Helix design, prediction and stability

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Recent work revealing that our knowledge is now sufficient to build a reasonable quantitative model for the helix/coil transition in heteropolypeptides represents a watershed in research into α-helix stability, prediction and design. The opportunity is presented to design specific α-helix propensity patterns that may be used both to modify thermodynamic properties of target proteins and peptides, and for *de novo* protein design. Despite these advances, the picture is not yet complete, and further studies of still poorly characterized factors are required to obtain a more precise understanding of α-helix stability.

Current Opinion in Biotechnology 1995, **6**:382–386

Introduction

The α-helix, the first protein structural feature to be described, has always been a popular object of study. The main reason for this is that an α-helix may be regarded as a suitable simplified model of a folded protein. In particular, most of the interactions found in proteins are also present in α-helices (i.e. electrostatics, hydrogen-bonding and hydrophobic interactions), and the α-helix offers the important advantage of a symmetrical and quite rigid nature that allows the projection of a three-dimensional problem onto a one-dimensional model. Moreover, it has been repeatedly shown that short monomeric polyalanine-based peptides and protein fragments may significantly populate helical conformations in aqueous solution. In the past few years, an important qualitative difference between efforts devoted to the study of α-helices and those devoted to other structural conformations has been the coalescence of theory and experimental data into a global quantitative model of α-helix stability in heteropolypeptides.

In this review, we first describe the main interactions known to contribute to α-helix stability in aqueous solution, then consider the incorporation of these energy contributions into different models of helix/coil transition theory, and finally discuss the possible use of approximate quantitative models to engineer the α-helix propensity of peptides and proteins. To conclude this review, we comment on possible future directions of this field of research.

Factors contributing to α-helix stability

α-Helix stability is modulated by a large number of physico-chemical factors in a delicate balance. Therefore, a quantitative description of these factors is the first step to gaining an understanding of helix stability. In recent years, a large number of experimental papers have analyzed the contribution to helix stability of many of these factors; these are listed below.

First, a reasonable estimation for the enthalpic contribution of main-chain–main-chain hydrogen-bonding to helix stability has been obtained from the calorimetric analysis of a polyalanine-based peptide [1]. Second, it has been found in many different model systems, both peptides [2–10,11•] and proteins [12–15], that not all the amino acids have the same tendency to participate in a helical conformation (intrinsic propensities). Similar results have been obtained in a statistical analysis of a protein database [16]. Third, the presence of residues with side chains that can make hydrogen bonds with the main-chain amide or carbonyl groups when located at the beginning (N-cap) or end (C-cap) of α-helices has been found to stabilize and nucleate the helical conformation in peptides [17–20,21•] and proteins [12,13,22,23,24•]. A similar effect can arise from the presence of blocking groups at the beginning or end of a polypeptide chain [19,25]. Fourth, several side-chain–side-chain interactions (other than electrostatic interactions [26–28]) at positions i, i+3 and i, i+4 in α-helices have been described recently. These include the following: hydrogen bonds [29]; aromatic rings with hydrophobic side chains [30•], sulphur-containing amino acids [31•] or the histidine ring [32]; and interactions between pairs of hydrophobic side chains [33•]. Fifth, residues bearing charged groups close to the helix ends also have been shown to influence α-helix stability by interacting with the helix macrodipole in a conformationally dependent manner [34–38]. And sixth, sequence motifs involving several specific interactions have been identified that may result in a cooperative effect.

Most of these motifs were discovered through the statistical analysis of protein structure databases [39,40,41•,42•]. One of them is the capping-box motif formed by a good capping residue (i.e. Ser, Thr, Asn and Asp;

Abbreviations

CD—circular dichroism; **NMR**—nuclear magnetic resonance.

three-letter amino acid code) and a Glu, Gln or Asp at position i+3 [39,40]. This motif contains a double reciprocal hydrogen bond resulting from the side chain of residue i+3 hydrogen-bonded to the main-chain amide of the capping residue and the side chain of this residue hydrogen-bonded to the amide of residue i+3. Studies of peptides in aqueous solution have assessed the local nature of this double hydrogen bond and its role in α-helix stabilization [17,18,43]. Another example of a motif is termed a hydrophobic staple and consists of a hydrophobic residue (i.e. Leu, Ile, Val, Met or Phe) before the capping residue (i.e. Ser, Thr, Asn or Asp), whose side chain interacts with that of another hydrophobic residue within the helix at position i+5 [41•,44••,45••]. As in the previous case, the motif is formed in peptides and stabilizes the α-helix [44••,45••]. More importantly, both the capping box and the hydrophobic staple show a strong cooperative effect when placed in phase [45••]. Another motif involving two hydrophobic residues at positions C–3 and C′ and a glycine at position C′ (i.e. a Schellman motif [42•]) has also been described. Experimental analysis of peptides containing this motif, however, has revealed neither a strong contribution to helix stability nor evidence of a role in helix formation, except in the presence of the helix-promoting solvent trifluoroethanol (AR Viguera, L Serrano, unpublished data). Motifs of this kind have been postulated to be important for the early steps in protein folding as they define the α-helix ends and the direction of the polypeptide chain entering or exiting the helix [41•,42•,45••].

The helix/coil transition applied to protein fragments

Recently, spectroscopic analysis, either by circular dichroism (CD) or nuclear magnetic resonance (NMR), has been carried out for a large number of short peptides encompassing α-helices of natural proteins. The results so far indicate that although short-range interactions are insufficient to determine a single definite α-helix structure in a peptide, they do determine α-helix propensities, experimentally observed as helical populations in peptides, which are different for every sequence and for each of the residues in the peptide. Thus, accurate predictions of α-helix stability require a statistical mechanics approach [46,47] in which all the possible helical conformations in a peptide and all the energy contributions are taken into account [48]. Using this approach, several papers have modified the classical helix/coil transition theory to include the effects of some of the factors that have been found recently (see above). Energy contributions that depend only upon the conformational state (helix or coil) of one amino acid can be introduced into the theory in a straightforward manner, as has been recently shown for the capping effects [21•]. On the other hand, the factors that rely on the conformational state of several adjacent amino acids, such as side-chain interactions or the macrodipole effect, are more complicated to model with the complete multiple sequence theory, as formulated by Lifson and Roig [47]. Some attempts to achieve this have used the one-sequence approximation [49], or modified versions of the complete theory to include specific interactions [21•,50,51]. These formalisms have been shown capable of describing changes in helicity, as observed by far-UV CD of purposely designed polyalanine-based peptides. Applying a statistical mechanics approach to describe the conformational properties of protein fragments demands the simultaneous consideration of all known energy contributions. The first attempt to do this was made by Finkelstein *et al.* [48], who provided the general principles to be followed. Even so, their model did not have the real predictive power for heteropolypeptides because of the lack of experimental data at that time, which resulted in a rather arbitrary parameterization.

Recently, a new modification of the helix/coil transition (an algorithm termed AGADIR) has been released, taking advantage of the great wealth of experimental information from the past three years. This information allows the parameterization of the known energy contributions to α-helix stability [52••]. The model has been tested extensively with CD and NMR data on protein fragments and seems to provide a consistent quantitative description of α-helix stability in short peptides [53•]. The last helix/coil transition formalism mentioned above, although it includes some non-standard assumptions (e.g. the use of a different reference state for each residue) renders results very similar to the complete model as formulated by Lifson and Roig [47] for polyalanine-based peptides shorter than 30 residues. Evidence for this is provided by fitting this model to the data on alanine and glycine helix propensities obtained by Baldwin's group [54] (see Table 1). To determine the dependence of the

Table 1. Nucleation (σ) and elongation (*s*) parameters at 0°C for Ala and Gly intrinsic propensities obtained from the fitting of different helix/coil transition approximations to the polyAla-based peptides of Baldwin and co-workers [54,56].

	Lifson–Roig*		AGADIR†			AGADIR (MS)‡			AGADIR§			AGADIR (MS)#		
Residue	σ	*s*	σ (ΔG)	*s*	ΔG	σ (ΔG)	*s*	ΔG	σ (ΔG)	*s*	ΔG	σ (ΔG)	*s*	ΔG
Ala	0.0029	1.54	0.0029	1.534	0.56	0.0029	1.534	0.56	0.00329	1.355	0.61	0.00285	1.43	0.60
Gly	0.0029	0.05	0.0029	0.0575	2.34	0.0029	0.05	2.41	0.00329	0.117	1.94	0.00285	0.12	1.94
			(–0.792)			(–0.792)			(–0.775)			(–0.792)		

The *s* and ΔG values for Ala and Gly are obtained by fitting several polyAla-based peptides studied at 0°C, pH 7.0 and 1 M NaCl [56]. *†‡The fitting has been carried out assuming the same elongation parameter for Ala and Lys, and without taking into consideration capping and blocking effects. §#The fitting has been done using an elongation of 1.06 for Lys and the capping and blocking effects described in AGADIR [52••,53•]. *Lifson–Roig approximation [56]. †§Fitting with AGADIR. ‡#Fitting with AGADIR multiple-sequence (MS) approximation. All ΔG values are in kcal mol^{-1}.

energy contributions on the theoretical framework used, the whole parameter set of AGADIR has been implemented recently in the complete multiple sequence approximation (V Muñoz, L Serrano, unpublished data). This approximation accounts for conformations with more than one non-overlapping helical segment, and the helicities of each residue are normalized with the molecular partition function. It is observed that the parameters implemented in AGADIR are independent of the approximation used, with the exception of a small increase in the value of the nucleation term ($\Delta G = -0.775$ to -0.792 kcal mol^{-1}; nucleation parameter $[\sigma] = 0.00329$–0.00286 at O°C).

The satisfactory success attained by this quantitative model suggests that the most basic terms contributing to α-helix stability are already known, although other less ubiquitous terms may still be discovered. It is also very encouraging that such a model is able to detect the experimentally observed bypassing of a putative stop signal, the capping-box motif, in a peptide encompassing α-helix 5 of the *Escherichia coli* chemotactic protein CheY [44••] (Fig. 1). A conclusion from this last observation is that it is not possible to use stereochemical rules to define start and stop signals in α-helices, but that α-helical ends are simply defined by an energy balance resulting from local and tertiary interactions.

The effects of some of the more relevant experimental conditions (e.g. pH and temperature) have been introduced recently into the model through classical equations and assumptions [55•]. Even so, ionic strength, which affects significantly the helical content of charged [26–29] and uncharged peptides [56], has not been considered in any model as yet. This is mainly because of the lack of an appropriate simplified theoretical description for ionic strengths >100 mM.

Engineering α-helix propensities

The ability to design sequences with specific patterns of α-helix propensity — understood as overall helical population and its distribution along the polypeptide chain of the corresponding isolated peptide — is of great interest. This capability would facilitate the design of mimetic peptides as well as *de novo* protein design. It would also make straightforward the enhancement of the stability of proteins of known three-dimensional structure by enabling the modification of residues located in the solvent-exposed face of α-helices (through higher intrinsic helical propensities or specific side-chain interactions). This has already been achieved by substituting polar residues with alanine residues [57•,58••]. Yet, using a complete model for the helix/coil transition, it should be possible to engineer the helical propensity of each residue in a polypeptide chain with relatively high precision. This would allow a polar surface to be maintained on the solvent-exposed face of the mutated α-helix, preventing aggregation problems and conserving or creating new functions. Using AGADIR to design mutations, a large increase in the helical

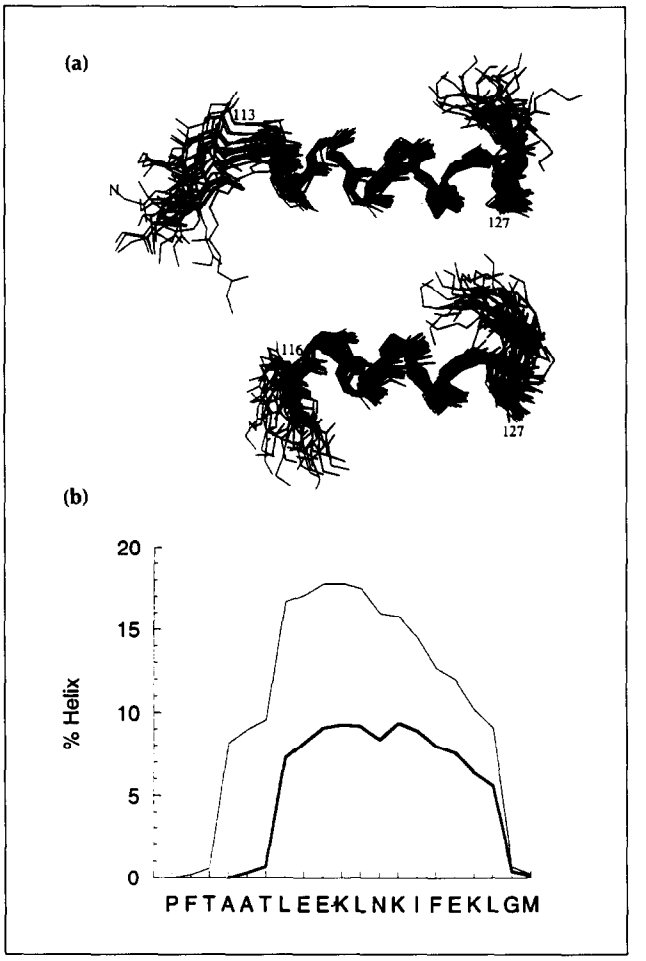

Fig. 1. Helix prediction for two peptides corresponding to portions of α-helix 5 of *E. coli* chemotactic protein CheY. **(a)** Superimposition of the best 25 structures, calculated on the basis of distance constraints derived from observed nuclear Overhauser enhancement (NOE) cross-correlations, for peptide 110–130 (top), which corresponds to the full length of helix 5 of CheY, and for a shorter version, peptide 113–130 (bottom). α-Helix 5 has an internal capping-box sequence fingerprint, residues Thr-Leu-Glu-Glu (at positions 115–118). Structures were calculated from data obtained in 30% trifluoroethanol, but the critical NOEs were also detected in aqueous solution [44••]. **(b)** AGADIR calculation of the helical population along the sequence for the two peptides in (a). Thin line, longer peptide; thick line, shorter peptide.

content of some natural peptides corresponding to α-helices has already been achieved ([53•]; V Muñoz, L Serrano, unpublished data). The effects of such mutations on the global stability and folding of proteins are actually being investigated in two different model proteins: the *E. coli* chemotactic protein CheY (V Muñoz, P Cronet, E Lopez-Hernandez, L Serrano, unpublished data), and the activation domain of human procarboxypeptidase A (V Villegas, AR Viguera, X Aviles, L Serrano, unpublished data).

Future perspectives

The design of an approximate quantitative model for α-helix stability represents a watershed. Nevertheless,

for the model to be refined, we must continue to study and look into other factors that affect α-helix stability (i.e. cooperative interactions, etc.). A comparison of the results from AGADIR and the experimental information on protein fragments also reveals certain limits for the two-state approximation (helix/coil) because other local structures, such as β-turns and β-strands, may also be present at experimentally observable levels [59]. This will become more apparent as greater resolution is achieved in the spectroscopic techniques. To overcome these limitations, a more complex theory accounting for conformations other than α-helices will be required in the future. On the other hand, the possibility of designing specific patterns of α-helix propensity for each residue in a polypeptide chain opens new and very attractive perspectives in the applied field of peptide and protein design.

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