3_{10} Helices in Peptides and Proteins As Studied by Modified Zimm-Bragg Theory

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Abstract: Recent experimental data suggest that the amount of 3_{10} helical conformation in peptides and proteins might be larger than previously expected (Millhauser, G. L. *Biochemistry* **1995**, *34*, 3873-3877). This led us to explore a principal assumption of Zimm-Bragg theory of the helix-coil transition, that only one helical state can occur in polypeptides. In the present work we modify Zimm-Bragg theory to include the 3_{10} helix as a competing helical state. Incorporation of the second helical state does not significantly change the nature of the helix-coil transition, preserving good agreement between theory and the large amount of relevant experimental data. The analysis of the model indicates that 3_{10} helices should be on average shorter than α-helices. Also shorter polypeptides are predicted to have a significant ratio of 3_{10} helical to α-helical hydrogen bonds. Moreover, as the total number of hydrogen bonds in the polypeptide decreases, the probability for a particular hydrogen bond to be in the 3_{10} state rather than the α-helical state increases. The present analysis provides somewhat unexpected support for the recent proposal of the 3_{10} helix as a thermodynamic intermediate in α-helix folding.

Introduction

Motions and structural transitions that proteins undergo intimately relate to their function and stability. Since α-helices are a significant element of protein architecture, the detailed investigation of the mechanism of formation of α-helices is of clear importance. Moreover, in one of the currently favored views of early events in protein folding, metastable fragments of secondary structure are formed early in folding and then coalesce to form tertiary structure.[1,2] This hypothesis emphasizes the importance of the study of early events in helix formation and understanding the factors governing the emergence and stabilization of helices.

More than 30 years ago the theory of helix-coil transitions in polymers was developed.[3-5] Theoretical models, most notably the Lifson-Roig and Zimm-Bragg models, were successful in describing this transition in polymers and gained wide acceptance. The theory was generalized to include a more realistic representation of the polymer and to consider the transition in greater detail by a number of researchers (see ref 6 for a review). Scheraga and his co-workers determined the fundamental parameters in these theories, the helix initiation and propagation parameters for individual amino acids, using the host-guest method.[7,8]

In the last few years a large number of synthetic peptides and peptides excised from proteins have been found to be in helical conformations in solution. Formation of helices by these peptides became an issue of extensive experimental study (e.g., see refs 9-11) and prompted further developments of classical helix-coil theories to include effects specific to biopolymers. Baldwin and co-workers[12] modified Lifson-Roig theory to account for the effects of N- and C-capping on helix content. Modification of the Zimm-Bragg model to consider specific side chain interactions was performed by Kallenbach and his colleagues.[13] Finkelstein et al. have shown that extension of the Zimm-Bragg model to include weak side chain interactions describes the formation of helical structures in short peptides with reasonable accuracy.[14]

Notable advances in understanding factors governing α-helix formation were achieved in these studies (see ref 15 for a review). However, as the majority of the research has focused on the formation of the α-helical hydrogen bond pattern, the potential interference and role of a different type of helical conformation have not been considered.

A body of evidence indicating the amount and importance of the 3_{10} helical conformation in biopolymers exists. Barlow and Thornton[16] reviewed all helices found in proteins. They have observed that, from all residues in any kind of helical conformation, approximately 1/10 are in the 3_{10} helical conformation. This fraction increases to almost 50% if the count is restricted to only short helices of five residues or less.

Double label ESR spectroscopic measurements were recently performed on Ala-substituted water soluble peptides. These experiments indicate that these short helical peptides, generally considered to be α-helical, might in fact be in 3_{10}, or a mixture of 3_{10} helical and α-helical conformations.[17,18] Given the

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Figure 1. Hydrogen bond pattern of α-helices and 3_{10} helices. A sequence of eight peptide groups is shown. Note that whereas the α-helix can be elongated by a 3_{10} helical turn, the opposite event is improbable. To form an $i,i+4$ hydrogen bond following an $i,i+3$ hydrogen bond requires the dissolution of an existing hydrogen bond (because of competition for the same acceptor). Thus, the entropically disfavored restriction of additional dihedral angles required for the formation of this interaction is not balanced by a sufficient enthalpic surplus of hydrogen bond formation, making this event of low probability.

ambiguity in distinguishing 3_{10} helical versus α-helical conformation by CD or NMR, these results strongly suggest that there may be a significantly larger population of 3_{10} helical hydrogen bonds in helical peptides than previously thought.

The 3_{10} helical conformation is also proposed to exist as a transient kinetic intermediate on the pathway of α-helix folding/unfolding. The formation of marginally stable 3_{10} helical conformations was observed in a number of molecular dynamics simulation studies of α-helix denaturation,[19,20] as well as in the free-energy calculation of the α-helical turn formation.[21] The analogous conjecture was put forward by Sundaralingam and Sekharudu[22] on the basis of their analysis of solvated α-helical segments found in protein crystal structures.

In order to explore the possible role of 3_{10} helical hydrogen bonds in the formation and equilibrium structure of helical peptides, we have extended the original Zimm–Bragg model of helix–coil transitions to include 3_{10} helical hydrogen bonds together with α-helical hydrogen bonds. Within the context of this model we have explored a range of questions regarding the role of 3_{10} helices in biopolymers. Questions of primary interest for us include the following. Does the inclusion of the second helical conformation dramatically change the nature of overall helix–coil transition, as predicted by the Zimm–Bragg model? Does the modified theory reproduce experimental data on 3_{10} helices? And finally, are there indications that the 3_{10} conformation might be an intermediate between coil and α-helical structure?

Methods

Qualitative Consideration of the Model. In a polypeptide, the α-helical hydrogen bond connects the amide hydrogen of the jth peptide group and the oxygen of the $j-3$ peptide group (Figure 1). A 3_{10} hydrogen bond occurs between the jth amide hydrogen and the oxygen of the $j-2$ group. In the Zimm–Bragg theory of the helix–coil transition, two parameters are used to characterize the transition. The equilibrium constant, s, the helix-propagation constant, is associated with adding a helical hydrogen bond to the already existing helical region. This free energy, associated with the propagation of the helix, is a balance between the enthalpic gain of hydrogen bond formation and acquiring the conformation stabilized by intramolecular as well as polypeptide–solvent interactions and the entropic loss of the large number of conformations available to a residue in the coil state. The addition of a hydrogen bond to an already existing helical region restricts two dihedral angles. A larger entropic price is paid for the formation of the first helical hydrogen bond than for subsequent ones. In the case of an α-helix, six dihedral angles are restricted. The decrease of the equilibrium constant for the formation of the first hydrogen bond is taken into account in Zimm–Bragg theory by the nucleation parameter, σ. The statistical weight for the first hydrogen bond is written as σs, and thus the statistical weight for the formation of a helix containing n hydrogen bonds is σs^n. From this simple consideration, it is clear that it should be easier (the statistical weight should be larger) to initiate a 3_{10} helix since fewer dihedral angles are restricted. Four dihedral angles would be restricted in this case, compared to six in the case of an α-helix.

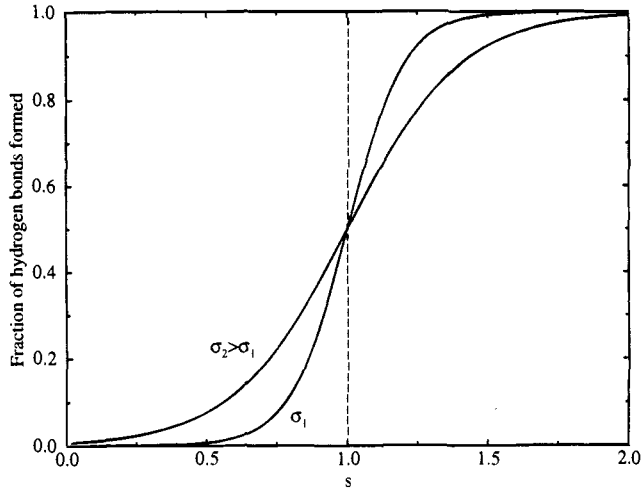

Figure 2. Schematic of the helix–coil transition as predicted by classical Zimm–Bragg theory. Two cases are shown corresponding to different values of the initiation parameter σ.

To gain some insight into how two different helical states might coexist in the same polypeptide, consider the following simplistic model. Suppose that the polypeptide is sufficiently long and the population of helices is sufficiently rare so that the two types of helices compete but do not interfere. In other words, the state of each residue can be coil or either sort of helix, but interconversion of helices and elongation of any sort of helix by a different kind of helical conformation are rare events. In this situation, coil to 3_{10} helix and coil to α-helix transitions may be described independently by Zimm–Bragg theory. The two transitions will be represented by similar curves, and $\theta_{3_{10}}/\theta_\alpha$ (where θ_ν is the portion of hydrogen bonds of helical type ν) will be a measure of the probability for a hydrogen bond to be 3_{10} helical versus α-helical. As the nucleation parameter, σ, should be larger for 3_{10} helices, this transition will be less cooperative. Therefore $\theta_{3_{10}}/\theta_\alpha$ is greater than unity when only a few hydrogen bonds exist and less than unity at the final stages of helix formation (Figure 2).

These qualitative considerations suggest that the 3_{10} helical conformation might be preferable at the initial stages of helix formation. Extending further the analysis of this model, one might note that since the Zimm–Bragg model predicts the average length of helices at the transition midpoint to be related to σ, as $\langle n\rangle \approx \sigma^{-1/2}$, 3_{10} helices should be on average shorter than their α-counterpart.

The realization that a smaller entropic price is paid for initiation of a 3_{10} helix leads to the prediction that formation of a 3_{10} helix should be less cooperative. This, in turn, leads to the suggestion that 3_{10} helices should be shorter than α-helices and should prevail in the initial but not at the later stages of helix formation.

The qualitative analysis just presented provides the basic findings from a more rigorous extension of the Zimm–Bragg theory. More accurate treatment is however essential to establish that this result is not a consequence of the oversimplification of the model or due to the neglect of important terms. In the following sections we consider the helix–coil transition more accurately.

General Formulation of the Model. Our treatment is essentially that of Zimm–Bragg with an additional assumption that a third state, a 3_{10} helix, can occur in the system. It is convenient to present here the set of assumptions of Zimm–Bragg theory.

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(1) A given state of the peptide group is represented by the state of the NH group alone. That is, by the statement of whether the hydrogen is bound or not to the oxygen of the preceding peptide group (in the original paper, the authors considered a polypeptide from C- to N-terminal and thus counted oxygen atoms).[5]

(2) The statistical weight of unity is assigned to each unbound segment (coil state).

(3) Every bound segment that follows a bound segment has a statistical weight of s (helix propagation).

(4) Sequences of less than μ consecutive unbound segments do not occur. For the α-helix μ is often taken to be 3.[5,23]

(5) Every bound segment following an unbound segment of allowed length μ has the weight of σs (helix initiation).

We assigned statistical weights to different possible conformations of the polypeptide chain in the spirit of the Zimm−Bragg model. From Figure 1, one can observe that an α-helix can be elongated by 3_{10} helical hydrogen bonds. Moreover, initiating a 3_{10} helix in this case involves the fixing of only two dihedral angles, and thus no additional entropic price is paid. Contrary to the 3_{10} helix, α-helices cannot start from the 3_{10} conformations, because (see Figure 1) one peptide group would be left in a coil conformation, which is forbidden by assumption 4 of the Zimm−Bragg model.

From these additional considerations we can construct an extension of the Zimm−Bragg model to include the 3_{10} state. Denoting a peptide group in the coil conformation as "0", in the 3_{10} helix as "1" and in the α-helical conformation as "2", the rules that we used for assigning statistical weights are the following: (1) For each "0" the statistical weight is 1 (coil). (2) A "1" following a "1" or a "1" following a "2" has a statistical weight of $s_{3_{10}}$ (3_{10} helix propagation). (3) A "2" following a "2" has a statistical weight of s while a "2" following a "1" has a weight of 0 (α-helix propagation). (4) If a "2" is preceded by a minimum of three "0"s, the weight is σs (α-helix initiation). Analogously, a "1" preceded by a minimum of two "0"s is assigned a weight of $\sigma_{3_{10}} s_{3_{10}}$ (3_{10} helix initiation). (5) If the number of preceding "0"s is less than 3 in the former and 2 in the latter case, the statistical weight is 0.

Following the above rules, we construct a matrix, the elements of which represent all possible statistical weights for the unit, and calculate the partition function and average properties of interest using the matrix method.[5] The dimension of the matrix that we used is 27 × 27 (the correlation of three consecutive units is considered, and each of them can be in one of three conformations, $3^3 \times 3^3 = 27 \times 27$). Along with the analogous matrix of dimensions 8 × 8 ($2^3 \times 2^3$—two possible states for each unit), Zimm and Bragg presented a simplified version of their theory containing a 2 × 2 matrix that included an interaction between neighboring units only. This simplified version became highly popular, and it is this treatment that is often used for comparison with the experiment nowadays. As an analogous simplification is not obvious for our case, we use a complete treatment including correlation of three consecutive links.

Choice of Parameters. A large number of experiments have been performed to quantitate the values of σ and s for helices. Although a range of values exist, and there is ongoing discussion regarding these in the literature,[15,24] we have used values suggested by Baldwin and his co-workers. The values of $\sigma = 0.00191$ and $s = 1.5$ were chosen for α-helix formation.[12,23]

The nucleation parameter accounts for the entropic loss due to the restriction of additional dihedral angles upon the formation of the first hydrogen bond. Four additional angles are fixed on initiating an α-helix and two angles when a 3_{10} helix is initiated. Thus, it is reasonable to expect that $\sigma_{3_{10}}$ will be on the order of $\sigma^{1/2}$ (the equilibrium constant is proportional to the exponent of the entropy difference, and the restriction of twice as many dihedral angles involves roughly double the entropy loss): $\sigma = 0.00191$ gives rise to $\sigma_{3_{10}} = \sigma^{1/2} = 0.04$. We used $\sigma_{3_{10}} = 0.01$ as a first iteration (see the discussion below concerning the influence of the value of $\sigma_{3_{10}}$ on the results of the model). The propagation constant s may be interpreted as an equilibrium constant for adding a residue in a helical conformation to an existing helix versus termination of the helix. The natural logarithm, $\ln(s)$, is thus proportional to ΔG_ν, the Gibbs free energy difference between a coil state and ν-helical state (ν is either 3_{10} or α) for the residue located at the end of the helical region. The majority of data suggest that the 3_{10} helical state is less stable than the α-helical state. The propagation constant for 3_{10} helices, $s_{3_{10}}$, can be expressed in relation to α-helix propagation as

$$s_{3_{10}} = \exp(-\beta \Delta G_{3_{10}}) = \exp(-\beta \Delta G_\alpha) \exp(-\beta \Delta\Delta G);$$
$$\Delta\Delta G = \Delta G_{3_{10}} - \Delta G_\alpha$$

Thus, $s_{3_{10}} = ks$, where k, less than unity, is a coefficient dependent on the difference in free energies of 3_{10} helical and α-helical states.

The relative stability of 3_{10} helices and α-helices was investigated in a number of recent computational studies. Tirado-Rives et al. explored the formation of 3_{10} helices and α-helices in an undecaalanine peptide.[25] A potential of mean force for the α-helix to 3_{10} helix transition in a decamer of the non-natural amino acid methylalanine (MeA), which contains an additional methyl group bonded to C_α, in different solvents was constructed by Smythe et al.[26] Finally, the preference for a particular helical conformation in Ala and MeA decamer peptides was investigated by Zhang and Hermans.[27]

These studies provide overall support for the stability of α-helical states over 3_{10} helices in alanine peptides, yielding a relative stabilization in the range of 1−1.6 (kcal/mol)/residue. However, they disagree significantly in their assessment of the role of different factors, such as solvation, which contribute to this stability. In addition, there is a significant discrepancy in the predicted relative stability of 3_{10} helices versus α-helices in MeA peptides.[26,27] It appears that the assessment of factors contributing to the stability of different helical conformations depends strongly on the force field and the protocol used in the calculation. For example, calculations on a blocked MeA_{10} peptide predicted the α-helix to be the preferable conformation when the AMBER[28] united atom or AMBER/OPLS[29,30] force field was used. However, the 3_{10} helix was found to be more stable in the same system when the AMBER all-atom force field was employed.[31] Force-field and protocol differences were also found to affect an assessment of the role of solvent, with different calculations leading to qualitatively different conclusions about whether solvation favored the α-helical or 3_{10} helical state in alanine-based helices. A number of these issues are discussed in a recent publication by Marshall and co-workers.[31] For these reasons, and on the basis of arguments we present below, we have focused on a range of parameters to describe the influence of 3_{10} helical states within the Zimm−Bragg helix−coil theory which are somewhat outside of these theoretical estimates.

Tobias and Brooks[21] constructed a free-energy surface for the folding of a single α-helical turn. From their calculations, a metastable state that resembled a 3_{10} turn was identified on the path between coil and helix. This state was less stable than the α-helical turn by approximately 0.6 kcal/mol. A more optimal 3_{10} turn, one obtained by some optimization of side chain−side chain contacts and details of the backbone configuration, should be more stable than the turn they characterized. Thus, according to this calculation, 0.6 (kcal/mol)/hydrogen bond represents an upper limit on the free-energy difference between two helical states.

An upper limit on the free-energy difference between α-helical and 3_{10} helical structures can also be estimated on the basis of characteristic time scales for coil to helix transitions and 3_{10} helix to α-helix transitions. This relationship yields an upper bound on the free-energy

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difference for 3_{10} helical/α-helical states which is consistent with that found by Tobias and Brooks.[21]

Many independent researchers have observed the formation of 3_{10} helical structures during the simulation of α-helix unfolding.[19-21,32-35] If such states represent transient intermediates to helix formation/dissolution, the characteristic time for the α-helix to 3_{10} helix transition should not be larger than the characteristic time for α-helix denaturation, i.e.,

$$\tau_{\alpha\rightarrow 3_{10}} \leq \tau_{\alpha\rightarrow \mathrm{coil}} \tag{1}$$

This forms the basis of our argument.

We now consider the thermodynamics for α-helix formation:

$$\text{coil (c)} \leftrightarrow \alpha\text{-helix } (\alpha)$$

$$K_{eq}^{c\rightarrow\alpha} = \frac{k_{c\rightarrow\alpha}}{k_{\alpha\rightarrow c}} = \frac{\tau_{\alpha\rightarrow c}}{\tau_{c\rightarrow\alpha}}$$

In this expression, $K_{eq}^{c\rightarrow\alpha}$ is the equilibrium constant for α-helix formation, $k_{c\rightarrow\alpha}$ and $k_{\alpha\rightarrow c}$ are the kinetic rates for the forward and backward reactions, correspondingly, and $\tau_{\alpha\rightarrow c}$ and $\tau_{c\rightarrow\alpha}$ are the characteristic times of helix denaturation and folding. The transition between α-helical and 3_{10} helical conformations can be considered in a similar fashion:

$$\alpha\text{-helix } (\alpha) \leftrightarrow 3_{10} \text{ helix } (3_{10})$$

$$K_{eq}^{\alpha\rightarrow 3_{10}} = \frac{k_{\alpha\rightarrow 3_{10}}}{k_{3_{10}\rightarrow\alpha}} = \frac{\tau_{3_{10}\rightarrow\alpha}}{\tau_{\alpha\rightarrow 3_{10}}}$$

Then, eq 1 can be rewritten in the following way:

$$\tau_{3_{10}\rightarrow\alpha}/K_{eq}^{\alpha\rightarrow 3_{10}} = \tau_{\alpha\rightarrow 3_{10}} \leq \tau_{\alpha\rightarrow c} = \tau_{c\rightarrow\alpha}K_{eq}^{c\rightarrow\alpha} \tag{2}$$

Rearrangement of eq 2 yields

$$K_{eq}^{\alpha\rightarrow 3_{10}} \geq \tau_{3_{10}\rightarrow\alpha}/\tau_{c\rightarrow\alpha}K_{eq}^{c\rightarrow\alpha}$$

Substituting $K_{eq}^{c\rightarrow 3_{10}}/K_{eq}^{c\rightarrow\alpha}$ for $K_{eq}^{\alpha\rightarrow 3_{10}}$, the equilibrium constant for α-helix formation, $K_{eq}^{c\rightarrow\alpha}$, cancels out. Now expressing $K_{eq}^{c\rightarrow 3_{10}}$ as $\sigma_{3_{10}}s_{3_{10}}^{n}$, a lower limit on the value of $s_{3_{10}}$ is obtained,

$$s_{3_{10}} \geq (\tau_{3_{10}\rightarrow\alpha}/\tau_{c\rightarrow\alpha}\sigma_{3_{10}})^{1/n} \tag{3}$$

for a polypeptide with n 3_{10} helical or $n-1$ α-helical hydrogen bonds. Thus, from the considerations just given, we arrive at a lower bound for the 3_{10} helix propagation parameter which depends on two characteristic time scales and the parameter for initiation of a 3_{10} helix from a coil state. Below we describe relevant ranges for these quantities.

The characteristic time for relaxation of a 3_{10} helix to its more stable α-helical counterpart, $\tau_{3_{10}\rightarrow\alpha}$, was estimated in two recent simulation studies. Unrestrained molecular dynamics simulations of alanine peptides were performed by Tirado-Rives et al.[25] and Zhang and Hermans.[27] In simulations of the Ala_{10} peptide by Zhang and Hermans,[27] the transition from an initial 3_{10} helical conformation to its α-counterpart took from 80 to 150 ps (three independent runs were performed). In a simulation of undecaalanine,[25] the analogous transition took 15 ps. Although these values differ by almost an order of magnitude, they provide some guidelines for this time scale, and we use them as limiting values in eq 3.

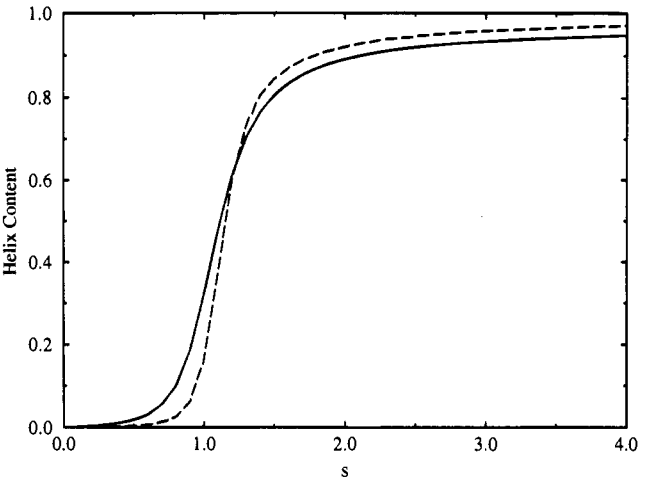

Figure 3. Dependence of the helix content (fraction of helical hydrogen bonds formed) on s as predicted by the original (dashed line) and modified (solid line) Zimm−Bragg theory. Calculations are for a polypeptide containing 30 peptide units. The modified theory uses $s_{3_{10}} = 0.85s$, which corresponds to a 0.1 (kcal/mol)/hydrogen bond free-energy difference between 3_{10} helical and α-helical states).

There is a lot of literature on the time scales for helix folding. Early estimates suggest that this process occurs on a microsecond time scale.[36] However, more recent fast-kinetic measurements suggest that the process may be faster by 1−2 orders of magnitude, placing $\tau_{c\rightarrow\alpha}$ in the range of 100 ns.[37] We consider these ranges to construct limiting cases for the upper bound on $s_{3_{10}}$.

Clearly, our model will show decreasing amounts of 3_{10} helix as $s_{3_{10}}$ decreases. Therefore, we consider a "worst-case" scenario by taking $\tau_{3_{10}\rightarrow\alpha} = 15$ ps for blocked undecaalanine ($n = 9$ α-helical hydrogen bonds), the helix-folding time, $\tau_{c\rightarrow\alpha} = 1$ μs, and $\sigma_{3_{10}} = 0.04$. With these parameters, eq 3 gives an estimate of $s_{3_{10}} \geq 0.45$. If we use the folding time suggested by recent experimental data (~100 ns),[37] and the time for the 3_{10} helix to α-helix transition in blocked decaalanine from the values of Zhang and Hermans, $\tau_{3_{10}\rightarrow\alpha} = 100$ ps, eq 3 yields a lower bound of $s_{3_{10}} \geq 0.66$.

Equation 3 provides a limit on possible values of $s_{3_{10}}$ that is independent of the α-helix propagation constant, s. As we noted above, the value of s obtained by different methods varies and is currently under discussion. The upper limit on the difference in stability of an α-helix versus a 3_{10} helix will depend on the choice of s. Again, considering the worst case, if s for alanine is assumed to be 1.5 (as proposed by Baldwin and co-workers and used in our analysis), upper limits on $\Delta\Delta G$ will be 0.72 and 0.49 (kcal/mol)/hydrogen bond in the two limiting estimates of $s_{3_{10}}$ just discussed. If, on the other hand, we use measured parameters obtained by Scheraga and co-workers ($\sigma = 8 \times 10^{-4}$, $s = 1.06$) with our limits on $s_{3_{10}}$, we find $\Delta\Delta G \leq 0.49$ (kcal/mol)/hydrogen bond in the first case and $\Delta\Delta G \leq 0.26$ (kcal/mol)/hydrogen bond in the second case.

Thus, from this analysis, we find that the difference in stability of α-helices and 3_{10} helices should be at most of the order of k_BT and likely is less than 0.3−0.4 (kcal/mol)/hydrogen bond. We explore the corresponding range of parameters in our model. We also discuss how the results are affected by larger differences in stability between α-helices and 3_{10} helices.

Results and Discussion

One question we address is whether the inclusion of the less cooperative 3_{10} state will alter the qualitative behavior of the Zimm−Bragg model, thereby bringing it out of agreement with a large body of experimental data. In Figure 3 we demonstrate that the overall sigmoid-like shape of the transition (fraction of helical states (both types) as a function of propagation parameter

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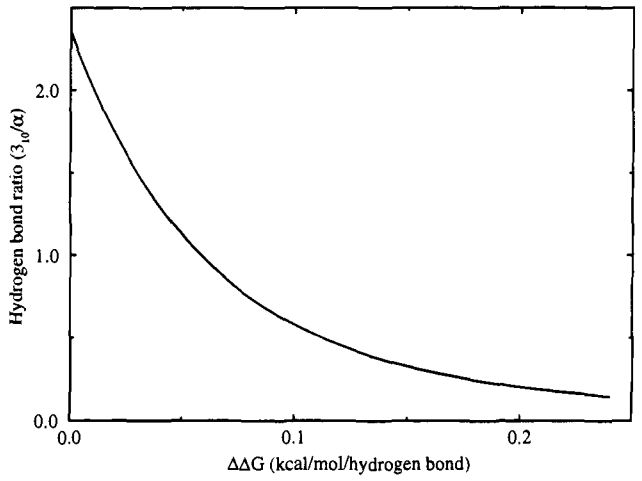

Figure 4. Ratio of the number of $i,i+3$ to $i,i+4$ hydrogen bonds as a function of the difference in stability of 3_{10} helical and α-helical states for a polypeptide of 20 peptide units.

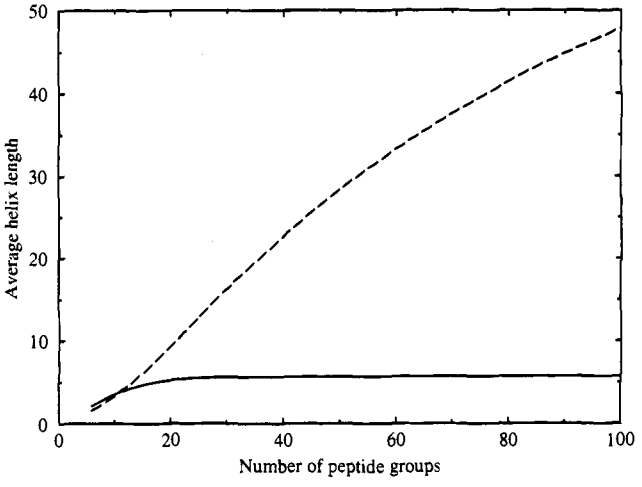

Figure 5. Dependence of the average length (number of contiguous hydrogen bonds) of the α-helix (dashed line) and 3_{10} helix (solid line) on the length of the polypeptide.

s) is practically unchanged from the original two-state model. This result is valid for the large range of relative stabilities of two helical types.

In Figure 4 we illustrate the dependence of the fraction of 3_{10} helical hydrogen bonds on the relative stability of the α-helical and 3_{10} helical states. For a polypeptide comprising 20 peptide groups, the ratio of 3_{10} helical to α-helical hydrogen bonds varies from 0.6 to 0.14 as $\Delta\Delta G$ ranges from 0.1 to 0.3 (kcal/mol)/hydrogen bond. As the length of the polypeptide increases, this ratio decreases as would be expected (data not shown). These findings suggest that shorter helices can contain a significant fraction of 3_{10} helical hydrogen bonds.

The dependence of the average length of the helical fragment (contiguous hydrogen bonds) on the total length of the polypeptide is shown for each type of helix in Figure 5. As the polypeptide becomes longer, the average length of the α-helix increases while the length of the 3_{10} helix remains practically constant at 5−7 hydrogen bonds (6−8 residues). The asymptotic length of the 3_{10} helix depends on $\Delta\Delta G$ and decreases to between one and two hydrogen bonds as $\Delta\Delta G$ approaches 0.7 (kcal/mol)/hydrogen bond. This feature, predicted by the model, is in agreement with the observation of Barlow and Thornton[16] that 3_{10} helices are on average shorter than α-helices and are more likely to be found in short polypeptides.

We may also examine more detailed aspects of helix composition. For example, we find that the majority of 3_{10}

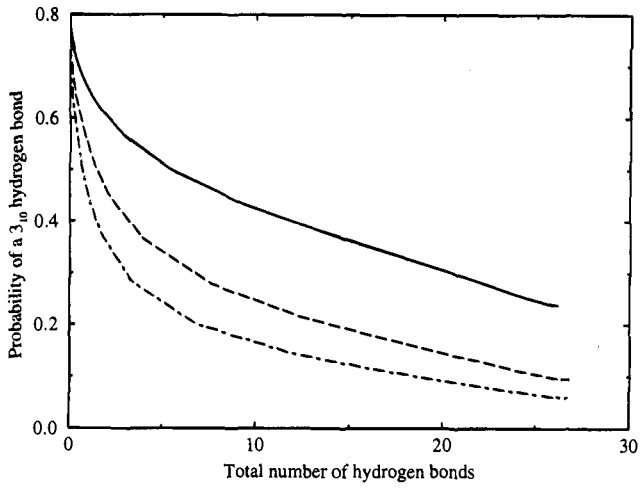

Figure 6. Probability for a hydrogen bond to be 3_{10} as a function of the total number of hydrogen bonds in a polypeptide of 30 peptide units. As the polypeptide unfolds and the total number of hydrogen bonds decreases, the probability for a hydrogen bond to be $i,i+3$ increases. Results are shown for three different values of $\Delta\Delta G$, 0.1 (solid line), 0.2 (dashed line) and 0.3 (kcal/mol)/hydrogen bond (dashed−dotted line). Half of the hydrogen bonds are in 3_{10} helical states when the total number of hydrogen bonds are 5.25, 1.4, and 0.7 for the three different values of $\Delta\Delta G$ chosen above.

helices, as described in the model, initiate from an existing α-helical segment rather than from the coil region, thus bypassing the entropic cost of initiation and leading to a relatively weak dependence of the results on $\sigma_{3_{10}}$, the nucleation parameter for the 3_{10} helix. This weak dependence is indicated by the small variation of the 3_{10} helical content and the average length of 3_{10} helices, which are within 5% of their values when $\sigma_{3_{10}} = 0.01$ for the range $0.005 < \sigma_{3_{10}} < 0.02$.

In a survey of helical structures occurring in proteins, Barlow and Thornton observed that only 24% of all 3_{10} helices emerge as an extension of an α-helix. Roughly 75% of all surveyed 3_{10} helices contain only three residues (one hydrogen bond).[16] A significant portion is suggested to occur as a junction between elements of secondary structure, primarily two β-strands. Helix−coil transition theories predict that helices containing a single turn are unlikely to emerge in isolation. In proteins, these single-turn helices might be stabilized by favorable interactions with adjacent elements of secondary or tertiary structure and represent, for example, a connection between them. This effect may bias the statistics toward a smaller percentage of 3_{10} helices starting from α-helices.

The most interesting prediction that follows from the analysis of the model is shown in Figure 6. As the polypeptide unfolds and the number of residues in any helical state decreases, the probability for a hydrogen bond to be in the 3_{10} helical state increases. From this figure it can be seen that, depending on the difference in stability between a 3_{10} helical and α-helical hydrogen bond, the first 1−5 hydrogen bonds are predicted to be $i,i+3$ rather than $i,i+4$ in a polypeptide of 30 peptide groups. In this estimate, we assumed that under denaturing conditions the difference in relative stabilities of the α-helix and 3_{10} helix, with respect to the unfolded coil state, is unchanged. In strong denaturing conditions, this difference might be expected to diminish significantly, or disappear. This would lead to a smaller difference between s and $s_{3_{10}}$, while preserving the difference between the initiation constants for the two helical types (which are determined by the different entropic costs upon the formation of the first hydrogen bond, and may be assumed independent of solvent conditions). This reduction, in turn, would result in a higher population of the

Table 1. Characteristics of 3_{10} Helical Structures for Different Values of Fundamental Parameters

	Baldwin:[12,23] $s = 1.5, \sigma = 0.001\,91$				Scheraga:[7,8] $s = 1.06, \sigma = 0.0008$		used in the model: $s = 1.5, \sigma = 0.001\,91$	
3_{10} helix parameters	$s_{3_{10}}^{max} = 0.45$		$s_{3_{10}}^{max} = 0.66$		$s_{3_{10}}^{max} = 0.45$	$s_{3_{10}}^{max} = 0.66$		
α-helix parameters	$\sigma_{3_{10}} = 0.01$	$\sigma_{3_{10}} = 0.04$	$\sigma_{3_{10}} = 0.01$	$\sigma_{3_{10}} = 0.04$	$\sigma_{3_{10}} = 0.02$	$\sigma_{3_{10}} = 0.02$	$\Delta\Delta G = 0.1$ kcal/mol	$\Delta\Delta G = 0.3$ kcal/mol
H-bond ratio 3_{10}:α ($N = 20$)	0.05	0.05	0.1	0.12	0.36	0.89	0.6	0.14
average length of the 3_{10} helix	1.3	1.5	1.8	1.8	1.7	2.5	6.0	2.5
first H-bonds that are of the 3_{10} type	1st, 15%	1st, 35%	1st, 25%	1st, 55% 2nd, 38% 3rd, 29%	1st, 38% 2nd, 25%	1st, 69% 2nd, 54% 3rd, 45% 4th, 38%	1st, 64% 2nd, 58% 3rd, 55% 4th, 52%	1st, 41% 2nd, 32%

3_{10} helical conformation. This result supports the proposal that the 3_{10} helix can be a thermodynamic intermediate on the α-helix folding pathway.

A mechanistic picture of α-helix formation, consistent with the results above, might be viewed as follows. At the initial stage of helix formation, under denaturing conditions, the probability that the first helical turn will be a 3_{10} hydrogen bond rather than α-helical is high (up to 70%). The newly formed helical region can then grow as a 3_{10} helix up to a certain length (1−5 hydrogen bonds), before its α-helical counterpart becomes thermodynamically preferred. The $i,i + 4$ hydrogen bond (α-helical) can then be formed at the N-terminus of the segment. An α-helix may then grow with the 3_{10} segment becoming possibly shorter and progressing toward the C-terminus. In this way, we predict that the C-terminus of longer, more stable helices should have a notable population of 3_{10} helical hydrogen bonds and that a significant amount of 3_{10} helical hydrogen bonds should exist in nascent folding units or short helical fragments of polypeptides.

In Table 1 we summarize the results of our model calculations when extreme values of the parameters, as discussed in the Methods section, are used. From the data present in this table, it can be seen that the qualitative picture of the coexistence of two helical types holds for the majority of the extreme cases. The quantitative description of the balance between different helical conformations, as predicted by the present treatment, awaits further experimental investigation on the α-helix stability and folding time scale.

Conclusion

The classical theory of the helix−coil transition assumes that a particular residue can be in only one of two states, coil or α-helix. Recent experimental data suggest, however, that a significant population of 3_{10} helices may be present in short alanine-based peptides. In this work, we have shown that, upon the addition of a different helical state, corresponding to a 3_{10} helix, to the Zimm−Bragg model, the sigmoid-like shape of the overall helix−coil transition is practically unchanged. From our analysis of this modified model, we find that 3_{10} helices should be on average shorter than α-helices. An average length of 3−7 residues is predicted when physically reasonable values for relative stability of 3_{10} helical and α-helical states are used. This relates well to the conclusions reached in an experimental study of the occurrence of 3_{10} helices in Aib-substituted peptides.[38] Furthermore, our analysis indicates that the 3_{10} helix should be the thermodynamic intermediate in α-helix folding, and possibly be the dominant species in short, marginally stable, helical peptides. The principal reason for this effect is the smaller entropic change, relative to that of the α-helix, upon the formation of the first hydrogen bond in the case of the 3_{10} helix.

We believe this observation to be especially interesting in light of the increased attention focused on the role and place of 3_{10} helical conformations in naturally occurring peptides and proteins.[39] Our findings provide a theoretical foundation for this hypothesis and yield new insights into the significance of 3_{10} helical hydrogen bonds in the early stages of α-helix formation in protein folding.

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