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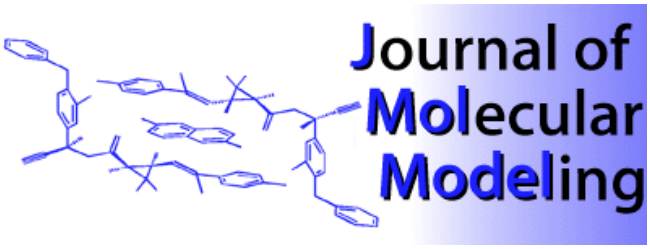

FULL PAPER

Effect of Stereochemistry (Z and E) and Position of α,β-Dehydrophenylalanine (ΔPhe) on β-turn Stability

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Abstract Several model peptides containing α, β-dehydrophenylalanine (ΔPhe) in both Z and E configurations were studied for β-turn stability at the AM1 level of theory. Both configurations of ΔPhe are well able to stabilize β-turns in the backbone. However, the β-turns for peptides bearing Z-ΔPhe are energetically more stable than the E-counterparts. The difference in energies between the global minima of these peptides having the Z and E configuration of ΔPhe, is dictated by the size and stereochemistry of residues flanking ΔPhe. One distinct feature of E-ΔPhe is that it pushes peptides to adopt a Type II β-turn with the ΔPhe residue in the (i + 1) position of the turn. This unique feature may be exploited in peptide design.

Keywords α,β–dehydrophenylalanine (ΔPhe), Z and E configuration, AM1, β-turn

Introduction

Amino acid residues possessing a double bond in the side chain (α, β-dehydroamino acids) have been found in peptides derived from microbes and fungi, [1, 2] for example, the antibiotics nisin, subtilisin, epidermin etc. [3, 4] Some enzymes like histidine ammonia lyase from bacteria and mammals, and phenylalanine ammonia lyase from plants, also contain α, β-dehydroamino acid residues. The most commonly occurring dehydroamino acids in nature are dehydrophenylalanine (ΔPhe), dehydrotryptophan (ΔTrp), dehydroalanine (ΔAla), dehydroleucine (ΔLeu), dehydrovaline (ΔVal), dehydroproline (ΔPro) and dehydroaminoisobutyric acid (γAbu). [2, 5]

There has been a great interest in recent years in the use of α, β-unsaturated amino acids in peptide design, because such residues are able to modulate the backbone conformation to produce stable folded structures like β-turns and α- and other helices. [6, 7] Furthermore, α, β-dehydro residues confer increased resistance to peptide degradation by enzymes. Among the various α, β-dehydroamino acids, most studies have centered on ΔPhe, perhaps because its synthesis is relatively easy. Of the two possible stereoisomers of ΔPhe, namely the Z and E forms (Fig. 1), all investigations have focussed only on the Z-isomer. This is mainly because synthetic routes leading to the Z-isomer are straightforward. Very recently, Inai *et. al.* have published a method for the synthesis of the E-isomer. [8] In small peptides bearing a single Z-ΔPhe, a Type II β-turn structure is generally seen, [9-11] while peptides containing more than one Z-ΔPhe residues tend to take a helical structure. [12-14] The stability of

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Table 1 *ϕ, ψ values for different types of β-turns [17]*

β-turn Type	i+1 residue		i+2 residue	
	ϕ	ψ	ϕ	ψ
I	-60	-30	-90	0
I'	60	30	90	0
II	-60	120	80	0
II'	60	-120	-80	0
III	-60	-30	-60	-30
III'	60	30	60	30

these helices is dependent on the number of saturated amino acids separating the two Z-ΔPhe residues.

We were interested in examining theoretically if the E-isomer, like its Z counterpart, could induce the same conformational preferences in the peptide backbone and what are the differences in stability of peptides containing Z and E-ΔPhe.

The last aspect of stability between peptides possessing a Z or E isomer of ΔPhe is dependent on the nature of the residue flanking the N and C terminals of ΔPhe. The stereochemistry (D or L) of the flanking residues will also influence the stability of the Z and E forms. To find answers to the above issues, we have carried out a systematic investigation of model peptides having Z- ΔPhe and E- ΔPhe, with an emphasis on β-turn propensity and stability. The following peptides were studied.

Ac-Gly-(Z or E)ΔPhe-Gly-NHMe (**P1**)
Ac-Ala-(Z or E)ΔPhe-Ala-NHMe (**P2**)
Ac-Leu-(Z or E)ΔPhe-Leu-NHMe (**P3**)
Ac-Ile-(Z or E)ΔPhe-Ile-NHMe (**P4**)
Ac-Val-(Z or E)ΔPhe-Val-NHMe (**P5**)
Ac-Phe-(Z or E)ΔPhe-Phe-NHMe (**P6**)
Ac-D-Val-(Z or E)ΔPhe-Val-NHMe (**P7**)
Ac-Val-(Z or E)ΔPhe-D-Val-NHMe (**P8**)

These flanking residues were selected in order to probe the effect of size and branching on the stability of the Z and E-isomers of ΔPhe.

For the above peptides, two β-turns can be conceived. The first (Ac-X-ΔPhe-X) with ΔPhe at the (i + 2) position (Fig. 2a), would involve the CO group of Ac in an intramolecular H-bond with the NH group of the residue succeeding ΔPhe. In the second β-turn (X-ΔPhe-X-NHMe), ΔPhe is at the (i + 1) position in the turn, and has the CO group of the residue preceding ΔPhe in an H-bond with NHMe (Fig. 2b). Each of the two β-turn sequences mentioned above could be formulated as Type I, Type II or Type III and their mirror images Type I', Type II' and Type III' respectively. Very obviously then, the Z and E-isomers will prefer one of the six turn types.

Methodology

Calculation of the heat of formation (ΔH_f) of the various peptides was done on an SGI O2 computer at the AM1 level of theory [15] using the MOPAC suite of programs (v 6.2) as an interface in Sybyl 6.4 (Tripos Inc. USA). The AM1 Hamiltonian was used, since it is known to reproduce hydrogen bonding best among the other semiempirical methods. [16] Each peptide was built with ΔPhe in the (i + 1) or (i + 2) position in turn Types I, II and III and their mirror images, with ϕ, ψ values taken from ref. 17 and presented in Table 1. Two optimization methods were carried out. In the "constrained" procedure, the optimization was run by flagging the ϕ, ψ angles of residues in the (i + 1) and (i + 2) positions of the β-turn to 0 (*i.e.* held fixed) in the Z-matrix, while all other degrees of freedom were optimized. In the "unconstrained" case, every degree of freedom in the molecule was optimized. Minimization was run with the EF algorithm to a GNORM of 0.01 and the PRECISE option. Since it is well known that all semi-

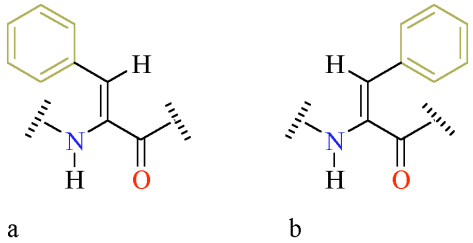

Figure 1 *The two stereoisomers of α, β-dehydrophenylalanine (a) Z-isomer (b) E-isomer*

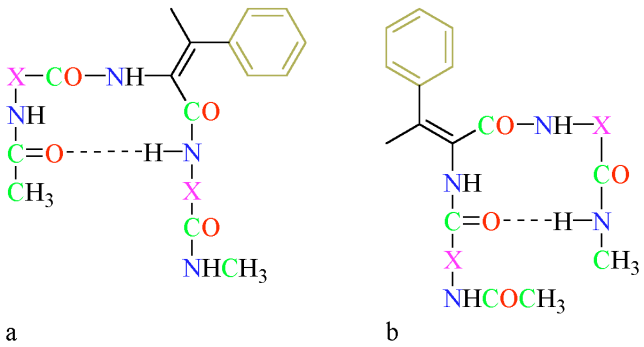

Figure 2 *Schematic representation of β turn (a) ΔPhe in i+2 position; (b) ΔPhe in i+1 position. X = Gly, Ala, Leu, Ile, Val, Phe*

Table 2 *Global and local minimum energy conformations obtained by 'constrained' minimization of model peptides having ΔPhe in Z and E configuration*

Peptide	ΔPhe in Z configuration		ΔPhe in E configuration	$\Delta\Delta H_f$ [a]
	Gl. Min.	Local Min. (up to 1 kcal·mol^{-1})	Gl. Min.	
P1	II' (i+2)	1. I (i+1), I' (i+1), II (i+1) 2. II' (i+1), II (i+2)	1. II (i+1) 2. II' (i+1)	-2.2
P2	1. I (i+1) 2. II' (i+1)	–	II' (i+1)	-1.0
P3	I (i+1)	III (i+1), II' (i+1)	1. II (i+1) 2. II' (i+1)	-3.9
P4	II (i+2)	I (i+2)	II' (i+1)	-4.0
P5	III (i+2)	I (i+1)	II' (i+1)	-3.5
P6	I' (i+2)	1. I (i+1), I (i+2) 2. II (i+2), III (i+2)	I (i+1)	-2.5
P7	I (i+1)	III (i+1)	II' (i+1)	-2.3
P8	II (i+2)	II (i+1), I' (i+1), III' (i+1)	II (i+1)	-1.9

[a] Difference in heat of formation between global minima of peptides having ΔPhe in Z and E configuration. The abbreviation I (i+1) refers to Type I β turn with ΔPhe at the (i+1) position of the turn. Thus, I (i+2), II (i+1), II (i+2), III (i+1) and III (i+2) have their related meanings. No local minima within 1 kcal of global minima were obtained for peptides having ΔPhe in E configuration.

empirical methods underestimate the barrier to rotation in peptides, the key word MMOK was used to allow for a molecular mechanics correction. Stable points on the potential energy surface (PES) of the molecule were identified by running a FORCE calculation and were confirmed by the presence of all real frequencies.

For each peptide, the global minimum referred to in the next section is the lowest energy structure from among the twelve possible conformations built from two β-turn sequences, each in three turn types and their mirror images.

Results and discussion

Minimization with "constraints"

In Table 2 is given the global minimum and local minima within 1 kcal mol^{-1} of global minimum of the various peptides (**P1**-**P8**) with ΔPhe in Z and E configurations. Only local minima upto 1 kcal mol^{-1} of the global minimum have been considered, since the population of states (as given by the Boltzmann factor $\exp(-\Delta E/k_bT)$) with energy higher than 1 kcal mol^{-1} , in the *ensemble* of conformations, is miniscule. The difference in the heat of formation ($\Delta\Delta H_f$) between the global minimum of each peptide with ΔPhe in Z and E configurations is also listed in this table.

It is seen that all peptides with ΔPhe in the Z configuration are more stable than their E counterparts. However, this difference in energy between the global minimum energy conformers of the peptides with ΔPhe in Z and E configurations ($\Delta\Delta H_f$) correlates with the size of the side chain of the amino acid flanking ΔPhe in case of aliphatic amino acids. Thus for Gly, this difference ($\Delta\Delta H_f$) is 2.2 kcal mol^{-1} (Table 2). In Ala, with a small hydrophobic side chain, this difference for some unexplainable reason is smaller at 1.0 kcal mol^{-1} (Table 2). For a γ-branch substituent like Leu, this difference rises to 3.9 kcal mol^{-1} , while for β-branching as in Ile and Val it is 4.0 and 3.5 kcal mol^{-1} respectively (Table 2).

A bulky side chain as in Phe, is not seen to destabilize the E-form greatly. The difference in the global energy minimum of the Z and E-forms ($\Delta\Delta H_f$) is of the same order as seen in Gly (Table 2). This is because the flat aromatic ring is able to find a conformation that minimizes steric interaction with ΔPhe.

Another feature that is apparent in Table 2, is that the E-stereoisomer of ΔPhe strongly prefers the (i + 1) position in a Type II β-turn, while Z-ΔPhe seems to adjust well at both the (i + 1) and (i + 2) positions, with no specific choice of turn type. The latter tendency agrees well with what has been observed in crystallographic studies of some related peptides. [7]

The effect of stereochemistry of the neighboring residues on the stability of Z and E-isomers is also markedly pronounced. In the peptides Ac-D-Val-ΔPhe-Val-NHMe and Ac-Val-ΔPhe-D-Val-NHMe, a D-isomer at both the N and C terminal ends of ΔPhe has a stabilizing effect on the E-isomer relative to the parent Ac-Val-ΔPhe-Val-NHMe as seen in Ta-

Table 3 *Global and local minimum energy conformations obtained by 'unconstrained' minimization of model peptides having ΔPhe in Z and E configuration*

Peptide	ΔPhe in Z configuration		ΔPhe in E configuration		ΔΔHf [a]
	Gl. Min.	Local Min (up to 1 kcal·mol^{-1})	Gl. Min.	Local Min. (up to 1 kcal·mol^{-1})	
P1	γ G1, ΔPhe & G3	–	1. II (i+1), Inv γ G1, γ G3 2. II' (i+1), γ G1, Inv γ G3	–	-1.7
P2	II' (i+1), Inv γ A1 & A3	1. Inv γ A1, γ ΔPhe 2. Inv γ A1 & A3	1. II' (i+1), Inv γ A1 & A3 2. γ A1, Inv γ A3	1. II (i+1), Inv γ A1 2. Inv γ A1, γ ΔPhe	-1.3
P3	II' (i+1), Inv γ L3	1. I (i+1) 2. I' (i+1) 3. III (i+1) 4. γ L3	1. II' (i+1) 2. Inv γ L3	γ L3	-2.4
P4	Inv γ I1 & I3, γ ΔPhe	Inv γ I1 & I3	Inv γ I1 & I3, γ ΔPhe	Inv γ I1 & I3	-1.9
P5	Inv γ V1, γ ΔPhe	-	Inv γ V1, γ ΔPhe	Inv γ V1	-1.9
P6	1. II' (i+1), Inv γ F1 & F3 2. Inv γ F1 & F3, γ ΔPhe	II (i+1), Inv γ F1, γ F3	Inv γ F1 & F3, γ DPhe	Inv γ F1 & F3	-2.2
P7	γ DVal & V3	1. Inv γ DVal, γ ΔPhe 2. I (i+1), γ DVal 3. II' (i+2), γ DVal	II' (i+1), γ DVal	γ DVal & V3	-1.3
P8	II (i+2), Inv γ V1	1. II (i+1), γ V1 2. Inv γ V1 3. Inv γ DVal	Inv γ V1, γ ΔPhe	II (i+1)	-1.6

[a] See comments under Table 2. The abbreviation γG1 means a γ-bend at Gly 1, Inv. γG1 denotes an inverse γ-bend at G1. Also see definitions under Table 2

ble 2. This effect is slightly greater when the D-amino acid is placed at the C-terminal side of ΔPhe.

It must be mentioned that quite a few β-turns for these peptides are not stationary points on their PES. This was indicated by the appearance of small imaginary frequencies in the FORCE calculation (*vide supra*). This could be a result of the constrained minimization. It is possible that these β-turns will be stable conformations if these sequences are part of larger oligopeptides or proteins.

Unconstrained Minimization

For all peptides unanimously, the minimization with removal of constraints produces structures that have much lower energies than in the constrained case. Here too, the Z-isomers are more stable than their E-partners. The global minimum energy structure, local minima restricted to 1.0 kcal mol^{-1} above the global minimum and difference in the heat of formation ($\Delta\Delta H_f$) between the global minimum of the Z and E-isomers are summed up in Table 3.

From Table 3, it is seen that β-turns are not always the global minimum conformation for some of these peptides and sometimes appear as local minima. The most notable cases are for the peptides Ac-Leu-ΔPhe-Leu-NHMe (**P3**) and Ac-Val-ΔPhe-Val-NHMe (**P5**), where no β-turn structure is found either as a global or local minimum. For these peptides, it seems that the most stable structures are γ-bends. The peptide ^{t}Boc-Ala-E-ΔPhe-Val-OMe has been shown by NMR and empirical energy calculations to adopt two consecutive γ-bends. The authors have not ruled out the possibility of a Type II β-turn conformation with ΔPhe at the (i + 2) position in the turn for this peptide. The structure of the peptide precludes the adoption of a β-turn with ΔPhe at the (i + 1) position. Based on the results for "unconstrained" minimization, it seems that for small peptides both Z and E forms of ΔPhe may lead to structures with either β-turns or γ-bends. However, for larger peptides or in the presence of a structured environment (*i.e.* receptor/enzyme) it is likely that β-turn structures may prevail.

Wherever β-turns were observed (as global or local minima, with ΔPhe at the (i + 1) position) for the other peptides, the φ,ψ values of residues involved in the turn deviate from ideal values, mainly because some of the residues are additionally forced into a γ-bend or an inverse γ-bend. The appearance of γ-bends and distortion of the β-turns is partly because of the absence of a structured environment in these calculations. Some distortion of β-turns has also been noted in crystallographic studies of peptides having Ile and Val on either side of ΔPhe. [7]

In contrast to the previous results with "constrained" minimization, the peptides bearing Z-ΔPhe in an unconstrained

minimization show a leaning to adopt a Type II β-turn (or its mirror image) with ΔPhe at the (i + 1) position of the turn, very much like the E-ΔPhe peptides.

Conclusions

In order to investigate whether the E-isomer of α, β-dehydrophenylalanine (ΔPhe) can modulate the φ, ψ angles of a peptide backbone in a β-turn motif, several model peptides were built and minimized with and without the application of constraints on the backbone dihedrals, using the AM1 Hamiltonian. It is seen that for these peptides having E-ΔPhe, β-turns occur either as the global minimum or as local minima within 1 kcal mol^{-1} above the global minimum. However, the β-turn structures with the Z-isomer of ΔPhe are more stable than the E-counterparts. Nevertheless, for all the peptides investigated, the E-configurations are never more than 4.0 kcal mol^{-1} higher in energy than the Z forms. The size, branching in the side chain and stereochemistry of the residues surrounding ΔPhe rule the difference in energies between the two isomers.

In conclusion, it can be said that E-ΔPhe offers one distinct advantage over Z-ΔPhe in inducing β-turns in peptides, *i.e.* E-ΔPhe is partial towards a Type II β-turn with the ΔPhe residue in the (i + 1) position in the turn. This property may be used advantageously in peptide design.

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