Peptide Structures

Mixed Helices—A General Folding Pattern in Homologous Peptides?**

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Oligomers composed entirely of unnatural monomers that form characteristic secondary structures have attracted considerable attention in the last years.[1] The motivation for work in this area ranges from gaining a better understanding of the structure and function of biomolecules and imitating them to developing polymers with novel properties. Considerable stimulation of research on these foldamers[2] came from the investigation of β-peptides.[3] Numerous secondary structures were found in these β-peptides as well as in homologous γ- and δ-peptides.[1,4]

In their studies on β-peptides Seebach and co-workers found a unique type of secondary structure, which they referred to as a "mixed helix".[5] Other authors have also described such mixed helices in the meantime.[6] In the familiar periodic helices all corresponding backbone torsion angles of the monomer constituents have the same values, and all peptide bonds form hydrogen bonds of the same type. In contrast, the periodicity of the mixed helices emerges at the level of dimer units. Here, the monomer constituents have alternating values for the backbone torsion angles, and the peptide bonds form hydrogen bonds of different types in an alternating way as well. The CO and NH groups of adjacent peptide linkages are involved in hydrogen bonds that are formed alternatingly in the forward and backward directions. This leads to the formation of alternating hydrogen-bonded rings of different size along the sequence (Figure 1). In the mixed helices of the β-peptides 10-membered 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.

The existence of mixed helices in β-peptides raises the question whether this folding pattern might also exist in the homologous γ- and δ-peptides and even in the native α-peptides. The transfer of the folding principle to the homologous peptides leads to the hydrogen-bonding patterns with $i\rightarrow(i+1)/i\leftarrow(i+3)$ interactions of the amino acids (Figure 1). Moreover, it is feasible that this principle can be

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[**] This work was supported by the Deutsche Forschungsgemeinschaft (Project HO2346/1 "Sekundärstrukturbildung in Peptiden mit nicht-proteinogenen Aminosäuren" and SFB 610 "Proteinzustände mit zellbiologischer und medizinischer Relevanz") and the Fonds der Chemischen Industrie.

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  DOI: 10.1002/anie.200353249

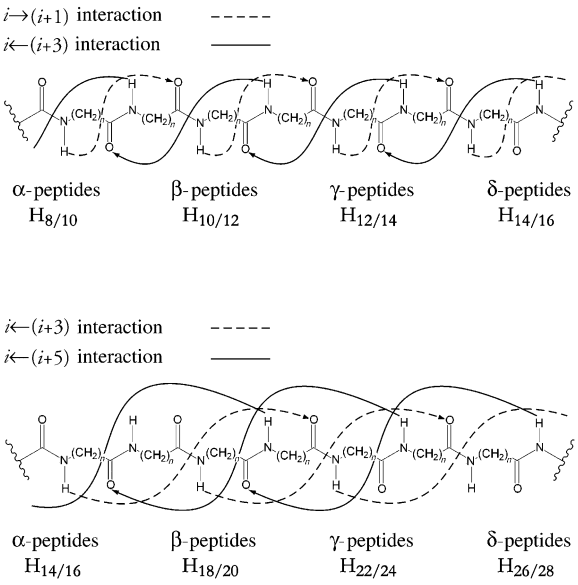


Figure 1. Alternative hydrogen-bonding patterns in mixed helices $H_{x/y}$ of homologous α- ($n=1$), β- ($n=2$), γ- ($n=3$), and δ-peptides ($n=4$). The index x/y denotes the number of atoms in the alternating hydrogen-bonded rings.

extended to mixed helices with still larger alternating ring systems, as for instance with an $i\rightarrow(i+3)/i\leftarrow(i+5)$ interaction of the amino acids (Figure 1). Finally, it would be interesting to find further structure alternatives for a given hydrogen-bonding pattern.

On the basis of theoretical methods it is possible to answer these questions. For this purpose the conformational space of hexamers of α-, β-, γ-, and δ-peptides was systematically searched for mixed helices having the hydrogen-bonding patterns shown in Figure 1. In this search the backbone torsion angles φ, θ, ζ, ρ, and ψ of the oligomers (cf. structures in Table 1) were systematically varied.[7] Thus, about 1.1×10^5, 1.7×10^6, 1.1×10^6, and 6.3×10^5 conformations were generated for the α-, β-, γ-, and δ-peptides, respectively. For the δ-peptides only mixed helices with the combination of the smaller rings according to Figure 1 were sought. Since boundary effects cannot be excluded in oligomers of six amino acids, the two possible orders of the alternating rings were considered in the hexamers. In the corresponding conformation pools the candidates for mixed helices were selected on the basis of general geometry criteria for the formation of hydrogen bonds. Dependent on the peptide and helix types, these were in between 5 and 30 conformations that fulfilled the hydrogen-bonding patterns in Figure 1. These structures were the starting points for geometry optimization[8a] at various levels of ab initio MO theory, which provide reliable results in the conformational analysis of peptides (HF/6-31G*, B3LYP/6-31G*).[9] The resulting stationary points on the potential energy surface were characterized by the eigenvalues of the matrix of force constants.

Both ab initio models show mixed helices as energy minima for all homologous peptides. For the β-, γ-, and δ-peptides there are even several representatives for the hydrogen-bonding patterns examined, which are denoted by superscripted Roman numbers on the helix symbol in Table 1. The mixed helix of the α-peptides shows a sequence of alternating 14- and 16-membered rings. Interestingly, the model structures of α-peptides with the smaller 8- and 10-membered rings could not be localized as energy minima. The basic patterns of the mixed helices in the various homologous peptides are characterized by the backbone torsion angles listed in Table 1. Figure 2 shows the most stable helices for

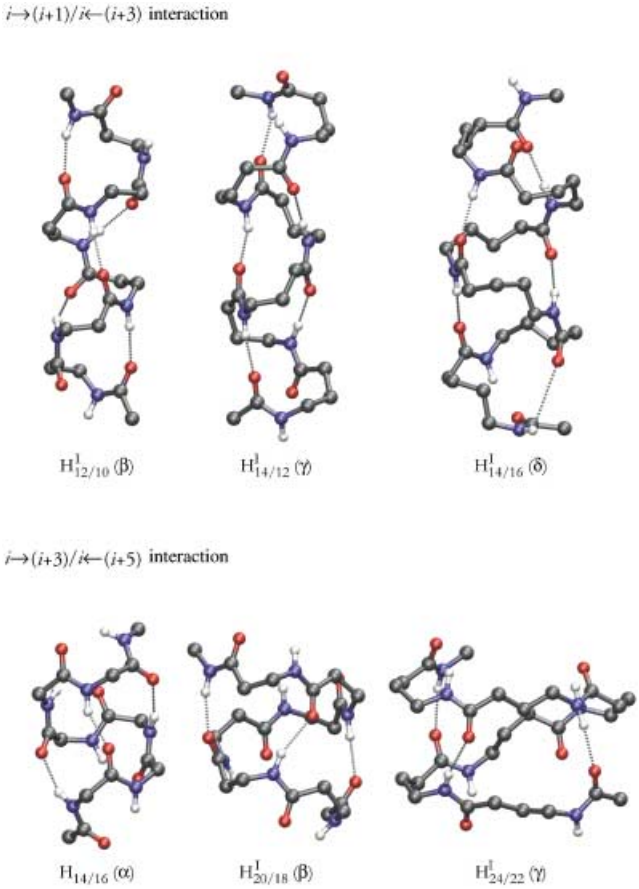
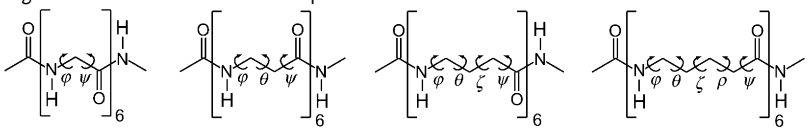


Figure 2. Most stable mixed helices in hexamers of homologous α-, β-, γ-, and δ-peptides for the investigated hydrogen-bonding patterns.

each peptide and hydrogen-bond type. Force field calculations (CHARMm23.1)[10] proved that the hexamer structures are maintained in sequences of up to 20 monomer constituents.

Even more impressive than the wide variety of mixed helices that are possible in the homologous peptides is the considerable stability of some of the structures in comparison to the periodic helices. This is most striking for the β-peptides (Table 1). In the case of the α-peptides, we selected the 3_{10}-helix characterized by 10-membered hydrogen-bonded rings for the energy comparison. For the β-peptides, the experimentally determined helices with 14- and 12-membered rings,[11] which are confirmed by our calculations as particularly stable, served as references to estimate the stability of the mixed-helix alternatives. The reference structure for the γ-peptides was an experimentally found and theoretically

Table 1: Basic patterns of mixed helices in hexamers of α-, β-, γ-, and δ-peptides characterized by the corresponding backbone torsion angles[a] φ, θ, ζ, ρ, and ψ, and the relative energies[b] based on the most stable periodic helix.



α-peptides β-peptides γ-peptides δ-peptides

Type[c]	φ	θ	ζ	ρ	ψ	ΔE HF	ΔE B3LYP	ΔE PCM
α-Peptides								
$H_{14/16}$	81				−67	**−36.2**	**−65.3**	46.4
	−81				85			
H_{10}[d]	−63				−20	0.0	0.0	**0.0**
β-Peptides								
$H_{12/10}^{I}$	−102	61			89	**−82.6**	**−79.5**	**−10.7**
	90	66			−111			
$H_{12/10}^{II}$	87	61			−96	−44.1	−54.6	31.0
	−27	−50			160			
$H_{10/12}^{III}$	179	−62			−21	−45.9	−56.0	26.6
	−93	51			87			
$H_{20/18}^{I}$	91	66			171	−69.5	−57.4	12.8
	−79	−57			149			
$H_{20/18}^{II}$	99	67			173	−35.3	−28.1	36.7
	−150	57			47			
$H_{20/18}^{III}$	153	162			69	−27.9	−20.9	39.5
	73	52			−144			
$H_{18/20}^{IV}$	79	−171			100	−0.5	−1.4	67.7
	110	−48			−43			
H_{14}	−148	61			−138	13.4	26.0	0.0
H_{12}	−87	92			−109	0.0	0.0	22.6
γ-Peptides								
$H_{14/12}^{I}$	−91	79	−80		162	19.9	13.7	49.1
	94	84	−73		−29			
$H_{14/12}^{II}$	65	56	−126		−53	46.7	45.8	80.2
	−64	−37	−61		143			
$H_{24/22}^{I}$	74	−177	−80		−168	12.3	9.9	40.9
	−125	62	−77		154			
$H_{24/22}^{II}$	117	−68	−174		128	44.5	45.8	68.1
	−89	−72	83		58			
$H_{24/22}^{III}$	94	79	−66		−102	72.0	67.1	87.0
	123	64	66		16			
H_{14}	138	−60	−65		141	**0.0**	**0.0**	**0.0**
δ-Peptides								
$H_{14/16}^{I}$	−172	159	−77	−68	131	**−1.9**	−10.0	89.5
	76	69	−167	82	−126			
$H_{16/14}^{II}$	113	−54	−62	167	159	2.4	**−14.2**	90.0
	−126	82	−66	−67	164			
H_{10}	98	−62	−68	169	−85	0.0	0.0	40.2
H_8	180	66	−142	69	−173	19.1	28.2	**0.0**

[a] Angles in degrees. For reasons of space, only the angles of the two central amino acids are given. The angles of all amino acids at all levels of approximation are given in the Supporting Information. [b] Relative energies at the HF/6-31G*, DFT/B3LYP/6-31G*, and PCM//HF/6-31G* levels (solvent water) in kJ mol^{-1}. The most stable structures are indicated. The total energies of the periodic reference helices are given in the Supporting Information. [c] $H_{x/y}$ denotes a mixed helix with alternating hydrogen-bonded rings having *x* and *y* atoms, respectively. H_x denotes periodic helices with hydrogen-bonded rings of *x* atoms. [d] 3_{10}-helix.

confirmed periodic structure with 14-membered rings.[4a,12] Finally, structures with 8- and 10-membered rings, respectively, which proved to be the most stable periodic structures according to our calculations, were the reference structures for the δ-peptides. Both ab initio MO models agree fairly well in their stability predictions. Only for the δ-peptides are the two mixed helices and the periodic reference helix are energetically equivalent at the Hartree–Fock level, although the mixed helices are distinctly more stable according to the density functional theory.

It might be supposed that the influence of the medium could be the reason for the larger number of experimentally determined periodic helices in homologous peptides in comparison to the single representative of a mixed helix found until now. The hydrogen bonds in the mixed helix are formed alternately in forward and backward directions along the sequence. Thus, only a small helix dipole should result. This is confirmed by the dipole moments of $\mu = 3.8$ D for the hexamers of the most stable mixed helix of the β-peptides and $\mu = 31.5$ D for the periodic helix alternative with 14-membered rings. Since the formation of mixed helices is at a disadvantage in polar media, it is more likely to occur in less polar media. In order to estimate the influence of the environment, the solvation energies were calculated for the solvent water based on the polarizable continuum model (PCM//HF/6-31G*).[8b] The results indicate that the mixed helices, in particular those of the α-, γ-, and δ-peptides, indeed become more unstable than the periodic folding alternatives. Only the most stable mixed helices of the β-peptides remain competitive in strongly polar solvents (Table 1).

The formation of mixed helices can also be influenced by the introduction of substituents into the monomer units.[13] Our calculations on models of mixed helices of β-peptides for all possible substitution patterns, which we will report on elsewhere, reveal that a mixed helix with alternate substituents at the α- and β-carbon atoms is especially stable. This substitution pattern is the same as that in the mixed helix found by Seebach and co-workers. Thus, mixed helices prove to be a novel and interesting alternative of general importance for determinining secondary structures in α-peptides and their homologues.

Received: November 4, 2003 [Z53249]

Keywords: ab initio calculations · conformational analysis · foldamers · peptides · secondary structure

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