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Helix–coil transitions re-visited

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Abstract

The thermally-induced helix–coil transition in polyamino acids is a good model for determining the helix-forming propensities of amino acids but not for the two-state folding/unfolding transition in globular proteins. The equilibrium and kinetic treatments of the helix–coil transition are summarized here together with a description of applications to various types of homopolymers and copolymers. Attention is then focused on the helix–coil transition in poly-L-alanine as an example of a non-polar polyamino acid. To render such a non-polar polymer water soluble, it is necessary to introduce polar amino acids such as lysines, but care must be taken as to the location of such polar residues. If they are attached as end groups, as in a triblock copolymer, they do not perturb the helix-forming tendency of the central poly-L-alanine block significantly, but if they are introduced *within* the sequence of alanine residues, then the hydration properties of the lysines dominate the behavior of the resulting copolymer, thereby leading to erroneous values of the parameters characterizing the helix-forming tendency of the alanines. Neutral but polar residues, such as glutamines, also exhibit hydration-dominating properties but less so than charged lysines. Some details of the calculations for an alanine/glutamine copolymer are presented here. It is concluded that random copolymers based on a neutral water-soluble host provide reliable information about the helix-forming tendencies of amino acid residues that are introduced as guests among such neutral host residues.

Keywords: Polyamino acids; Helix-probability profiles; Helix-forming propensity; Equilibrium and kinetic treatment; Random copolymers; Role of hydration

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1. Introduction

The helix–coil transition received early attention as a simplified model for conformational changes in globular proteins [1]. While subsequent analysis [2] indicated that such a simplified model cannot capture the cooperative features of the folding/unfolding transition of globular proteins, the recent revived interest in helix–coil transitions has focused on the intrinsic tendencies of individual amino acid residues to adopt a helical vs. a non-helical conformation, thereby facilitating the prediction of locations of helical segments within the amino acid sequence in globular proteins.

The helix–coil transition has been studied by theoretical and experimental methods. The theoretical approach has proceeded along three independent paths, a phenomenological or statistical mechanical one [1], a molecular mechanical one [3–7], and an empirical consideration of the relative frequency of occurrence of the α-helical conformation in globular proteins [8–13]. The experimental approach has made use of various types of synthetic polymers [14–16] (homopolymers, specific-sequence copolymers, regular-repeating sequence copolymers, block copolymers, and random copolymers) and of natural and mutated globular proteins [17]. This article is concerned primarily with all of these approaches, and the information that they provide about the intrinsic tendencies of amino acid residues to adopt a helical or a non-helical conformation. Additional details are provided in a recent review [18].

The intrinsic tendency reflects the interatomic interactions between a side chain of an amino acid residue and its own backbone, including the peptide bonds of the neighboring residues. Interactions involving more than one residue are denoted as extrinsic properties. There is reason to believe that intrinsic interactions are generally dominant in helix formation [19,20], although extrinsic interactions can skew the results. A helical conformation of a residue is one in which its dihedral angles ϕ and ψ (for rotation about its N–C^{α} and C^{α}–C′ bonds, respectively) adopt a narrow range of values (around $\phi = -60°$, $\psi = -40°$) in a Ramachandran diagram [21]. A non-helical conformation is an energy-weighted ensemble of all *other* regions of the Ramachandran diagram, i.e., the statistical-coil conformation (frequently, but erroneously, referred to as a random coil).

2. Theoretical approach

2.1. Phenomenological theory

2.1.1. Initial equilibrium treatment

The first phenomenological theoretical treatment of the helix–coil transition in a homopolymer was that of Schellman [22], to whom this article is dedicated. Despite its gross over-simplification, relying on only two-states (perfect helix and complete coil) instead of 2^N states per chain, where N is the chainlength, this treatment captured the essential qualitative features of the helix–coil transition, e.g. the dependence of the transition curve on the chainlength, the instability at the ends of the helix and its greater stability in the middle, etc.

2.1.2. Equilibrium matrix treatment

A more rigorous, statistical–mechanical treatment of the helix–coil transition in a homopolymer was based on the one-dimensional Ising model. It was first developed by Zimm and Bragg [23], and shortly thereafter by Lifson and Roig [24], and others [1]; a simplified form of the theory was obtained by Zimm and Bragg [23] and by Poland and Scheraga [25]. A summary of helix–coil transition theories has been presented by Poland and Scheraga [1].

In the simplified form of the theory [25], leading to a quadratic secular equation for the matrix used to generate the partition function Z, the latter is given by

$$Z = (0 \quad 1)\mathbf{W}^N \begin{pmatrix} 1 \\ \\ 1 \end{pmatrix} \tag{1}$$

where the statistical-weight matrix for a residue is

$$\mathbf{W} = \begin{pmatrix} s & 1 \\ \\ \sigma s & 1 \end{pmatrix} \tag{2}$$

with s being a temperature-dependent equilibrium constant for the conversion from a coil state to a helical state at the end of a long helical sequence, and σ being a nucleation parameter. The quantity s is generally greater then 1 at low temperature, and decreases to values less than 1 as the helix converts to the coil with increasing temperature. The solution of the quadratic secular equation provides the fraction α of coil states [25] as

$$\alpha = \frac{1}{2}\left\{1 + \frac{1-s}{[(1-s)^2 + (4\sigma/s)]^{1/2}}\right\} \tag{3}$$

with $1-\alpha$ being the fraction of helical states.

This equation can reproduce helix–coil transition curves for homopolymers as a function of temperature, where the temperature dependence arises primarily from the temperature dependence of s.

The Zimm–Bragg and Lifson–Roig theories deal only with hydrogen bonding interactions within the backbone of the helix. Subsequent to the development of these theories, attempts were made to incorporate interactions involving the side chains, e.g. hydrophobic interactions in the helix [26] and within the coil [27]; helix–helix interactions [28], sequence-specific interactions [29], and N- and C-capping [30–32] have also been incorporated.

When considering a specific-sequence copolymer, rather than a homopolymer, a quantity of interest is $P_h(i)$, the probability that the residue at position i (being a type-A amino acid) is helical [33], where

$$P_h(i) = \frac{1}{Z}(0 \quad 1)\left[\prod_{j=1}^{i-1}\mathbf{W}_A(j)\right]\frac{\partial \mathbf{W}_A(i)}{\partial \ln s_A(i)} \times \left[\prod_{j=i+1}^{N}\mathbf{W}_A(j)\right]\begin{pmatrix}1\\1\end{pmatrix} \tag{4}$$

with

$$Z = (0 \quad 1)\left[\prod_{j=1}^{N}\mathbf{W}_A(j)\right]\begin{pmatrix}1\\1\end{pmatrix} \tag{5}$$

and

$$\mathbf{W}_A(j) = \begin{pmatrix} s_A(j) & 1 \\ \sigma_A(j)s_A(j) & 1 \end{pmatrix} \tag{6}$$

A more detailed model for a specific-sequence copolymer, with focus on the hydrogen-bonding states of individual backbone NH and CO groups, has been presented by Gō et al. [34], and applied to myoglobin and hen egg-white lysoyzme. Even more detailed models, involving more states per amino acid residue, have been treated by Tanaka and Scheraga [35]. In these applications to globular proteins the theory is, strictly speaking, applicable to determining $P_h(i)$ in the unfolded form, i.e. the propensity of residue i to adopt the helical conformation [33]. The Ising model, which considers only nearest-neighbor interactions in the Zimm–Bragg and Lifson–Roig theories, has also been extended to take medium-range interactions (between the ith and the $(i+k)$th residues, for $k \leqslant 4$) into account, and applied to predict the helical, extended, and coil probabilities of 37 proteins [36].

The helix–coil transitions in many specific-sequence copolymers have been studied, [9,29,33,37–43] and helix-probability profiles have been computed with Eq. (4). Examples of these are: $^{+}H_3N$-KETAAAKFERQHMDSSTSA-OH and other S-peptide derivatives [29], 27 homologous sequences of cytochrome c proteins [9], and α-lactalbumin which was shown to have a similar helix-probability profile as that of the homologous hen egg-white lysoyzme [44].

A regular-repeating sequence copolymer, e.g., poly-(ABC) can be treated as a homopolymer by an analog of Eq. (1), in which the statistical-weight matrix $\mathbf{W}$ is obtained as the product of the statistical-weight matrices of residues A, B and C. The resulting single matrix, then, effectively converts the regular-repeating sequence copolymer to a homopolymer in which the individual 'single' residues are ABC units. An example of such a copolymer [45] is Ac-$(Ala_2\ Gln\ Ala_2)_3$-Tyr-NH_2, but with an analysis of the transition data having been made for a specific-sequence copolymer rather than as a regular-repeating sequence copolymer.

An example of a (tri)-block copolymer is (D,L-Lysine)$_x$ (L-alanine)$_y$ (D,L-Lysine)$_x$, in which the D,L-Lysine blocks served to render the L-alanine block soluble in water [46]. The thermally-induced helix–coil transition of the L-alanine block was then treated as that of a homopolymer with Eq. (1).

In treating random copolymers, advantage is taken of the observation [19,20] that short-range interactions play a dominant role in determining the conformation of each amino acid residue in a globular protein. This concept underlies the frequently used short-range interaction models [8–13,35] for 'predicting' the conformational preferences of the residues in the unfolded form of a globular protein [33]. It also provides the basis for using random copolymers to determine the values of the Zimm–Bragg parameters, σ and s, that characterize the *intrinsic* tendency of each of the 20 naturally occurring amino acids to adopt the α-helical relative to the statistical-coil conformation (see Ref. [20] and citations therein). In random copolymers, the various long-range interactions in each member of the ensemble contribute to the helix stability *of that member*, but are averaged out over the whole ensemble [47]. In specific-sequence copolymers, on the other hand, such position-dependent long-range interactions are not averaged out, and hence contribute significantly to the helix stability [29,47]. Thus, random copolymers are a convenient and legitimate vehicle with which to obtain the intrinsic values of σ and s.

The theory of Lehman and McTague [48] provides an exact treatment of the melting behavior of a random copolymer but, for ease of computation in analyzing experimental data, resort is had to an appropriate treatment [49] that can be carried out to any desired degree of approximation, p, and the approximate results converge to the exact ones with increasing p. Actually, for random copolymers of *amino acids*, the first one or two approximations ($p=1$ or 2, respectively) suffice to give results that agree with those from the theory of Lehman and McTague. The $p=1$ and 2 approximations correspond to treatments originally presented by Lifson [50] and Allegra [51], respectively. Refs. [49] and [52] provide the equations for application of the exact and approximate methods for evaluating σ and s from experimental helix–coil transition curves for random copolymers.

2.1.3. Kinetics of helix–coil transition

Schwarz [53,54], McQuarrie et al. [55], and Poland and Scheraga [56] have treated the kinetics of the helix–coil transition. Schwarz considered only the initial rate of the process, and limited his treatment to single breaks at the ends and in the interior of helical sequences and the reverse reactions. On the other hand, McQuarrie et al. calculated the total time dependence of the change in helix content for the special case in which the perturbation is so large that the reverse reaction is negligible. They found that the change to a new equilibrium state is not a simple relaxation process. Poland and Scheraga also limited their calculations to the initial rate of the helix–coil transition when the system is suddenly perturbed from equilibrium. They used the Lifson–Roig model in which *three* consecutive residues must be in a helical state before a hydrogen bond can be formed. The other two theories [53–55] were based on the Zimm–Bragg model in which *every* helical state contributes a hydrogen bond. Even though Poland and Scheraga generated all possible initial reactions, not just the simplest possible reactions treated by Schwarz, the two sets of results are similar because Poland and Scheraga found that reactions on helix ends dominate the initial rate, which is what Schwarz assumed in the first place. Several subsequent theoretical treatments of the kinetics of the helix–coil transition have been carried out, the most recent being that of Buchete and Straub [57]. These authors used a mean-field approximation to derive mean first-passage times for helix formation from the Zimm–Bragg model as a function of σ and s. This approach allowed for the possibility of multiple helix nucleation sites and multiple helical domains, with estimates of the time scale for helix propagation in the range of ns to μs, suggested by Brooks [58] and Klimov et al. [59], and measured by Thompson et al. [60].

2.2. Molecular theory

The foregoing statistical mechanical treatments of the helix–coil transition were phenomenologi-

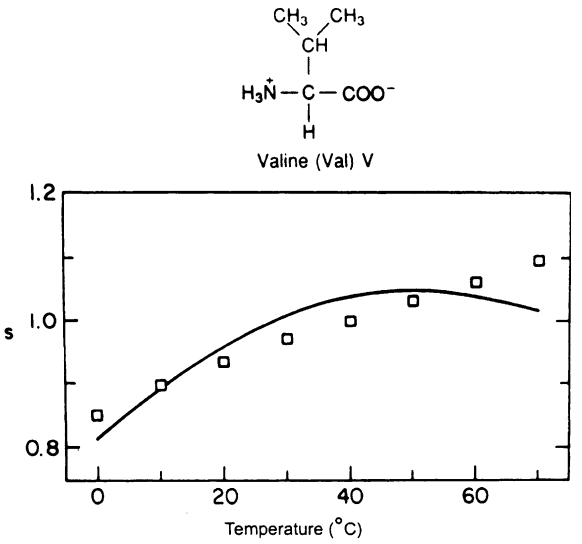

Fig. 1. Variation of the Zimm–Bragg parameter *s* with temperature for poly-L-valine [6].

cal. The problem has also been treated by molecular mechanics [3–7]. The quantities σ and *s* of the Zimm–Bragg theory were computed for several homopolymers by using an early version [61] of our current [62] force field. This treatment provided an understanding of how inter-atomic interactions contribute to the relative stabilities of the helix and coil forms. For example, whereas *s* normally decreases with increasing temperature, the opposite is true for L-valine, for which *s* increases as the temperature rises [6] (Fig. 1) because of side chain–side chain hydrophobic interactions in the helix form. A balance of hydrophobic interactions in the helix and coil forms was shown to be responsible for the observed temperature dependence of *s* for other non-polar amino acid residues [7].

3. Experimental approach

Various types of homopolymers, specific-sequence copolymers, regular-repeating sequence copolymers, block copolymers and random copolymers have been used to obtain values of the Zimm–Bragg parameters σ and *s*, and their temperature dependence. Values of *s* over the temperature range of 0–60 °C have been obtained for all 20 naturally occurring amino acid residues [63], using a host–guest technique with random copolymers based on a water-soluble neutral host (Fig. 2). The values of *s* and σ at 20 °C for the naturally occurring amino acids are presented in Table 2 of Ref. [18]. Corresponding values at a single temperature (usually room temperature) have been obtained with specific-sequence copolymers [37–41], with some differences from those obtained with random copolymers [63] or block copolymers [46]. The sources of these differences are discussed in the next section.

4. Helix-forming tendencies of the amino acids

The determination of σ and *s* for the naturally-occurring amino acids in water by examination of the helix–coil transition in homopolymers had been frustrated by the low solubility and helix-instability of such homopolymers. Therefore, to circumvent these problems, we had to resort to various types of copolymers. For example, poly-L-alanine was brought into the aqueous phase by incorporating this homopolymer between two blocks of poly-D,L-lysine, and the thermally-induced helix–coil transitions of various chain-lengths of the poly-L-alanine block were examined [46]. While this technique worked for poly-L-alanine, it does not work for most other homopolymers which are not helical in water or, if helical, do not melt in the range of 0–100 °C.

This problem was alleviated by use of the host–guest technique with random copolymers, in which the amino acid of interest was incorporated as a guest among non-ionic host residues whose values of σ and *s* were known [19,20,63]. Thus, the values of σ and *s* of the guest residues are computable [49,52] from the difference between the melting curves of the host homopolymer and the host–guest copolymer. In the particular case of L-alanine, for which σ and *s* could be obtained from both triblock [46] and random copolymers [64], similar values were obtained from transition curves of both types of copolymers, as illustrated in Table 1. The similarity of these values for two different types of copolymers attests to the validity of the underlying assumptions, primarily the dom-

inance of intrinsic (short-range) interactions [19,20].

In an alternative study of L-alanine by Marqusee et al. [37], the solubility problem was surmounted by incorporating lysine residues *within* the poly-L-alanine sequence as, for example, in their regular-sequence copolymer 3K(I), Ac-AAAAKAAAAKAAAAKA-NH_2. From thermally-induced helix–coil transition curves of 3K(I), it was concluded [37] that L-alanine is a very strong helix-former with a value of s close to 2 at room temperature rather than close to 1, as implied by the thermodynamic parameters of Table 1 (also see Fig. 2). However, as shown by molecular mechanics calculations of Vila et al. [42,65,66], the observed high helix content of 3K(I) (72% at 1 °C, pH 7, from experiment [37] and 60% at 1 °C, pH 6, from simulations [66]) is due to the L-lysine, and not the L-alanine, and

Table 1
Thermodynamic parameters for L-alanine

	From triblock copolymers [46]	From random copolymers [64]
$\Delta H°$, cal/mol[a]	-190 ± 40	-242 ± 21
$\Delta S°$, eu[a]	-0.55 ± 0.12	-0.70 ± 0.07
σ	0.00014	0.0008

[a] These thermodynamic components of $\Delta G°$ characterize the temperature dependence of s.

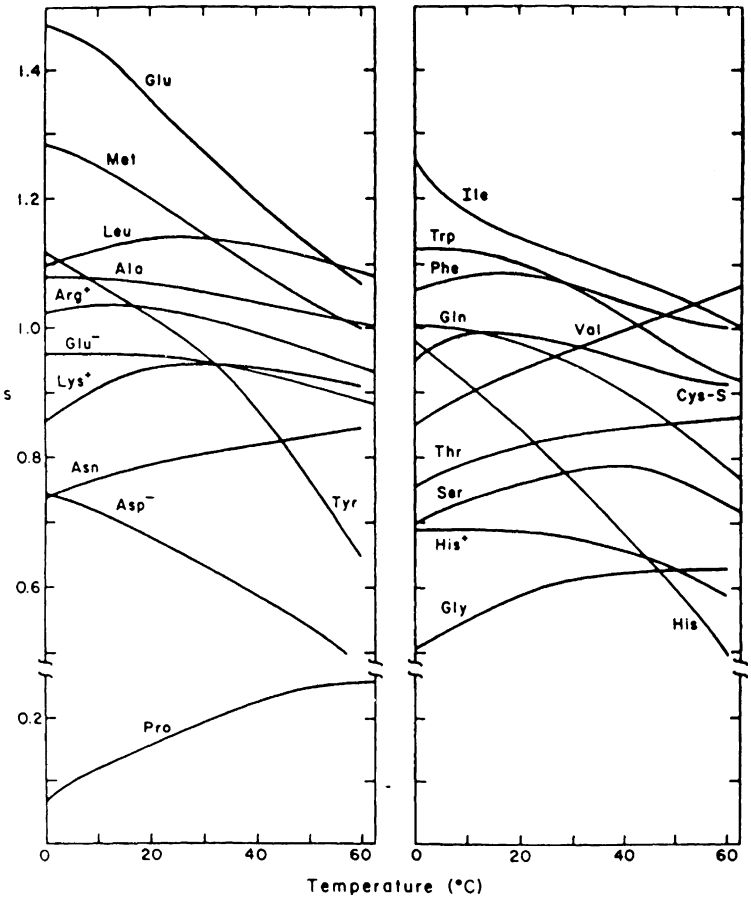

Fig. 2. Plots of s vs. T for the 20 naturally occurring amino acid residues, including some in ionized form. The data were obtained with the host–guest technique [63].

Table 2
Summary of the EDMC[a] runs for the peptide 3Q(I)

Number of generated Conformations	Number of accepted conformations	Lowest energy (kcal/mol)	Helical fraction $\theta_{coupling}$ (%)	Helical fraction $\theta_{dihedral}$ (%)	Experimental helical fraction (%)
107 352	3477	−353.98	20[b]	37[c]	49[d]

[a] Electrostatically drivin Monte Carlo [71].

[b] From an empirical relation introduced by Pardi et al. [72] based on the identification of helical secondary structure by segments of several successive spin–spin coupling constants $^3J_{HN\alpha}$ less than 6 Hz. In this relation, residues with Boltzmann averaged vicinal coupling constants ($^3J_{HN\alpha}$) less than 6 Hz contribute with weights of 0.90, 0.80, 0.75 if the number of consecutive residues is four, three, or two, respectively, while a single residue contributes with a weight of 0.55.

[c] Computed at 1 °C, pH 6, as a ratio between the Boltzmann averaged number of residues in the helical conformation and the total number of residues (16 for the peptide under consideration). The assignment of a residue to a helical state was based on its dihedral angles, ϕ and ψ. A residue was considered to be in the helical state if both ϕ and ψ assumed the canonical values (-60 ± 20, -40 ± 20).

[d] Experimental value [45] for Ac-$(AAQAA)_3$-Y-NH_2) obtained from CD measurements at 0 °C.

depends strongly on the lysine content, in agreement with experimental results of Williams et al. [43] who used a preformed scaffold to nucleate the helix. The computations of Vila et al. [65,66] showed that the L-lysine residues introduced into the L-alanine sequence (to make it soluble) sequester the water away from the CO and NH groups of the backbone, thereby enabling them to form internal hydrogen bonds. This solvation effect dictates the conformational preference and, hence, modifies the *apparent* conformational propensity of the L-alanine residues. Recently, Garcia and Sanbonmatsu [67] have come to a similar conclusion from computations on such copolymers containing charged arginine instead of charged lysine. All studies [37–41], in which charged residues were incorporated inside the non-polar sequence, presumably were influenced by the competition for water between the charged side chains and the backbone CO and NH groups.

It appears that uncharged polar residues, such as L-glutamine [42], which have also been introduced within an L-alanine sequence to solubilize it [45], can exhibit a similar competition between the neutral side chain and the backbone CO and NH groups. This is demonstrated here by the results of simulations at 1 °C, pH 6, with the sequence Ac-AAAAQAAAAQAAAAQA-NH_2 [3Q(I)], an analogue of 3K(I). In these simulations, more than 100 000 conformations were generated and energy-minimized, and the total free energy was then computed by the procedure described in previous publications [66,68–71]. Details of the results obtained in these simulations are provided in Table 2. From this Table, it can be seen that there are no significant differences between the Boltzmann averaged value of the helix content for 3Q(I) and the helix content determined experimentally by CD for the peptide Ac-$(AAQAA)_3$-Y(NH_2) [45]. Table 3 shows the resulting Boltzmann averaged values of the vicinal coupling constants, $^3J_{HN\alpha}$ for each amino acid in the sequence. For comparison, Table 3 also includes the corresponding Boltzmann averaged values of the vicinal coupling constants computed [66] for the copolymers 3K(I) at 1 °C, pH 6, as well as the experimental values of $\langle^3J_{HN\alpha}\rangle$ for 3K(I) as determined by NMR [75] at 2 °C, pH 5. It can be inferred from the values in Table 3 that replacement of the charged lysines in the sequence by glutamines (values computed at pH 6) leads to a significant decrease of the helix content.

Shalongo et al. [76] carried out a series of NMR experiments on the sequence Ac-$(AAQAA)_3$-(NH_2) that show an average helix content at 0 °C that is in agreement with the experiments of Scholtz et al. [45] at 0 °C, with our estimated value of 37% helix content at 1 °C, pH 6, and with our earlier theoretical calculations on Ac-$(AAQAA)_3$-Y(NH_2) [42]. However, their analysis of the distribution of residue helicity showed that the helix content near the N-terminus is greater

Table 3
Computed[a] Boltzmann averaged values of the vicinal coupling constants ($^3J_{HN\alpha}$)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Sequence																
3K(I)[b]	**3.7**	**4.2**	**4.3**	**4.8**	**5.0**	**4.7**	**4.7**	**4.5**	**5.0**	**5.0**	**4.7**	**4.8**	**4.8**	**5.2**	6.4	**5.9**
3K(I)[c]	7.3	6.7	6.9	6.8	6.1	6.0	**5.1**	**5.0**	**5.9**	**5.2**	**5.2**	**4.9**	**5.1**	**5.5**	**5.3**	**5.5**
3Q(I)[d]	6.7	6.9	7.7	7.8	**5.6**	6.9	**4.3**	6.0	6.9	6.9	**5.5**	7.5	6.9	**4.0**	**4.0**	6.7

[a] The theoretical values of the coupling constants at pH 6 were computed at 1 °C and pH 6, using the Karplus relation: [73,74] $^3J_{HN\alpha} = 6.4\cos^2\delta - 1.4\cos\delta + 1.9$, where $\delta = |\phi - 60|$ (in degrees). Values of the coupling constants less than 6 Hz are in boldface type to facilitate the comparison.

[b] Experimental value of $\langle^3J_{HN\alpha}\rangle$ as determined [75] by NMR at pH 5 and 2 °C.

[c] Values of $\langle^3J_{HN\alpha}\rangle$ corresponding to the peptide 3K(I) computed [66] at pH 6 and 1 °C.

[d] Computed values of $\langle^3J_{HN\alpha}\rangle$ at pH 6 and 1 °C corresponding to the peptide 3Q(I).

than that at the C-terminus. A similar helix profile has also been observed for Ac-$(AAQAA)_3$-(NHMe) in molecular dynamics simulations by Shirley and Brooks [77]. In our simulations, on the other hand, the helix content at the C-terminus appears to be higher than that at the N-terminus. This difference can be attributed to both (a) an extra alanine residue in the 3Q(I) peptide; and (b) a shift in the glutamine positions. In the sequence studied by Shalongo et al. and by Shirley and Brooks there are two alanine residues before the first glutamine at the N-terminus, while the 3Q(I) peptide contains a row of four alanines before the first glutamine. With four, instead of two alanines, at the N-terminus in 3Q(I), the first glutamine residue is further removed from this longer alanine sequence and, hence, provides less shielding of the alanine CO and NH groups, thereby reducing the helix content at the N-terminus of 3Q(I).

Our results are in agreement with recent calculations [65] showing that a single charged lysine residue has a larger effect on the solvation preference of the CO and NH groups than a single glutamine residue. These results can easily be rationalized by recognizing that lysine is a more polar residue than glutamine; however, we cannot dismiss other important effects such as side-chain entropy, charge–dipole interactions, etc.

All the evidence accumulated through our theoretical calculations with both charged [65,66] or highly polar neutral residues (this work) inserted within a sequence of alanines tends to show that the competition for solvation between the side-chain charged or polar neutral residues and the main-chain CO and NH groups introduces a significant effect on the apparent intrinsic helix-forming tendency of the alanine residues in these copolymers. Similar effects can be expected from context-dependent interactions in attempts to determine helix propensities from globular proteins. Thus, random copolymers appear to be a convenient and legitimate method with which to obtain the intrinsic helix-forming tendency parameters s and σ for the naturally occurring amino acids [49,52,63].

5. Conclusions

If proper attention is paid to the role of extraneous residues, introduced to solubilize an otherwise insoluble homopolymer, then studies of the thermally-induced helix–coil transition can provide quantitative information about the helix-forming propensities of the amino acid residues. However, since the statistical mechanics of the helix–coil transition is based on a short-range (Ising) model, the helix–coil transition is not a good model for either the equilibrium or kinetics properties in the folding/unfolding of globular proteins. In globular proteins, the essentially two-state transition arises from a balance between short- and long-range interactions [2]. On the other hand, helix–coil transition theory can provide information about the probability of occurrence of helical runs in the unfolded form of globular proteins [9,33].

6. Addendum

We have recently learned about a paper by Dyer et al. [78] describing studies of the folding and unfolding dynamics of α-helix nucleation in two model helical peptides. The 'results reveal that α-helix nucleation occurs on a sub-microsecond time-scale with a substantial enthalpic barrier'.

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