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Theoretical Prediction of the Basic Helix Types in α,β-Hybrid Peptides

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Abstract: *This study provides a complete overview on all possible helical folding patterns, their stabilities, and their detailed molecular structure in the novel foldamer class of α,β-hybrid peptides on the basis of ab initio molecular orbital (MO) theory. The results indicate a considerable intrinsic potential of backbone folding. As found for other peptide foldamers, representatives of mixed or β-helices are most stable in more apolar media, whereas polar environments favor the helices with the hydrogen bonds pointing in only one direction. The theoretical results confirm the hydrogen-bonding patterns found in the first experimental studies on these hybrid peptides. Selecting special backbone substitution patterns, the secondary structure potential of the α,β-hybrid peptides could be of great importance for a rational peptide and protein design.* © 2006 Wiley Periodicals, Inc. Biopolymers (Pept Sci) 84: 408–413, 2006

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Keywords: *peptide foldamers; alternate hybrid peptides; secondary structure formation; peptide helices; ab initio MO theory*

INTRODUCTION

Oligomers of nonproteinogenic amino acids have attracted considerable attention in the last several years because of their capacity to form ordered secondary structures.[1–3] Recently, a novel type of such peptide foldamers was suggested whose sequence is alternately composed of α- and β-amino acid constituents. Nuclear magnetic resonance (NMR) studies on these α,β-hybrid peptides indicate the formation of various definite helix structures.[4–6] A first X-ray study provides explicit structure data for a helix with 11-membered hydrogen-bonded rings.[7] Some peptides of this type show even biological activity.[8–10] For

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an understanding of the conformational properties and as a basis for secondary structure design, knowledge on the intrinsic backbone folding patterns to be expected in this novel class of peptide foldamers could be useful. From the very beginning, the synthetic efforts in the field of peptide foldamers were successfully accompanied by theoretical conformational analyses employing the methods of ab initio molecular orbital (MO) theory. On the basis of theoretical calculations, it was possible to obtain a complete overview on the characteristic secondary structure elements in several classes of peptide foldamers as, for instance, in β, γ, δ, aminoxy, and hydrazino peptides.[11–22] The most stable structures determined by the theoretical calculations agree very well with the typical secondary structures found in experimental studies. In some cases, novel and unusual folding patterns were predicted,[14–17,22] which were experimentally confirmed later.[23–27] Thus, the reliability of ab initio MO theory is sufficient for the prediction of the typical folding patterns, their stabilities, and their geometries in the novel foldamer class of α,β-hybrid peptides.

METHODS

Looking at a sequence of alternating α- and β-amino acids, three basic types of helices with hydrogen-bonding interactions between non-neighboring peptide bonds are formally possible (Figure 1):

1. helices with all hydrogen bonds in a backward direction along the sequence,
2. helices with all hydrogen bonds in a forward direction of the sequence, and
3. helices with the hydrogen bonds alternately changing in backward and forward direction, which are sometimes named "mixed" or β-helices.[17,22,28,29]

In all three cases, the helical periodicity appears at the level of dimer units. This differs from the familiar peptide helices of α-peptides and other homologous peptides, where the periodicity is realized at the monomer level provided that all hydrogen bonds have the same orientation. Only the mixed helix type with its alternating change of the hydrogen-bond directions exhibits the periodicity also in the homologous α, β, γ, and δ peptides at the dimer level.[17]

A detailed look at the three basic helix types shows two different hydrogen-bonding patterns for each type. In the simplest helices with backward hydrogen-bond orientations, the hydrogen bonds are either formed by 1 ← 4 amino acid interactions between α-amino acid constituents and β-amino acid constituents and vice versa, or alternatively, by 1 ← 5 amino acid interactions between two α-amino acid and between two β-amino acid constituents (Figure 1). In the first case, 11-membered hydrogen-bonded

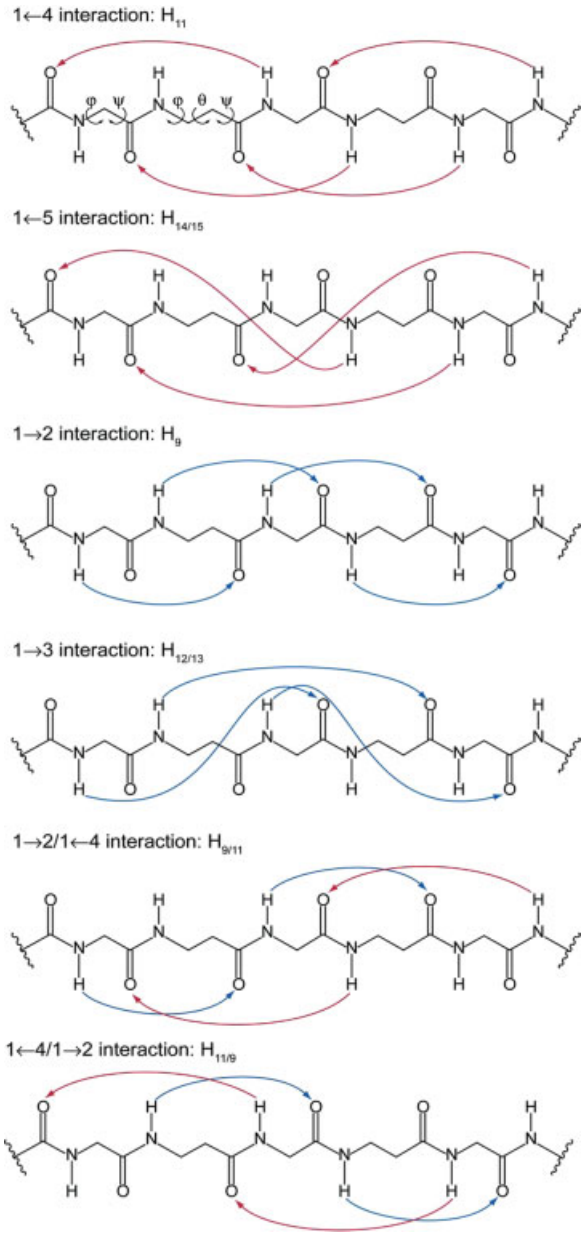

FIGURE 1 Hydrogen bonding patterns for α,β-hybrid peptide helices with backward, forward, and alternating backward/forward hydrogen-bond directions along the sequence.

pseudocycles result from the two interactions (H_{11}), in the second case alternating 15- and 14-membered pseudocycles appear ($H_{14/15}$). A comparable situation exists for the two helix types with forward orientation of the hydrogen bonds. The first helix is realized by 1 → 2 amino acid interactions between α- and β-amino acid constituents and vice versa (Figure 1), leading to 9-membered hydrogen-bonded pseudocycles (H_9). The helix type with 1 → 3 amino acid interactions between the same types of amino acid constituents, α or β (Figure 1), is characterized by alternating 12- and 13- membered pseudocycles ($H_{12/13}$). The hydrogen-bond-

Table I HF/6-31G* Backbone Torsion Angles[a] of All Helix Conformers in Octamers of α,β-Hybrid Peptides

Helix[b]	φ	θ	ψ	Helix[b]	φ	θ	ψ
H_{11}	71.9		20.2	$H_{16/18}^{I}$	86.2		−53.7
	93.7	−79.1	90.2		−64.2	−60.2	172.7
	70.0		19.0		91.2		−57.5
	97.8	−76.9	85.2		−72.7	−60.8	155.1
	70.5		18.1		119.8		−91.6
	99.3	−77.3	84.0		−58.3	−49.8	125.7
	74.7		13.3		159.4		−140.5
	105.5	−76.1	85.7		−30.6	−51.4	151.4
$H_{14/15}$	−72.3		−23.9	$H_{16/18}^{II}$	86.6		−46.4
	−115.9	78.5	−130.2		−159.4	63.9	100.8
	−73.9		−23.6		101.2		−67.6
	−118.8	77.7	−124.6		−170.9	58.3	75.4
	−71.9		−29.2		121.2		−139.5
	−117.2	80.6	−121.1		−106.1	57.2	51.6
	−93.8		1.1		157.7		−148.6
	−132.9	68.6	−113.4		−106.8	61.8	49.0
$H_{12/13}$	−138.9		15.4	$H_{18/16}^{I}$	135.3		−147.5
	69.4	44.3	67.0		−66.2	−60.0	111.2
	103.5		96.7		130.8		−150.6
	64.2	40.1	63.4		−70.9	−55.2	138.7
	96.3		101.1		95.7		−120.4
	66.2	41.7	52.9		−81.3	−64.9	170.9
	99.3		108.6		76.4		−132.4
	62.8	46.8	91.4		−78.6	−57.8	153.5
$H_{9/11}$	147.6		−42.5	$H_{18/16}^{II}$	126.4		−158.9
	−90.9	55.2	91.4		−132.2	56.5	24.5
	132.9		−61.1		109.9		−143.9
	−92.5	58.2	87.5		−131.7	57.1	44.9
	133.8		−58.8		83.8		−84.0
	−93.4	58.4	88.9		−178.3	64.4	36.0
	131.4		−59.8		82.1		−84.7
	−91.2	55.0	88.8		179.2	64.8	25.7
$H_{11/19}$	64.0		−150.9				
	−77.5	−59.3	97.7				
	59.1		−148.8				
	−78.6	−59.8	96.8				
	59.3		−149.6				
	−78.1	−59.4	97.2				
	58.0		−149.5				
	−75.2	−63.0	109.1				

[a] Torsion angles in degrees.
[b] See Figure 1.

ing patterns of the two mixed helix types are realized by interactions only between α- and β-amino acid constituents. The backward orientations result from 1 ← 4 amino acid interactions leading to 11-membered pseudocycles, the forward orientations from 1 → 2 interactions leading to 9-membered pseudocycles. Although of the same size, the structures of the alternating 9- and 11-membered pseudocycles are different in the two helix alternatives $H_{9/11}$ and $H_{11/9}$ (Figure 1). The detailed description given here concerns helices with the smallest possible pseudocycles formed between non-nearest neighbor peptide bonds. Increasing the sequence distances between the interacting amino acids leads to hydrogen-bonding patterns with still larger pseudocycles. The helices H_{18} and $H_{21/22}$ are the representatives with the next possible ring sizes for the backward helices, H_{16} and $H_{19/20}$ for the forward helices, and $H_{16/18}$ and $H_{18/16}$ for the mixed helices.

To obtain a complete overview on all possible helix types, we performed a systematic conformational search on blocked α,β-hybrid peptide octamers. Although it is known that a rigidification of the backbone by acyclic or cyclic substituents may favor secondary structure formation, we

Table II Relative Energies and Free Enthalpies of All Helix Conformers in Octamers of α,β-Hybrid Peptides at Various Levels of Ab Initio MO Theory

Helix[a]	HF/6-31G*[b]	DFT/6-31G*[b]	PCM//HF/6-31G*[b,c]	ΔG[b,d]
H_{11}	76.8	81.9	9.6	60.2
$H_{14/15}$	101.1	103.7	15.8	81.4
$H_{12/13}$	126.7	130.6	65.0	111.1
$H_{9/11}$	63.8	55.1	47.9	51.2
$H_{11/9}$	14.6	18.0	**0.0**[e]	10.1
$H_{16/18}^{I}$	28.9	23.4	49.8	29.7
$H_{16/18}^{II}$	42.0	23.1	70.6	41.6
$H_{18/16}^{I}$	**0.0**[f]	**0.0**[g]	21.7	**0.0**[h]
$H_{18/16}^{II}$	50.1	35.5	60.0	50.4

[a] See Figure 1.
[b] In kJ/mol.
[c] Dielectric constant $\varepsilon = 78.4$.
[d] HF/6-31G* level.
[e] $E_T = -2057.724165$ a.u.
[f] $E_T = -2057.735525$ a.u.
[g] $E_T = -2069.939317$ a.u.
[h] $G_T = -2057.091093$ a.u.

selected an unsubstituted backbone. Special substitution patterns would enforce folding into one or only a few special secondary structure elements. Thus, information on all principally possible folding patterns of the peptide backbone, which we want to obtain, gets lost. Numerous studies[1–3] on peptide foldamers demonstrate that backbone substitution does not so much concern the principal types of the intrinsic backbone folding patterns, but more the stability relationships between the most stable of them.

There are further advantages to our strategy. The greater number of folding alternatives, which we can expect from our general conformational search, opens up the possibility for synthetic chemists to think about special substitution patterns to favor the one or the other helix type. Finally, the pool of all theoretically predicted helix conformers represents a good support for experimental structure analyses of these peptides. Thus, experimental information from NMR studies might well be related to the theoretically predicted structures.

To obtain a complete overview on all helix conformers in α,β-hybrid peptides, we followed a strategy already employed for other peptide foldamers.[14,16,17] A pool of 145,152 conformations was generated by a systematic variation of the backbone torsion angles (φ,ψ) of the α- and (φ,θ,ψ) of the β-amino acid constituents of blocked octamers in intervals of 30° (Figure 1). From this pool, all conformations fulfilling the described hydrogen-bonding patterns were selected on the basis of general geometry criteria for hydrogen bonds. These conformations were the starting points of complete geometry optimizations on the basis of ab initio MO theory (HF/6-31G*). Structures keeping the helical hydrogen-bonding patterns of Figure 1 were confirmed as minimum conformations by the determination of the eigenvalues of the force constants matrix. The obtained vibration frequencies also enabled the determination of the free enthalpy differences. The effects of correlation energy were estimated by reoptimization of the HF/6-31G* conformers at the B3LYP/6-31G* level of density functional theory (DFT). The influence of an aqueous environment was described by a polarizable continuum model (PCM//HF/6-31G*). The solvation energy considers the electrostatic, van der Waals, and cavitation energy contributions.

All quantum chemical calculations were performed employing the Gaussian03[30] and the Gamess-US[31] program packages.

RESULTS AND DISCUSSION

Our calculations show that all the above-described helix types (Figure 1) with backward (H_{11}, $H_{14/15}$) and alternating hydrogen-bond orientations ($H_{11/9}$, $H_{9/11}$) are confirmed at all approximation levels. For the helices with forward orientations of the hydrogen bonds, only the $H_{12/13}$ type is predicted. Like in α-peptides, the formation of helices with hydrogen bonds pointing into the forward direction of the sequence is obviously disfavored. Helix conformers with still larger pseudocycle sizes were only found for the mixed helices ($H_{16/18}$, $H_{18/16}$) of the octamers. For these hydrogen-bonding patterns, there are even two conformational alternatives denoted by superscript Roman numerals in the order of decreasing stability. The torsion angles of the various helices calculated at the HF/6-31G* level of ab initio MO theory are given in Table I. The corresponding B3LYP/6-31G* data are part of the Supplemental file.

The energy data for the single molecules in Table II show a preference of the mixed or β-helices over the

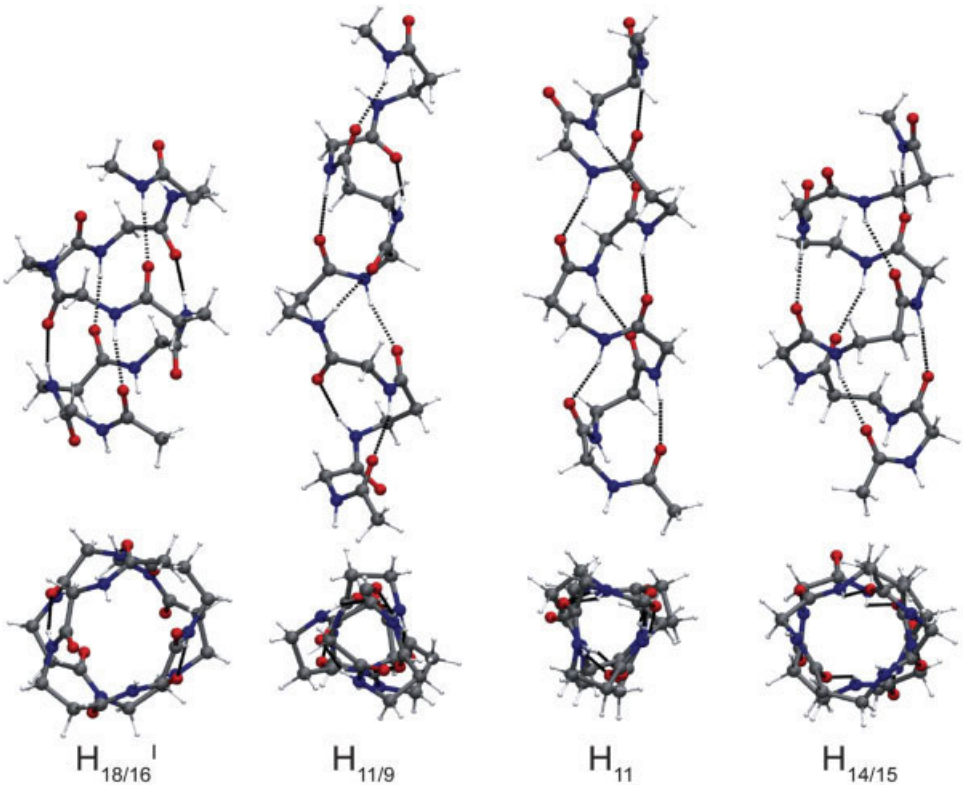

FIGURE 2 The most stable helix conformer in vacuum ($H_{18/16}^{I}$) and the three most stable helix conformers in an aqueous environment ($H_{11/9}$, H_{11}, $H_{14/15}$) in blocked octamers of α,β-hybrid peptides according to ab initio MO theory.

other helix types. The HF and DFT energy data, which are in close correspondence, indicate the mixed helices $H_{18/16}^{I}$ and $H_{11/9}$ distinctly favored in vacuum. The same trend is reflected by the free enthalpy values. However, in an aqueous environment, the stability of the other helix types increases considerably due to their higher total dipole moments. Now, the $H_{11/9}$ helix is most stable. This helix type was indeed confirmed in a recent NMR study on α,β-hybrid peptides with acyclic backbone substituents.[6] The helices H_{11} and $H_{14/15}$ with backward hydrogen-bond directions are also rather stable. Their hydrogen-bonding patterns were found by NMR measurements on α,β-hybrid peptides with cyclic amino acid backbones.[4] The theoretically predicted geometry data for H_{11} correspond well to the recently published X-ray data.[7] A further experimental study on another class of α,β-hybrid peptides suggests a helix with 13-membered hydrogen-bonded pseudocycles,[5] which obviously corresponds to the $H_{12/13}$ hydrogen-bonding pattern of our study. According to the theoretical results, the stability of this folding pattern is distinctly lower than that of the other helix types. Possibly, the very special backbone structure of the investigated derivative, where the C(α) and C(β) atoms of the β-amino acid constituents are part of a cyclopropane ring, favors this helix type. Figure 2 depicts the most stable helices in vacuum and in an aqueous environment.

CONCLUSIONS

Our ab initio MO study indicates a considerable intrinsic potential of backbone folding in the novel foldamer class of α, β-hybrid peptides. As found in other peptide foldamer classes, mixed or β-helices are most stable in more apolar media, whereas polar environments favor the helices with the hydrogen bonds pointing in only one direction. The theoretical results confirm the hydrogen-bonding patterns found in the first experimental studies on these foldamers and provide the corresponding detailed molecular structures together with those for further helix representatives. Selecting special backbone substitution patterns, the secondary structure potential of the α,β-hybrid peptides could be of great importance for a rational peptide and protein design.

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Supplemental file: Table with the torsional angles of all helices at the B3LYP/6-31G* level of ab initio MO theory, pdb-files of all predicted α,β-hybrid helices.

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