Comparison of Fully Optimized α- and 3₁₀-Helices with Extended β-Strands. An ONIOM Density Functional Theory Study

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Abstract: We compare the structures and energies of β-strands, α-helices, and 3_{10}-helices for capped polyalanines, acetyl(ala)$_N$NH$_2$, for values of N from 2 to 18, using completely optimized mixed DFT/AM1 calculations. Non-pairwise additive cooperativity is manifest from the variation of the relative energies, helical strain, dipole moments, and H-bond lengths of both types of helices, but especially for the α-helices. While the gas-phase 3_{10}-helices are more stable for small polyalanines, largely due to the additional H-bond, the α-helices become relatively more stable as the polyalanines increase in size.

Protein folding and the factors that control the stable folded structures remain among the most daunting problems facing biochemistry today. To understand the factors that influence and control the eventual folded structures, one needs to be able to evaluate the energetic contributions of various H-bonding motifs to the relative energies of the various secondary structures that are commonly found in proteins. One of these secondary structures, the α-helix, derives its stability from the cooperative interactions of the H-bonds in its three chains of H-bonding interactions. The 3₁₀-helix, which forms two H-bonding chains, also occurs in proteins. 3₁₀-Helical fragments commonly occur near the ends of α-helices.[1] Experimental studies indicate that helices tend to be unstable for short peptides. For example, Kemp has reported dramatic increases in helical propensity for solubilized polyalanines as they become larger.[2]

Comparison of the energies of α- and 3₁₀-helices to extended structures without H-bonds can be important to the understanding of the relative energies of these structures. When studied as a function of peptide size, these energies provide important insights into the effects of H-bond cooperativity on these relative energies. While solvation energies make significant contributions to the relative energies in solution, the energetics of the gas-phase systems must be understood to determine these contributions. Several experimental studies have appeared on the conversion between these two secondary structures in solution,[3–8] as well as in the gas phase.[9–13]

Theoretical studies of the relative energies of α- and 3₁₀-helices, as well as other secondary structures, have been reported.[14–21] Notably, recent studies have compared fully relaxed helices containing up to 10 residues,[15] and infinitely long peptides using periodic models.[21] However, to the best of our knowledge, no theoretical calculations that consider fully optimized geometries of helical peptides containing more than 10 residues have yet appeared.

We present the results of mixed DFT/AM1 ONIOM calculations on the capped polyalanines, acetyl(ala)$_N$NH$_2$ for up to $N = 18$. As we shall see from the discussion below, capped α-helical polyalanines containing fewer than 12 amino acid residues are only predicted to be stable for acetyl(ala)$_8$NH$_2$ and acetyl(ala)$_{10}$NH$_2$.

Calculational Details

We used the ONIOM[22,23] method as programmed in the G98[24] and G03[25] suites of computer programs. ONIOM divides the system into

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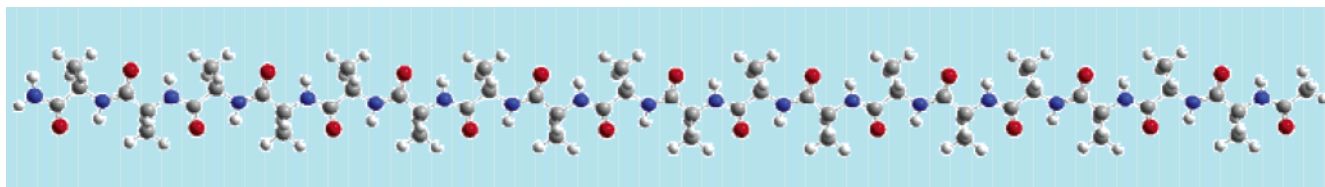(23) Vreven, T.; Morokuma, K. *J. Chem. Phys.* **2000**, *113*, 2969.

Figure 1. $\beta(18)$.

up to three segments which can be treated at different levels of calculational complexity. Thus, one can treat the essential part of the system at the high level, while the less critical parts of the system might be calculated at the medium or low level. For this study we only used two levels (high and medium). We treated the cores of the helices or β-strands (equivalent to a corresponding peptide containing only glycines) at the high level, with only the methyl groups that distinguish alanine from glycine at the medium level. The high level used hybrid DFT methods at the B3LYP/D95(d,p) level. This method combines Becke's three-parameter functional[26] with the nonlocal correlation provided by the correlation functional of Lee, Yang, and Parr.[27] In the ONIOM method, there are unsatisfied valences in the high level at the interface between it and the medium level. These valences were satisfied by using the default method of capping them with a hydrogen atom in the direction of the connecting atom in the medium level with a C–H distance of 0.723886 times the C–C distance. We used the AM1[28] semiempirical molecular orbital method for the ONIOM medium level.

All geometries were completely optimized in all (up to 561) internal degrees of freedom. Most structures are too large for the calculations of vibrational frequencies. However, vibrational calculations on several of the smaller structures (up to $N = 12$) confirmed that the reported geometries are true minima on the potential energy surfaces (PES), as there are no imaginary vibrational frequencies. A detailed discussion of the vibrational spectra will be presented elsewhere.

In a previous study of five 17-amino acid peptides,[29] we found little difference in relative energies between this procedure and another where the side chains (in this case, the methyls) were subsequently optimized using DFT, with the (previously optimized) peptide chain held fixed.

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The current procedure also gave relative energies that agreed well with complete DFT optimizations for a series of five small 3₁₀-helical peptides.[30]

Results and Discussion

To simplify the presentation, we shall adopt the nomenclature $3_{10}(N)$, $\alpha(N)$, and $\beta(N)$ to indicate the respective structures containing N alanines. Each peptide has one CO and one NH_2 from the capping groups in addition to those of the alanines. When we wish to distinguish between the H-bonds in the three H-bond chains of $\alpha(N)$, we shall designate the interactions in the chains (counting from the acetyl end) as M, $M+1$, and $M+2$, where M can be any integer ≥ 0. Similarly, those in the two chains of $3_{10}(N)$ can be designated by M and $M+1$. Thus, $\alpha(18)$ would contain three chains of six, five, and five H-bonds, while $3_{10}(18)$ would contain two chains: one each of nine and eight H-bonds.

β-Strands. As in a previous report,[29] we calculated the optimized structures of extended β-strand conformations of the polyalanines to provide suitable references for the helical structures. The β-strands generally assume a conformation (Figure 1) where the methyl groups on alternating ala residues are almost completely on opposite sides of the molecule, while the methyl of the acetyl group remains roughly coplanar with the C's and N's that form the backbone. The backbone is slightly puckered with CCNC (ω), CNCC (ϕ), and NCCN (ψ) dihedral angles of about 177°, −167°, and 171°, respectively. Aside from the increase in chain lengths, the structures differ little from each other as N increases.

3₁₀-Helices. The structures of the 3₁₀-helices (Figure 2) present no real surprises. We found minima on the potential energy surfaces for all $3_{10}(N)$ from $N = 2$ to 18. The smallest structure, $3_{10}(2)$, contains only one H-bond. While it does represent an energy minimum, the corresponding β-strand is more stable by almost 10 kcal/mol. The helices become more tightly wound as they become longer due to the shortening of the H-bonds that accompanies the stronger cooperative interactions. The dihedral angles, NCCN (ψ), near the center of the helices increase from about −19° to about −22°, while the CNCC dihedrals (ϕ) decrease from about −62 to −60° for $3_{10}(5)$ and $3_{10}(18)$, respectively. The dihedral angles about the peptide C–N bond (ω) deviate more from planarity for these helices than for the β-strands or α-helices. They tend to be about 174° near the center of the helices. The dihedrals for the amide C–N's nearest the acetyl end are about 165°.

α-Helices. Polyalanines could only be completely optimized to α-helical structures for α(8), α(10), and α(≥12). α(≤5) could not be optimized to any structure containing an α-helical motif. α(6), α(7), α(9), and α(11) all formed hybrid α/3₁₀-helical structures (see discussion below).

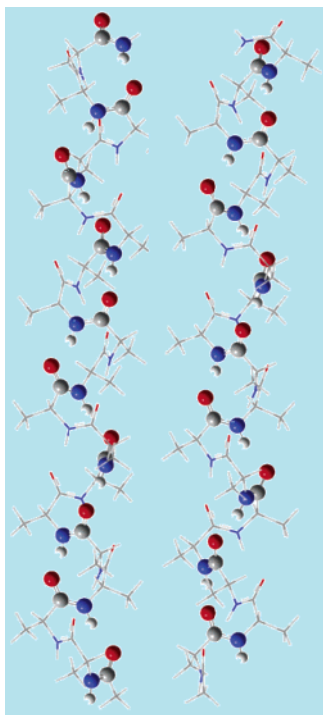(30) Wieczorek, R.; Dannenberg, J. J., submitted for publication.

Figure 2. $3_{10}(18)$, emphasizing the two H-bonding chains.

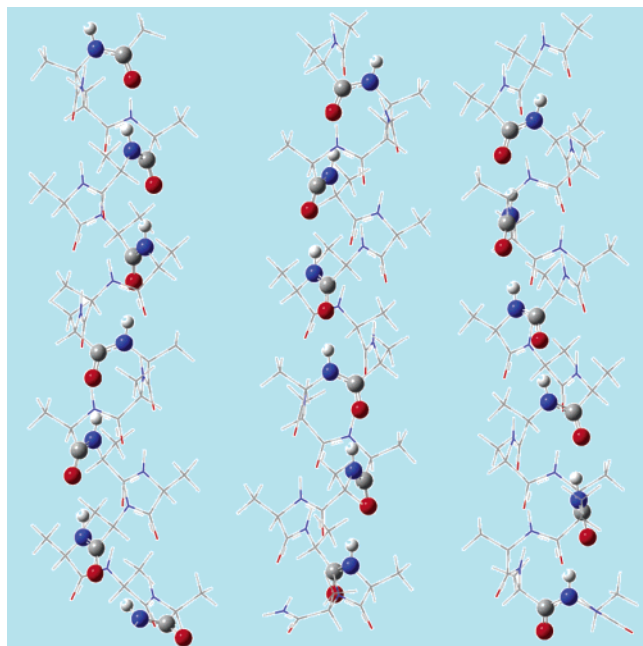**Figure 3.** $\alpha(18)$, emphasizing the three H-bonding chains.
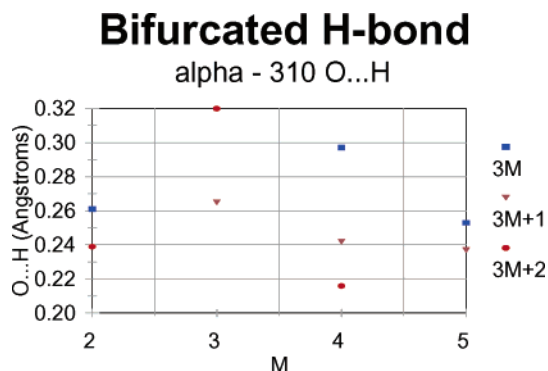

Figure 4. Difference in O···H distance in the first (bifurcated) H-bond between the 3_{10}-like and α-like interactions as a function of M within the H-bonding chains of the α-helices.
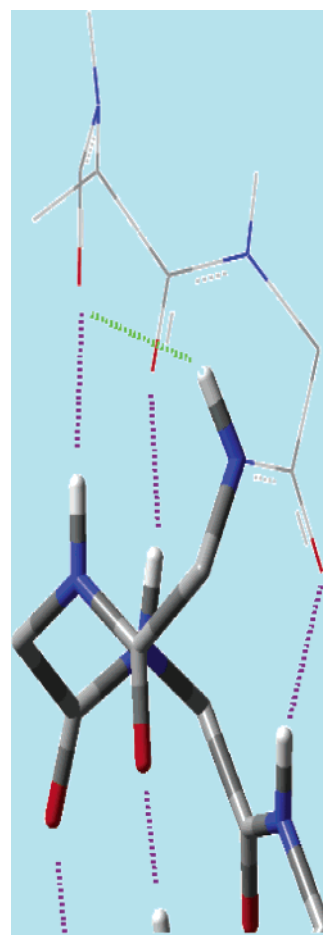

Figure 5. Detail of the acetyl end of $\alpha(18)$, illustrating the two H-bonds to the first C=O. The 3_{10} interaction is in green.

All of the α-helical structures (Figure 3) that we were able to completely optimize contain one H-bond reminiscent of a 3_{10}-helix that links the terminal acetyl C=O to the third alanine N–H. As the same C=O also interacts with the fourth alanine to form a normal α-helical H-bond, the O-atom forms a pair of H-bonds (see Figure 4). This feature makes the local geometry near this end of the peptide atypical of the rest of the helix. Thus, the H-bond lengths and angles (C=O···H and O···H–N), as well as the dihedrals of the peptide backbone, differ substantially from those in the rest of the helix. For all the α-helices in this study, the 3_{10} H-bond is shorter than the α H-bond to the terminal C=O. However, the difference between

these two H-bond O···H's tends to decrease as N increases for $\alpha(12)$ and larger (corresponding to $M \geq 3$), although it increases upon going from $M = 2$ to $M = 3$ (see Figure 5). While there are not many data points for each H-bonding chain with $M \geq 3$, this tendency becomes more apparent when one compares the H-bond lengths within the H-bonding chains. Since this structural feature exists in all eight α helices that we studied, it may be a general characteristic of α-helices, at least for those formed from peptides terminated with an acetyl at their amino end. However, the observation that the α-helical O···H distances decrease relative to the 3_{10} ones in the bifurcated H-bonds suggests that the former are becoming relatively stronger as N
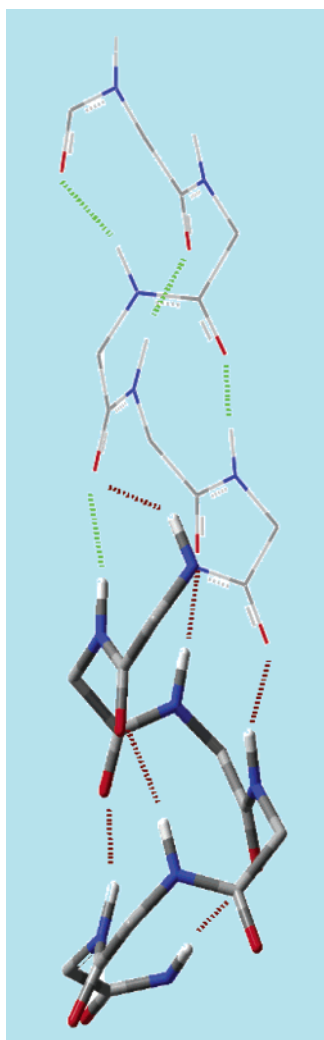
Figure 6. Structure of hybrid(11). The α part is shown as tubes and the 3₁₀ part as wire frame.

increases. Thus, the 3₁₀ H-bond could disappear for α-helices of sufficiently large size.

The helical dihedral angles CNCC (ϕ) near the center of the helices decrease from about $-61°$ to $-58°$ as N increases from 12 to 18, while the NCCN (ψ) angle remains roughly constant between $-45°$ and $-46°$.

Hybrid Structures. Our attempts at obtaining optimized structures for $\alpha(N)$, where $N = 6, 7, 9$, and 11, all led to hybrid 3₁₀/α helical structures (see Figure 6 for an example). In these structures, the first one to three C=O's starting from the acetyl end form 3₁₀-helical H-bonds, the second, third, or fourth C=O forms both 3₁₀- and α-helical H-bonds, while the remaining H-bonds are typical of an α-helix. Hybrid structures somewhat analogous to these have been reported for a crystalline heptapeptide[31] and an undecapeptide in solution.[32]

Determination of whether any other hybrid structures might exist as local minima on the respective PESs go beyond the bounds of this study. Nevertheless, we note that other hybrid structures should exist as local minima on the PES. The $\alpha(N)$

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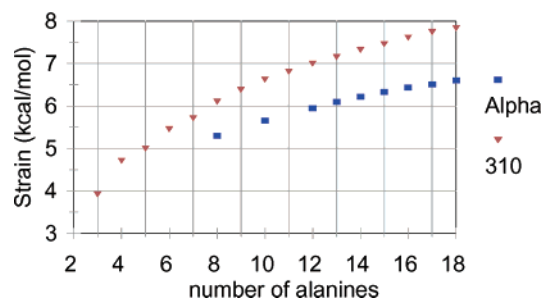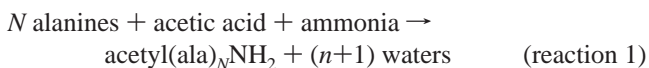
Figure 7. Helical strain per H-bond for helical peptides.

structures are less stable than the 3₁₀(N) structures for most values of N explored in this study. Hybrid structures should logically have energies between the pure α and 3₁₀ structures. As the value of N increases, more such conformations should exist. Furthermore, structures analogous to these are likely intermediates along the reaction path for interconversion between α- and 3₁₀-helices.

Helical Strain. Distortion from the extended β-strand structure to form either of the two helical conformations that we consider will necessarily cause some steric strain. This increase in energy caused by this strain will be more than compensated for by the H-bonds that form within the helical structures. A stable H-bonding distance will be established when decreasing the O···H distance would induce as much additional strain as it stabilizes the H-bond. We previously estimated this strain for $\alpha(17)$ peptide at about 6.7 kcal/mol[29] from the H-bonding interaction energies of the appropriate chains of H-bonding formamides that had been previously calculated,[33] together with an estimate of the vibrational contribution to the difference in enthalpies in the β-strand and α-helical structures. We use the same method to calculate the strain energies of the two helical forms as a function of N. As can be seen from Figure 7, the strain energies per H-bond are always larger for 3₁₀- than for α-helices. Also, the strain per H-bond increases with the size of the helix. More induced strain can be compensated for by the stronger and shorter H-bonds in the larger helices (see discussion of cooperativity below). We note that the helical strain calculated for $\alpha(17)$ is very slightly different from our previous report, as we used a slightly different method to calculate the energy of the helix.

Relative Energies and Cooperativity. In principle, one can imagine the capped polyalanines formed from alanines, acetic acid, and ammonia from the following reaction:

$$N \text{ alanines} + \text{acetic acid} + \text{ammonia} \rightarrow$$
$$\text{acetyl(ala)}_N\text{NH}_2 + (n+1) \text{ waters} \qquad \text{(reaction 1)}$$

From this reaction, we calculate the polymerization energy of the peptide according to eq 1:

$$E_{\text{polymerization}} = E_{\text{peptide}} - E_{\text{CH}_3\text{COOH}} - E_{\text{NH}_3} - NE_{\text{alanine}} -$$
$$(N + 1)E_{\text{H}_2\text{O}} \quad (1)$$

The energy used for alanine is that of the most stable conformation without the internal H-bond. The energies of

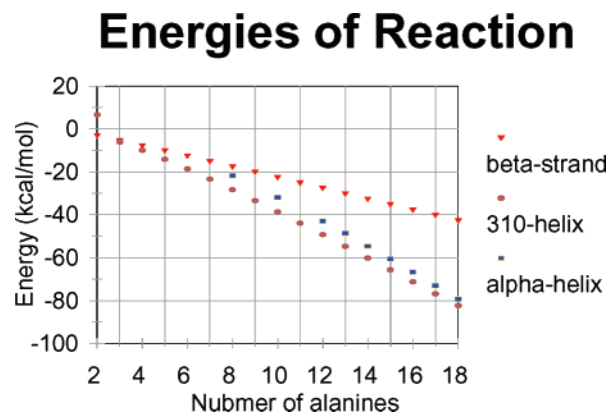(33) Kobko, N.; Dannenberg, J. J. *J. Phys. Chem. A* **2003**, *107*, 10389.

Energies of Reaction

Figure 8. Energies of polymerization calculated using eq 1 for the different conformations of the capped polypeptides.

Energy per Peptide Interaction
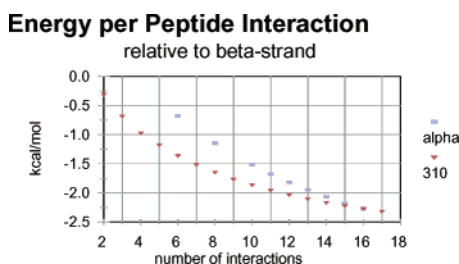### relative to beta-strand

Figure 9. Energy of helices relative to β-strand per H-bonding peptide interaction. There is one more H-bond in 3_{10}-helices than in isomeric α-helices.

Incremental stabilities

Figure 10. Increase in polymerization energy (from eq 1) upon addition of one alanine residue.

Average O...H Distance

Figure 11. Average O,H distance as a function of the number of alanine residues for helical structures.

Minimum O...H Distances in Helices

Figure 12. Minimum O,H distances in helical structures.

reaction 1 for the completely extended β-strands, α-helices, and 3_{10}-helices as a function of N in the generic formula acetyl-$(ala)_N NH_2$ are presented in Figure 8. The data indicate that the 3_{10}-helices are stable relative to the β-strands for all values of N beginning with $3_{10}(3)$. This structure contains two H-bonds. As previously noted, α-helices are minima on the PESs for $\alpha(8)$, $\alpha(10)$, and all $\alpha(N)$ where $N \geq 12$. $\alpha(9)$ and $\alpha(11)$ could not be optimized as stable α-helical minima. They both spontane-ously rearranged to isomeric hybrid $\alpha/3_{10}$-structures. The other α-helices all have some structural ambiguities near the ends. The smallest stable α-helix, $\alpha(8)$, has six H-bonds. Thus, each of the three H-bonding chains contains two H-bonds. Figure 8 immediately illustrates several important points. First of all, the downward curvature of the stabilities of both helices indicates the influence of H-bond cooperativity on the relative energies. On the other hand, those of the β-strands are quite linear, indicative of a simple group additivity relationship. Second, a rough extrapolation of the curve for the relative energies of the α-helices as N decreases suggests that α-helical structures with $5 < N < 8$ would be stable versus β-strands if minima corresponding to such structures were not unstable with respect to the corresponding mixed $\alpha/3_{10}$-helices. Third, although the 3_{10}-helices are more stable in the gas phase than the isomeric α-helices for all values of N that we studied, the greater downward curvature for the α-helix suggests that it would become more stable for N greater than about 20.

Cooperativity. Both the 3_{10}- and α-helices exhibit significant amounts of cooperativity. As mentioned above, the curvatures of Figure 8 strongly suggest cooperative H-bonding.

The same data, replotted in Figure 9 as the energy of interaction per H-bond between ala residues, show that the energy per interaction becomes increasingly more negative as N increases. The cooperativity is clearly much stronger for the
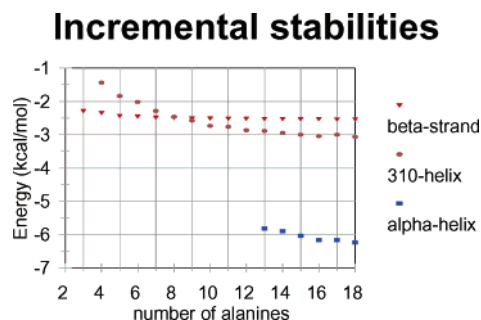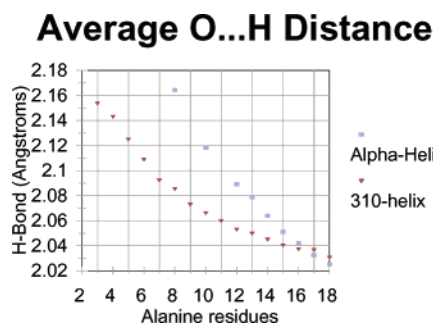
α- than for the 3_{10}-helices. Figure 9 shows the energies per H-bond becoming equivalent for the two helical conformations for $N = 18$. However, the most compelling data that illustrate this point come from a comparison of the incremental stability for each conformation upon addition of another alanine residue (Figure 10). We obtain these values from the difference in the polymerization energy (calculated using eq 1) for two peptides containing N and $N - 1$ alanines. The β-strands have virtually constant incremental stabilities, while those for the 3_{10}-helices appear to be approaching an asymptotic value of about -3 kcal/mol for $N = 18$. However, the incremental stabilities for the α-helices are more negative than -6 kcal/mol for $N = 18$, more than twice that of the 3_{10}-helices. Values for α-helices where $N \leq 12$ are not included, as $\alpha(7)$, $\alpha(9)$, and $\alpha(11)$ are not stable.

The cooperativity is further illustrated by the data plotted in Figures 11–13. The O···H distance is generally taken to be an indication of H-bond strength. We have previously demonstrated an approximately inverse proportional relationship for these quantities for the range of normal H-bond lengths in chains of H-bonding formamides.[33] Figure 11 displays the average O···H distance as a function of N for both kinds of helices. While the average O···H distance decreases monotonically for both kinds
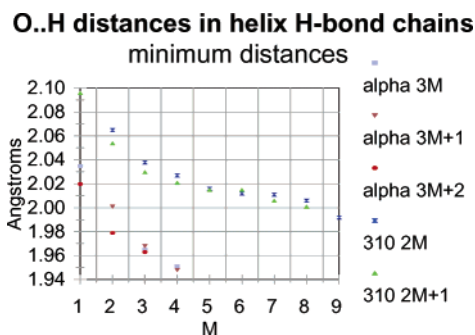
O..H distances in helix H-bond chains
minimum distances

Figure 13. Shortest O⋯H distances in each H-bonding chain for helical structures,

C=O lengths in helices
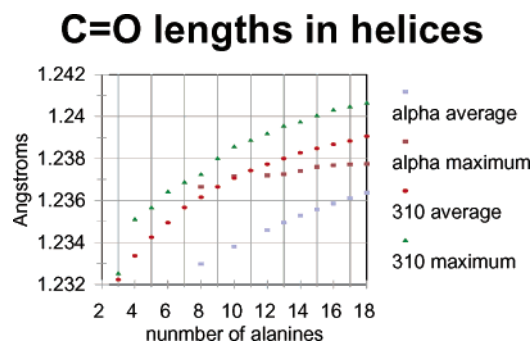
Figure 14. Variation of H-bonding C=O distances with peptides size.

Dipole Moment per C=O

Figure 15. Dipole moments per carbonyl group of the three structures as a function of number of C=O's.

of helix, the decrease is much steeper for the α-helices. In fact, the average O⋯H distances of the α-helices become less than those for 3_{10}-helices for values of $N = 17$ or more, despite the observation that the 3_{10}-helices are still more stable. However, one must remember that the 3_{10}-helices all have one more internal H-bond than their isomeric α-helical counterparts.

In our previous studies of formamide chains[33] and α-helices,[29] we found the H-bonds nearest the center of the H-bonding chains to be the shortest. The shortest O⋯H distance in each helical structure is plotted vs N in Figure 12. Here, one sees that the shortest distances decrease with N, but that the curves are not monotonic. These same data are replotted in Figure 13 to show the comportment of the O⋯H distances in the three individual H-bonding chains of the α-helices and the two individual H-bonding chains of the 3_{10}-helices. These chains can be identified as having a number of H-bonds equivalent to M, $M + 1$, and $M + 2$ for α-helices and M and $M + 1$ for 3_{10}-helices, as previously noted. Plotted in this manner, the shortest O⋯H distances clearly decease monotonically within each H-bonding chain.

The C=O distances for the H-bonding carbonyls lengthen as the peptides increase in size (see Figure 14). The C=O's nearest the center of the peptides have the longest distances. Here, the 3_{10}-helices show a greater change than the α-helices. While cooperativity results in longer C=O distances, another factor might be the helical strain. The greater strain in the 3_{10}-helices may be partially manifest as lengthened C=O bonds.

The behavior of the dipole moments of the three kinds of secondary structures further illustrates the cooperativity inherent in the helical structures. Figure 15 illustrates the dipole moment per C=O group. The dipole moment of $\beta(N)$ increases almost linearly with N, as can be seen from the almost constant dipole per carbonyl. On the other hand, those of both helices continue to increase (to their presumably asymptotic limits) as the peptide
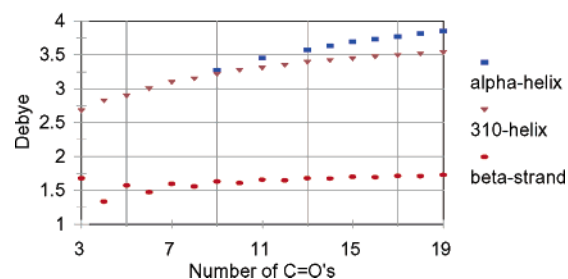
grows longer. This increase is significantly greater for $\alpha(N)$ than for $3_{10}(N)$. While any assignment of the component dipoles within a molecule must be arbitrary, as they cannot be individually measured, the component along the helical axis could not increase by more than the vector sum of the individual dipoles for purely electrostatic interactions. The observed increase of dipole moment per carbonyl indicates that non-additive cooperative effects must be important for both helical motifs, but especially for $\alpha(N)$.

The nature and the origin of the cooperativity, and the differing extents to which it is observed in the two different kinds of helices, deserve considerable discussion. Cooperativity can be defined in several different ways. If the stabilization energy of three (or more) species be greater than expected from sum of the equivalent direct pairwise interactions, the interaction can be defined as cooperative. Such an analysis does not take long-range (non-nearest neighbor) pairwise interactions into account as direct interactions. For example, a series of three equidistant, linearly arranged dipoles all oriented in the same direction will have a greater electrostatic stabilization than twice the interaction of two equivalent dipoles. The series of three dipoles will have two 1−2 and one 1−3 pairwise interactions. Thus, the 1−3 interaction produces a cooperative component to the stabilization, which is purely electrostatic in this model. Cooperativity can extend beyond the contributions of pairwise interactions. Such enhanced cooperativity (sometimes called non-pairwise additivity) cannot be attributed to electrostatic interactions (which are purely pairwise additive). Extended hydrogen bonding in peptide structures recalls H-bonding in many molecular crystals and aggregates where nonadditive cooperativity plays a significant role.[34,35] In fact, these nonadditive cooperative H-bonding interactions have been shown to determine the preferred polymorph in crystals of acetic acid,[36] urea,[37] and cyclohexane-1,3-dione[38] (which crystalizes as the less stable enol). We have recently shown that chains of formamide molecules show an extraordinary degree of cooperativity and, especially, nonadditive cooperativity in H-bonding chains.[33] In particular, the strongest H-bonds (the two nearest the center) of an H-bonding chain of 15 formamides are 3 times as strong as that of a formamide dimer, whereas pairwise additivity, as predicted by electrostatic interaction of the dipole moments, predicts them to be only 1.5 times that of the dimer. Put another way, the calculated cooperativity is 200% of the

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stabilization of the formamide dimer, or 4 times the 50% predicted by the electrostatic model.

The nonadditive component of the energetic cooperativity can, in principle, be due to one or more effects that are difficult to distinguish from one another. Several effects that are frequently invoked are polarizabilty, charge transfer, and covalent interaction. All of these effects should result in geometric distortions in real molecules. Comparing the effects of graded electric fields with those of extended H-bonding can distinguish polarizability from the latter two effects.[39] Using this technique, we have shown that the H-bonding in chains of the enols of cyclohexane-1,3-dione, which is an excellent example of what Gilli calls resonance assisted H-bonding,[40] cannot be explained by a combination of electrostatic and polarization effects.[39] Distinguishing between charge-transfer and covalent effects presents another problem. While charge-transfer of less than an entire electron implies a covalent interaction, the converse is not true. Put differently, while charge transfer can be taken as evidence of a covalent interaction, lack of charge transfer cannot be taken as evidence for the absence of such an interaction.

Alternatively, cooperativity can be addressed from changes in physical properties. For example, the shortening of an H-bonding interaction upon formation of additional H-bonds might be considered structural evidence of nonadditive cooperativity.[41] The change in dipole moment can be seen as another measure of nonadditive cooperativity. For molecules that only interact electrostatically, the dipole moment of an aggregate will simply be the vector sum of the dipole moments of the individual molecules. Dipole moments in excess of this sum constitute evidence for nonadditive cooperative interactions.

The cooperativity in the formation of the 3_{10}- and (especially) the α-helical structures of this study meets all the relevant tests for nonadditivity. In particular, the H-bonds shorten while the C=O bonds lengthen with increasing peptide size. The strongly nonlinear relationships of both the energy of polymerization (especially the incremental energy of polymerization) and the dipole moments with increasing peptide size further confirm the nonadditive nature of the cooperativity in the helices.

The α-helices have shorter and stronger H-bonds, and larger increases in dipole moment, with increasing peptide size than their 3_{10}-helical counterparts (particularly when the number of interactions per H-bonding chain is considered). Nevertheless, the above discussion cannot entirely explain the marked difference in cooperativity for the two kinds of helices that we have considered. Let us individually consider several possible explanations for these observations: (1) the α-helices are more polarizable; (2) covalent interactions in the α-helices are greater than for the 3_{10}-helices; (3) the two H-bonding chains in the 3_{10}-helices reach the asymptotic limits of their cooperativity for smaller peptides than the 3-H-bonding chains of α-helices; and (4) the differential extents of strain in the two helical types can impede covalent interaction within the peptide H-bonds of the 3_{10}-helices more than their α counterparts.

Our optimized geometries for the two helical types confirm the well-known detail that the C=O groups and the H-bonds in

the α-helices are better aligned with the helical axes than for their 3_{10}-helical conformations. Thus, the projection of an H-bond near the center of $\alpha(18)$ on the helical axis is 0.98, while that of a similar H-bond in $3_{10}(18)$ is 0.79. As the polarizability of the H-bond (and the C=O) should be greatest along its axis, the effect of the overall polarizability of the helix should be greater on the H-bonds of the α- rather than 3_{10}-helices. Thus, the nonadditive cooperative effect due to polarizability should be better for α-helices.

The H-bonds in the α-helices are shorter within H-bonding chains of equivalent length (M) of the α- than the 3_{10}-helices. These observations might be due, at least in part, to the higher polarizability along the α-helical axis, as discussed above. Nevertheless, the shorter H-bonding distances would increase the π-orbital overlap between N and O atoms across the H-bonds, which would enhance any covalent interactions between H-bond donors and acceptors. The continuous π-orbital conjugation (except for the H's) throughout each H-bonding chain suggests that resonance-assisted H-bonding[42] might be operative within each chain.

We estimate the strain energy per H-bond to be about 1 kcal/mol greater for the more tightly wound 3_{10}- than for the α-helix (see Figure 7). Greater strain energy should make the H-bonds more difficult to form, hence longer than in the presence of less strain. To the extent that the increased strain lengthens the H-bonds of the 3_{10}-helix relative to the α-helix, the effects of both the pairwise electrostatic and all of the nonadditive contributions mentioned in the three preceding paragraphs would be attenuated for the 3_{10}-helices.

Cooperative interactions in H-bonding chains must eventually reach asymptotic limits, or the energy of breaking an H-bond near the center of an infinite chain would, itself, approach infinity. Since the average chain lengths are slightly more than 50% longer in 3_{10}- than in α-helices, as the 3_{10}-helices have one more H-bond and two rather than three chains, these should reach their asymptotic limit at shorter peptide length than those of the α-helices. Our previous report on formamide chains shows that chains of 9 and 10 formamides are already close to the asymptotic limit for the interaction energies to be H-bonding. These two chains contain 17 H-bonds between them, as do the two chains of $3_{10}(18)$.

Each of the first three effects can act in concert with the others. For example, the increased polarization shortens the H-bonds, allowing more efficient overlap for covalent interaction. Both of these increase the overall dipole moment (and by inference the local electric field at the H-bond), which results in further polarization, etc. Thus, rationally assigning the relative importance of each would be quite difficult.

Solvation Effects. We do not consider solvation explicitly in the present study. Nevertheless, we believe that some discussion of the expected effects of solvation would be appropriate. Each peptide linkage, -CONH-, has one H-bond donor at the NH and (potentially) two H-bond acceptors at the C=O. The numbers of available H-bonding sites (i.e., those not involved in intramolecular H-bonds) differ for the three secondary structures considered. The $\beta(N)$ structures contain no intramolecular H-bonds. Thus, all H-bonding donor and acceptor sites are potentially exposed to solvent. For the two helical

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structures, only the H-bonding sites at the ends are completely exposed to solvent. The $3_{10}(N)$ structures have two exposed −H's at one end and two exposed C=O's at the other, while the $\alpha(N)$'s have three at each end. Thus, the $\alpha(N)$ structures can potentially form one more H-bond to solvent as donor and two more as acceptor than can the $3_{10}(N)$'s. This structural difference should render the $\alpha(N)$ structures more soluble in H-bonding solvents (such as water) than the $3_{10}(N)$'s.

In principle, the C=O's involved in the intrahelical H-bonds could form additional H-bonds using the other lone pairs of the O's as H-bond acceptors. However, interaction of a solvent molecule, such as water, to form a stable H-bond with one of these C=O's would likely disrupt the helical structure of polyalanine (or any other helix made from amino acid residues with alkyl side chains). If water molecules simply interact with the sides of the helix without penetrating the spaces between the methyl groups, the interaction would likely be less stabilizing than the water/water interactions disrupted, and thus, hydrophobic. Further work needs to be done in this area in order to provide quantitative measures of the interactions of these and similar helices with individual water molecules. Nevertheless, it is worthy of note that Hecht has designed de novo artificial non-natural proteins that fold in bundles of four α-helices that mutually interact due to hydrophobic effects.[43] His further observation that larger α-helices are more stable and form more stable de novo proteins also supports our observations about cooperativity in the H-bonding within the helices.

The two factors discussed above suggest that the α-helix would be better solvated than the 3_{10}-helix, as the former has more available H-bonding sites and the latter (which is longer, but thinner) will have more surface on the sides of the helix, and thus more hydrophobic sites.

Conclusions

The results of our calculations show that $3_{10}(N)$, which have one more internal H-bond than their α counterparts, are more stable than $\alpha(N)$ for short peptides, but $\alpha(N)$ become more stable for larger values of N. Both kinds of helices exhibit substantial cooperative interactions, including significant components of nonadditive cooperativity that cannot be explained by purely electrostatic interactions. This cooperativity is much more substantial for $\alpha(N)$ than for $3_{10}(N)$ due to a combination of factors (higher polarizability, increased covalent interactions, less strain, and more H-bonding chains) that symbiotically interact. The more potent cooperativity of $\alpha(N)$ is manifest energetically (by increased stability per H-bond, particularly for incremental changes in N), structurally (by decreasing O···H distances as N increases), and from dipole moments (the greater upward curvature of dipole vs N). The helical strain energy per H-bond (which is greater for 3_{10}- than α-helices) increases with N in response to the stronger, shorter H-bonds that result from the cooperative interactions.

Supporting Information Available: Cartesian coordinates of the relevant structures. This material is available free of charge via the Internet at http://pubs.acs.org.

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