

Design of peptides with α , β -dehydro-residues: synthesis and crystal structure of a tripeptide *N*-benzyloxycarbonyl- Δ Val- Δ Phe-L-Ala-OCH₃

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Abstract

In order to develop the design rules for producing specific conformations of peptides with α , β -dehydro-residues a peptide Cbz- Δ Val- Δ Phe-Ala-OCH₃ was synthesized in solution phase. The crystal structure has been determined by X-ray diffraction method. The structure was refined to an *R*-value of 0.050. The peptide adopts a type I β -turn conformation with backbone torsion angles of two corner residues, $\phi_1 = -53.9(6)^\circ$, $\psi_1 = -33.0(6)^\circ$, $\phi_2 = -73.7(5)^\circ$ and $\psi_2 = -12.2(6)^\circ$. The conformation is stabilized by an intramolecular 4 \rightarrow 1 hydrogen bond involving NH of Ala residue as a donor and carbonyl oxygen atom of Cbz group as an acceptor. The torsion angles, $\chi_1^{1,1} = 172.8(6)$ and $\chi_1^{1,2} = -6.9(9)$ of Δ Val residue indicate that its side chain is planar while the torsion angles, $\chi_2^1 = -9.0(9)$, $\chi_2^{2,1} = -43.4(10)$ and $\chi_2^{2,2} = 130.1(9)$ show that the side chain of Δ Phe deviates considerably from the planarity. This is the first sequence in which Δ Val and Δ Phe are introduced at adjacent positions and the structure reveals clearly that the side chain of Δ Phe is a relatively less rigid than that of Δ Val. The molecules are packed in columns parallel to *c*-axis.

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1. Introduction

The studies of peptides containing α , β -dehydro-residues have shown that the α , β -dehydro-residues induce predictable conformations in peptides [1]. However there are still many more combinations (various dehydro-residues) that need to be investigated for possibly new forms of conformations. Therefore, a new combination of Δ Val and Δ Phe has been used for determining the structure of a pseudo-tetrapeptide. It is well known from previous studies that the peptides containing two consecutive Δ Phe residues at positions 2 and 3 generate an unfolded S-shaped structure in which all residues have torsion angles ϕ , ψ centred at ± 60 , ± 30 [2]. It has also been reported that the two consecutive Δ Phe residues at positions 3 and 4 induce folded conformations resulting in a 3_{10} -helix [1]. So far, the effects of combinations of different types of dehydro-residues have

not been investigated and hence the corresponding design rules are not available. We report here the synthesis, crystal structure and molecular conformation of a new combination of two different types of dehydro-residues in a pseudo-tetrapeptide Cbz- Δ Val- Δ Phe-Ala-OCH₃. The structure reveals that the peptide Cbz- Δ Val- Δ Phe-Ala-OCH₃ adopts a type I β -turn conformation that has been observed for the first time with dehydro-residues.

2. Experimental

The peptide Cbz- Δ Val- Δ Phe-Ala-OCH₃ (4) was synthesized using the following steps:

2.1. Synthesis of Cbz- Δ Val-OH (1)

The compound (1) was synthesized by 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

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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 by drop which yielded a white solid material. It was filtered and recrystallised from benzene. The solid product of Cbz- Δ Val-OH (1) was obtained with a yield of 67%.

2.2. Synthesis of Cbz- Δ 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 isobutylchloro-formate (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 Cbz- Δ Val- Δ Phe-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. Thereafter, the reaction mixture was poured over the crushed ice, the resultant product was washed with 5% sodium bicarbonate and water and finally recrystallised from acetone–water mixture to yield 70% of compound (3).

2.4. Synthesis of Cbz- Δ Val- Δ Phe-Ala-OCH₃ (4)

To a solution of compound (3) (1.5 g, 3.9 mmol) in dichloromethane (DCM) (10 ml), Ala-OCH₃·HCl (0.66 g, 4.7 mmol) was added followed by triethylamine (TEA) (0.65 ml, 4.7 mmol). This mixture was stirred for 72 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. ¹H-NMR of Cbz- Δ Val- Δ Phe-Ala-OCH₃ (4)

In order to confirm the correctness of the final synthesis of the peptide, ¹H-NMR spectra were recorded in CDCl₃ with 400 MHz Bruker DRX 400 instrument and the following results were obtained: δ 1.52 (m, 6H, C ^{γ} 1, C ^{γ} 2, Δ Val); δ 3.74 (s, 3H, OCH₃); δ 4.63–4.67 (m, 2H, –CH₂, Cbz); δ 5.33, δ (bd, NH, Ala); δ 6.31–6.33 (bd, 1H, NH Δ Val); δ 7.26–7.28 (m, 11H, Ar, Cbz, Δ Phe, C ^{β} Δ Phe); δ 7.52 (s, 1H, NH Δ Phe). The observed ¹H NMR spectra

clearly indicated the presence of peptide Cbz- Δ Val- Δ Phe-Ala-OCH₃ in the solution.

2.6. Structure determination

The peptide Cbz- Δ Val- Δ Phe-Ala-OCH₃ was crystallised from its solution in 80:20 acetone–water mixture at room temperature (298 K) by slow evaporation. The unit cell parameters and X-ray intensity data were collected on CAD4 single crystal diffractometer with Cu K α radiation. The unit cell parameters were refined by a least-squares fit of 25 high angle ($25 \leq \theta \leq 40^\circ$) reflections. These reflections were centred individually on the diffractometer. Lorentz and polarisation corrections were applied. The absorption correction was not made because the crystal dimensions were small ($0.4 \times 0.3 \times 0.2$ mm³). The structure was solved with direct methods using the program SHELXS 97 [3]. The coordinates of non-hydrogen atoms were refined anisotropically using program SHELXL 97 [4]. The coordinates of hydrogen atoms were obtained from difference Fourier maps and were included in the subsequent cycles of refinement using isotropic temperature factors of non-hydrogen atoms to which they were attached. However, the temperature factors for hydrogen atoms were not refined. The final R-factor for 2326 observed reflections ($I \geq 2\sigma(I)$) was 0.050. The crystallographic data and parameters of refinement are given in Table 1. The atomic scattering factors used in these calculations were those of Cromer and Mann [5] for non-hydrogen atoms and Steward, Davidson

Table 1
The details of intensity data collection and refinement for Cbz- Δ Val- Δ Phe-Ala-OCH₃

| | |
|---|--|
| Molecular formula | C ₂₆ H ₂₉ N ₃ O ₃ |
| Molecular weight | 479.52 |
| Crystal system | Hexagonal |
| Space group | P6 ₁ |
| <i>a</i> (Å) | 12.295(1) |
| <i>b</i> (Å) | 12.295(1) |
| <i>c</i> (Å) | 30.177(1) |
| <i>V</i> (Å ³) | 3950.6(5) |
| <i>Z</i> (molecules/unit cell) | 6 |
| <i>D_c</i> (g cm ^{–3}) | 1.21 |
| <i>F</i> (000) | 1524 |
| Total no. of independent reflections | 2422 |
| No. of observed reflections ($I \geq 2\sigma(I)$) | 2326 |
| Radiation (λ , Cu K α /Å) | 1.5418 |
| μ_r | 0.71 |
| Instrument used | Enraf-Nonius CAD4 |
| Mode of data collection | ω – 2θ |
| Crystal Dimension (mm ³) | $0.4 \times 0.3 \times 0.2$ |
| <i>R</i> | 0.050 |
| <i>R_w</i> | 0.128 |
| <i>S</i> (Goodness-of-fit) | 0.954 |
| Temperature (K) | 293 |
| Weighting details | Calc. $w = 1/[\sigma^2(F_o^2) + (0.0944P)^2 + 0.0000P]$ where $P = (F_o^2 + 2F_c^2)/3$ |

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