Design of Secondary Structures in Unnatural Peptides: Stable Helical γ-Tetra-, Hexa-, and Octapeptides and Consequences of α-Substitution

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The secondary structures of oligomeric organic molecules are known to reveal fascinating architectures that can be responsible for their aesthetic beauty as well as their biological function. Nowhere are these features more prominently evident than in peptides and proteins, where the Holy Grail of structure and function can be related in many instances to the presence of the venerable α-helical motif.[1,2] The α-helix is ubiquitous in oligomers of natural α-amino acids,[2] and a great deal of scholarly research has been devoted to the fundamentals of their design and secondary structures.[3]

Recent work published by Seebach[4,5] and Gellman,[6] as well as efforts in our laboratory,[7] have revealed that *β*-peptides can also adopt helical structures in solution, as evidenced mainly by NMR, molecular modeling, and CD measurements.[8,9] Other unnatural oligomers are also known to exhibit interesting secondary structures.[10]

We report that *γ*-peptides derived by homologation of L-alanine and L-valine form stable right-handed helical secondary structures in organic solvents as evidenced by detailed NMR studies. Remarkably, a mere tetramer unit "*γ*-Ala-*γ*-Val-*γ*-Ala-*γ*-Val" is sufficient for helix formation, a trend which is observed in the corresponding hexamer and octamer as well. We also show that α-substitution in these *γ*-peptides can further stabilize the helical structure, provided that the spatial orientation of the α-group is compatible with the main peptide backbone.[11]

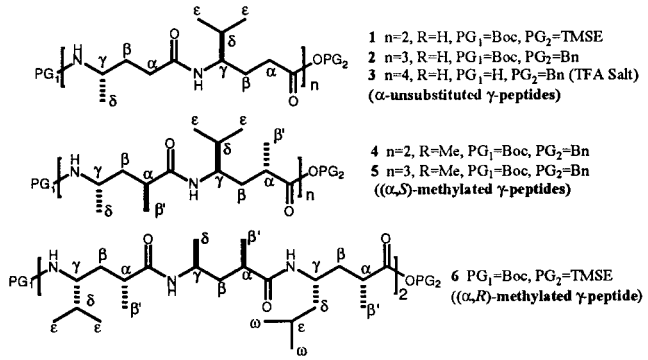

Figure 1. Chemical structures of three types of *γ*-peptides

The homologous *γ*-peptides were synthesized by following standard methods of chain extension[12] and peptide coupling[13] (Figure 1). The *γ*-peptides **1**−**5** were then studied by 2D-NMR in pyridine-d_5, which gave much better resolutions compared to CD_3OH or $CDCl_3$. ^1H NMR resonances and sequence assignments were done by COSY, TOCSY, and ROESY techniques for the tetramer **1** and hexamer **2** (as their *N*-Boc 2-(trimethylsilyl)ethyl or benzyl esters), for the Boc-deprotected octamer (TFA salt) **3,** and for the (α,*S*)-methyl-substituted analogues **4** and **5**. Sequential assignments and structure determination was based on key interresidue NOEs.[13] Families of structures for each molecule were determined using a restrained molecular dynamics simulated annealing protocol[14,15] that included NOE-derived distances as well as NOE and coupling-constant-derived dihedral restraints. Figure 2 shows the right-handed 2.6_1 helical structures of the tetramers **1** and **4** and hexamers **2** and **5** as well as octamer **3** as 20 superimposed structures. Taking the latter as a representative example of a helical structure in solution, the motif exhibits well-defined $(i + 3)$NH···O═C(i) H bonds, generating 14-membered rings with a pitch of ca. 5 Å. Temperature-dependence experiments[16] for peptides **1**, **2**, **4**, and **5** in pyridine-d_5 and DMSO-d_6 titration experiments[10b,17] in pyridine-d_5 or $CDCl_3$ for peptides **1** and **2** corroborate the 2D-NMR assignments. Thus, the chemical shifts of the NH protons of residues 3−6 in the hexapeptide **2** taken as a representative example are only weakly affected over the range 273−323 K, indicating a relatively stable interresidue

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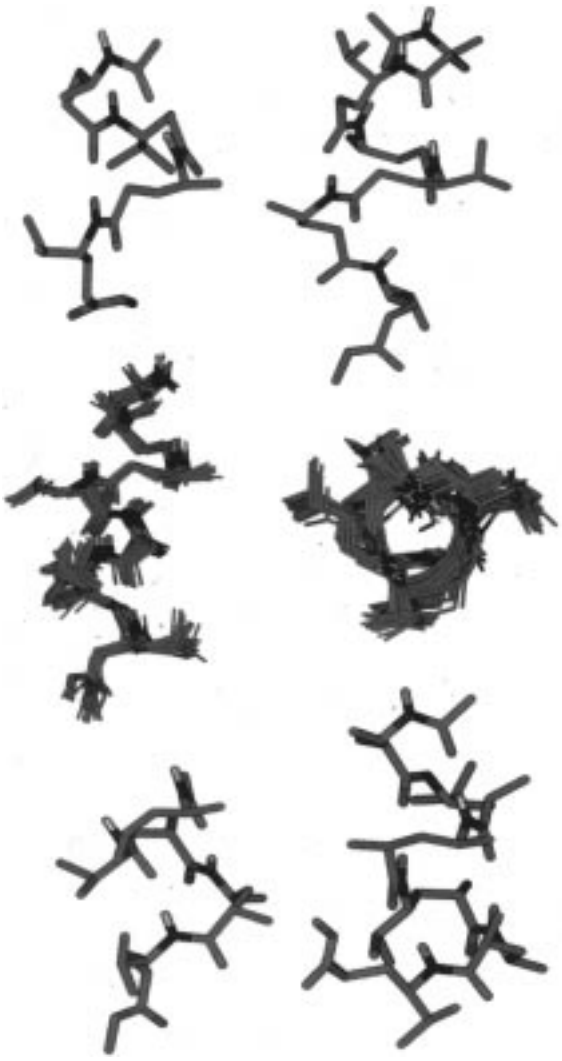

Figure 2. Right-handed 14-helical structures of tetramer **1** (top left), hexamer **2** (top right), octamer TFA salt **3** (middle, side view and top view of 20 superpositions), (α,*S*)-methylated tetramer **4** (bottom left), and hexamer **5** (bottom right). Structures are derived from NMR constraints. Green = carbon, red = oxygen, blue = nitrogen.

Table 1. Temperature Dependence ($-d\delta/dT$) of ^{1}H NMR Chemical Shifts of the NH Protons of γ-Peptides **1** and **2** and (α,*S*)-Methylated γ-Peptides **4** and **5** (1 mM in Pyridine-d_5)[a]

peptides	γ-Ala(1)	γ-Val(2)	γ-Ala(3)	γ-Val(4)	γ-Ala(5)	γ-Val(6)
1	15.1	12.6	5.4	5.0		
2	14.4	13.3	5.4	3.9	5.8	5.8
4	12.3	10.6	1.7	2.9		
5	10.5	9.1	3.1	2.4	3.0	3.1

[a] Values are expressed in ppb K^{-1}.

H-bonded structure within the helix (Table 1). Interestingly, the (α,*S*)-methylated analogues of tetramer **4** and hexamer **5** showed smaller NH shifts compared to their unsubstituted precursors **1** and **2**, thus indicating a higher order of helix stability, possibly due to more favorable side-chain hydrophobic interactions.[11] The NH protons of γ-Val(4) and γ-Ala(3) are the least shifted in the presence of increasing concentrations of DMSO-d_6, thus reflecting the relative stability of the interresidue H bonds in the 14-helix

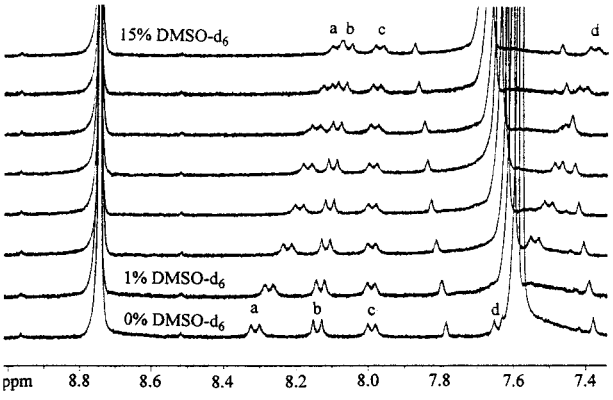

Figure 3. Chemical shift movements of amide protons of tetramer **1** (1 mM in pyridine-d_5, 25 °C) in the presence of increasing concentrations of DMSO-d_6 (1−15% v/v): (a) Val-2; (b) Val-4; (c) Ala-3; (d) Ala-1.

of tetramer **1** (Figure 3). Circular dichroism experiments[13] did not reveal the characteristic patterns exhibited by helical structures of α- and β-peptides, an observation also corroborated by Seebach[5] and Balaram.[18]

The presence of a stable helical structure, even at the level of a tetramer such as **1**, is unprecedented for unnatural peptides. Equally of interest is the consequence of α-substitution in the tetra- and hexapeptides **1** and **2**. Inspection of models predicted that an (α,*S*)-methyl substituent would not alter the 14-helix because of its favorable spatial orientation vis-à-vis the main peptide backbone. Not surprisingly, the (α,*R*)-methylated analogue **6** in a related series did not adopt a helical secondary structure, no doubt due to unfavorable nonbonded interactions of the α-substituent in a 14-helix.[5,19]

The facility with which γ-peptides can assume stable helical structures in protic and nonprotic solvents such as methanol[5] and pyridine is of interest in many contexts. In addition to the purely architectural and design aspects, such unnatural γ-peptides can exhibit biological activity in their own right. Potentially more relevant, however, is the prospect that such metabolically longer-lived peptides can replace topologically similar segments in larger peptide units formed by natural α-amino acids. Insertion of such surrogate motifs in nonfunctional regions of biologically active oligopeptides in which relevant natural segments are maintained would offer a powerful tool toward the engineering of novel functionally useful hybrid proteins.[20]

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Supporting Information Available: ^{1}H NMR chemical shifts and NOEs data, 2D-NMR ROESY spectra, variable-temperature ^{1}H NMR data, DMSO-d_6 titration NMR data, representative procedures, physical constants, ^{1}H NMR spectra of key peptides, and CD spectra (52 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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