Abstract

X-ray crystallographic studies of designed peptides provide definitive proof of the success of a design strategy and yield essential structural information that can be utilized in the future design of biologically and structurally important polypeptides. The ability to create locally folded, hydrogen bonded structures in short peptide oligomers is important for peptide design strategies, which rely on the use of folding nuclei in the construction of protein secondary structure modules like helices and β-hairpins. The realization that backbone homologated amino acids, specifically and residues can be incorporated into folded polypeptide structures, has stimulated considerable recent interest in the areas of peptide mimetic and foldamer design. Polypeptides with unnatural, modified backbones provide an entry to novel classes of intramolecularly hydrogen bonded helical structures, which are without precedent in conventional peptides composed of amino acid residues. The design of hybrid sequences, which contain , , and amino acid residues, greatly expands the diversity of peptide structure space. The insertion of additional backbone atoms into amino acid residues enhances the number of torsional variables that describe local residue conformation. For example, in the case of residues, four torsional variables describe the conformational space, = (Ci-1-Ni-C i-C i), 1 = (Ni-C i-C i-C i), 2 = (C i-C i-C i-Ci), and = (C i-C i-Ci-Ni+1), as compared to two Ramachandran angles , , for an residue. In developing the structural chemistry of residues, it has proved fruitful to explore the conformational properties of residues which are conformationally constrained, either by backbone cyclisation or by the use of gem-dialkyl substitution. In these approaches, conformational choices at selected positions are biased, using local stereochemical constraints that limit the range of accessible backbone torsion angles. Recent trends in peptide research focus on the incorporation of β-, γ- and higher homologues of the α-amino acid residues in designed peptides as they confer significant proteolytic stability. X-ray crystallographic studies of such modified peptides containing non-protein residues are essential, since information on the geometric and stereochemical properties of modified amino acids can only be gathered from systematic structural studies of synthetic peptides incorporating them.

This thesis reports a study of the structures and conformations of designed peptides containing stereo chemically *unconstrained* γ-amino acid residues, derived from naturally occurring proteinogenic α-amino acid residues by backbone homologation. The structures described in this thesis contain the backbone homologated, unconstrained γ-amino acid residues, 4Val, 4Leu and 4Ile and the 3-monosubstituted γ-amino acid residue pregabalin (Pgn). The crystal structure determination of peptides permitted the characterization of intramolecularly hydrogen bonded helices in hybrid sequences and homooligomeric γ- peptides. The studies enabled the precise determination of conformational and geometric parameters of three backbone homologated γ-amino acid residues, 4Val, 4Leu and 4Ile and one monosubstituted unconstrained γ-amino acid residue, pregabalin (Pgn). A detailed analysis of the backbone conformations and intramolecular hydrogen bond parameters of γ- amino acid residues in different hetero- and homooligomeric helices is also provided.

This thesis is divided into 7 chapters.

Chapter 1 provides a brief introduction to the stereochemistry of polypeptide chains, description of backbone torsion angles of α-, β- and γ- amino acid residues and the major secondary structures of α-, β-, γ- and hybrid peptides. Some previous studies on unconstrained backbone homologated γ-residues are briefly reviewed, followed by a concise introduction to X-ray diffraction and the methods of structure solution.

Chapter 2 describes the characterization of the □□ C12 helix in oligopeptides containing the unconstrained □ residue, □4(R)Val. The crystal structure determinations of [Aib-□4(R)Val]n oligomers viz. Boc-[Aib-□4(R)Val]2-OMe, Boc-[Aib-□4(R)Val]4-OMe, Boc-[Aib-□4(R)Val]5- OMe and Boc-[Aib-□4(R)Val]8-OMe reveal the formation of C12 helical structures in the solid state. The structures of (□□)n peptides ranging in length from 4 to 16 residues permit characterization of the conformational parameters for □-residues. A comparison of the structures of 4 and 8 residue peptides containing repeat (□□)n, (αβ)n and (□□)n sequences is also described. The structure determination of the (□□)n sequences, Boc-[Aib-□(*S*)Val]n-OMe (n = 2 and 4) and the (αβ)n sequences, Boc-[Aib-β3(*R*)Val]n-OMe (n = 2 and 4), permitted a comparison of the intramolecular hydrogen bonding patterns in peptide helices. The (□□)n sequences formed 310 helical structures stabilized by C10 hydrogen bonding rings, while the corresponding (□□)n sequence yielded the backbone expanded C12 helix. In the case of the (αβ)n series the tetrapeptide yielded two consecutive C11 hydrogen bonds corresponding to a C11 helix. In contrast, the (αβ)4 octapeptide yielded a mixed C14/C15 helical structure generated by successive *three* residue turns of the type αβα and βαβ.

Chapter 3 presents the crystallographic characterization of an (□□□)4 dodecapeptide **Boc-[Aib-γ4(*R*)Val-γ4(*R*)Val]4-OMe**. This study was intended to establish whether helical folding could be maintained in sequences containing contiguous γ4(*R*)Val residues. Specifically, an (αγγ)n sequence composed of successive two residue, 4→1 hydrogen bonded turn can result in a helical structure in which the αγ and γα sequences form C12 hydrogen bonded turns while the γγ segment forms a C14 turn. The crystal structure of the 12-residue peptide **Boc-[Aib-γ4(*R*)Val-γ4(*R*)Val]4-OMe** described in this chapter does indeed demonstrate formation of a mixed C12/C14/C12 helix. Three independent molecules are observed in the crystallographic asymmetric unit permitting an analysis of the conformational and hydrogen bond parameters in this hybrid helical structure.

Chapter 4 addresses the question whether (□□)n helices can be characterized in crystals in sequences composed of *unconstrained □ and □ residues*. The crystal structures of 4 □□ hybrid peptides, **Boc-Leu-γ4(*R*)Val-Val-OH**, **Boc-[Leu-γ4(*R*)Val-Val]2-OH**, **Boc-[Leu- γ4(*R*)Leu]2-OMe** and **Boc-[Leu-γ4(*R*)Val]5-OMe** are presented. In all four cases folded intramolecularly hydrogen bonded structures are obtained. C12 intramolecular hydrogen bonding is observed in all 4 peptides, suggesting that helical folding is readily obtained in(□□)n and (□□□)n sequences *even in the absence of local backbone constraints*. Preorganization of □ residues does not appear to be a necessary condition for initiating helical folding in hybrid peptides containing □-residues.

Chapter 5 follows up the results obtained in the preceding chapters and examines the conformational properties of homooligomeric (□□)n sequences. The structures of the □- peptides Boc-[□4Val]n-OMe

(n = 2-6, 8), Boc-[□4Leu]n-OMe (n = 4) and Boc-[□4Ile]n-OMe (n = 6, 10) determined in crystals by X-ray diffraction, are described. The tetrapeptides Boc- [□4(*R*)Leu]4-OMe and Boc-[□4(*S*)Leu]4-OMe reveal incipient C14 helical structures of opposite handedness. The hexapeptide Boc-[□4(*R*)Ile]6-OMe and decapeptide Boc-[□4(*R*)Ile]10-OMe fold into C14 helices stabilized by 4 and 8 intramolecular hydrogen bonds respectively, which are backbone expanded analogues of the □-peptide 310 helices. The foldability of unconstrained homooligomeric □-peptides, is in sharp contrast to the tendency of their □- counterparts to form extended, sheet like structures. The structures presented in this chapter constitute the first reported characterization of C14 helix in homooligomers of unconstrained □-residues.

Chapter 6 describes the crystal structures of two pregabalin zwitterions, **(*S*) Pgn** and **(*R*, *S*) Pgn**, five derivatives, one γγ dipeptide, **Boc-Gpn-(*R*, *S*) Pgn-OH** (Gpn, gabapentin) and two hybrid isomeric pentapeptides with the template **Boc-Aib-Xxx-Leu-Phe-Val-OMe** [where, Xxx = (S)Pgn and γ4(R)Leu]. Five derivatives of pregabalin, **Boc-(*R*, *S*) Pgn-OH**, **Boc-(*R*, *S*) Pgn-Cyclohexylamide**, **Boc-(*R*, *S*) Pgn-NHMe**, **Piv-(*S*) Pgn-NHMe** and **Piv-(*R*, *S*) Pgn-NHMe**, reported in this chapter do not possess any intramolecular hydrogen bonds. The γγ dipeptide, **Boc-Gpn-(*R*, *S*) Pgn-OH** forms a C9 hydrogen bond, involving the Gpn residue. Pregabalin (Pgn) is a γ3 substituted analogue and is isomeric with γ4Leu, described in Chapters 4 and 5. Two model pentapeptide sequences were examined to compare the conformational characteristics of γ3 and γ4 monosubstituted γ-residues. The crystal structures of two isomeric pentapeptides, **Boc-Aib-(S)Pgn-Leu-Phe-Val-OMe** and **Boc-Aib- γ4(*R*)Leu-Leu-Phe-Val-OMe** are reported. In the case of the Pgn containing pentapeptide two polymorphic crystal forms are obtained, containing three independent peptide molecules. All three independent molecules fold into short helical segments, with C12 hydrogen bonds at αγ and γα segments and C10 at αα segments. The γ4Leu pentapeptide yielded a conformation almost identical to that observed in the γ3 (Pgn) pentapeptides.

Chapter 7 presents a summary of the results obtained and highlights the major, conclusions. Stimulated by the structural results obtained in this study a detailed analysis of γ residue containing helical turns was also carried out by extracting the structures from the CCDC (Cambridge Crystallographic Data Centre) database.

Appendix presents a series of tables listing the backbone torsion angles and hydrogen bond parameters of the peptides relevant for the present study. The structures reported in this thesis are listed below (for the structures deposited in the Cambridge Crystallographic Data Centre, the respective CCDC numbers are indicated in parentheses):

1. Boc-[Aib-α(*S*)Val]2-OMe [C24 H44 N4 O7] (**881183**)
2. Boc-[Aib-α(*S*)Val]4-OMe [C42 H76 N8 O11] (**882909**)
3. Boc-[Aib-β3(*R*)Val]2-OMe [C26 H48 N4 O7. 2H2O (2O)] (**881179**)
4. Boc-[Aib-β3(*R*)Val]4-OMe [C46 H84 N8 O11] (**881180**)
5. Boc-[Aib-□4(*R*)Val]2-OMe [C28H52N4O7] (**881181**)

6. Boc-[Aib-α4(*R*)Val]4-OMe [C50 H92 N8 O11] (**881182**)

7. Boc-[Aib-α4(*R*)Val]5-OMe [C61 H112 N10 O13] (**881177**)

8. Boc-[Aib-α4(*R*)Val]8-OMe [C78H144N12O15. 2H2O] (**881178**)

9. Boc-[Aib-α4(*R*)Val-α4(R)Val]4-OMe [C94 H172 N16 O19] (**909490**)

10. Boc-Leu-α4(*R*)Val-Val-OH [C23H43N3O6] (**881185**)

11. Boc-[Leu-α4(*R*)Val-Val]2-OH [C41H76N6O9] (**881186**)

12. Boc-[Leu-α4(*R*)Leu]2-OMe [C34H64N4O7. 2H2O (O)] (**930729**)

13. Boc-[Leu-α4(*R*)Val]5-OMe [C71H132N10O13. 2H2O (2O). 2CH3OH (CO)] (**922799**)

14. Boc-[α4(*S*)Leu]4-OMe [C38H72N4O7 . 0.5 H2O (0.5 O)] (**922802**)

15. Boc-[α4(*R*)Leu]4-OMe [C38H72N4O7 . 0.5 H2O (0.5 O)] (**922800**)

16. Boc-[α4(*R*)Ile]6-OMe [C54H102N6O9] (**930731**)

17. Boc-[α4(*R*)Ile]10-OMe [C86H162N10O13] (**930730**)

18. Pregabalin Zwitterion (*S*) Pgn [C8H17NO2]

19. Pregabalin Zwitterion (*R*, *S*) Pgn [C8H17NO2]

20. Boc-(*R*,*S*) Pgn-OH [C13H25NO4]

21. Boc-(*R*, *S*) Pgn-Cyclohexylamide [C19H36N2O3]

22. Boc-(*R*, *S*) Pgn-NHMe [C14H28N2O3]

23. Piv-(*S*) Pgn-NHMe [C14H28N2O2]

24. Piv-(*R*, *S*) Pgn-NHMe [C14H28N2O2]

25. Boc-Gpn-(*R*, *S*) Pgn-OH [C22H40N2O5]

26. Boc-Aib-(*S*) Pgn-Leu-Phe-Val-OMe (Form-I) [C38H63N5O8. H2O] (**971416**)

27. Boc-Aib-(*S*) Pgn-Leu-Phe-Val-OMe (Form-II) [C38H63N5O8. H2O (O)] (**971417**)

28. Boc-Aib-α4(*R*)Leu-Leu-Phe-Val-OMe (Form-I) [C38H63N5O8] (**971418**)