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Forming Stable Helical Peptides Using Natural and Artificial Amino Acids

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Contents

1. Helical peptide structures

The backbone conformation of a polypeptide can be most succinctly described by the three torsion angles, ω, ψ and φ, of the backbone (Figure 1). The partial double bond character of the amide bond ensures that the C, O, N and H of the amide lie in a plane, with ω usually close to 180° (or exceptionally, for proline, close to 0°).

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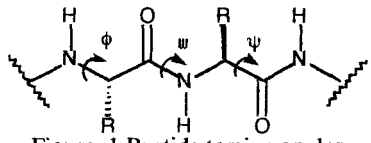


Figure 1 Peptide torsion angles

For amino acids other than glycine, geometrical constraints imposed by the side-chain and the carbonyl group also restrict the values of ϕ and ψ that can be adopted; repeating values of $\phi = -57°$, $\psi = -47°$ result in the backbone adopting an α-helical conformation (Figure 2).[1,2]

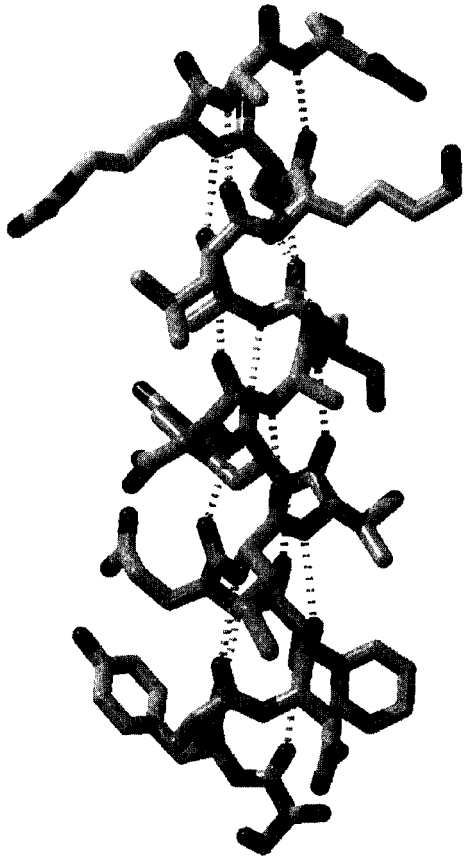


Figure 2 α-Helix:
Residues 15-33 of cytochrome C reductase[3]

The carbonyl groups and amide protons are orientated in opposing directions, meaning that the hydrogen bonds run in the same direction along the surface of the helix. This creates hydrogen bonds between the C=O of *i* and the NH of *(i+4)* residues, which stabilise the structure. The helix rises 5.4 Å *per* complete turn, or 1.5 Å *per* single residue, the hydrogen bonds repeating as the helix rises; a regular repeat of 3.6 residues *per* complete turn allows the *i,(i+4)* hydrogen bond interaction described. α-Helices found in nature are right-handed, due to the chirality of the amino acids; L-amino acids are not capable of stacking in a left-handed form, due to the close and destabilising interaction that would occur between the *i* residue carbonyl oxygen, and the *(i+4)* residue β-carbon.

As each amide bond has an associated dipole, an overall macrodipole is created that runs along the axis of the helix.[1] This macrodipole means that even in a longer sequence, an effective charge resides at the ends of

the helix. The terminal amino group of a sequence is termed the *N*-cap, and is slightly positively charged in character, whilst the carboxylic acid is termed the *C*-cap, and is slightly negatively charged. The effect arises from the charges associated with these functional groups in monomeric amino acids.

The majority of ordered structures in proteins and peptides are α-helix and β-sheet motifs; overall, about 30% of residues in proteins occur in α–helices .[1,4] α-Helical motifs play crucial roles in many biological processes; for example, the key receptor-binding component in peptide hormones, such as neuropeptide Y[5] or calcitonin,[6] is frequently an α-helix:[7] sequence-specific DNA-binding proteins often employ α-helical motifs as the DNA recognition element:[8] the hemolytic activity of peptides such as melittin[9] and alamethicin[10] are believed to be linked to their helical structure.[11] α–Helices also form important structural elements of many proteins,[2] and motifs such as the four-helix bundle[12] and coiled-coil[13] are common. It has been suggested that helix formation is an early step in protein folding, which subsequently guides the rest of the process[14,15,16] although this hypothesis is under debate.[17,18,19]

Other helical structures are occasionally found in proteins and in naturally occurring peptides. The 3_{10} helix is more tightly wound, with hydrogen bonds between the *i* and *(i+3)* residues, and torsion angles in the region $\phi = -70°$, $\psi = -5°$ (Figure 3). This geometry creates a helix in which the *i,(i+3)* α-carbon atoms, and hence the side-chains, are exactly aligned; the resulting steric clash renders this conformation less favourable than the α-helix. The 3_{10} helix is therefore less common, occurring in about 3-4% of residues in protein crystal structures, principally at the ends of α-helices.[2,4] There is no energy barrier between 3_{10} and α-helical structures, and the two forms can therefore easily interconvert;[20] indeed, the 3_{10} helix has recently been proposed as an intermediate in the folding pathway for α-helices.[21,22]

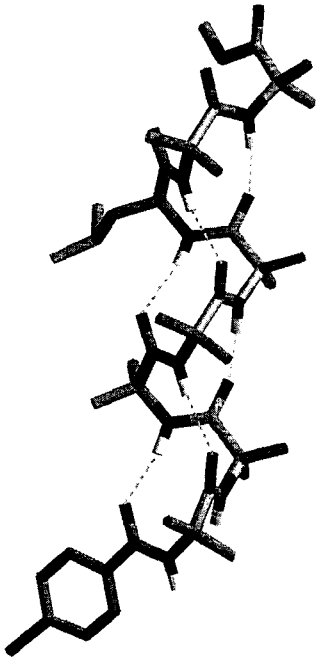


Figure 3 3_{10} helix[23]
Aib-rich peptide

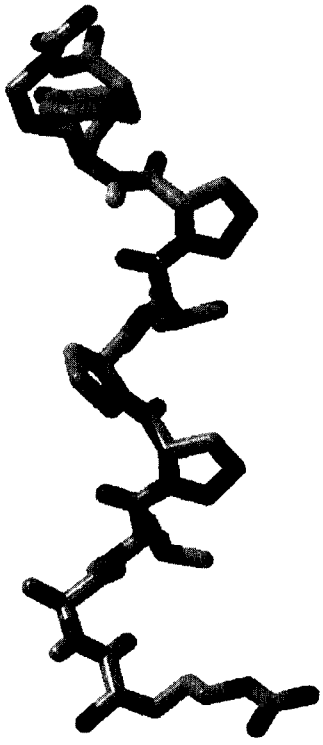


Figure 4 Poly(Pro) type II helix:[24]
Pro-rich ligand to the SrcSH3 domain

The type II helix is a left-handed helix with 3.0 residues per turn and the *i* and *(i+3)* residues in register. In general, the type II helix forms from poly(Pro) sequences; the predominance of proline residues means that this structure cannot be stabilised by internal hydrogen bonding, and must instead depend on backbone solvation. The poly(Pro) type II helix has recently been shown to be a key structural element of ligands for the signal transduction adaptor domain, SH3.[25] Type II helices containing both Gly and Pro residues are also found in polypeptides such as collagen.[26]

The π-helix is loosely wound, with torsion angles of $\phi = -57°$, $\psi = -70°$, and hydrogen bonds between the *i* and *(i+5)* residues. It is entropically and thermodynamically disfavoured, and is therefore rare, although it has occasionally been observed in crystal structures.[27] Finally, the alternating L- and D-amino acid sequence of the channel-forming polypeptide antibiotic gramicidin, which adopts β-sheet-like torsion angles, results in a wider β-helix structure, right-handed, with 6.7 residues per turn, through which ions can pass.[28]

Short polypeptides do not, in general, form ordered structures in aqueous solutions, although organic solvents, particularly trifluoroethanol (TFE), stabilise helical conformations.[29,30] In order to study the role that an isolated helix might play in any of the biological systems described above, it is necessary to design peptides or peptidomimetics that will adopt a stable helical conformation in water. This problem has been extensively studied by structural biologists, and a considerable weight of experimental data, together with theoretical calculations, has made it possible to predict and design peptide sequences that will form stable helices.[31,32,33] Organic and peptide chemists can now design and synthesise peptidomimetics of defined conformation; the use of peptidomimetic templates[34] and the design of "peptide nanostructures"[35] have recently been reviewed.

Our aim is firstly to provide an overview of how the properties of the 20 proteinogenic amino acids affect peptide helicity. Several excellent recent reviews[2,31,32,33] cover this in greater depth. Our main focus, however, is upon the use of non-natural methods for producing short peptides of defined helical structure. The review is comprehensive up to the end of 1997.

2. Helix propensities of the 20 proteinogenic amino acids

(i) Helix-coil transition theory

The transition between a helical conformation and a random coil is not a simple two-state transition. In a population of peptides with a 50% helical content overall, some peptides will exist in a random coil and some as complete helices, with a range of states existing between (Figure 5).

Figure 5 Fraying in partly helical peptide molecules

The helix-coil transition was first successfully analysed by Zimm and Bragg[36]and by Lifson and Roig.[37] Although the two approaches use different initial assumptions, in practice the two theories are very similar, as shown by Qian and Schellman.[38] The peptide is considered to fold via two processes; nucleation of the helix, governed by a nucleation parameter (σ in Zimm-Bragg notation), and subsequent elongation of the helix, which depends on the helical propensity (s) of each amino acid. For a peptide helix of n residues, the equilibrium constant for the helix-coil transition, K_{eq} is given by:[39,40]

$$K_{eq} = \sigma s^{n-2}$$

The nucleation of the helix, which involves the first four amino acids of the helix adopting a helical conformation, is an entropically disfavoured process; moreover, in the Zimm-Bragg and Lifson-Roig theories, these four residues are not initially stabilised by hydrogen bonds to subsequent residues in the helix. The value of s is consequently low, about 10^{-3}. As early studies indicated that s values for each amino acid would be around unity,[41] it was assumed for a long time that peptides with fewer than 20 amino acids could not form stable helices.

The Zimm-Bragg and Lifson-Roig analyses do not take into account additional factors which serve to stabilise helical conformation, and it has since been conclusively shown that short peptide sequences can be designed to adopt stable helical conformations. These factors, and the short peptide sequences, are discussed below. Nevertheless, these central assumptions of the helix-coil transition remain valid, and recent work has focussed on the analysis of experimental results by the helix-coil theory to determine s-values and the contributions to the free energy of helix formation for the 20 proteinogenic amino acids.

(ii) Statistical analysis of protein structures

Initial studies on the factors governing helix stability focused on examining the frequency with which different amino acids occur in α-helix, β-sheet or random coil motifs in protein structures. Based on such statistical analyses, the helical propensity of a given residue, P_α, was derived by Chou and Fasman.[42] P_α is equivalent to the normalised probability of finding the residue within a helix: helix-forming residues have $P_\alpha > 1$. The probability that a given amino acid will appear within the main body of a helix, P_{mid}, or at the N-cap or C-cap, has also been derived by Richardson and Richardson.[2,43]

(iii) Studies of small helical peptides

The advent of routine synthesis of short peptides has made possible systematic studies of the helical properties of such peptides. Allied to this, reliable methods for analysing peptide conformation have also recently been developed. The most commonly used method for determining the secondary structure of peptides is circular dichroism spectroscopy (CD).[44,45] The observed CD signal at 222 nm may be used to measure the amount of helical structure present in a peptide[46] and values of σ and s computed from this using Lifson-Roig theory.[47] CD spectroscopy has the advantages of speed, ease and simplicity. More detailed structural analysis may be carried out using NMR spectroscopy; NH exchange data have been used to measure helix content and the parameters of the helix-coil transition,[48] and to prove that the two-state model is not applicable to small helical peptides.[49] In favourable cases complete solution structures of helical peptides may be obtained.[50] ESR studies on doubly spin labelled peptides have also been used to probe helical conformation.[21]

The major breakthrough in the study of the helical propensities of amino acids using small peptides came when Marqusee and Baldwin designed a 17-residue peptide which adopted a stable helical structure in

water.[51] The helical stabilisation in this case arises partly from ionic interactions between Glu and Lys residues, and partly from the apparently high helix propensity of Ala. The marginal stability of α-helical structure in water means that single amino acid substitutions will have a large effect on the helical content of the peptide; by substituting different residues into the host peptide and measuring the helical content by CD, it has therefore been possible to determine s values for all 20 amino acids. Subsequent work by the Baldwin, Kallenbach and Stellewagen groups[32,52-62] has been carried out using a range of host peptides (Figure 6) to examine the effects of position within the helix and other factors such as intra-side chain interactions.

Host peptide	Sequence	Reference
AEK	Ac-Y-EAAAK-EAXAK-EAAAK-A-NH_2	(51, 52)
AK	Ac-Y-KAAXA-KAAXA-KAAXA-K-NH_2	(47, 53, 54, 55)
E_4K_4	succinyl-YS-EEEE-KKKK-XXX-EEEE-KKKK-NH_2	(56, 57)
AQ	Ac-AAQAA-AAQAA-AAQAA-Y-NH_2	(58, 59. 60, 61)
AXA	succinyl-YS-EEEE-KAKK-AXA-EEAE-KKKK-NH_2	(62)

Figure 6 Model host peptides for studying helical propensities of amino acids
X indicates where the amino acid under study is substituted
A = Ala, E = Glu, K = Lys, Q = Gln Y = Tyr, S = Ser

(iv) Studies of helical regions of proteins and polypeptides

The earliest studies on the s-values of the 20 proteinogenic amino acids were carried out by Scheraga.[41] Random copolymers of hydroxybutylglutamine (HBLG) or hydroxypropylglutamine (HPLG) with each amino acid in turn were synthesised, and values of σ and s derived from the CD data.

Subsequent studies have focussed on mutagenesis of solvent-exposed residues in helical domains of proteins. For example, the Ala32 residue located towards the end of the second α-helix of barnase was substituted by each of the other 19 amino acids and the free energy of unfolding of each mutant protein determined by urea denaturation studies;[63] this enabled a scale of relative free energy differences (ΔΔG) for the 20 amino acids to be assembled. In the same way, mutants of Ser44 (located in the middle of the 39-50 helix of T4 lysozyme) and Val131 (located in the middle of the 126-134 helix of T4 lysozyme) were studied.[64,65] A model protein system, consisting of an α-helical peptide designed to form a homodimeric coiled coil similar to that found in leucine zipper motifs, has also been used; after measuring the thermodynamic stability of the parent system, guest residues were substituted into solvent-accessible positions on the outside of the coiled coil, and ΔΔG values determined.[66]

These experiments have the advantage that protein folding, unlike the helix-coil transistion, is considered to be a simple two-state process; the ΔΔG values may therefore be determined directly. Conversely, however, it is not possible to derive s values directly, and the extent to which interactions with the rest of the protein architecture contribute to the helix-forming or helix-breaking propensities of the guest residue is unclear.

(v) Hydrophobic amino acids

The studies of small helical peptides, the statistical analysis of protein structures, and the majority of studies on protein helices, indicate that Ala is a strongly helix-stabilising residue; the s-value has in some cases been estimated to be as high as 2[53] and Ala has the strongest preference of any amino acid for a mid-helix

location.[2,43] Indeed, the AEK, AK and AQ hosts (Section 3(ii)) have been designed to take advantage of the high helix propensity of alanine, with the Lys, Gln and Glu residues included to improve aqueous solubility, prevent aggregation and in some cases impart additional stabilisation from electrostatic and hydrogen bonding interactions. It is believed that the methyl side-chain reduces the conformational freedom of Ala, biasing it towards a helical conformation; however, in contrast to most other amino acids, the methyl group is small and steric clashes with other side-chains do not arise. In addition, there is no loss of side-chain entropy on helix formation.

On the basis of ESR studies, it has recently been suggested that Ala-based peptides form 3_{10} helices, rather than α-helices.[67] This work remains controversial; NMR studies[68] appear to confirm this observation, but vibrational CD[69] and molecular modelling,[70] as well as subsequent ESR studies[71] seem to indicate that these peptides are, in fact, α-helical.

For other hydrophobic amino acids, the stabilisation of the helical structure due to hydrophobic interactions is in competition with the entropic cost of restricting side-chain rotamers within the helix,[72,73] and with possible steric interactions between side-chains. Studies on small helical peptides and solvent-accessible protein helices indicate that Leu and Met are helix-stabilising, whereas bulky and β-branched[73] residues such as Phe and Tyr, and in particular Val and Ile, are helix-destabilising.[54,57,65,66,74] However, within the hydrophobic core of proteins, Phe and Ile must have a favourable effect upon α-helix stability, as they occur with reasonable frequency in the middle of protein helices.[2,33] In this context, a recent study of helicity of short peptides in micelles demonstrated that the helical propensity of amino acids in membrane-like environments is governed by their hydrophobicity and not by the steric bulk of the side-chain;[75] in these experiments, Ile and Val ranked as the best helix promoters.

In certain instances, hydrophobic interactions between these residues have been shown to stabilise helical structure, even in isolated peptides. For example, stabilising interactions between Tyr and either Val or Leu have been found in variants of the AK peptide.[76] This work has shown that both possible interactions are most stabilising when the spacing between residues is *i,(i+4)* (Figure 7) with the tyrosine towards the *N*-terminus. The stabilisation is thought to arise from the exclusion of water from the non-polar surface formed by the hydrophobic residues. This interaction is less stabilising in internal positions in the sequence, where the helix breaking effects of Tyr would be stronger.

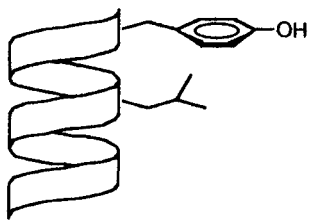


Figure 7 Tyr-Leu interaction

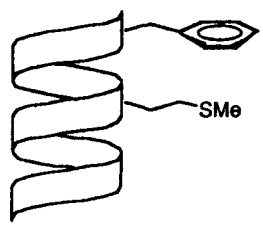


Figure 8 Phe-Cys interaction

A similar interaction is found between Phe and Cys or Met. This type of interaction is commonly found in proteins, and was therefore studied in a model Ala-based peptide.[77] The substitution of only one of the pair of residues gave a decrease in helical content in comparison to a model alanine based system, but when the residues were in an *i,(i+4)* spacing (Figure 8), with both Cys and Met in the *C*-terminal position, the helicity was found to be higher. The interaction is thought to arise between the electronegative sulfur atom with the

slightly electropositive aromatic protons. There is also some mutual shielding for the two hydrophobic residues. Hydrophobic interactions have also been observed between Phe and Ile residues at *i,(i+4)* spacing within an analogue of the 76-87 helical domain of myohemerythrin, which contains the sequence -Ile-Lys-Glu-Lys-Phe-.[78] ROESY cross-peaks between the Phe aromatic and Ile methyl resonances of this sequence were observed, and a sharp drop in helicity occurred when either Phe or Ile were mutated.

(vi) Capping box effects

Although the main body of the α-helix is stabilised by intrahelical hydrogen bonding, there will necessarily be four amide NH groups at the N-terminus of the helix, and four C=O groups at the C-terminus, that cannot participate in such hydrogen bonding. Presta and Rose [79] have recently proposed that helices may be stabilised when residues such as Asn, Asp, Glu, Gln, Ser, Thr and His are placed at the N-terminus, as hydrogen bonding between the side-chains of these residues and the free NH groups may occur. In the same way, when residues such as Asn, Arg, Gln, Lys and Ser are placed at the C-terminus, hydrogen bonding between side-chains and the C=O and NH groups may occur. This prediction is reinforced by the frequency of occurrence of such residues at the *N*-cap and *C*-cap positions.[43] Many protein crystal structures illustrate these 'capping box' interactions,[80,81] for instance between $Ser O_\gamma$ and the NH of the backbone 3 residues away (Figure 9), and peptide studies[82-88] and protein mutagenesis[89] have confirmed the hypothesis. It is believed that acylation or succinylation of the N-terminus can also stabilise helical conformation by hydrogen bonding between the acyl C=O and the backbone NH.[79,88]

Figure 9 Ser capping box: residues 12-15 from haemoglobin V[90]

Gly and Pro are inevitably found to have a negative effect on α-helix stability when incorporated in an internal position; however, Gly is found very frequently at both the *C*-cap and *N*-cap of α-helices, and Pro is frequently found at the *N*-terminus .[43] Gly has no side chain, and so the entropic cost for this residue of folding is higher than for other, more constrained residues. This greater cost in turn means that initiating or joining a helix is less likely for glycine. It has been suggested that the conformational flexibility of Gly creates a turn at the *C*-cap,

allowing subsequent residues to hydrogen bond to the C=O groups. By contrast, the lack of an amide hydrogen in Pro means intrahelical hydrogen bonding is not possible when this residue is placed in mid-helix: when it is found mid-helix, the steric effects of the ring cause the helix to kink or bulge.[2,91] At the *N*-terminus of the helix, Pro is frequently found adjacent to the *N*-cap; it is believed that it positions residues such as Asp or Asn correctly for the stabilising "capping-box" interactions described above.[92] It has recently been suggested that a similar effect may occur when Pro occurs adjacent to the C-cap residue.[93] Finally, the conformational rigidity of Pro may encourage helix nucleation, by constraining the first C=O groups of the helix into the correct orientation. The Lifson-Roig helix-coil theory has recently been modified to take account of helix capping interactions.[94]

(vii) Side-chain main-chain interactions

The alignment of the amide bonds in a helix results in the formation of a macrodipole, with a small positive charge at the *N*-terminus and negative charge at the *C*-terminus. Helix macrodipoles have been invoked to explain certain features of protein structure and function, such as altered pK_A values for residues at the termini of helices; however, there is controversy over whether the macrodipole effect is large enough to be significant.[95] Nevertheless, attempts have been made to stabilise helical structure by positioning residues of opposite charge to the macrodipole at the termini. In one example, $(Ala)_{20}$-$(Lys)_{20}$-Phe and $(Glu)_{20}$-$(Ala)_{20}$-Phe peptides were studied.[96] It was found that altering the pH to allow the polar side chains to become charged resulted in the stabilisation of the helical structure when the charge was at the "correct" terminus. Under the appropriate pH conditions, the helix was propagated further into the lysine or glutamic acid blocks. Similar results have also been obtained by substituting polar residues at various positions within the AQ peptides;[32,60,61] again, positive charges stabilised the *C*-cap, and negative charges the *N*-cap. More specific work has been done on the effect of incorporation of histidine residues at the *C*-terminus of the AQ peptides.[59] This has also been found to be stabilising, depending on the precise positioning of the residue in the sequence and the pH.

The effect of altering the spacing group between a charged side chain terminus and the helix backbone has also been investigated, by incorporating basic residues with alkyl side chains of varying length (Lys, Orn and shorter side chain residues) into a host peptide.[97] It was found that longer side chain residues such as Lys have higher helix propensities, whilst the shorter side chains were found to be more destabilising. It is thought that the major destabilising force is the side chain amine competing in hydrogen bonding with backbone amide groups. The effect of bringing the amine group closer in space to the helix backbone could then increase this competition. A similar effect has also been noted with Asp and Glu; Glu has a generally higher helix propensity than Asp, which is a helix breaker. However, Asp(COO^-) is found to be helix-stabilising at the N-terminus, again due to electrostatic stabilisation of the helix macrodipole.[60]

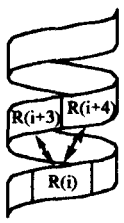


Figure 10 Idealised intrahelix interactions

(viii) Electrostatic and hydrogen-bond interactions between side-chains

Ion-pair and hydrogen bond interactions between amino acid side-chains are found in many protein structures; within an α–helix, residues with *i,(i+3)* and *i,(i+4)* spacing should be able to interact in this way (Figure 10). The initial studies on the AEK host[51] studied the effects of *i,(i+3)* and *i,(i+4)* spacing, and orientation, of Glu-Lys and Lys-Glu ion pairs, and found that the *i,(i+4)* spacing resulted in highly effective helix stabilisation. Further studies with AEK,[52] with an Arg-containing variant of AEK,[98] and with variants of the E_4K_4 host[99] (which was itself designed to be stabilised by *i,(i+4)* hydrogen bonding interactions) confirmed these results. Stabilisation of model hosts by Gln-Glu,[61] His-Asp,[100] Gln-Asp,[101] Gln-Asn,[102] Glu-Lys, Asp-Lys and Glu-His[103] and Asp-Arg[104] interactions has also been demonstrated; shorter side-chains, in general, show stronger interactions, but tend to be more orientation-specific because of restrictions on the possible geometry. These helical peptides retain their conformational stability even up to 2.5M NaCl[61,100,102] indicating that most of the stabilisation arises from hydrogen bonding and not from electrostatic interactions. In addition, the peptides are stable down to pH2, where the interaction must be between Glu^0-$Lys(NH_3^+)$ (Figure 11). Charged groups placed at *i,(i+5)* spacing were shown to have no effect on the stability of these peptides, and were therefore used as controls. However, it should be noted that a recent molecular dynamics simulation of the AQ host[105] showed that stabilisation of the unusual π-helix structure by *i,(i+5)* hydrogen bonding interactions may be possible. Finally, interactions between aromatic and charged residues have also been observed; stabilisation of helicity by a Trp-His pair was recently studied.[106]

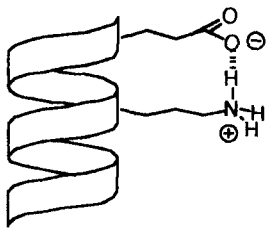


Figure 11a $Glu(COO^-)$-$Lys(NH_3^+)$ interaction at pH7

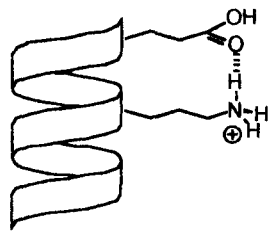


Figure 11b Glu^0-$Lys(NH_3^+)$ interaction at pH2

It should be noted that in general the role of such interactions in stabilising protein structures remains controversial; mutagenesis studies on α-helical domains within proteins[107] have frequently failed to find evidence for side-chain hydrogen-bond mediated stabilisation, but database analyses have shown correlation between side-chain interactions and helical structure.[108]

(ix) Amphipathic helices

α-Helical peptides in which one face of the peptide bears predominantly polar side-chains and the opposite face non-polar side-chains are classed as amphipathic. This group of peptides is important, as they will tend to form helical structures at the interfaces of polar and non-polar environments, for instance at lipid-water interfaces, or between the hydrophobic core and the solvent accessible exterior of a protein. Amphipathic helices may be identified by inspection of a helical wheel diagram (Figure 12), essentially a projection of the helix down the helix axis, or by computational methods based on calculated hydrophobic moments. Examples include hormones such as endorphins, antibiotics such as the magainins, and the apolipoproteins, and have recently been divided into classes based on their structural and biological properties.[109] It has been shown that it is possible to design amphipathic α-helical peptides *de novo*, with suitable arrangements of polar and non-polar

amino acids, which successfully mimic all the structural and biological characteristics of the naturally occurring peptides.[7] These ideas have been extended further in the TASP (Template-Assisted Synthetic Protein) approach to *de novo* protein architecture design;[110] amphiphilic helices have been assembled on macrocyclic templates to form four-helix bundles which contain a significantly higher degree of helicity in comparison to the free peptides. Finally, an 18-member sequence was designed to give an amphiphilic helix, containing four methionine residues on the hydrophobic face.[111] Oxidation converted the methionines into hydrophilic residues, switching the peptide to a β-sheet structure.

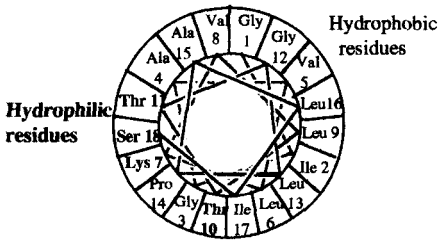


Figure 12 Helical wheel projection of an amphipathic helix: residues 1-18 of melittin[1]

(x) Comparison of small peptide studies, protein studies and predictive methods

The greater understanding of the factors governing α-helix stability discussed in the preceding sections have, in turn, led to more powerful computer algorithms for prediction of helical secondary structure.[93,112,113] The most recent of these algorithms are based on helix-coil transition theory, but include parameters for the energetic contributions of interactions such as capping interactions and side-chain-side-chain interactions, obtained both by calculation and by analysis of the helicity measurements from small peptide and mutagenesis experiments.[114,115,116]

Comparisons between the results of the small peptide, protein, homopolymer, predictive and statistical methods for studying α-helix formation have recently been made.[31,32,33,62,117] In general, the rank order of helical propensities of the 20 proteinogenic amino acids is similar; however, there are discrepancies between the ΔΔG values relative to Gly derived by the different approaches. (Figure 13). Overall, the results from different host peptides tend to be comparable. The rank order of helical propensities is also similar to those derived from protein mutagenesis studies and to the statistical results, and there is good correlation to the computational methods, particularly to AGADIR. The frequencies with which different amino acids appear as *N*-caps also correlate well with the stabilisation of host peptides by capping interactions. However, there are discrepancies in ΔΔG values relative to alanine between the host peptide and protein studies; it is thought that these may arise when the folding process in the protein under study is not dependent on helix formation.[118] Recent studies comparing one domain of ribonuclease T_1 with a 17-residue peptide corresponding to this domain[117,119,120] have shown that where the context is the same in both peptide and protein, the helix propensities are identical.

A more serious discrepancy arises between the results from the host peptide and protein studies, where Ala is regarded as being highly helix-stabilising, and the studies on HBLG and HPLG, where the s-value of Ala is found to be about 1.[41] These latter studies were reinforced by recent results obtained using artifical helix-inducing templates[121] (see below), where s values for Ala of 1.02 were reported. It has been suggested that the

conformations of block copolymers such as HBLG are intrinsically stabilised by polar side-chain interactions, and that including Ala would disrupt these interactions;[122] conversely, it has been argued that the Ala-based model peptides do not take sufficient account of the stabilisation of Ala by the Lys side chains.[121,123] It is clear that the helical propensities of amino acids are strongly influenced by their context, in ways that are not yet fully understood.

AQ/AK	ΔΔG kcal mol^{-1}	AXA	ΔΔG kcal mol^{-1}	T4 lysozyme	ΔΔG kcal mol^{-1}	HBLG	ΔΔG kcal mol^{-1}	P_{mid}
Ala	-1.88	Ala	-1.05	Ala	-0.96	Met	-0.42	Ala
Arg	-1.67	Glu$^-$	-0.84	Leu	-0.92	Ile	-0.39	Leu
Leu	-1.60	Leu	-0.69	Met	-0.86	Trp	-0.37	Met
Lys	-1.52	Ile	-0.67	Ile	-0.84	Phe	-0.36	Gln
Met	-1.37	Arg	-0.64	Gln	-0.80	Ala	-0.35	Lys
Gln	-1.31	Met	-0.53	Arg	-0.77	Arg	-0.33	Arg
Glu$^-$	-1.20	His	-0.51	Lys	-0.73	Tyr	-0.32	Glu
Ile	-1.18	Lys	-0.47	Tyr	-0.72	Gln	-0.30	Phe
Tyr	-1.3 to -1.1	Val	-0.32	Val	-0.63	Cys	-0.30	Ile
Ser	-1.10	Ser	-0.29	Trp	-0.58	Glu$^-$	-0.29	His
His	-1.07	Asp$^-$	-0.27	Phe	-0.59	Val	-0.28	Trp
Cys	-1.06	Gln	-0.25	His	-0.57	Lys	-0.27	Asp
Asp$^-$	-1.00	Asn	-0.20	Thr	-0.54	Leu	-0.23	Val
Asn	-0.99	Thr	-0.17	Glu$^-$	-0.53	His	-0.22	Thr
Trp	-1.1to-0.97	Gly	0.00	Ser	-0.53	Thr	-0.19	Asn
Phe	-0.95	Phe	0.06	Asp$^-$	-0.42	Asn	-0.16	Tyr
Val	-0.83	Trp	0.06	Cys	-0.42	Ser	-0.15	Cys
Thr	-0.56	Cys	0.12	Asn	-0.39	Asp$^-$	-0.08	Ser
Gly	0.00	Tyr	0.90	Gly	0.00	Gly	0.00	Gly
Pro	+3.00	Pro	1.51	Pro	2.50	Pro	0.70	Pro

Figure 13 Rank order of helical propensities, ΔΔG values relative to Gly, and ΔG_m for the AK/AQ,[55] AXA[62] and HBLG[41] host peptides, T4 lysozyme,[64] and P_{mid} values[2] (adapted from refs 32, 62).

3. Helix stabilisation using unnatural residues

(i) Hydrophobic, α,α-disubstituted and dehydro amino acids

The effects of hydrophobic side-chains upon helix stability have been tested by incorporating unnatural hydrophobic amino acids. Substitution of the straight-chain, non-proteinogenic amino acids Abu, Nva and Nle (Figure 14) in the host peptides AK and E_4K_4 showed them to be as strongly helix-stabilising as Ala.[124,125] This is expected; as these amino acids are not β–branched, the loss of conformational freedom on restriction of the side-chain conformation will not be so acute, and there will be no destabilising steric interactions with adjacent side-chains on the helix. In contrast, the inclusion of β-branched amino acids such as Tle (Figure 14) was found to be more destabilising than either Ile, Val or Gly. These results were largely confirmed when Abu, Nva, Nle and Tle were included in the helical domains of T4 lysozyme by mutagenesis.[126] In certain contexts,

Tle was found to have a stabilising effect on the helical domains, possibly because, with such a conformationally rigid residue, there will be little entropic penalty to packing the side-chain in a hydrophobic region of the protein.

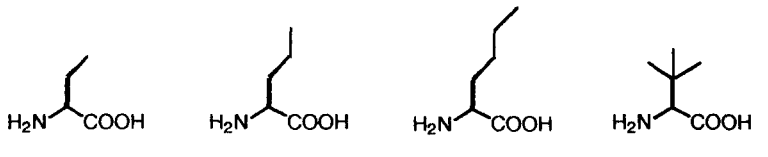


Figure 14

Stabilisation of α-helices by using residues capable of π-stacking has also been examined.[127] Pairs of ε-(3,5-dinitrobenzoyl)-Lys residues were substitued in a variant of the AEK host, at different spacings (Figure 15). The overall results showed again that the *i,(i+4)* spacing was the most effective stabilising arrangement. Increasing the percentage of water, up to 90%, increased the helical content. Pairs of ε-acyl-Lys residues in the same *i,(i+4)* spacing had no stabilising effect, indicating that the majority of the stabilisation arises from π-π interactions and that the effect from the Lys side chain aliphatic portion was negligible in comparison.

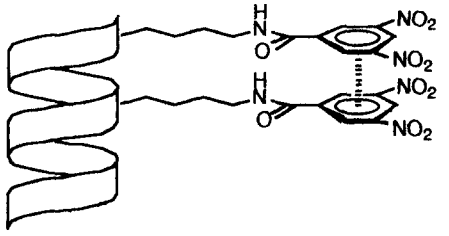
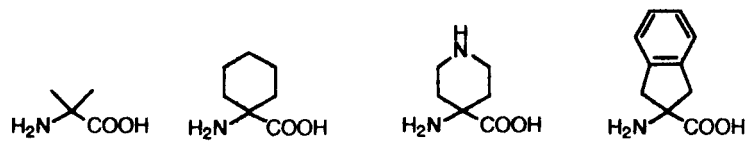


Figure 15 ε-(3,5-dinitrobenzoyl)-Lys interactions

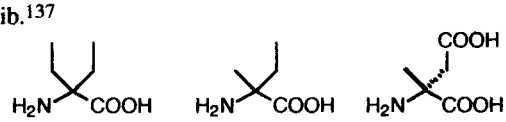
By far the most frequently used hydrophobic helix-stabilising amino acid is α-aminoisobutyric acid (Aib: Figure 16).[128] Although this residue is not one of the 20 proteinogenic amino acids, it frequently occurs in microbial peptides, such as alamethicin.[129] Aib is restricted to a small range of conformational space ($\phi = \pm 60 \pm 20°$, $\psi = \pm 30 \pm 20°$) because of the steric effects of the α,α-disubstituted structure; as a result, peptides containing Aib tend to be helical, and both α-helix and 3_{10} helix structures are possible. Extensive crystallographic studies have indicated that where fewer that 50% of the residues in a peptide are Aib, the peptide will be α-helical; peptides with fewer than 8 amino acids and peptides with greater than 50% Aib residues will be 3_{10} helical.[129] Structures that are neither all α-helical nor all 3_{10} helical are also possible.[20] It should be noted that whereas L-amino acids form right-handed helices, these achiral molecules have no overall preference, and can form both left- and right-handed helices. Any chiral amino acids in the sequence will determine the screw sense of the helix, with greater levels of L-amino acids directing folding to the right-handed form.[130] 1-Amino-1-cycloalkanecarboxylic acids, such as 1-amino-1-cyclohexylcarboxylic acid (Ch) and aminoindane carboxylic acid (Ind) (Figure 16), have a similar stabilising effect on 3_{10} and α-helical conformation.[131,132,133] An amino homologue of Ch, Pip, designed to increase the water solubility of such helical peptides, has recently been reported.[134]



Aib (α-aminoisobutyric acid) | Ch (1-amino 1-cyclohexylcarboxylic acid) | Pip (4-aminopiperidine-4-carboxylic acid) | Ind (aminoindane carboxylic acid)

Figure 16

Homologues of Aib, such as diethyl glycine (Deg) and dipropyl glycine (Dpg) (Figure 17) were initially believed, on the basis of simple peptide structures and calculations, to induce an extended conformation;[128] however, recent structural studies on 5-10 residue peptides has shown that they may also induce 3_{10} and α-helical conformations.[135] Other α,α-disubstituted structures such as Iva[136] and (*R*)-Asp(2-Me) also promote helical structure: indeed, in certain peptides protonated (*R*)-Asp(2-Me) has been shown to have a greater α-helix stabilising effect than Aib.[137]



Deg (diethylglycine) | Iva | (R)-Asp(2-Me)

Figure 17

A detailed study by CD, nmr and X-ray diffraction of a non-polar host peptide (Figure 18), substituted with ten different α,α-disubstituted amino acids, showed that both (*R*)- and (*S*)-enantiomers of a β-tetralin-derived amino acid (Figure 19(a)) showed more α-helix stabilisation than Aib or Ala.[138] Finally, α,β-dehydroamino acids, particularly ΔPhe (Figure 19(b)), have been found to stabilise 3_{10} helices.[139,140]

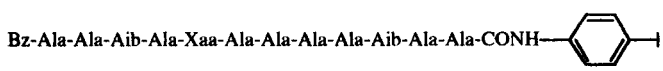
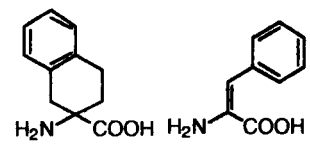


Figure 18



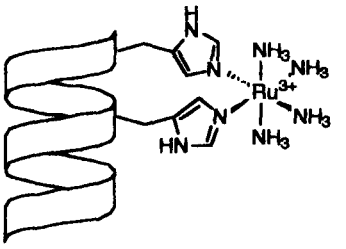
ΔPhe

(a) (b)

Figure 19

(ii) Electrostatic interactions between side-chains

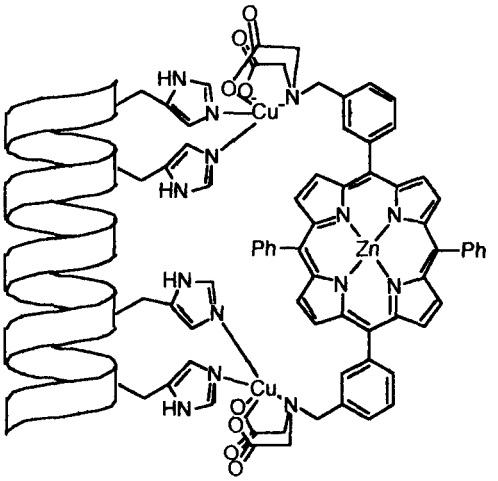
It is possible to generate electrostatic interactions between side-chains using methods other than hydrogen bonds. Transition metals are frequently found in enzyme active sites, bound using basic, acidic, or in some cases sulfur-bearing side chains. These types of interactions can also be used in the stabilisation of helical peptides, by the binding of metals by appropriate residues.



Peptide: Ac-AEAAAKEAAAKHAAAHA-NH_2

Figure 20 Binding of Ru(III) by His[142]

This effect has recently been demonstrated using a modified AEK host peptide.[141] When His-Cys or His-His residue pairs were substituted in this host in an *i,(i+4)* arrangement, the peptides changed from about 50% helical to about 90% helical on the addition of Cu^{2+}, Zn^{2+} or Cd^{2+} ions. When ruthenium salts were added to the His-His peptides,[142] an exchange-inert complex was formed (Figure 20), which also imparted remarkable stability to the helix. Thermal denaturation studies showed that the metal gave a further 1kcal mol^{-1} of stabilisation to the helix.



Peptide: Ac-YEAHAKAHAAAAAAHAEAHAKK-NH_2

Figure 21 Peptide-strapped zinc porphyrin assembly[143]

Stabilisation of α-helical structure in this manner has recently been used to construct a helical peptide-strapped zinc porphyrin assembly.[143] An alanine-rich peptide containing two *i,(i+4)* His-His pairs was ligated to a water-soluble porphyrin via Cu^{2+} chelation (Figure 21). Preliminary results indicated that the rigid porphyrin template further enhanced the peptide helicity, relative to the Cu^{2+}-chelated peptide. It is also possible to use unnatural residues to give better metal binding properties. The synthesis of four amino acid analogues bearing metal binding aminodiacetic acid groups (Figure 22) has been performed.[144] Pairs of these residues have been incorporated into variants of the AEK host peptide of varying lengths, with different separation gaps, *i,(i+4)* and *i,(i+3)*. Preliminary results have shown that several peptides are significantly stabilised by the addition of

Cd^{2+} ions, and that the *i,(i+4)* spacing is optimal. In the case of the smallest peptide, an 11-mer, this resulted in an increase from 0% to 82% helical content at 4°C and pH 7.9.

An attempt to stabilise helical structure by the interaction between a Lys $-NH_3^+$ group and a modified Lys, ε-acylated with a crown ether has been made (Figure 23) [145]. Several different separations between these residues were tried, including the *i,(i+4)* spacing successful with other methods. No stable helical structures were detected, even in less polar solvents; however, the peptides were short (7 residues) and it is possible that this approach may be successful with longer peptides.

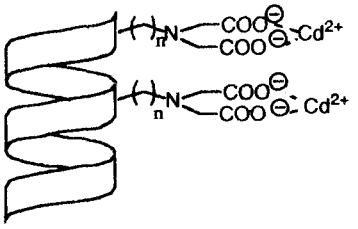


Figure 22 Aminodiacetic acid side chain groups

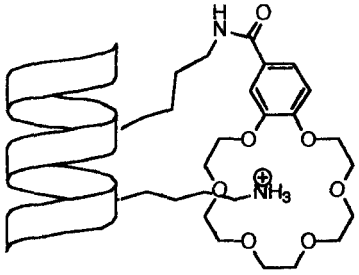


Figure 23 Crown ether-charged Lys interaction

(iii) Disulphide bond formation between side-chains

It is also possible to stabilise helical structures by means of formal covalent bonds between residues separated in the sequence. The commonly employed natural method of performing this task is to use disulphide bonds. This is illustrated in the example of charybdotoxin,[146] where the tertiary structure of the protein is locked using disulphide bonds. In nature, however, this method is not usually employed to stabilise single helices.

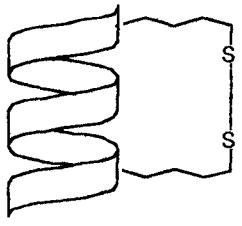


Figure 24 Disulphide *i,(i+7)* bonds

The synthesis of a short, alanine based peptide containing two designed cysteine homologues has been performed, giving rise to helices stable at 60°C after disulphide bond formation.[147] Both (D)- and (L)-2-amino-6-mercaptohexanoic acid derivatives were synthesised and incorporated in an *i,(i+7)* fashion (Figure 24) into four peptides of varying length, including the AK host, with the (D)-enantiomer towards the *N*-terminus. Analysis was then performed in the Acm- protected, deprotected and oxidised states, under conditions of varying temperature. The results showed that although reasonably helical as the acyclic, Acm- protected form (expected due to the high alanine content), the cyclic, disulphide linked peptides were almost completely helical at 0°C, and still retained significant (>48%) helicity at higher temperatures.

A recent study of an N-terminal fragment of parathyroid hormone related protein (PTHrP) indicated that *i,(i+3)* disulphide bridges may also promote α-helix formation.[148] [(D)-Cys^5, (L)-Cys^8]PTHrP(1-12)-$CONH_2$ was synthesised and the oxidised, cyclic form (Figure 25) was shown by NMR and CD measurements to be α-

helical between residues 3 and 9 in a micellar environment, and to contain one turn of the helix in water. In contrast to the host peptides normally utilised for the study of helix propensities, linear (S-Acm-protected) [(D)-Cys^5, (L)-Cys^8]PTHrP(1-12)-$CONH_2$ has no inherent helix-forming properties, and had no ordered structure at all in an aqueous environment. This *i,(i+3)* disulphide bridge, therefore, is inducing an α-helical conformation rather than merely stabilising it. Cyclic [(L)-Cys^5, (L)-Cys^8]PTHrP(1-12)-$CONH_2$ adopted a γ-turn conformation.

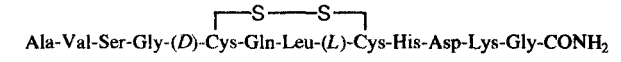


Figure 25

(iv) Lactam bridge formation between side-chains

It is possible to form rigid peptide structures using covalent bonds that would not be encountered in proteins. The successful stabilisation of α-helices by hydrogen bonding interactions between -NH_2 and -COOH groups at *i,(i+4)* spacing (section 3(viii)) has prompted many groups to introduce lactam bridges between Lys and Asp or Lys and Glu at *i,(i+4)* spacing (Figure 26).

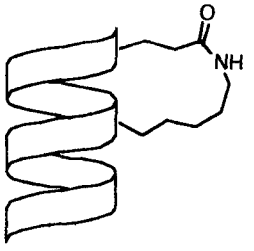
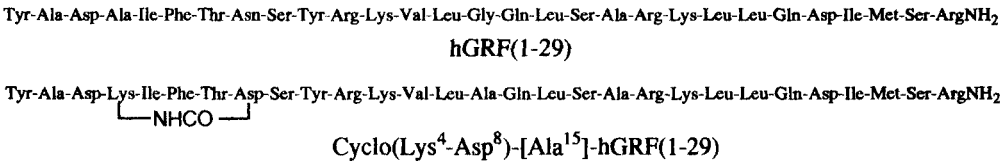


Figure 26 Amide *i,(i+4)* bonds

Lactam bridges of this type were first used in attempts to stabilise the α-helical conformations of peptide hormones. The most systematic study of this kind has been carried out on analogues of human growth hormone releasing factor (hGRF).[149] The biological potency resides in the N-terminal portion of hGRF (Figure 27), which is largely α-helical; Lys-Asp, Asp-Lys, Lys-Glu, Glu-Lys and Glu-ornithine (Orn) lactam bridges were therefore incorporated into the N-terminal sequence at various points to stabilise the helicity. The greatest stabilisation was shown, by CD and NMR, to arise from the Lys-Asp bridges;[150] in general, the more stable helices were also the most biologically active. Stabilisation of α-helical structure by the formation of Lys-Asp *i,(i+4)* bridges has also been demonstrated for the peptide hormones neuropeptide Y (NPY)[151] and PTHrP.[152] The N-H exchange rate of an 11-residue peptide stabilised with a Lys-Asp *i,(i+4)* bridge has been studied by NMR, and an s value of 1.7±0.2 for Ala derived from these measurements.[153]



hGRF(1-29)

Cyclo(Lys^4-Asp^8)-[Ala^{15}]-hGRF(1-29)

Figure 27

Stabilisation of helical structure by Lys-Glu *i,(i+4)* lactam bridges has been systematically studied in a model peptide[154] (Figure 28). The peptide was found to be slightly helical in aqueous solution, but showed greater helicity in 50% trifluoroethanol/water solution. It was suggested that the stabilising effect of these bridges is small, compared to linear model peptides. This is possibly due to the size of the macrocycle defined by amide bond formation. Given the separation in space of the two residues in a helix, the formation of an eight membered chain between them is unlikely to give sufficient conformational direction to form the first turn of the helix. A similar model peptide, with Lys-Asp *i,(i+4)* lactam bridges (Figure 28) was highly helical in aqueous buffer[155] and strongly resistant to thermal and chemical denaturation, reinforcing the hypothesis that the shorter *i,i+4* bridge imparts greater stability.

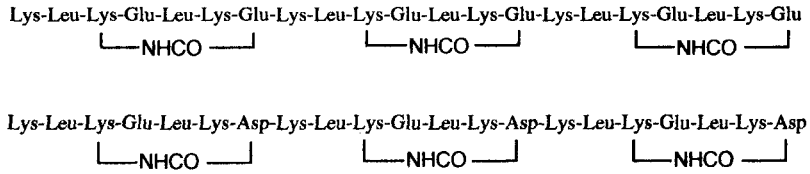


Figure 28

However, in a study of the stabilisation of the α-helical conformation of human calcitonin (hCT) by Lys-Asp and Asp-Lys *i,(i+4)* lactam bridges[156] only a slight increase in the helical content of the peptide was seen, dependent on the positioning of the amide bridge, and the ordering of the bridging residues. The analogue containing the Asp-Lys bridge was found to have only low helix content even under helix promoting (TFE rich) conditions. The most stabilised variant of the system was found to have the Lys-Asp *i,(i+4)* lactam bridge situated towards the N-terminus, reflecting the hypothesis that the N-terminus is the more significant in helix formation.

Introducing two overlapping lactam bridges into a peptide should stabilise the conformation even further. A hexapeptide containing two Lys-Asp *i,(i+4)* lactam bridges has recently been synthesised (Figure 29) and demonstrated by NMR and CD to form a rigid α-helix in water and water/TFE.[157]

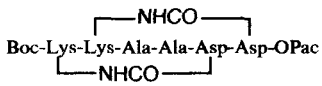


Figure 29

Further studies on the ideal ring size for the stabilisation of α-helical structure have also been carried out on hGRF.[149] The Lys-Asp bridges initially investigated by this group were all 20-membered rings; in order to study cyclic analogues with ring sizes as small as 18, Orn or (*S*)-diaminopropionic acid (Dpr) residues were used in addition to Lys. For larger rings up to 24, spacer residues such as Gly, β-alanine or γ-aminobutyric acid were introduced between the -COOH of the Glu or Asp and the -NH_2 of Dpr, Orn or Lys (Figure 30). In contrast to the results described above, the most biologically active analogues were those with 21- or 22-membered rings, although all analogues with greater than 19-membered rings were shown by CD to possess substantial α-helicity.

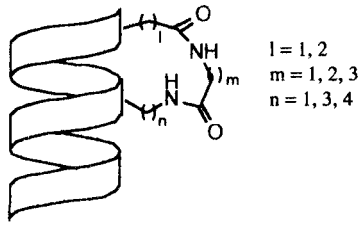


Figure 30 Amide *i,(i+4)* bonds, with varying linker lengths

Longer and more complex lactam bridges have recently been described. A heptapeptide containing a (*D*)-Glu-Lys *i,(i+7)* lactam bridge has been synthesised (Figure 31), and reported to adopt a helical conformation.[158] The (*D*)-stereochemistry at Glu was necessary in order to bridge between the *i* and *i+7* positions. The synthesis of a tripodal side-chain bridge, spanning the *i, (i+3)*and *(i+7)* positions (Figure 31) has also been reported.[159] Finally, there is a single report of a Glu-Lys *i,(i+3)* lactam bridge being used to stabilise the α-helical conformation of apolipoprotein E.[160]

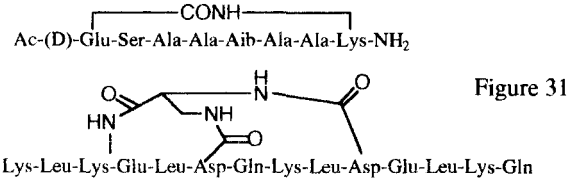
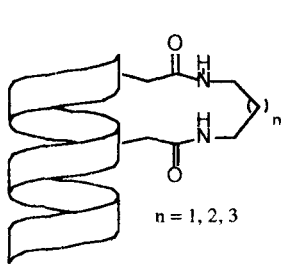


Figure 31

Overall, the results of these studies suggest that the stabilisation of α-helical conformation by lactam bridges is highly context-dependent. It must be borne in mind that, in cases where the biological potency of the resulting hormone analogues is measured, this does not necessarily correspond to the α-helical stability of the analogue; the biologically active conformation is not always known, and may be a bent helix[149] or a turn.[156] Nevertheless, further work will be necessary to elucidate the conditions under which a lactam bridge will stabilise a helical structure, and under which a different conformation will be more favoured.

(v) Other covalent bonds between side-chains.

The structural repertoire available for the stabilisation of helical conformations would be greatly increased if side-chain linkages other than amide bonds were available; moreover, non-natural side-chain bridges may well impart proteolytic stability to such peptide analogues. Two such non-natural linkages have recently been reported. In one study,[161] Glu residues at *i,(i+7)* spacing within the C-terminal helix of apamin, or the S-peptide derived from RNAseA, were linked by α,ω-diaminoalkanes (Figure 32). Greatest helical stabilisation, as determined by NMR and CD measurements, was obtained with 1,4-diaminopropane or 1,5-diaminopentane linkers. For comparison, these peptides were also constrained with the *i,(i+7)* disulphide linkers studied by Schultz.[147] The α,ω-diaminoalkane tethers were found to be superior in stabilising α-helical conformation; this was attributed to the destabilising effect of the (D)-2-amino-6-mercaptohexanoic acid residue on the helix. Stabilisation of helical conformation using two rigid, overlapping *i,(i+7)* bridges has also been attempted (Figure 33).[162] Preliminary CD measurements indicated the presence of helical structure, possibly distorted.



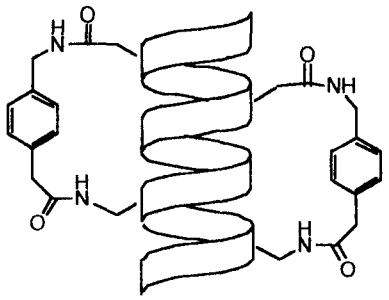


Figure 32 α,ω-Diaminoalkane tethers[161] Figure 33 Overlapping *i,(i+7)* bridges[162]

4. Templates for helix formation

(i) Templates and caps for individual helices

As described above (Section 2(i)), the nucleation of a helix is an entropically disfavoured process. A preorganised template, in which the orientation of the first four amide bonds of the helix is fixed in a rigid structure, should therefore promote helix formation and prevent fraying of the ends. The first such template to be described[163] was a simple tripeptide mimetic with the hydrogen bond between the *i* C=O and the *(i+4)* NH replaced by a covalent bond (Figure 34). Preliminary NMR evidence suggested that such a template adopted a relaxed helical structure, but no further details have been published. Cyclic hydrazides have also been proposed as helical templates, but their effectiveness is still unproven.[164]

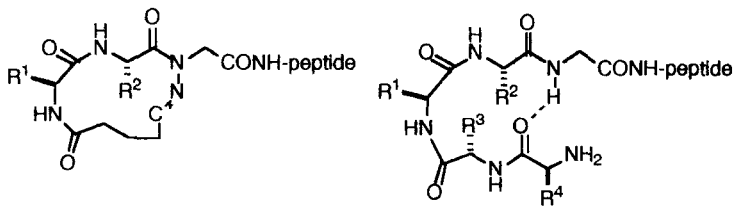


Figure 34 Covalent mimic (a) of the first turn of the helix (b)

A series of preorganised templates, designed to take advantage of the known *N*-capping properties of Pro, have been successfully synthesised and studied by Kemp.[165] The first of these to be reported was a rigid tricyclic template derived from proline. This template has been shown by NMR to adopt three distinct conformations, one of which, shown in Figure 35, places all three carbonyl groups in the correct orientation for initiation of the folding process. With peptides attached to the C-terminus of the template, hydrogen bonding between these carbonyl groups and the relevant amide N-H stabilises both the helical conformation of the peptide and locks the template in the requred orientation. The template can induce helicity, even in short peptides or in sequences with no helical preference; it also acts as a reporter of helical conformation, as only a single conformation of the template is seen by NMR when a helical conformation is reached. With the use of this template, therefore, deduction of the value of s for a particular residue is simplified.[166]

Alanine-rich hexapeptides nucleated by Ac-Hel_1-OH have been used to determine the s values of Ala and Lys.[121,167] In contrast to the studies of host peptides, the s value of Ala was found to be 1.01-1.02, consistent with the values reported by the Scheraga group.[41] It was therefore suggested that the unusual stability of hosts such as AK and AEK arises, not from the helix-forming propensity of Ala itself, but from the

stabilisation of the host peptide conformation by interactions between the ε-NH_3^+ of the Lys residues with the *(i-4)* backbone carbonyl group. The small side-chain of the Ala residues within these hosts would favour Lys-backbone interactions, whereas more bulky residues would prevent such interactions.

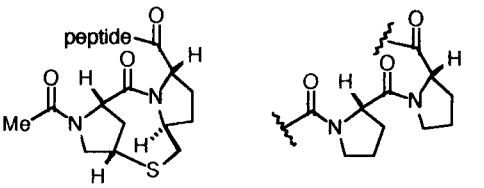


Figure 35 Kemp helix template molecule Ac-Hel]-OH, and Pro-Pro.

Other helix inducing templates have been developed by this group. A tri-proline template (Figure 36) has been studied; however, the increased flexibility of this template rendered it ineffective as a reporter of helicity.[168] Constraint of this latter template with an additional thioether bridge (Figure 36) results in a template that appears by NMR to initiate a 3_{10} helix in the region of the template, with the peptide becoming more α-helical further away.[169]

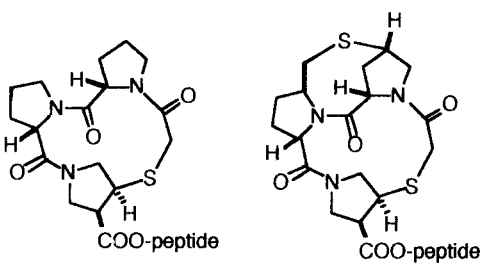
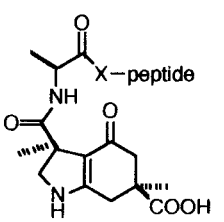


Figure 36

A different template has also recently been reported,[170] and the conformation of template-hexapeptide conjugates examined by CD and NMR. Interestingly, this template only induces significant helical conformation when the peptide is attached via an ester linkage (Figure 37; X = O).



X = O or NH

Figure 37

Attempts to nucleate the conformation of the helical fragment of HIV gp41 transmembrane protein using a structurally simpler Kemp's triacid imide (Figure 38) were unsuccessful.[171] However, a more rigid multicyclic template (Figure 38) nucleated helical conformation of a 9-residue peptide.[172] Although preliminary molecular modelling studies suggested that this template should induce a π-helix, the crystal structure revealed that additional hydrogen bonding to one water molecule displaced the conformation to a regular α-helix.

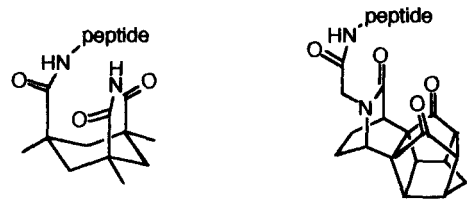
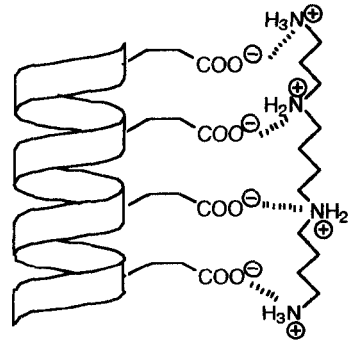


Figure 38

Surprisingly little research has been done on the design and synthesis of flexible peptidomimetics that can act as *N*-cap or *C*-cap residues. A 15-residue peptide has been synthesised with a series of sulfur-containing residues, isosteric with the Asp side-chain, at the *N*-terminus.[173] Increased helicity relative to the uncapped peptide was seen in all cases, particularly for the peptide capped with a sulfonic acid-bearing residue. The synthesis of a group capable of capping the *C*-terminus of a peptide has also been reported,[174] involving the synthesis from a tripeptide of a moiety bearing a stable positive charge, capable of stabilising the macrodipole. No results concerning the efficiency of this group have been reported as yet.

(ii) Helix receptors

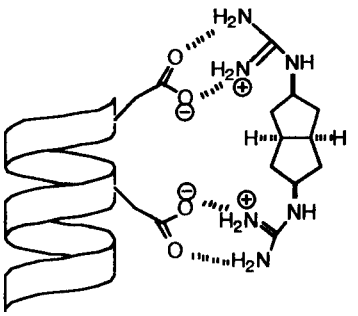
As many peptides adopt helical conformations on binding to receptors[5] or DNA,[6] another method for the stabilisation of helical structure would be to produce a synthetic receptor for a helical conformation. It was recently reported[175] that spermine could induce a random coil-to-helix transition in a synthetic peptide with Glu residues at *i,(i+3)* and *i,(i+4)* spacing (Figure 39).



Ac-Tyr-**Glu**-Gln-Ala-Ala-**Glu**-Gln-Gln-**Glu**-Ala-Ala-Gln-**Glu**-Ala-$CONH_2$

Figure 39 Interactions between spermine and a Glu-contining peptide

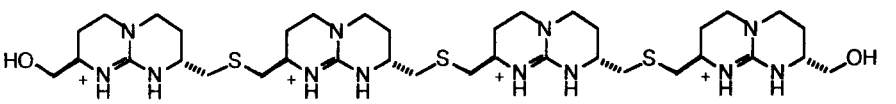
Guanidinium receptors have also been used to stabilise the helicity of suitable peptides. A rigid bisguanidinium receptor, designed to bridge one turn of a helix, was shown to stabilise the helicity of peptides with Asp residues at *i,(i+3)* and *i,(i+4)* spacing (Figure 40) to an extent.[176]



i, i+3 peptide: Ac-Ala-Ala-Gln-**Asp**-Ala-Ala-**Asp**-Ala-Ala-Ala-Ala-Ala-Gln-Ala-Ala-Tyr-$CONH_2$
i, i+4 peptide: Ac-Ala-Ala-Gln-**Asp**-Ala-Ala-Ala-**Asp**-Ala-Ala-Ala-Ala-Gln-Ala-Ala-Tyr-$CONH_2$

Figure 40

A more flexible, tetraguanidinium receptor was able to stabilise the helicity of peptides with Asp residues at *i,(i+3)* spacing (Figure 41). Molecular modelling indicated that the receptor was able to wrap around the peptide helix, forming hydrogen bonds to each Asp -COOH,[177] in a manner analogous to spermine.



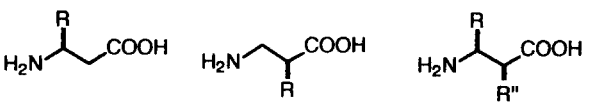
Ac-Ala-Ala-Ala-**Asp**-Gln-Leu-**Asp**-Ala-Leu-**Asp**-Ala-Gln-**Asp**-Ala-Ala-Tyr-$CONH_2$

Figure 41

5. Miscellaneous

Until recently, studies of peptide helices have focussed on the α, 3_{10} and π-helical structures that may be formed using α-amino acids. Using non-natural monomers that will give different peptide backbone structures, however, opens up the possibility of synthesising novel peptide architectures. Although helical structures that may form in this way will have no counterpart in proteins, they may still have interesting molecular recognition properties in biological and other systems, and the use of non-natural monomers will make them promising leads for drug design.

Two groups have recently investigated the peptide architectures formed by β-peptides. Seebach[178] has extensively explored the structural properties of peptides built from acyclic, 2-substituted, 3-substituted, or 2, 3-substituted, β-amino acids (Figure 42). Despite the conformational flexibility of these monomers, β-peptides as short as 6 residues adopt stable helical conformations in MeOH and pyridine, as determined by NMR. (*S*)-β-Peptides with identical substitution patterns adopt a left-handed 3_1 helical conformation, in which the pitch of the helix is about 5 Å and the *i* and *(i+3)* side-chains are in register.



β^3 β^2 $\beta^{2,3}$

Figure 42

Gellman[179] has reported that cyclic β-amino acids will also form stable helical structures. In this work, peptides containing up to six *trans*-2-aminocyclohexanecarboxylic acid (*trans*-ACHC) (Figure 43) monomers were synthesised. Their solution structures in methanol were determined using NMR, and a crystal structure of the hexameric peptide was also obtained. These peptides also adopt a 3_1 helical conformation. In contrast, peptides derived from *trans*-2-aminocyclopentanecarboxylic acid (*trans*-ACPC) adopt a 2.5_{12} helix.[180]

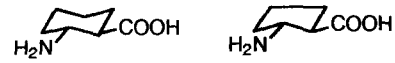


Figure 43

Longer-chain amino acids have also been investigated. Peptides containing mostly α-amino acids, with a central β-alanyl-γ-aminobutyryl segment, have been crystallised and found to adopt regular 3_{10} helical structures.[181] Peptides containing vinylogous amino acids (Figure 44) have also been synthesised,[182] and adopt helical conformations similar to 3_{10} structures, and peptides formed from chiral *N*-substituted glycines have been shown to form stable helical conformations, consistent with the formation of a poly(Pro) type I helix.[183]

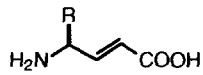


Figure 44

Finally, the design of nonpeptide templates that mimic the orientation of the side-chains in α-helices are of increasing importance, as such structures could also be important leads for drug design. Surprisingly, only one group has made major advances in this area, with the synthesis of templates based on 1,6-substituted[184] and 1,1,6-substituted[185] indanes (Figure 45). Molecular modelling indicated that the side-chains of these templates overlaid the side-chains in the *i*, *(i+1)* (and *(i-1)*) positions of an α-helix very closely, and several of the templates, designed to mimic neuropeptides, bound with micromolar affinity to the appropriate receptors.

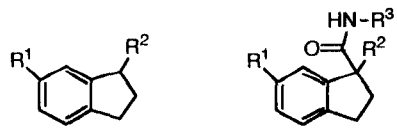


Figure 45 1,6- and 1,1,6-indane templates. R^1, R^2 and R^3 are amino acid side-chains.

6. Summary

Considerable progress has been made in understanding the factors governing helical stability of short peptides and designing stable peptide helices, but the picture is not yet complete. Bioorganic chemists have an important contribution to make; the design of unnatural amino acids, peptide mimetics and helix templates that can be incorporated into short peptides enables the current hypotheses of the properties of amino acids that favour or disfavour helix formation to be rigorously tested. It is clear, when the discrepancies between helical propensities derived from Ala-based host peptides and from the Ac-Hel templates are considered, that s-values are more dependent on context, sequence and inter-residue interactions than previously predicted. It may well

be that the different stabilising factors used in each model to produce a short, helical peptide suitable for study will inevitably impose their own set of helix preferences on the s-values determined.

The ability to design unnatural amino acids, mimetics and templates that will produce short, stable helical peptides is also of crucial importance for medicinal chemists. A portfolio of techniques that could ensure that (almost) any sequence of amino acids adopted a helical structure would enable the interactions of the side-chains of the residues in protein or peptide helices with any biological receptor - protein, DNA, RNA - to be studied, independently of the possible role of those side-chains in stabilising the structure. Moreover, many of the stabilised peptide helices so far produced have had impressive biological properties, and may become therapeutically useful. One of the most promising approaches to this goal appears to be the design of artificial *N*-cap residues to stabilise the first turn of the helix, and templates to mimic the first turn of the helix: this reflects the importance of such motifs in stabilising helices in proteins. Formation of a covalent linkage between appropriate side-chains is also important for helix stabilisation: again, this reflects the importance of the various side-chain-side-chain interactions that stabilise naturally occurring peptide helices. However, it should be borne in mind that enhanced helical stability does not necessarily mean improved biological activity, or vice versa; the preferred conformation of a peptide at a receptor may well differ from the conformation predicted from its sequence, or determined in solution. Finally, the design of non-peptide scaffolds that will display amino acid side-chains in the spatial arrangments produced by helices will undoubtably generate many lead candidates for drug discovery programmes. The scope for biological and medicinal chemists to make an impact in this area is considerable.

7. Addendum

Since the submission of this manuscript, further advances in this area have been reported. The helix-stabilising propensity of alanine continues to be debated. Recent results from the Kemp group[186] using helix templates suggest that the helix-stabilising propensity of Ala is an artifact arising from side-chain interactions in Lys-rich peptides: these studies have been contradicted by NMR studies from the Kallenbach group[187] on Dpr-rich peptides, wherein the side-chains are reported to be too short to enhance helicity directly. A further series of α-helix cap templates have been reported,[188] and hydrazone linkages have been shown to be effective for the stabilisation of α-helices.[189] The introduction of covalent *i,(i+4)* bridges using Grubbs' ring-closing metathesis methodology has been reported.[190]

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Biographical sketch

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Alethea B. Tabor was born in Hertfordshire. She received her B. A. in Natural Sciences from the University of Cambridge in 1985, and her Ph. D. from the University of Cambridge in 1989, under the supervision of Professor Andrew Holmes. After postdoctoral work with Professor Stuart Schreiber at Harvard University, she joined the Department of Chemistry at Edinburgh University as a lecturer, before moving to her present post as a Lecturer in Chemistry at University College London in 1994. Her research interests are in the design, synthesis and biological properties of unnatural and conformationally constrained peptides and amino acids.