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Recent advances in helix–coil theory

Andrew J. Doig

Department of Biomolecular Sciences, UMIST, P.O. Box 88, Manchester M60 1QD, UK

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Abstract

Peptide helices in solution form a complex mixture of all helix, all coil or, most frequently, central helices with frayed coil ends. In order to interpret experiments on helical peptides and make theoretical predictions on helices, it is therefore essential to use a helix–coil theory that takes account of this equilibrium. The original Zimm–Bragg and Lifson–Roig helix–coil theories have been greatly extended in the last 10 years to include additional interactions. These include preferences for the N-cap, N1, N2, N3 and C-cap positions, capping motifs, helix dipoles, side chain interactions and 3_{10}-helix formation. These have been applied to determine energies for these preferences from experimental data and to predict the helix contents of peptides. This review discusses these newly recognised structural features of helices and how they have been included in helix–coil models.

Keywords: α-Helix; Random coil; Protein stability; Protein structure; Zimm–Bragg; Lifson–Roig

1. Introduction

It is a pleasure to be a part of this tribute to John Schellman. He has made numerous fundamental contributions to biophysical chemistry. In particular, he was the first person to analyse the thermodynamics of the helix–coil transition [1], thus founding the field that is the subject of this review. Peptides that form helices in solution do not show a simple two-state equilibrium between a fully folded and fully unfolded structure. Instead they form a complex mixture of all helix, all coil or, most frequently, central helices with frayed coil ends. In order to interpret experiments on helical peptides and make theoretical predictions on helices, it is therefore essential to use a helix–coil theory that considers every possible location of a helix within a sequence. The purpose of this review is to cover how helix–coil theories have been developed in the last 10 years, principally by including additional structural features of the helix, such as the distinct preferences that amino acids have for particular locations in the helix, or helix dipole interactions. I do not consider in detail the results obtained from applying the models to empirical data, such as the values of preferences

E-mail address: andrew.doig@umist.ac.uk (A.J. Doig).

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for helix interiors, or the origins of helix energetics, such as the influence of solvation on helix preferences. These areas are well covered in reviews [2–8], as well as numerous papers cited later in the text.

The first wave of work on helix–coil theory was in the late 1950s and early 1960s, and this has been reviewed in detail by Poland and Scheraga [9]. Scheraga and co-workers, in particular, continued to use and develop these models to determine helix preferences with host/guest polypeptides. In their work, long polypeptides of hydroxybutyl-L-glutamine or hydroxypropyl-L-glutamine containing 100s of amino acids were randomly substituted with low amounts of a guest amino acid. Application of helix–coil theory gave helix preferences of the guest [10].

In 1992, Qian and Schellman [11] reviewed current understanding of helix–coil theories. Since this time there has been a great deal more interest in the field, primarily driven by two kinds of experimental result. Firstly, following the pioneering work of Marqusee and Baldwin [12], peptide models have become available that form stable α-helices, despite typically having only 15–20 amino acids. Their sequences are mostly alanine, thus giving a relatively context-free environment to insert interactions of interest. Secondly, examination of protein crystal structures has shown that amino acids have distinct preferences for different environments within the helix. For example, Argos and Palau [13], Richardson and Richardson [14] and Presta and Rose [15] showed in the 1980s that amino acid preferences for the first positions in the helix are entirely different from interior preferences. Helix–coil theory was therefore developed to (i) provide a quantitative interpretation of the new experimental results from short peptide models and (ii) include the new interactions missing from the older models. It is the purpose of this review to examine this recent work. First, I briefly cover the problem of the helix–coil transition and the older Zimm–Bragg (ZB) and Lifson–Roig (LR) models. A more detailed explanation can be found in the Poland and Scheraga [9] or Qian and Schellman [11] reviews. Then I cover new structural features of the helix and how they are included within helix–coil models.

2. Structure of the α-helix

Proteins are built of regular local folds of the polypeptide chain called secondary structure. The α-helix was first described by Pauling, Corey and Branson in 1950 [16], and their model was quickly supported by X-ray analysis of haemoglobin [17]. Irrefutable proof of the existence of the α-helix came with the first protein crystal structure of myoglobin, in which most secondary structure is helical [18]. α-Helices were subsequently found in nearly all globular proteins. It is the most abundant secondary structure, with ~30% of residues found in α-helices [19].

A helix combines a linear translation with an orthogonal circular rotation. In the α-helix the linear translation is a rise of 5.4Å per turn of the helix and a circular rotation is 3.6 residues per turn. Side chains spaced $i,i+3$, $i,i+4$ and $i,i+7$ are therefore close in space, and interactions between them can affect helix stability. Spacings of $i,i+2$, $i,i+5$ and $i,i+6$ place the side chain pairs on opposite faces of the helix, avoiding any interaction. The helix is primarily stabilised by $i,i+4$ hydrogen bonds between backbone amide groups.

The conformation of a polypeptide can be described by the backbone dihedral angles φ and ψ. Most φ,ψ combinations are sterically excluded, leaving only the broad β region and narrower α region. One reason why the α-helix is so stable is that a succession of the sterically allowed α φ,ψ angles naturally position the backbone NH and CO groups towards each other for hydrogen bond formation. It is possible that a succession of the most stable conformation of an isolated residue in a polymer with alternative functional groups could point two hydrogen-bond donors or acceptors towards each other, making secondary structure formation unfavourable. One reason why polypeptides may have been selected as the polymer of choice for building functional molecules is that the sterically most stable conformations also give strong hydrogen bonds.

The residues at the N-terminus of the α-helix are called N′–N-cap–N1–N2–N3–N4, etc., where the N-cap is the residue with non-helical φ,ψ angles immediately preceding the N-terminus of

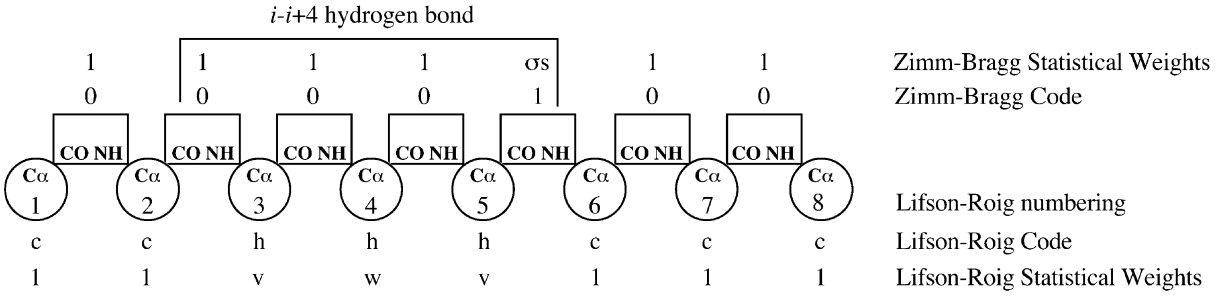

Fig. 1. Zimm–Bragg and Lifson–Roig codes and weights for the α-helix.

an α-helix and N1 is the first residue with helical ϕ,ψ angles [14]. The C-terminal residues are similarly called C4–C3–C2–C1–C-cap–C′, etc. The N1, N2, N3, C1, C2 and C3 residues are unique because their amide groups participate in $i,i+4$ backbone–backbone hydrogen bonds using either only their CO (at the N-terminus) or NH (at the C-terminus) groups. The need for these groups to form hydrogen bonds has powerful effects on helix structure and stability [15].

3. Helix–coil theory

The simplest way to analyse the helix–coil equilibrium, still occasionally observed, is the two-state model, in which the equilibrium is assumed to be between 100% helix conformation and 100% coil. This is incorrect and its use gives serious errors. This is because helical peptides are generally most often found in partly helical conformations, often with a central helix and frayed, disordered ends, rather than in the fully folded or fully unfolded states.

3.1. Zimm–Bragg model

The two major types of helix–coil model are (i) those which count hydrogen bonds, principally ZB [20], and (ii) those that consider residue conformations, principally Lifson–Roig [21]. In the ZB theory the units being considered are peptide groups and they are classified on the basis of whether their NH groups participate in hydrogen bonds within the helix. The ZB coding is shown in Fig. 1. A unit is given a code of 1 (e.g. peptide unit 5 in Fig. 1) if its NH group forms a hydrogen bond, and 0 otherwise. The first hydrogen-bonded unit proceeding from the N-terminus has a statistical weight of σs, successive hydrogen bonded units have weights of s, and non-hydrogen-bonded units have weights of 1. The s-value is a propagation parameter and σ is an initiation parameter. The most fundamental feature of the thermodynamics of the helix–coil transition is that the initiation of a new helix is much more difficult than the propagation of an existing helix. This is because three residues need to be fixed in a helical geometry to form the first hydrogen bond, while adding an additional hydrogen bond to an existing helix requires that only one residue is fixed. These properties are thus captured in the ZB model by having σ smaller than s. The statistical weight of a homopolymeric helix of a N hydrogen bonds is σs^{N-1}. The cost of initiation, σ, is thus paid only once for each helix, while extending the helix simply multiplies its weight by one additional s-value for each extra hydrogen bond. The complete helix–coil equilibrium is handled by determining the statistical weight for every possible conformation that contains a helix, plus a reference weight of 1 for the coil conformation. The population of each conformation is given by the statistical weight of that conformation divided by the sum of the statistical weights for every conformation (i.e. the partition function). Thus, the greater the statistical weight, the more stable the conformation. Partition functions are extremely powerful concepts in statistical thermodynamics, since they include all properties of an equilibrium. Any property of the equilibrium can be extracted from the partition function by applying the appropriate mathematical

function. This is analogous to quantum mechanics, where any property can be determined from a system by applying an operator function to a wavefunction. In this case the properties could be the mean number of hydrogen bonds, the mean helix length, the probability that each residue is within a helix, etc. Statistical weights can be regarded as equilibrium constants for the equilibrium between coil and the structure (as the reference coil weight is defined as 1). They can therefore be converted to free energies as $-RT\ln(\text{weight})$.

3.2. Lifson–Roig model

In the LR model, each residue is assigned a conformation of helix (h) or coil (c), depending on whether it has helical ϕ,ψ angles. Every conformation of a peptide of N residues can therefore be written as a string of N 'c's or 'h's, giving 2^N conformations in total. Residues are assigned statistical weights depending on their conformations and the conformations of surrounding residues. A residue in an h conformation with an h on either side has a weight of w. This can be thought of as an equilibrium constant between the helix interior and the coil. Coil residues are used as a reference and have a weight of 1. In order to form an $i,i+4$ hydrogen bond in a helix, three successive residues need to be fixed in a helical conformation. M consecutive helical residues will therefore have $M-2$ hydrogen bonds. The two residues at the helix termini (i.e. those in the centre of chh or hhc conformations) are therefore assigned weights of v. The ratio of w/v gives the approximate effect of hydrogen bonding [1.7:0.036 for Ala [22] or $-RT\ln(1.7/0.036) = -2.1$ kcal mol^{-1}]. A helical homopolymer segment of M residues has a weight of v^2w^{M-2} and a population in the equilibrium of v^2w^{M-2} divided by the sum of the weights of every conformation (i.e. the partition function). In this way the population of every conformation is calculated and all properties of the helix–coil equilibrium are evaluated. The LR model is easier to handle conceptually for heteropolymers, since the w and v parameters are assigned to individual residues. The substitution of one amino acid at a certain position thus changes the w- and v-values at that position. In the ZB model, the initiation parameter σ is associated with several residues and s with a peptide group, rather than a residue. It is therefore easier to use the LR model when making substitutions. Indeed, most recent work has been based on this model. A further difference is that the ZB model assigns weights of zero to all conformations that contain a chc or chhc sequence. This excludes a very large number of conformations that contain a residue with helical ϕ,ψ angles but with no hydrogen bond. In LR theory, these are all considered. The ZB and LR weights are related by the following formula [11]: $s = w/(1+v)$; $\sigma = v^2/(1+v)^4$.

The treatment of peptide conformations is based on Flory's isolated-pair hypothesis [23]. This states that while ϕ and ψ for a residue are strongly interdependent, giving preferred areas in a Ramachandran plot, each ϕ,ψ pair is independent of the ϕ,ψ angles of its neighbours. Pappu et al. examined the isolated-pair hypothesis in detail by exhaustively enumerating the conformations of poly(Ala) chains [24]. Each residue was considered to populate 14 mesostates, defined by ranges of ϕ,ψ values. By considering all 14^N mesostate strings, all conformations were considered for up to seven alanines. The number of allowed conformations was found to be considerably fewer than the maximum, thus showing that the isolated-pair hypothesis is invalid. The chains mostly populated extended or helical conformations, as many partly helical conformations are sterically disallowed. Such effects are not included in helix–coil theories, thus presenting a considerable challenge for the future. Helix–coil theories assign the same weight (1) to every coil residue; steric exclusion means that this should vary and be lower than 1 in many cases.

3.3. Single sequence approximation

Since helix nucleation is difficult, conformations with multiple helical segments are expected to be rare in short peptides. In the one-, or single-helical-sequence approximation, peptide conformations containing more than one helical segment are assumed not to be populated and are excluded from the partition function (i.e. assigned statistical

weights of zero). As peptide length increases, the approximation is no longer valid, since multiple helical segments can be long enough to overcome the initiation penalty. The single-sequence approximation also breaks down when a sequence with a high preference for a helix terminus is within the middle of the chain. The error from using the single-sequence approximation therefore shows a wide variation with sequence and could be potentially serious if a sequence has a high preference to populate more than one helix simultaneously. Conformations with two or more helices may also often include helix–helix tertiary interactions that are ignored in all helix–coil models.

4. Extension of the helix–coil models

The majority of recent work has been based on the LR model [21]. In the original model, weights are assigned to residues in the centre of hhh triplets (a weight of w for propagation) or in the centre of chh or hhc triplets (a weight of v for initiation). Residues in all other triplets have weights of 1. LR-based models have been extended by assigning weights to additional conformations. In general, this work has been motivated by the discovery of additional features that affect helix stability in protein crystal structures. Their inclusion within helix–coil theory has allowed the measurement of the effect of these features on helix stability.

4.1. N- and C-caps

The first residue in a helical (h) conformation at the N-terminus is called N1 and the preceding residue, the last in a non-helical (c) conformation before the beginning of the helix, is called the N-cap. Argos and Palau [13] first showed that N-cap preferences differ from other helical positions, although the terminology was originated by Richardson and Richardson [14]. N-capping can therefore be included in LR theory by assigning a weight of n to the central residue in a cch triplet [25]. Application of the model to experimental data for which the N-terminal amino acid of a helical peptide was varied [26] allowed the determination of the n-values, and hence free energy of N-capping (as $-RT\ln n$) of all the amino acids [27].

Similarly, the C-cap is the first residue in a non-helical conformation (c) at the C-terminus of a helix. C-cap weights (c-values) are assigned to central residues in hcc triplets. Application of the model to experimental data for which the C-terminal amino acid of a helical peptide was varied allowed the determination of the c-values, and hence free energy of C-capping (as $-RT\ln c$) of all the amino acids [27].

A problem with the original definitions of the capping weights above is that they apply to isolated h or hh conformations that are best regarded as part of the random coil. A helical hydrogen bond can only form when a minimum of three consecutive h residues are present. The most stabilising class of N-caps (Asp, Asn, Ser, Cys and Thr) accept hydrogen bonds to the NH groups of the N3 residue. This is clearly impossible if an N3 residue does not exist, which is the case if there are not three or more consecutive h residues. The Doig et al. model [25] has the flaw that sequences such as Asp–Ala–Gly have a high population for the chc conformation, as Asp has a high N-cap preference. This is incorrect, however, as the Asp N-cap preference results from hydrogen bonding to the N3 position, so should not be apparent in chc and chhc conformations. Andersen and Tong [28] and Rohl and Baldwin [6] therefore changed the definition of the N-cap to apply only to the c residue in a chhh quartet. The N-cap residues in chc or chhc conformations have weights of zero in the Andersen–Tong model and 1 in the Rohl–Baldwin model. These modifications give different N-cap and helix interior energies after fitting.

4.2. Capping boxes

The N-terminal capping box [29] includes a side chain–backbone hydrogen bond from N3 to the N-cap ($i,i-3$). For example, a Ser–X–X–Glu sequence has a high preference for a helix N-terminus, as the Ser side chain accepts a hydrogen bond from the Glu backbone NH, while the Ser backbone NH donates a hydrogen bond to the Glu side chain. This is included in the LR model by assigning a weight of $w\times r$ to the chhh confor-

mation, where r is the weight for the Ser backbone to the Glu side-chain bond [6].

4.3. Side chain interactions

As helices have 3.6 residues per turn, side chains spaced $i,i+3$ or $i,i+4$ are close in space. Side chain interactions are thus possible when four or five consecutive residues are in a helix. They are included in the LR-based model by giving a weight of $w \times q$ to hhhh quartets and $w \times p$ to hhhhh quintets. The side chain interaction is between the first and last side chains in these groups; the w weight is maintained to maintain the equivalence between the number of residues with w weighting and the number of backbone helix hydrogen bonds.

Scholtz et al. [30] used a model based on the one-helical-sequence approximation of the LR model to quantitatively analyse salt-bridge interactions in alanine-based peptides. Only a single interaction between residues of any spacing was considered, although this was appropriate for the sequences they studied.

Shalongo and Stellwagen [31] also proposed incorporating side-chain interaction energies into the LR model, using a clever recursive algorithm. In our model we have considered only $i,i+3$ and $i,i+4$ side chain interactions [32]; Shalongo and Stellwagen more generally consider side-chain interactions of any spacing. In their implementation of the Lifson–Roig formalism, however, they change the definition of the propagating and initiating (capping) weights, such that the physical meaning of these parameters is lost and the number of propagating residues (w) no longer correlates with the number of hydrogen bonds formed. Their capping parameters are associated with residues in helical conformations, instead of coil. In proteins, however, N- and C-cap residues have non-helical dihedral angles [14]. This loss of physical meaning means that their energies for substituting residues at capping and interior positions in peptide helices are not directly transferable to helices in proteins.

4.4. N1, N2 and N3 preferences

The N1, N2 and N3 helix positions are unique because their amide NH groups do not participate in $i,i+4$ backbone–backbone hydrogen bonds. The helix N-terminus shows significantly different residue frequencies for the N-cap, N1, N2, N3 and helix interior positions [13,14,33]. Penel et al. [34] made a detailed survey of the structures adopted by the amino acids at N1, N2 and N3 and identified many new structural features. They found that the most significant structural preferences can be explained by short-range hydrogen bonding to the free N-terminal NH groups, as foreseen by Presta and Rose [15], with the strongest trends being the N2 amino acids Gln, Glu, Asp, Asn, Ser, Thr and His preferentially forming i,i or $i,i+1$ hydrogen bonds to the backbone. A complete theory for the helix should therefore include distinct preferences for the N1, N2 and N3 positions.

In the original LR model, the N1 and C1 residues are both assigned the same weight, v. Shalongo and Stellwagen [31] separated these as v_N and v_C. Andersen and Tong [28] did the same and derived complete scales for these parameters from fitting experimental data, although some values were tentative. The helix initiation penalty is $v_N \times v_C$, and so v_N- and v_C-values are all small (~ 0.04).

We added weights for the N1, N2 and N3 ($n1$, $n2$ and $n3$) positions as follows. The $n1$-value is assigned to a helical residue immediately following a coil residue. The penalty for helix initiation is now $n1 \cdot v$, instead of v^2, as v remains the C1 weight. An N2 helical residue is assigned a weight of $n2 \cdot w$, instead of w. The weight w is maintained in order to keep the useful definition of the number of residues, with a w weighting being equal to the number of residues with an $i,i+4$ main-chain–main-chain hydrogen bond. The $n2$-value is an adjustment to the weight of an N2 residue that takes into account the structures that can be adopted by side chains uniquely at this position. Similarly, an N3 residue is now assigned the weight $n3 \cdot w$, instead of w. Residues in the centre of the rare hch conformation have a weight of 1. Application of the model to peptides designed to probe the N1 position (CH_3CO–XAAAAQAAAAQAA-GY–NH_2 [35]) and the N2 position (CH_3CO–AXAAAAKAAAAKAAGY–NH_2 [36]) has given N1 and N2 preferences for most amino acids for these positions, and these agree well with prefer-

ences observed in protein structures. Petukhov et al. similarly obtained N1, N2 and N3 preferences for non-polar and uncharged polar residues by applying AGADIR (see below) to experimental helical peptide data, and found almost identical results [37,38].

4.5. Helix dipole

The three non-hydrogen-bonded NH groups at the N-terminus of the helix give a net positive charge; similarly, the three CO groups of C1, C2 and C3 give a negative charge at the C-terminus [39–41]. There is therefore a general trend for negative side chains to be favoured at the N-terminus of the helix and positive groups at the C-terminus. Helix dipole effects were added to the LR model by Scholtz et al. [30], although they used the one-sequence approximation, so that only one or no dipoles in total are present. In LR models, helix dipole effects are subsumed within other energies. For example, N-cap, N1, N2 and N3 energies include a contribution from the helix dipole interaction, so the energy of interaction of charged groups at this position with the dipole should not be counted in addition.

4.6. chc and chhc conformations in the random coil

The assignment of weights to very short helical segments chc and chhc requires careful consideration. While each such conformation is disfavoured, as they are residues that pay the entropic cost of restriction to helical ϕ,ψ space without forming stabilising bonds, the large number of places in which they can form in the peptide means that they make a significant contribution to the random coil population. The original LR model assigns these conformations weights of v and v^2, implicitly assuming that the initiation penalty for helix formation also gives the correct weights for these coil conformations. If, however, residues have more conformational freedom within a chc conformation than within the N1 or C1 positions of the helix, which seems reasonable, then the weights of chc and chhc should be higher. The root of the problem is that the c and h conformations are not well defined. The helix–coil parameters are determined by fitting a model to experimental data, rather than by rigorously defining an area of the Ramachandran map as helical and assessing the population within that area.

In our N1N2N3 model, an isolated helical residue, in the centre of a chc conformation, is assigned a weight $n1$, rather than v. This is because the most important structures adopted by residues at N1 are hydrogen bonds between the N1 side chain and the N1 backbone NH group [34]. These interactions can form if only a single residue is in an h conformation. All N1 residues commencing any helical segment have the weight $n1$. The N2 residue in a chhc sequence is assigned the weight $n2{\cdot}v$ for the same reason: N2 structures are typically hydrogen bonds between the N2 side chain and the N2 backbone NH group [34]. These can therefore form even if only two consecutive helical residues are present.

Andersen and Tong [28] gave weights of zero to chc and chhc conformations. While it seems strange to state that such conformations are never populated, they did find a better fit to experimental data than when using the Doig et al. model [25]. This may be because they also assigned N-cap weights to a minimum of three helical segments, as discussed above. Weights for these coil conformations thus vary greatly. As all the models fit well to experimental data, it is unclear at present which choice is best.

4.7. 3_{10}- and π-helices

The ideal α-helix has a periodicity of 3.6 residues per turn, encloses 13 atoms in a ring by formation of an $i,i+4$ C=O···H–N hydrogen bond, and is thus a 3.6_{13}-helix. The 3_{10}-helix is a more tightly wound helix, stabilised by $i,i+3$ C=O···H–N hydrogen bonds, while the π-(4.4_{16})-helix is wound less tightly, with $i,i+5$ C=O···H–N hydrogen bonds. The Lifson–Roig formalism can easily be adapted to describe helices of other co-operative lengths [42]. To treat the 3_{10}-helix–coil transition, the helical conformation, h_α, is replaced with h_τ, reflecting the different ϕ,ψ angles of 3_{10}-helical residues. The fundamental difference between a 3_{10}-helix and an α-helix is

that the 3_{10}-helix has an $i,i+3$ hydrogen bonding pattern, rather than the $i,i+4$ pattern characteristic of the α-helix. For a given number of units in helical conformations, a 3_{10}-helix consequently has one more hydrogen bond than an α-helix. An equivalent description of this difference is that initiation is easier for a 3_{10}-helix than for an α-helix: one fewer unit needs to be fixed in a helical conformation before the first hydrogen bond is formed. To include this difference in the 3_{10}-helix theory, one of the α-helical initiating residues (i.e. the central unit of either the $h_\alpha h_\alpha c$ or the $ch_\alpha h_\alpha$ triplet) must become a 3_{10}-helix-propagating residue. We arbitrarily chose to assign the propagating statistical weight, w_τ, to the central unit of the $h_\alpha h_\alpha c$ triplet such that the helix-propagating unit i is associated with the hydrogen bond formed between the CO of peptide $i-2$ and the NH of peptide $i+1$. The remainder of the statistical weights applicable to the α-helix–coil theory are maintained.

The models described above for the α-helix–coil and 3_{10}-helix–coil transitions can be combined to describe an equilibrium including pure α-helices, pure 3_{10}-helices and mixed α-/3_{10}-helices [42]. In this model, three conformational states are possible, 3_{10}-helical (h_τ), α-helical (h_α) and coil (c). Stretches of residues in h_α conformation are treated as in the pure α-helix model, and stretches of residues in h_τ conformation are treated as in the pure 3_{10}-helix model. Mixed helices consist regions of α- and regions of 3_{10}-helical structure, and transitions between the two types of helices. We defined two additional parameters, t_N and t_C, to describe the junction from 3_{10}- to α-helix and from α- to 3_{10}-helix, respectively. The pure 3_{10}-helix and the mixed α-/3_{10}-helix models were subsequently extended to include side chain interactions [43]. 3_{10}-Helices have only $i,i+3$ side chain interactions, while both $i,i+3$ and $i,i+4$ are possible in mixed helices.

Sheinerman and Brooks [44] independently produced a model for the α/3_{10}/coil equilibrium, based on the ZB formalism rather than the LR model. They similarly extended the classification of conformations from α/coil to α/3_{10}/coil. Their model differs from ours primarily in that it does not include additional parameters for junctions between α- and 3_{10}-helical segments, and that it allows a 3_{10}-helix to extend only from the C-terminus of an α-helix. N-terminal 3_{10}-helical extensions to α-helices are often observed in crystal structures, however [19,45].

In a π-helix, formation of an $i,i+5$ hydrogen bond requires that four units be constrained to the π-helical conformation, h_π. The π subscript designates the conformation and weights describing the π-helix, the dihedral angles of which are distinct from α- and 3_{10}-helices. Assigning statistical weights to individual units requires consideration of the conformations of the unit itself and its three nearest neighbours [42]. The initiating statistical weight, v_π, is assigned to a helical unit when one or more of its two N-terminal and nearest C-terminal neighbours are in the coil conformation. The definition of helix-initiating units as the two N-terminal and one C-terminal units of each helical stretch is again arbitrary. Units in a π-helical conformation with three helical neighbours are assigned the propagating statistical weight, w_π. A π-helix-propagating residue, i, is thus associated with the hydrogen bond between the NH of residue $i+2$ and the CO of residue $i-3$. Some recent work suggests that the π-helix may be of more than theoretical interest [46–49].

4.8. AGADIR

AGADIR is an LR–based helix–coil model developed by Serrano, Muñoz and co-workers. The original model [50] included parameters for helix propensities excluding backbone hydrogen bonds (attributed to conformational entropy), backbone hydrogen bond enthalpy, side chain interactions and a term for coil weights at the end of helical sequences (i.e. caps). The single-sequence approximation was used. The original partition function assumed that many helical conformations did not exist, as all conformations in which the residue of interest is not part of a helix were excluded [32,50]. These were corrected in a later version, AGADIRms, which considers all possible conformations [51]. If AGADIR and LR models are both applied to the same data, to determine a side chain interaction energy, for example, the results are similar, showing that the models are now not

significantly different [51,52]. The treatment of the helix–coil equilibrium differs in a number of respects from the ZB and LR models, and these have been discussed in detail in by Muñoz and Serrano [51]. The minimal helix length in AGADIR is four residues in an h conformation, rather than three. The effect of this assumption is to exclude all helices that contain a single hydrogen bond; only helices with two or more hydrogen bonds are allowed. In practice, this probably makes little difference, as chhhc conformations are usually unfavourable and hence have low populations. Early versions of AGADIR considered that residues following an acetyl at the N-terminus or preceding an amide at the C-terminus were always in a c conformation; this was changed to allow these to be helical [53].

The latest version of AGADIR, AGADIR1s-2 [53], includes terms for electrostatics [53], the helix dipole [53,54], pH dependence [54], temperature [54], ionic strength [53], N1, N2 and N3 preferences [37] and capping motifs, such as the capping box, hydrophobic staple, Schellman motif and Pro-capping motif [53]. The free energy of a helical segment, $\Delta G_{\text{helical-segment}}$, is given by $\Delta G_{\text{helical-segment}} = \Delta G_{\text{Int}} + \Delta G_{\text{Hbond}} + \Delta G_{\text{SD}} + \Delta G_{\text{dipole}} + \Delta G_{\text{nonH}} + \Delta G_{\text{electrost}}$, which are terms for the energy required to fix a residue in helical angles (with separate terms for N1, N2, N3 and N4), backbone hydrogen bonding, side-chain interactions, excluding those between charged groups, capping and helix dipole interactions, respectively. Electrostatic interactions are calculated with Coulomb's equation. Helix dipole interactions were all electrostatic interactions between the helix dipole or free N- and C-termini and groups in the helix. Interactions of the helix dipole with charged groups located outside the helical segment were also included. pH dependence calculations considered a different parameter set for charged and uncharged side chains and their pK_a values. The single-sequence approximation (see above) is used again, unlike in AGADIRms.

AGADIR is at present the only model that can give a prediction of helix content for any peptide sequence, thus making it very useful. It can also predict NMR chemical shifts and coupling constants. In order to do this, it must include estimates of all the terms that contribute to helix stability, notably the 400 possible $i,i+4$ side-chain interactions. Since only a few of these interactions have been accurately measured, the terms used cannot be precise. Further determination of energetic contributions to helix stability is therefore still needed.

4.9. Lomize–Mosberg model

Lomize and Mosberg also developed a thermodynamic model for calculating the stability of helices in solution [55]. Interestingly, they extended it to consider helices in micelles or a uniform non-polar droplet to model a protein core environment. Helix stability in water is calculated as the sum of main-chain interactions, which is the free energy change for transferring Ala from coil to helix, the difference in energy when replacing an Ala with another residue, hydrogen bonding and electrostatic interactions between polar side chains and hydrophobic side-chain interactions. An entropic nucleation penalty of two residues per helix is included. Different energies are included for N-cap, N1–N3, C1–C3, C-cap, hydrophobic staples, Schellman motifs and polar side-chain interactions, based on known empirical data at the time (1996). Hydrophobic interactions were calculated from decreases in non-polar surface area when they are brought in contact. Helix stability in micelles or non-polar droplets is found by calculating the stability in water, then adding a transfer energy to the non-polar environment.

4.10. Extension of the Zimm–Bragg model

Following the discovery of short peptides that form isolated helices in aqueous solution, Vásquez and Scheraga extended the ZB model to include helix dipole and side chain interactions [56]. The model is very general, as it can include interactions of any spacing within a single helix. It was applied to determine $i,i+4$ and $i,i+8$ interactions. Long-range interactions, beyond the scope of LR models, can thus be included. Roberts [57] and Gans et al. [58] also refined the ZB model to include side chain interactions.

5. Applications

The use of a helix–coil model is essential to quantitatively interpret results on helical peptides. They have therefore been widely used to determine the forces that affect helix stability, including interiors [5,22,59–62], N-caps [25–27], C-caps [27,63,64], N1 [35], N2 [36], N3 [37,38], capping boxes [65–67] and side chain interactions [30–32,52,68–75]. The results compare well to those made in proteins by site-directed mutagenesis.

In general, helix–coil parameters are extracted from experimental data by measuring the helix–coil content of a peptide that contains the interaction of interest, and in which the parameters for all other terms in the sequence are known. For example, the sequence Ac–AKAVAAAKAVAAA-KAKAGY–NH_2 was designed to measure the side-chain interaction energy between Val and Lys [74]. Two Val–Lys $i,i+4$ side chain interactions are present. This peptide had a helix content of 34%. The control sequence, Ac–AKVAAAAK-VAAAAKAKAGY–NH_2, is identical, except the two Val residues are moved one place towards the N-terminus, thus removing the Val–Lys interactions. It had a helix content of 25%, thus showing that the Val–Lys interactions are stabilising, as they increase the helix content by 9%. All the parameters required to predict the helix content of the control peptide using an LR-based model [22] were already measured, namely the w-, n-, c- and v-values of Ala, Lys, Val, Gly and Tyr. The predicted helix content of 23% was in excellent agreement with experiment, providing confidence that the model and parameters are correct. The helix content of sequence with the Val–Lys interactions could not be predicted, since the weight for this interaction (p-value) was unknown. It was found by determining the Val–Lys p-value that gave a prediction in agreement with the experimental helix content. In this case it was 1.6, giving a Val–Lys energy of $-RT\ln 1.6 = -0.25$ kcal mol^{-1}.

The models can predict helix contents for individual residues, as well as the mean helix content (typically measured by circular dichroism), so have been used to interpret hydrogen exchange data [76] and C13 chemical shifts at individual residues [77]. Knowledge of all the energies present in a sequence allows the prediction of the helix content of a peptide. In our experience, LR models are accurate to within a few percent. AGADIR is the only current method that can give a prediction for any sequence.

6. The future

There are several ways in which helix–coil models can continue to be developed. Firstly, additional interactions can be included, such as $i,i+7$ side chain interactions between side chains separated by two turns of the helix, or more structurally complex C-capping motifs. Secondly, conformations in addition to helix and coil can be included. This approach has begun by considering α-helix, 3_{10}-helix and coil, although it would clearly be preferable to consider the β conformation, as this is the next most frequent. Sheet–coil models are inherently more difficult to develop, as essentially any residue can form an interaction with any other in a sheet, unlike helices that contain only short-range interactions. Even the simplest sheet–coil models [78–81] are far more complex than helix–coil models. Thirdly, the simple division into helix or coil conformations could be made more sophisticated by considering rotameric states for each residue. Fourthly, tertiary structure could be included. Qian made a start to this problem by developing a model for coiled-coils that included a parameter for the interaction between two helices [82]. Finally, the treatment of the random coil needs to be improved, as the isolated-pair hypothesis appears to be invalidated by local steric effects [24]. This may be possible through the use of simulations of helical peptides or denatured proteins (for example [83–93]).

Ultimately, we would like to be able to calculate the stability of every possible conformation of a peptide or protein, thus solving the protein folding problem. The problem that all of these developments run into is that the calculation of the partition function rapidly becomes very complex and unwieldy. Time will tell whether advances in theory and computer power will allow us to approach the partition function of a small protein

with tertiary structure, or whether the method is already near to its practical limit.

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