The nature of the α-helix

The first determination of the structure of a protein, that of myoglobin in 1958, put an end to the idea that there might be a simple code linking protein structure to form and function. In retrospect, that is not surprising: the varied and diverse functions of proteins could not possibly be accommodated by a simple structural rule, as is the case for DNA and the double helix. That does not mean, of course, that protein structures have no common elements. The α-helix and the β-sheet are by far the most common. There are also less abundant, but no less widespread, elements such as the β-reverse turn and the 3_{10}-helix which has the same handedness as the α-helix, but is more tightly coiled and has a larger pitch.

Such units of secondary structure are the building-blocks of the various protein folds. Even so, our understanding of the construction of even the most basic elements of secondary structure is still far from complete. The existence of the α-helix was first predicted by Linus Pauling in 1951 (ref. 1), but it remains a topic of intense investigation, as three papers in this issue of *Nature Structural Biology* demonstrate.

Helix determination

It is well established that the substitution of arginine for lysine in the sequences of short peptides increases the stability of their helical conformations *in vitro*, despite the similarity of the structure and charge of these amino-acid residues. Fiori, Lundberg & Millhauser[2] have now probed this phenomenon further, asking what effect such substitutions have on the conformational equilibrium between the α- and 3_{10}-helix. Their work has produced the surprising result that not only does substitution of arginine for lysine preferentially stabilize the α-helical conformation but that this stabilizing effect is evident at parts of the peptide some distance from the substitution site.

The sixteen-residue peptide studied by Fiori and colleagues contains three lysine residues evenly spaced along its length. As expected, substitution of all three lysine residues by arginine results in a strong shift in the structure of the amino-terminal portion of the peptide, from a mixture of the 3_{10}- and α- conformations to a largely α-helical conformation. What had not been anticipated was that, when the lysines were altered one at a time, the substitution with the greatest effect on the N terminus of the peptide would be that of the most C-terminal lysine. In fact, the shift in N-terminal helicity produced by changing only the most C-terminal lysine matched that found in the fully substituted peptide.

This effect is not reduced by increasing the ionic strength of the peptide solution, so that subtle differences of the charge distribution of the two amino acids cannot be the cause. Fiori and colleagues suggest that the ability of the arginine residue to form a hydrogen bond to the amide carbonyl of the residue preceding it by three positions in the sequence, stabilizes a single turn of the α-helix. They argue that this turn of α-helix then acts as a 'capping' or 'nucleating' structure which, in turn, will favour the production of further turns of α-helix extending some distance from the capping structure itself.

The fact that extensive changes in the conformation of a peptide helix can be achieved by the subtle alteration of one end of it suggests a mechanism for transmembrane signalling. At its simplest, it could be postulated that the alteration in length of a peptide changing between α- and 3_{10}- helix could act as a mechanical trigger, almost like a piston. It is however dangerous to extrapolate from experiments carried out in aqueous solution to events occurring within the hydrophobic environment of biological membranes. Another paper in this issue[3] clearly demonstrates that the properties of the amino acids, familiar enough in aqueous solution, undergo radical changes when moved into hydrophobic environments.

Hydrophobic helices

Comparison of sequences known or predicted to pass through cell membranes indicates that helix structure is governed by parameters that are significantly different from those in aqueous solution. Transmembrane helical regions are rich in bulky residues (for example, isoleucine and valine) classified as 'helix-destabilizing' for peptides in aqueous solution. Using amino-acid substitutions within a short twenty residue peptide designed as a model for transmembrane helices, and various detergent-rich media to simulate the membrane environment, Li and Deber[3] characterize the 'transmembrane' helical propensity of the thirteen uncharged, naturally occurring amino acids.

In aqueous solution, the 'guest' residues substituted into the 'host' peptides show helical propensities that correspond well with those calculated by Chou and Fasman[4] in the 1970s. In the presence of detergent micelles, on the other hand, the helical propensity of the thirteen residues is significantly different from that in water. In these environments, helicity is to a large extent controlled by the side-chain hydrophobicity of 'guest' residues. Indeed, peptide helicity is effectively proportional to the hydropathy of the hydrophobic core of the peptides.

Glycine and proline 'guest' residues do not conform to this general rule: the peptide backbone is exposed at glycine residues, and the cyclic nature of proline results in three non-hydrogen carbonyl groups, as well as conformational strain on the α-helix. Isoleucine and valine, which are helix-destabilizing in water, stabilize helices in these hydrophobic environments; Li and Deber suggest that they may help to prevent the premature folding of transmembrane domains before insertion. Once in the membrane environment, these same residues would then promote the formation of the transmembrane domain.

Helix prediction

Further extension of the classical work of Chou and Fasman on the helical propensity of amino acids is the subject of a third paper in this issue, in which Munoz and Serrano[5] outline a method for predicting the solution behaviour of helical peptides. This work builds on the large body of literature dealing with the thermodynamic properties of small peptides and proteins and of their component amino acids. Using a synthesis of this data, they have developed an algorithm to predict the α-helical content of peptides which is firmly based on the chemical and physical properties of the amino acid residues.

This work also illustrates the difficulties increasingly encountered when attempting to predict peptide conformations from amino-acid sequences. A portion of the protein ubiquitin is predicted to be helical, and indeed the isolated peptide does have significant helical content in solution, but within the native protein it forms part of a β-sheet. It is clear that factors other than the intrinsic helix-forming ability of the sequence are important in determining the final conformation *in situ*. Nonetheless, Munoz and Serrano point out that the best results are achieved when predicting the conformation of peptides derived from protein sequences. They suggest that in these peptides large numbers of interactions have evolved to define the final conformations so that small errors in the predicted parameters are less critical than in artificial peptides where conformation may be determined by a few critical effects.

There is much more to be done before people can say that they fully understand the structure and function of α-helices, but this bevy of papers, tackling the problem from a number of different angles, certainly points the way for further research.

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