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Side-Chain Control of Folding of the Homologous α-, β-, and γ-Peptides into "Mixed" Helices (β-Helices)

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Abstract: *A systematic analysis of the substituent influence on the formation of the unique secondary structure type of ''mixed'' helices in the homologous α-, β-, and γ-peptides was performed on the basis of ab initio molecular orbital theory. Contrary to the common periodic peptide helices, mixed helices have an alternating periodicity and their hydrogen-bonding pattern is similar to those of β-sheets. They belong, therefore, to the family of β-helices. It is shown that folding of peptide sequences into mixed helices is energetically preferred over folding into their periodic counterparts in numerous cases. The influence of entropy and solvents on the formation of the various competitive mixed and periodic helix types is discussed. Among the oligomers of the various homologous amino acids, β-peptides show the highest tendency to form β-helices. The rules of substituent influence derived from the analysis of a wide variety of backbone substitution patterns might be helpful for a rational design of mixed helix structures, which could be important for mimicking membrane channels.* © 2005 Wiley Periodicals, Inc. Biopolymers (Pept Sci) 80: 675–687, 2005

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INTRODUCTION

Contrary to the periodic structure of common peptide and protein helices, e.g., the α- and the 3_{10}-helices, where the corresponding backbone torsion angles of all amino acids have the same values, mixed helices show the periodicity at the level of dimer units, i.e., the values of the corresponding backbone torsion angles of the monomers change alternately and adjacent peptide linkages are involved in hydrogen bonds that are formed alternately in the forward and backward directions of the sequence. Consequently, the resulting alternate hydrogen-bonded rings are of different size (Figure 1). Because of the similarity of the hydrogen-bonding pattern of mixed helices to that of parallel β-sheet structures, these helices are classified as β-helices (Figure 2).[1–4] The most prominent representative of a β-helix in α-peptides is the membrane channel-forming peptide gramicidin A[5–7] with alternating 20- and 22-membered hydrogen-bonded rings. Immediately after the discovery of gramicidin A, further types of β-helices were suggested for

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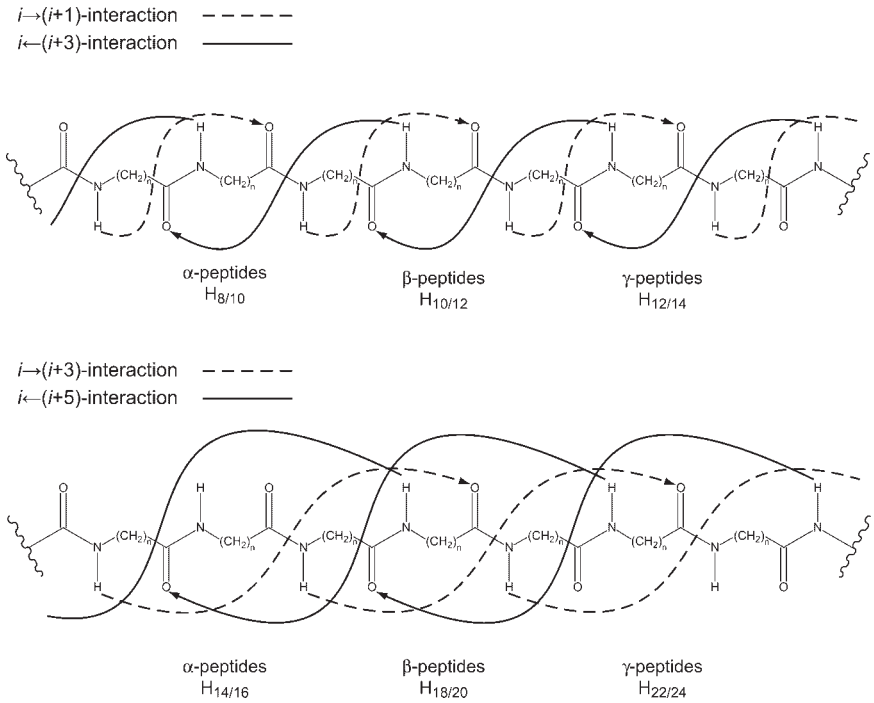


FIGURE 1 Alternative hydrogen-bonding patterns in mixed helices $H_{x/y}$ of homologous α- ($n = 1$), β- ($n = 2$), and γ-peptides ($n = 3$). The index x/y denotes the number of atoms in the alternating hydrogen-bonded rings.

α-peptides on the basis of general structure ideas, but only recently such secondary structures with alternating 14- and 16-membered hydrogen-bonded cycles were experimentally found.[3,4] In this context, it should be mentioned that antiparallel double-strand β-helices are also possible.[4,8] Besides, the relationships between nanotube assemblies of cyclopeptides with alternately D,L-substituted monomers deserve attention.[9,10]

The concept of β-helices was originally confined to α-peptides. This changed with the finding of a ''mixed'' helix in oligomers of β-amino acids (β-peptides) by Seebach and coworkers.[11–13] In this helix, 10-membered hydrogen-bonded rings with an interaction between the amino acids i and $(i + 1)$ in the forward direction are followed by 12-membered rings with an interaction between the amino acids i and $(i + 3)$ in the backward direction of the sequence [$i \rightarrow (i + 1)/i \leftarrow (i + 3)$ interaction, Figure 1]. This secondary structure type of β-peptides was confirmed in other experimental studies in the meantime.[14–16]

On the basis of ab initio molecular orbital (MO) theory, we could recently[17] extend the concept of mixed helices in several points:

1. Stable mixed helix conformers with $i \rightarrow (i + 1)/i \leftarrow (i + 3)$ amino acid interactions are also possible in the homologous γ- and δ-peptides (Figure 1).
2. Mixed helices with still larger alternating ring systems, as for instance with $i \rightarrow (i + 3)/i \leftarrow (i + 5)$ amino acid interactions (Figure 1) are thinkable in all homologous peptides.
3. There are structure alternatives with differing backbone torsion angles for the same hydrogen-bonding pattern in β-, γ-, and δ-peptides.

Remembering the outstanding role of gramicidin A as a membrane channel-forming compound, it may be useful to look for possibilities of a stabilization of this unusual and unique secondary structure type in all homologous peptides. In this way, novel types of membrane-channel forming peptides become accessible. It is well known from α-peptides that the side chains of the amino acid residues have a significant influence on the secondary structure formation, which is for instance documented by the propensity scales for the proteinogenic amino acids to form helical, sheet, and turn structures.[18–20] Gramicidin A itself is a good example for the substituent influence on the secondary structure formation, since an alternating sequence of D- and L-amino acids seems to be a basic requirement for the formation of the channel-like structure. Like in gramicidin A, experimental and theoretical data for β-peptides demonstrate the sensitivity of secondary structure formation to substituents.[21–24] Thus, folding into the two most important periodic folding patterns of β-peptides with

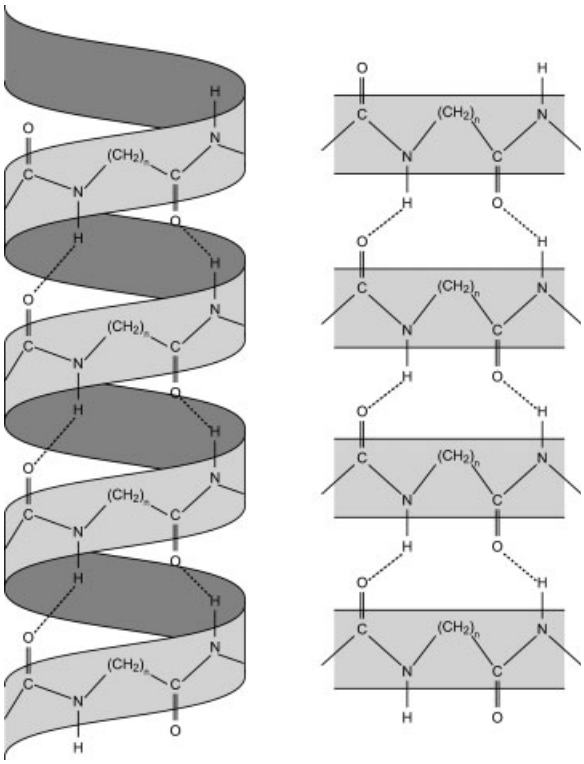


FIGURE 2 Structural similarities of mixed helices (β-helices) of homologous peptides and parallel β-sheets.

14- and 12-membered hydrogen-bonded rings (H_{14}, H_{12}) is clearly influenced by the substitution type of the backbone.[21,23,25] There are also hints that the mixed helix found in β-peptide sequences is favored by alternating β^2- and β^3-substituted amino acids.[11,22,24,26]

In this article, we present a systematic analysis on the substituent influence on folding into mixed helices for α-, β-, and γ-peptides employing ab initio MO theory. The data are compared with those for the most important periodic structures that are competitive in folding. Ab initio MO theory has been rather successful in the actual field of peptide foldamers to describe secondary structure formation and provided hints for interesting novel secondary structure types.[26–32]

METHODS

The starting point of our calculations were the various unsubstituted mixed helix conformers with $i \rightarrow (i + 1)/i \leftarrow (i + 3)$ and $i \rightarrow (i + 3)/i \leftarrow (i + 5)$ amino acid interactions, respectively, found for the homologous α-, β-, and γ-peptides in our recent study.[17] After generation of the selected substitution patterns in blocked hexamers of α-peptides, trimers and hexamers of β-peptides, and tetramers of γ-peptides, respectively, the geometries of all structures were completely optimized at the HF/6-31G* level of ab initio MO theory. In numerous studies, this approximation level has proved to be reliable for the description of peptide conformations.[33–35]

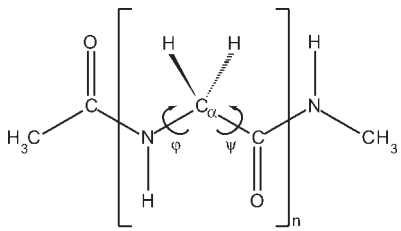
The resulting optimized structures were checked for maintenance of the corresponding helix type and confirmed as minimum conformations by the determination of the eigenvalues of the matrix of force constants. The vibration frequencies arising from these calculations were used for the estimation of the free enthalpies and the entropies of the various helix types at the standard temperature of 300 K on the basis of statistical thermodynamics. Single-point calculations on the optimized HF/6-31G* structures were performed to estimate the influence of the solvents methanol and water employing a polarizable continuum model (PCM//HF/6-31G*). The solvation energy considers the electrostatic, van der Waals, and cavitation energy contributions. The various substituted periodic structures of the homologous peptides, which were selected as reference structures for the stability comparisons, were treated in the same way.

The quantum chemical calculations were performed employing the Gaussian03[36] and the Gamess-US[37] program packages.

RESULTS AND DISCUSSION

Mixed Helices of α-Peptides

Our recent search for mixed helices with the hydrogen-bonding patterns of Figure 1 on blocked glycine hexamers **1** ($n = 6$) provided only one conformer with $i \rightarrow (i + 3)/i \rightarrow (i + 5)$ amino acid interactions.[17] (See Scheme 1.) In this $H_{14/16}$ structure with torsion angles of $\varphi_1 = 80^\circ$, $\psi_1 = -60^\circ$, $\varphi_2 = -60^\circ$, and $\psi_2 = 80^\circ$ in the periodic dimer unit, 14- and 16-membered hydrogen-bonded rings are alternating (Figures 1 and 3). The angle values predicted on the basis of simpler models for this helix type were $\varphi_1 = 125^\circ$, $\psi_1 = -85^\circ$, $\varphi_2 = -80^\circ$ and $\psi_2 = 100^\circ$,[1] and $\varphi_1 = 120^\circ$, $\psi_1 = -82^\circ$, $\varphi_2 = -92^\circ$ and $\psi_2 = 110^\circ$,[2] respectively. Mixed helices with an alternation of the smaller 8- and 10-membered pseudocycles arising from $i \rightarrow (i + 1)/i \leftarrow (i + 3)$ amino acid interactions are impossible



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SCHEME 1

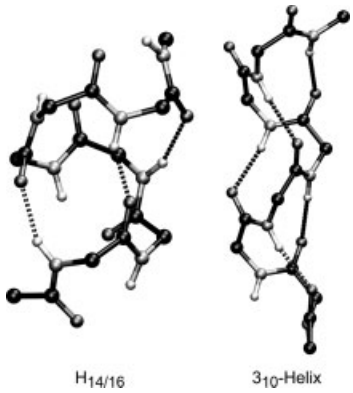


FIGURE 3 Mixed $H_{14/16}$-helix of α-peptides in comparison to the 3_{10}-helix.

for steric reasons. In Table I, the HF/6-31G* stabilities of the right-handed mixed helix conformers of the blocked unsubstituted and the α-methylsubstituted hexamers **1** are given and compared with the data for the corresponding periodic 3_{10}-helices. The total energies are available as a Supplemental file. It can be seen that a very high folding tendency into the two energetically equivalent right- and left-handed mixed helices exists for the unsubstituted hexamer. The mixed helix conformer is by 65.3 $kJ \cdot mol^{-1}$ more stable than the corresponding 3_{10}-helix structure. The left- and right-handed helices of substituted hexamers are only approximate mirror images and are energetically different. However, it is always possible to derive the energies for the left-handed helices from the data for the right-handed ones in Table I, since the mirror image of a substituted right-handed helix corresponds exactly to the left-handed helix bearing the substituents with the opposite configuration. Obviously, the tendency to form mixed helices decreases after introduction of R-, or alternatively, S-configured substituents in all hexamer constituents. Although the energy difference between the most stable mixed helix and the 3_{10}-helix is small, the latter is always more stable in these cases. Contrary to this, the formation of mixed helices in α-peptides is supported by an alternating R- and S-substitution of the monomers. Beginning the sequence with an R-amino acid, the right-handed mixed helix predominates and is by about 50 $kJ \cdot mol^{-1}$ more stable than the 3_{10}-helix conformer (Table I).

Helix formation is often discussed solely on the basis of energy data. It could be useful to estimate the free enthalpy differences between the competitive secondary structures and the influence of entropy contributions at standard temperature. Table II provides information on the differences of the free enthalpies, the enthalpies including the zero-point vibration energies and thermal corrections, and the entropies between the mixed helix conformers of α-peptides and the corresponding 3_{10}-helices. The values for the free enthalpy differences confirm the stability order, which was originally obtained on the basis of the total energies, also for a temperature of 300 K. However, the entropy influence is in favor of the periodic 3_{10}-helices for all substitution patterns. Obviously, β-helices are states of higher order than the periodic secondary structures.

Because of the alternating hydrogen-bond patterns, mixed helices have only rather small dipole moments in comparison to their periodic counterparts. Therefore, they are energetically disadvantaged in a polar environment. Estimation of the solvent influence for the solvents methanol and water at the PCM//HF/6-31G* level of ab initio MO theory confirms the preference of the corresponding 3_{10}-helix conformers (Table I). Nevertheless, the existence of the gramicidin A membrane channel as

Table I Relative Energies[a] of the Right-Handed 3_{10}-Helix and the Mixed $H_{14/16}$-Helix of Unsubstituted and Methylsubstituted Hexamers of 1 at the HF/6-31G* and at the PCM//HF/6-31G* Levels of Ab Initio MO Theory

	H_{10}[b]			$H_{14/16}$		
Substitution[c]	HF	PCM (MeOH)	PCM (H_2O)	HF	PCM (MeOH)	PCM (H_2O)
U	65.3	**0.0**	**0.0**	**0.0**	32.4	46.4
R	35.9	32.2	30.6	0.8	73.1	74.6
S	**0.0**	**0.0**	**0.0**	7.6	83.6	84.7
RS	53.6	**0.0**	**0.0**	**0.0**	25.5	26.3
SR	59.2	3.0	2.8	79.6	98.5	99.4

[a] In $kJ \cdot mol^{-1}$, Supplemental file with the total energies available from the authors (see end of text before acknowledgements).

[b] 3_{10}-Helix.

[c] U: unsubstituted; R: R-configuration; S: S-configuration; RS and SR: alternating RS- or SR-configurations of the methyl substituents.

Table II Relative Enthalpies,[a] Free Enthalpies,[a] and Entropies[a] for the Right-Handed Mixed and 3_{10}-Helices of Unsubstituted and Substituted Hexamers 1 of α-Peptides

Substitution[b]	$H_{14/16}$	H_{10}[c]
U		
ΔH	**0.0**	30.2
ΔG	**0.0**	19.6
ΔS	−35.5	**0.0**
R		
ΔH	6.2	35.8
ΔG	24.7	44.7
ΔS	−62.3	−29.8
S		
ΔH	13.9	**0.0**
ΔG	32.0	**0.0**
ΔS	−60.6	**0.0**
RS		
ΔH	**0.0**	49.2
ΔG	**0.0**	38.7
ΔS	−35.0	**0.0**
SR		
ΔH	82.6	54.6
ΔG	86.3	44.5
ΔS	−47.6	−1.3

[a] Relative enthalpies and free enthalpies in $kJ \cdot mol^{-1}$; relative entropies in $J\ mol^{-1} \cdot K^{-1}$. Supplemental file with the absolute values available from the authors (see end of text before acknowledgements).

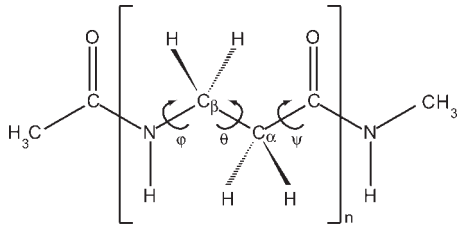
[b] U: unsubstituted; R: R-configuration; S: S-configuration; RS and SR: alternating RS- or SR-configurations of the methyl substituents.

[c] 3_{10}-Helix.

an $H_{20/22}$-helix in an apolar membrane environment and a single-strand $\beta^{4.4}$-helix[4,8] found in NMR studies on oligonorleucine sequences in deuterated chloroform, which corresponds to the $H_{14/16}$-conformer in our calculations, indicate the possibility of mixed helix formation in α-peptides. It might be interesting to speculate on transitions between mixed and periodic helix alternatives dependent on changes of the environment.

Mixed Helices of β-Peptides

Three types of mixed $H_{12/10}$-helices (I, II, III) with alternating 12- and 10-membered hydrogen-bonded rings were found in our study[17] on unsubstituted β-peptide hexamers **2** ($n = 6$). (See Scheme 2.) Most stable was the conformer I with torsion angles of $\varphi_1 = -100°$, $\theta_1 = 60°$, $\psi_1 = 90°$, $\varphi_2 = 90°$, $\theta_2 = 60°$, and $\psi_2 = -110°$ in the periodic dimer unit that corresponds to the mixed helix found in the Seebach group



2

SCHEME 2

with torsion angles of $\varphi_1 = -100°$, $\theta_1 = 60°$, $\psi_1 = 90°$, $\varphi_2 = 90°$, $\theta_2 = 70°$, and $\psi_2 = -70°$.[38] Conformer I is much more stable than the periodic H_{14}-helix of β-peptides (Table III). In Figure 4, the three $H_{12/10}$-helices are visualized together with the three rather stable periodic secondary structure alternatives H_6, H_{12}, and H_{14}. In order to get an overview on the substituent influence on mixed helix formation in β-peptides, all trimers **2** ($n = 3$) of the three basic mixed helix types with monomethylsubstituted amino acid constituents were subject of examination. Since in short oligomers boundary effects cannot be excluded, both possible orders of the alternating hydrogen-bonded rings, 10/12 and 12/10, respectively, were considered.

To characterize the various substituted derivatives, substituents in the 2-position (α-position) of an amino acid monomer are denoted by an uppercase ''A'' for the S-configuration and a lowercase ''a'' for the R-configuration. The corresponding notations for substituents in the 3-position (β-position) are an uppercase ''B'' for the S- and a lowercase ''b'' for the R-configuration. Confining our calculations to monosubstituted amino acid constituents and considering the dimer periodicity, a two-letter code is sufficient to distinguish between the various substituted derivatives. Thus, the notation AB12/10 for a periodic dimer unit of **2** means an S-configured methyl group in the 2(α)-position of the first monomer and an S-configured methyl group in the 3(β)-position of the second one. The hydrogen-bonded rings alternate in the order 12/10.

The spider plots of Figure 5 provide the complete information on the stabilities of all right-handed mixed helix trimers $H_{10/12}$ and $H_{12/10}$ of the types I, II, and III with monosubstituted constituents together with the stabilities of the corresponding periodic β-peptide structures H_6 with 6-membered hydrogen-bonded rings.[26] The periodic H_6 secondary structure type was selected for comparison, since it tolerates all substituents, whereas most of the corresponding H_{14}-helices could not be localized as minimum conformations at the trimer level. The stabilities are

Table III Relative Energies[a] of the Right-Handed Mixed $H^{I}_{12/10}$- and $H^{I}_{20/18}$-Helices of Substituted Hexamers of 2 in Comparison to the Right-Handed Periodic H_{12} and H_{14} β-Peptide Helices at the HF/6-31G* and PCM//HF/6-31G* Levels of Ab Initio MO Theory

	$H^{I}_{12/10}$			$H^{I}_{20/18}$			H_{12}			H_{14}		
Substitution[b,c]	HF	PCM (MeOH)	PCM (H_2O)	HF	PCM (MeOH)	PCM (H_2O)	HF	PCM (MeOH)	PCM (H_2O)	HF	PCM (MeOH)	PCM (H_2O)
U	**0.0**	**0.0**	**0.0**	13.1	19.1	23.5	82.6	30.3	33.5	96.0	8.0	10.7
AA	**0.0**	**0.0**	**0.0**	—	—	—	72.2	31.8	25.6	—	—	—
aa	57.6	53.3	51.7	—	—	—	126.0	76.7	75.8	81.0	14.7	8.3
BB	**0.0**	25.7	25.6	22.2	56.0	55.9	53.0	24.8	23.3	—	—	—
bb	62.8	70.6	76.6	36.6	67.8	67.8	139.2	124.5	124.0	47.8	**0.0**	**0.0**
Aa	47.7	54.4	52.9	—	—	—	97.7	49.8	47.4	124.3	69.7	69.3
aA	**0.0**	**0.0**	**0.0**	13.9	21.5	22.7	95.2	44.8	40.5	—	—	—
Ab	**0.0**	**0.0**	**0.0**	—	—	—	90.4	50.6	51.1	90.5	47.9	47.4
aB	22.0	25.3	29.4	26.3	36.5	38.7	73.3	26.4	26.1	—	—	—
BA	**0.0**	**0.0**	**0.0**	33.8	42.6	42.7	92.8	37.9	37.6	—	—	—
ba	134.4	123.2	121.0	—	—	—	161.6	117.0	115.0	94.6	20.7	18.1
Ba	0.1	**0.0**	**0.0**	—	—	—	66.6	20.5	19.1	—	—	—
bA	30.3	30.0	29.9	**0.0**	10.9	13.6	87.5	45.6	44.5	—	—	—
Bb	**0.0**	**0.0**	**0.0**	30.8	33.7	35.8	111.1	65.8	63.8	—	—	—
bB	96.6	94.1	94.3	55.2	66.0	68.5	115.0	72.9	72.7	—	—	—

[a] In $kJ \cdot mol^{-1}$; Supplemental file with the total energies available from the authors (see end of text before acknowledgements).
[b] For substitution pattern notation, see text.
[c] Dashes denote structures where the helix type is not kept after geometry optimization.

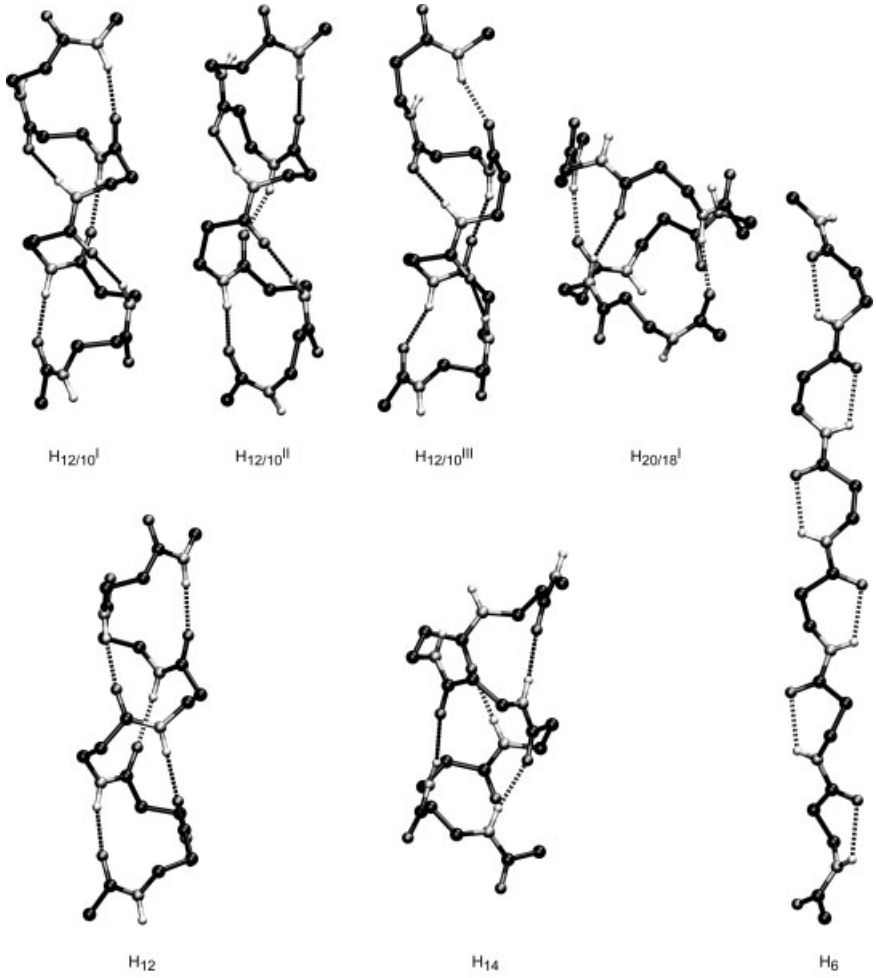


FIGURE 4 Mixed helix types (first line) and selected periodic secondary structures (second line) of β-peptides.

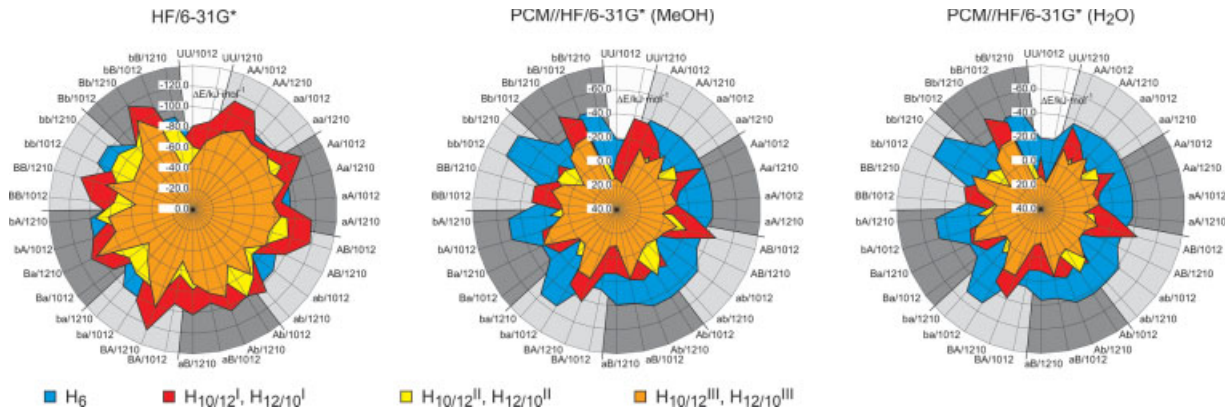


FIGURE 5 Spider plots of the relative energies (in $kJ \cdot mol^{-1}$) of the three different right-handed mixed helix types of β-peptides with alternating 10- and 12-membered hydrogen-bonded rings in trimer **2** for various substitution patterns in comparison to the corresponding periodic H_6 secondary structures. The two alternative possibilities of the order of the hydrogen-bonded rings ($H_{10/12}$ and $H_{12/10}$) are considered. References for the energy comparison are the corresponding extended β-peptide sequences, i.e., structures with negative relative energies are more stable than the extended conformations. For the notation of the substituent patterns, see text.

Table IV Relative Enthalpies,[a] Free Enthalpies,[a] and Entropies[a] for the Right-Handed Mixed and Periodic Helix Alternatives of Unsubstituted and Substituted Hexamers I of β-Peptides

Substitution[b,c]	$H_{12/10}$	$H_{20/18}$	H_{12}	H_{14}
U				
ΔH	**0.0**	8.6	80.9	88.6
ΔG	**0.0**	7.5	70.2	77.1
ΔS	−38.6	−34.9	−2.8	**0.0**
AA				
ΔH	**0.0**	—	70.8	—
ΔG	**0.0**	—	62.0	—
ΔS	−37.6	—	−8.1	—
aa				
ΔH	58.2	—	126.0	74.5
ΔG	60.4	—	128.6	63.3
ΔS	−45.0	—	−46.4	**0.0**
BB				
ΔH	**0.0**	18.6	50.1	—
ΔG	**0.0**	20.7	37.9	—
ΔS	−41.8	−48.9	−1.1	—
bb				
ΔH	64.5	31.7	140.0	38.9
ΔG	68.3	24.0	141.3	26.5
ΔS	−54.5	−16.3	−46.2	**0.0**
Aa				
ΔH	47.8	—	96.6	118.7
ΔG	48.8	—	87.4	104.2
ΔS	−52.1	—	−17.8	**0.0**
aA				
ΔH	**0.0**	11.6	93.6	—
ΔG	**0.0**	12.0	85.0	—
ΔS	−48.7	−50.0	−19.8	—
Ab				
ΔH	**0.0**	—	89.8	84.7
ΔG	**0.0**	—	87.0	73.2
ΔS	−38.5	—	−29.0	**0.0**
aB				
ΔH	22.7	23.7	71.5	—
ΔG	25.5	27.2	64.7	—
ΔS	−47.6	−50.1	−15.7	—
BA				
ΔH	**0.0**	31.4	91.1	—
ΔG	**0.0**	32.4	81.5	—
ΔS	−36.5	−39.8	−4.3	—
ba				
ΔH	136.6	—	162.3	87.5
ΔG	143.0	—	161.9	76.6
ΔS	−58.2	—	−35.4	**0.0**
Ba				
ΔH	3.2	—	68.4	—
ΔG	4.3	—	61.6	—
ΔS	−26.4	—	**0.0**	—
bA				
ΔH	34.8	**0.0**	90.4	—
ΔG	38.0	**0.0**	86.0	—
ΔS	−33.5	−22.6	−7.6	—
Bb				
ΔH	**0.0**	25.9	110.2	—
ΔG	**0.0**	22.0	109.4	—
ΔS	−13.0	**0.0**	−10.3	—
bB				
ΔH	98.2	51.5	114.2	—
ΔG	105.1	56.9	112.5	—
ΔS	−35.9	−31.0	−7.1	—

[a] Relative enthalpies and free enthalpies in $kJ \cdot mol^{-1}$; relative entropies in $J \cdot mol^{-1} \cdot K^{-1}$. Supplemental file with the absolute values available from the authors (see end of text before acknowledgements).

[b] For substitution pattern notation, see text.

[c] Hyphens denote structures where the helix type is not kept after geometry optimization.

given as relative energies referred to the corresponding extended peptide conformations. Thus, it is possible to compare the folding tendencies of all substituted peptide derivatives into the various helix alternatives starting from an extended peptide chain. All extended conformations were optimized keeping the backbone torsion angles fixed at 180°. The numerical data for the total energies and the backbone torsion angles for all trimers and the information on those trimer conformers that do not keep the mixed helix structure are available as a Supplemental file. It is possible to derive the information on all left-handed folding alternatives for a given substitution pattern from the spider plots of Figure 5, since a substituted left-handed conformer has the same energy as the right-handed conformer bearing the substituents with the opposite configuration. The HF/6-31G* data of Figure 5 demonstrate a considerable folding potential into mixed helices for several substitution patterns of the mixed helix type I. Thus, it can be seen that right-handed mixed helices are favored in the derivatives AB10/12 and BA12/10, which correspond to the mixed helix found by the Seebach group, but also in derivatives with the substitution patterns AA12/10, AA10/12, BB10/12, BB12/10, Bb12/10, bB10/12, Aa10/12, and aA12/10, respectively. These

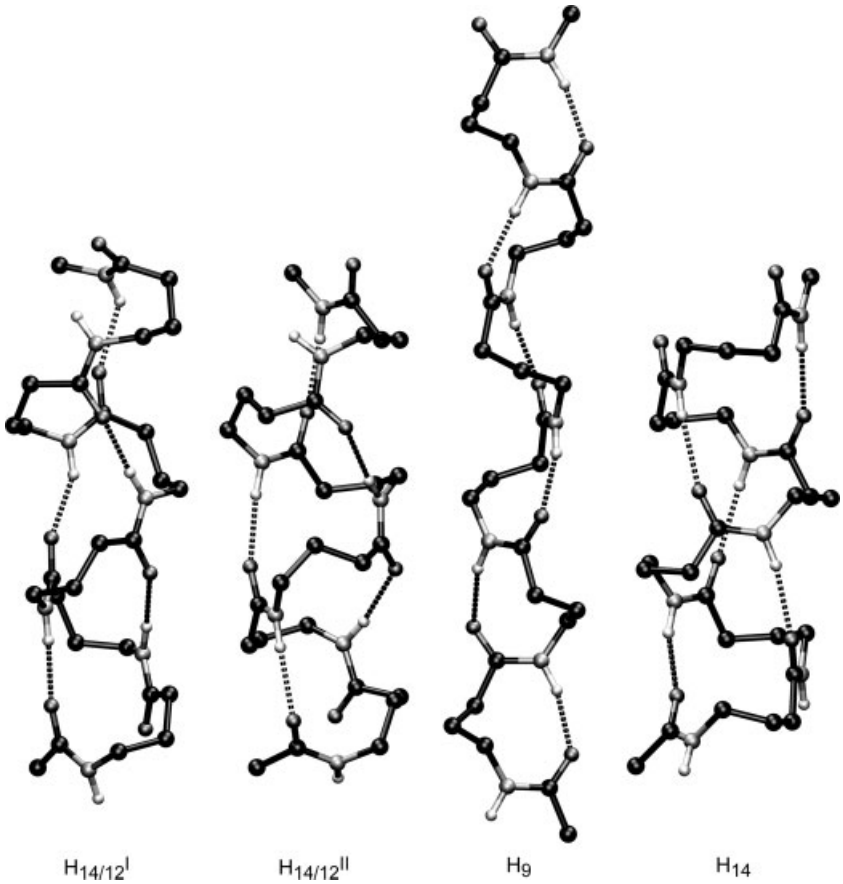
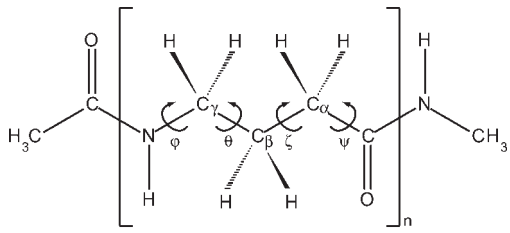


FIGURE 6 Mixed $H_{14/12}$-helices of γ-peptides in comparison to the periodic H_9- and H_{14}-helices.

conclusions may be transferred to the corresponding left-handed helices with the opposite configurations of the substituents. The mixed helix alternatives II and III are generally less stable than helix type I for most of the substitution patterns. Only the derivatives Ab10/12 for type II and Ba12/10 for III have stabilities comparable to those of the other competitive structures. As expected, the stability of all mixed helices, in particular for the types II and III, decreases in polar environments. Nevertheless, the spider plots for the solvents methanol and water in Figure 5 show that some of the substituted mixed helices of type I like Bb12/10 and AB10/12 are still rather stable in these media.

The calculations on the trimers indicate important general trends of substituent effects in mixed helix formation. For an estimation of the influence of the sequence length on the helix formation, it could be interesting to extend the study to the hexamers **2** ($n = 6$). Now, there is also the opportunity to compare the stabilities of the mixed helices with those of the periodic H_{14}- and H_{12}-helices experimentally found in β-peptides.[21,25] Besides, it becomes possible to examine the formation of mixed helices with the still larger alternating 20- and 18-membered hydrogen-bonded rings (Figures 1 and 4), which are only possible in longer sequences. Table III provides the energy data for substituted mixed helix hexamers and the corresponding periodic structures, which indicate the considerable stability of mixed helices at the HF/6-31G* level of ab initio MO theory.

With respect to the influence of various substitution patterns on mixed helix formation, the conclusions drawn from the trimer data can be maintained. More-



3

SCHEME 3

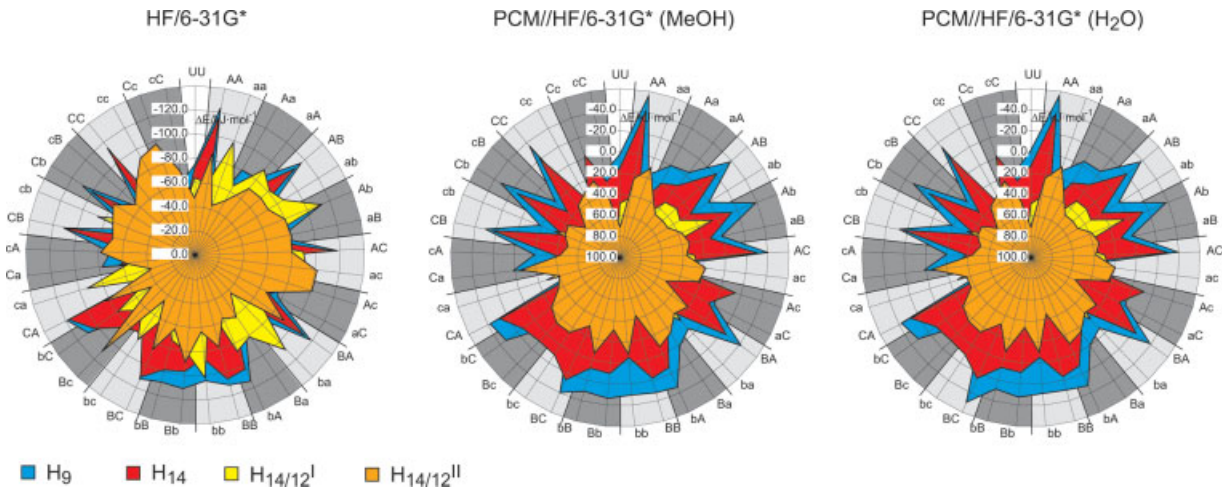


FIGURE 7 Spider plots of the relative energies (in $kJ \cdot mol^{-1}$) of the two different right-handed mixed γ-peptide helices $H^{I}_{14/12}$ and $H^{II}_{14/12}$ for various substitution patterns of tetramer **3** in comparison to the periodic right-handed H_9- and H_{14}-helices. References for the energy comparison are the corresponding extended γ-peptide sequences, i.e., structures with negative relative energies are more stable than the extended conformations. For the notation of the substituent patterns, see text.

over, the general rules of the substituent influence on the formation of the periodic H_{12}- and H_{14}-helices, which were derived from the conformational properties of blocked β-peptide monomers in one of our former studies,[23] are confirmed by the direct study of these helices at the hexamer level and extended by consideration of further substituent patterns. It is rather impressive that several substituted mixed helices of the $H_{12/10}$ type keep their stability advantages over their periodic counterparts also in polar solvents. Thus, for the substitution types AA, Ab, Ba, Bb, and last but not least, for BA, the substitution type of the experimentally found mixed helix, β-helix formation is still preferred. Obviously, the tendency to form mixed helices is much greater in β- than in α-peptides.

The energy data in Table III demonstrate that mixed helices with the larger alternating 20- and 18-membered hydrogen-bonded rings (Figures 1 and 4) are also more stable than the competitive periodic secondary structures in vacuo or in an apolar environment. However, in most cases the mixed $H_{12/10}$-helices with the smaller ring sizes are preferred. Only the right-handed $H_{20/18}$-helix with the bA substitution pattern is superior over the corresponding right-handed $H_{12/10}$-helix and has approximately the same energy as the left-handed bA12/10 conformer.

As in the case of α-peptides, the free enthalpies and entropies were estimated for the various helix types of β-peptides. Table IV provides the differences of the free enthalpies, the enthalpies with inclusion of the zero-point vibration energies, and the thermal corrections and the entropies for the main types of mixed and periodic β-peptide helices. Although the preference of mixed helices for the above-mentioned substitution patterns is also kept at the free enthalpy level, it is striking that the periodic helices H_{14} and H_{12} have greater entropy values than the corresponding mixed helices. In particular, the formation of periodic H_{14}-helices is favored by entropy effects. As already discussed for the mixed helices of α-peptides, the mixed helices of β-peptides represent higher-ordered states than their periodic counterparts.

Mixed Helices of γ-Peptides

Several mixed helix conformers were localized in our recent ab initio study[17] for unsubstituted γ-peptide hexamers **3** ($n = 6$) (see Scheme 3). Thus, two folding alternatives (I, II) with alternating 14- and 12-membered hydrogen-bonded rings [$i \rightarrow (i + 1)/i \leftarrow (i + 3)$ amino acid interactions, Figure 1] and even three (I, II, III) with alternating 24- and 22-membered rings [$i \rightarrow (i + 3)/i \leftarrow (i + 5)$ amino acid interactions, Figures 1 and 6] were found. Contrary to the situation in β-peptides, all mixed γ-peptide helices are less stable than the periodic folding alternatives at the HF/6-31G* level of ab initio MO theory. Thus, it seems to be relatively improbable to get mixed helices in γ-peptide sequences.

In our estimation of substituent effects, we considered tetramer structures **3** ($n = 4$) (see Scheme 3) of the two $H_{14/12}$-helices and selected the corresponding derivatives of the periodic H_{14}-helix, which has been experimentally found in γ-peptide sequences,[39] and the periodic H_9-helix, which is rather stable according

Table V Relative Energies[a] of the Right-handed Mixed $H^{I}_{14/12}$- and $H^{II}_{14/12}$ -Helices of Substituted Tetramers of 3 in Comparison to the Right-Handed Periodic H_9 and H_{14} γ-Peptide Helices at the HF/6-31G* and PCM//HF/6-31G* Levels of Ab Initio MO Theory

	$H^{I}_{14/12}$			$H^{II}_{14/12}$			H_9			H_{14}		
Substitution[b,c]	HF	PCM (MeOH)	PCM (H_2O)	HF	PCM (MeOH)	PCM (H_2O)	HF	PCM (MeOH)	PCM (H_2O)	HF	PCM (MeOH)	PCM (H_2O)
UU	10.6	46.6	50.9	27.3	69.2	73.2	3.1	12.9	14.9	**0.0**	**0.0**	**0.0**
AA	62.8	97.7	97.3	37.9	79.4	79.8	8.4	14.6	17.6	**0.0**	**0.0**	**0.0**
aa	24.6	64.9	65.3	—	—	—	100.7	101.8	102.2	—	—	—
Aa	25.2	59.7	59.8	—	—	—	31.3	23.6	24.0	—	—	—
aA	**0.0**	36.2	36.0	19.9	51.0	53.1	23.2	15.8	16.1	9.2	**0.0**	**0.0**
Ab	0.7	44.0	42.3	32.9	75.4	75.8	15.6	24.2	23.3	**0.0**	**0.0**	**0.0**
aB	58.4	93.9	91.6	31.7	71.0	71.6	52.2	51.2	50.4	—	—	—
Ac	5.0	34.1	33.3	**0.0**	36.3	35.4	13.3	**0.0**	**0.0**	—	—	—
aC	40.8	70.5	68.4	34.9	65.3	64.9	32.2	19.7	17.7	—	—	—
BB	67.1	102.5	100.1	32.6	75.5	74.6	5.3	14.3	13.1	**0.0**	**0.0**	**0.0**
bb	9.3	48.9	48.6	—	—	—	25.3	37.4	35.1	6.9	1.8	0.7
Bc	47.6	88.7	88.2	**0.0**	46.9	47.1	27.6	23.3	22.5	—	—	—
bC	32.1	70.9	68.8	—	—	—	7.8	19.3	20.2	1.7	**0.0**	**0.0**
Ca	22.8	62.4	60.8	—	—	—	15.4	36.7	26.6	0.8	**0.0**	**0.0**
cA	5.1	52.3	53.6	**0.0**	44.4	46.1	30.2	40.8	40.7	8.4	20.9	22.1
CC	43.9	81.4	79.7	37.7	73.3	73.1	**0.0**	14.9	8.4	3.3	**0.0**	**0.0**
cc	54.9	96.7	94.5	21.9	66.5	67.4	86.1	74.7	81.4	—	—	—
Cc	9.1	33.0	33.0	**0.0**	25.3	25.4	17.8	**0.0**	**0.0**	—	—	—
cC	55.6	81.5	79.7	25.9	47.2	47.4	55.9	28.8	28.0	—	—	—

[a] In $kJ \cdot mol^{-1}$. Supplemental file with the total energies available from the authors (see end of text before acknowledgements).
[b] For substitution pattern notation, see text.
[c] Dashes denote structures where the helix type is not kept after geometry optimization.

Table VI Favorable Substitution Patterns for the Formation of Right-Handed Mixed Helices in α-, β-, and γ-Peptides

Peptide	Helix Type[a]	Substitution Pattern[b]
α	$H_{14/16}$	U, RS
β	$H^{I}_{12/10}$	U, BA, BB, Bb, AA, Aa
	$H^{II}_{12/10}$	ba
	$H^{III}_{12/10}$	Ba
	$H^{I}_{20/18}$	bA
γ	$H^{I}_{14/12}$	aA, Ab
	$H^{II}_{14/12}$	Ac, Bc, Cc, cA

[a] See Figure 1.
[b] See text for nomenclature.

to our former calculations,[29,40] as folding alternatives for the energy comparison (Figure 6). Our nomenclature for the substituted γ-peptide derivatives has to be supplemented by an uppercase ''C'' for an S-configured substituent in the 4-position (γ-position) of a γ-amino acid constituent and by a lowercase ''c'' for an R-configured substituent in this position. Considering only monomethylsubstituted amino acid constituents and the dimer periodicity, the two-letter code can be maintained.

Figure 7 shows the spider plots of the stabilities for the various right-handed methyl-substituted γ-peptide tetramers. In Table V, the relative energies for the mixed and periodic helix alternatives of the most important substituted γ-peptide tetramers are explicitly given. A complete overview on the numerical geometry and energy data for all derivatives is again available as a Supplemental file. The spider plots at all approximation levels demonstrate that mixed helices in γ-peptide sequences, if they could be formed at all, need an apolar environment for their formation. Most promising are the substituent patterns aA and Ab for the formation of right-handed $H^{I}_{14/12}$-helices, whereas right-handed $H^{II}_{14/12}$-helices are favored by the substitution patterns Ac, Bc, cA, and Cc. The $H^{I}_{14/12}$-helix tolerates the various substitution patterns, but the $H^{II}_{14/12}$-helix is rather sensitive to substituent effects. In particular, R-substituents in the β-position of the first γ-amino acid constituent of the dimer units and R-substituents in the α-position of the second γ-amino acid constituent of the dimer units destroy the right-handed mixed helix conformation.

It may be useful to also give some hints on the substituent influence on the formation of the two rather stable periodic γ-peptide structures H_9 and H_{14} from our comparative study because the secondary structures of γ-peptides have not yet been so intensively investigated as those of β-peptides until now. Since there are only minor differences between the backbone torsion angles of the two helices, which are in a similar relation as the 3_{10}- and α-helices of α-peptides, the substituent influence on both helices is rather similar. Independent of the actual stability, the H_9 structure is kept for all substitution patterns, whereas the experimentally found H_{14} γ-peptide helix[39] is more influenced by substituents (Figure 7). The right-handed γ-peptide helices H_9 and H_{14} are clearly disadvantaged by R- and favored by S-substituents in the α-position of the amino acid constituents. R-substituents in the γ-positions are only accepted in a few cases. Substituents in β-position show an indifferent behavior. Generally, it seems to be difficult to support the formation of H_9- and H_{14}-helices in γ-peptides selectively by special substitution patterns.

CONCLUSIONS

Our quantum chemical analysis of the substituent influence on the folding of sequences of homologous α-, β-, and γ-peptides demonstrates considerable possibilities to enforce the formation of the unique secondary structure type of ''mixed'' or β-helices by special backbone substitution patterns. In numerous cases, folding of homologous peptide sequences into mixed helices is superior over that into periodic structures with the greatest probability to get mixed helices in β-peptides. The predominance of periodic peptide helices in peptides and proteins seems to be essentially caused by the influence of polar environments.

Our study provides both information on the substituent influence on the mixed helix formation in the various classes of homologous peptides and on the formation of mixed helix alternatives within the same class of homologous peptides. Table VI summarizes the substitution patterns that should be preferred to get mixed helix types in the various classes of homologous peptides. This information might be helpful for chemists in the rational design of peptide structures with membrane channel-forming properties.

SUPPLEMENTAL FILE

Comprehensive material of tables with the total energies and geometry data of all α-, β- and γ-peptide conformers, with the absolute values for the free enthalpies, enthalpies and entropies, and with the relative energies, which the spider plots of the β- and γ-peptides are based on, are available from the authors (http://www.biochemie.uni-leipzig.de/aghofmann).

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