)
ψ_1	$N_1-C_1^{\alpha}-C_1'-N_2$	−41.1(8)
ω_1	$C_1^{\alpha}-C_1'-N_2-C_2^{\alpha}$	−178.0(5)
ϕ_2	$C_1'-N_2-C_2^{\alpha}-C_2'$	−73.0(8)
$\chi_2^{1,2}$	$N_2-C_2^{\alpha}-C_2^{\beta}-C_2^{\gamma 2}$	−64.1(11)
$\chi_2^{1,1}$	$N_2-C_2^{\alpha}-C_2^{\beta}-C_2^{\gamma 1}$	64.5(8)
ψ_2	$N_2-C_2^{\alpha}-C_2'-N_3$	−3.8(9)
ω_2	$C_2^{\alpha}-C_2'-N_3-C_3^{\alpha}$	165.0(6)
ϕ_3	$C_2'-N_3-C_3-C_3'$	−62.0(7)
χ_3^1	$N_3-C_3^{\alpha}-C_3^{\beta}-C_3^{\gamma}$	−10.8(11)
$\chi_3^{2,2}$	$C_3^{\alpha}-C_3^{\beta}-C_3^{\gamma}-C_3^{\delta 2}$	18.0(12)
$\chi_3^{2,1}$	$C_3^{\alpha}-C_3^{\beta}-C_3^{\gamma}-C_3^{\delta 1}$	168.5(8)
ψ_3	$N_3-C_3^{\alpha}-C_3'-N_4$	−15.5(8)
ω_3	$C_3^{\alpha}-C_3'-N_4-C_4^{\alpha}$	172.2(5)
ϕ_4	$C_3'-N_4-C_4^{\alpha}-C_4'$	−119.7(7)
ψ_4	$N_4-C_4^{\alpha}-C_4'-O_5^{\mathrm{T}}$	−4.6(10)
$\chi_4^{1,1}$	$N_4-C_4^{\alpha}-C_4^{\beta}-C_4^{\gamma 1}$	69.2(9)
$\chi_4^{1,2}$	$N_4-C_4^{\alpha}-C_4^{\beta}-C_4^{\gamma 2}$	−58.6(9)
χ_4^2	$C_4^{\alpha}-C_4^{\beta}-C_4^{\gamma 1}-C_4^{\delta}$	168.9(9)

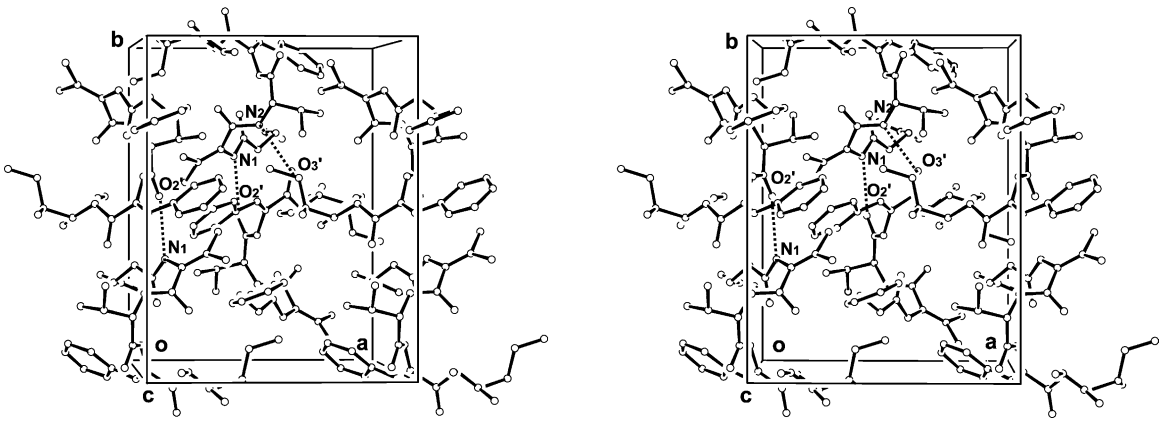

Fig. 2. Stereoview of the crystal packing of peptide Z-ΔVal-Val-ΔPhe-Ile-Ome. The hydrogen bonds are indicated by dotted lines. The atoms involved in the intermolecular hydrogen bonds are labeled.

$(i+3)$ positions can be accommodated in a 3_{10}-helical conformation without serious distortions.

3.3. Crystal packing

The molecular packing in the crystals is shown in Fig. 2. The C=O and N–H groups that are not involved in intramolecular hydrogen bonds are located at the opposite ends of the helices. This makes possible the formation of intermolecular hydrogen bonds between consecutive molecules, which results in a head to tail alignment and forms helical columns with a hydrogen-bonding pattern similar to that of a long 3_{10}-helical chain.

The parameters of hydrogen bonds in the structure are listed in Table 4. As seen from Fig. 2, the side chains of ΔVal and ΔPhe belonging to neighboring columns, interdigitate to form strong hydrophobic contacts. In the adjacent part that runs parallel to the hydrophobic channel, a polar environment is generated with NH and carbonyl groups which is responsible for intermolecular hydrogen bonds.

4. Conclusions

These results show that the combination of dehydro-residues induce folded conformations in peptides. It may be mentioned here that, the substitution of a ΔVal at $(i+1)$ position induces a type II β-turn conformation [7]. It may also be noted that a peptide with a ΔPhe at $(i+2)$ position adopts a β-turn II conformation [8]. Furthermore, if a ΔPhe is substituted at $(i+2)$ position with a branched β-carbon residue either at $(i+1)$ or at $(i+3)$ positions, the peptide is found in a distorted β-turn II conformation [10a,b]. Still further, a peptide containing a ΔPhe at $(i+2)$ position with branched β-carbon residues, such as Val and Ile at both $(i+1)$ and $(i+3)$ positions, adopts an unfolded conformation [9]. However, the presence of more than one ΔPhe residues in the sequence invariably produces a 3_{10}-helical conformation [11,12].

Finally, as observed in the present structure, the substitutions of ΔVal at $(i+1)$ and ΔPhe at $(i+3)$ positions with any residue at $(i+2)$ and $(i+4)$

Table 4
List of hydrogen bonds (estimated standard deviations are given in parentheses)

Type	D–H···A	D···A (Å)	D–H···A (°)	Symmetry
Intermolecular	$N_1–H_1\cdots O_2'$	3.099(7)	161	$-x, 1/2+y, 3/2-z$
Intermolecular	$N_2–H_2\cdots O_3'$	2.978(7)	167	$-x, 1/2+y, 3/2-z$
Intramolecular	$N_3–H_3\cdots O_0'$	2.764(6)	170	x, y, z
Intramolecular	$N_4–H_4\cdots O_1'$	3.047(7)	169	x, y, z

positions induce a 3_{10}-helical conformation in the peptides. Although, ΔVal at $(i+1)$ position prefers a type II β-turn conformation [7] and a ΔPhe surrounded with branched β-carbon residues such as Val and Ile is more compatible in the unfolded structure [9] but their combination produces a folded conformation in a manner similar to that of substituting two or more ΔPhe residues.

5. Supplementary material

Supplementary material (Tables 5–8), have been deposited with the British Library Document Supply Centre as supplementary publication number sup26696.

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