J|A|C|S
ARTICLES
Published on Web 06/17/2003

H-Bonding Cooperativity and Energetics of α-Helix Formation of Five 17-Amino Acid Peptides

Robert Wieczorek† and J. J. Dannenberg*

Contribution from the Department of Chemistry, City University of New York, Hunter College and the Graduate School, 695 Park Avenue, New York, New York 10021

Received March 24, 2003; E-mail: jdannenberg@gc.cuny.edu

Abstract: Five peptides, each containing 17 amino acids, have been completely geometrically optimized in their α-helical and β-strand forms using a mixed DFT/AM1 procedure. B3LYP/D95** was used for the entire helical structures, while AM1 was initially used to optimize the side chains, followed by reoptimization at the DFT level. The energetic and structural results show (1) that the helices are favored over the strands by 29.5 to 37.4 kcal/mol; (2) that alkyl groups on the amino acid side chains favor helix formation even in the absence of solvent; (3) that C−H···O hydrogen bonds contribute to the relative stability of the helices that contain amino acids (val, leu and ile) with β-hydrogens in their alkyl side chains; (4) that formation of these helices entails approximately 6.6 kcal/mol of strain within the backbone per hydrogen bond; and (5) that H-bond cooperativity is essential for the α-helix to become more stable than a corresponding β-strand. This last observation strongly suggests that pairwise potentials are inadequate for modeling of peptides and proteins.

Introduction

Despite the growing interest in protein folding and protein structures, very little quantitative information has been available on the nature of the H-bonding interactions that play a fundamental role in the energetics of peptide structures. H-bonding interactions are generally treated using pairwise potentials, which has been criticized in the literature.[1] Such potentials do not properly account for cooperative H-bonding interactions. To properly treat the protein-folding problem, one needs to understand the effects of structural modifications upon the energetics of proteins including those influencing primary, secondary, and tertiary structural properties. While the influence of environmental factors, such as solvation, certainly plays an important role in protein-folding modeling, we emphasize the necessity of understanding the intrinsic energetics of peptides (i.e., in the absence of solvation) in order to better differentiate the importance of the intrinsic and environmental influences. In this paper, we examine the effects of H-bond cooperativity and amino acid sequence (primary structure) upon the relative energies of α-helices to β-strands (secondary structure).

Very little has been reported on the relative energies of different peptide secondary structures as a function of peptide chain length and amino acid composition. The preference of certain peptides for the α-helical structure over the β-strand or random coil has often been variously attributed to entropy and solvation effects (including hydrophobic interactions). However, Kemp has recently shown that enthalpic factors including those attributable to cooperative H-bonding can also be important.[2] The importance of hydrogen bonding cooperativity to the determination of the secondary structures of peptides has been discussed previously in the literature.[3] However, most studies have used small molecules (generally amides) as models. The little quantitative information available is only for the smallest peptides. We have recently reported that hydrogen bonding chains of formamides exhibit an extraordinarily high cooperative effect on the energy of their interactions. The two central H-bonds of the chain of 15 formamides are 2.9 times as strong as that of the dimer. The H-bonding distances are inversely proportional to the magnitude of the H-bonding interaction energies.[4,5] This latter observation is consistent with previous reports.

Despite the suggestive evidence that H-bond cooperativity should provide enthalpic stabilization to α-helical structures, no molecular orbital studies on completely optimized α-helical peptides have appeared until now. These helices presumably need to be sufficiently large for H-bond cooperativity to overcome the unfavorable steric interactions that favor the open β-strand or random coil structures. Thus, attempts at completely optimizing helical peptides containing up to nine alanine residues using DFT techniques have provided only 3_{10}, not α-helices in the absence of solvation.[6] Since 3_{10}-helices have two, rather than three, H-bonding chains, each chain is longer for 3_{10}-helical peptides of the same length than for α-helices.

† On leave from Faculty of Chemistry, University of Wroclaw, F. Joliot-Curie 14, Wroclaw, Poland.

(1) Vendruscolo, M.; Domany, E. *J. Chem. Phys.* **1998**, *109*, 11101.
(2) Kennedy, R. J.; Tsang, K.-Y.; Kemp, D. S. *J. Am. Chem. Soc.* **2002**, *124*, 934.
(3) Guo, H.; Gresh, N.; Roques, B. P.; Salahub, D. R. *J. Phys. Chem. B* **2000**, *104*, 9746.
(4) Kobko, N.; Paraskevas, L.; del Rio, E.; Dannenberg, J. J. *J. Am. Chem. Soc.* **2001**, *123*, 4348.
(5) Kobko, N.; Dannenberg, J. J. Submitted for publication.
(6) Bour, P.; Kubelka, J.; Keiderling, T. A. *Biopolymers* **2002**, *65*, 45.

10.1021/ja035302q CCC: $25.00

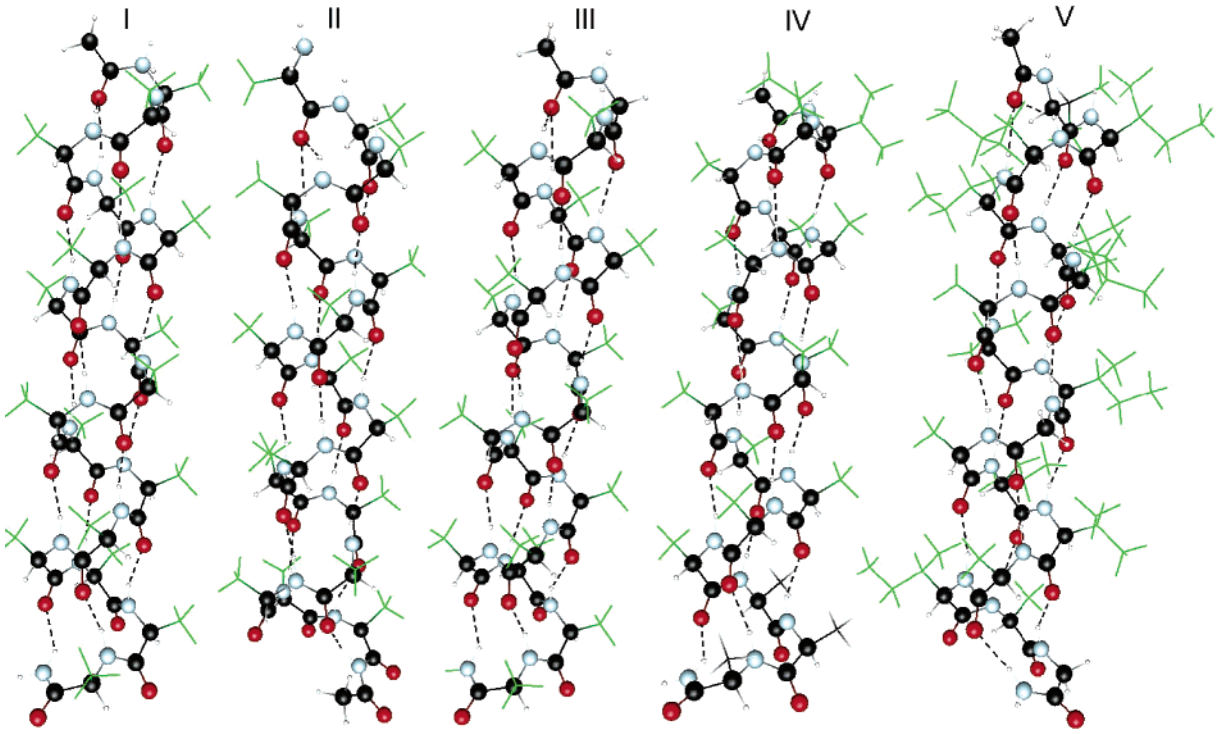

Figure 1. Structures of the five optimized α-helical peptides.

Since no α-helical peptides had been completely optimized using MO methods, no accurate theoretical measure of the energies of α-helix formation had previously been possible. Here, we present the energetic and structural properties of five completely optimized α-helical peptides containing 17 amino acid residues each in both the α-helical and β-strand forms. To the best of our knowledge, this is the first report describing completely optimized structures of peptides of similar complexity. Previous reports on quantum mechanical optimizations have concentrated on either peptides too small to form α-helices or helical or β-sheet structures that were only partially optimized. As there are too many reports, we cite only some representative recent examples.[6−19] Notable exceptions are the optimizations of several 3_{10}-helical peptides and one α-helical peptide containing nine amino acid residues (either Ala or Pro),[6] our previous report of fully optimized 3_{10}-helices of pentapeptides,[20] and an attempt to optimize a complete protein that was not run to complete geometric convergence.[21] The peptides that we consider here are (a and b) two different isomers of acetyl-$(Ala)_{17}NH_2$, **I** and $(Ala)_{17}$NHacetyl **II**; (c) Acetyl-Gly-$(Ala)_{16}NH_2$, **III**; (d) Acetyl-Val-Val-$(Ala)_{15}NH_2$, **IV**; and (e) acetylVILIV-LAVIGALVAIAGNH_2, **V** (see Figure 1). The isomers, **I** and **II**, can be interconverted by exchanging the terminal acetyl group with an H-atom at the other terminus. The geometries of these peptides are completely optimized using the ONIOM[22,23] method in which the helical core is calculated at the DFT B3LYP/D95** level, while the methyl side groups are calculated using the AM1[24] semiempirical molecular orbital method. We have shown elsewhere that this combination gives energetic results virtually indistinguishable from the pure DFT optimizations for calculations on five small peptides containing five amino acids each.[25] We emphasize that, at this level of calculation, the entire hydrogen bonding system is described exclusively at the DFT level. This, and the fact that we use a semiempirical molecular orbital method (AM1), rather than molecular mechanics for the lower level in ONIOM, should minimize the effect upon the hydrogen bonding network of the kinds of problems that occur at the interface of the two calculational levels that has recently been discussed by Karplus.[26]

Calculational Details

We used the ONIOM method as programmed in the Gaussian 98[27] suite of computer programs. ONIOM divides the system into 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

(7) Elstner, M.; Jalkanen, K. J.; Knapp-Mohammady, M.; Frauenheim, T.; Suhai, S. *Chem. Phys.* **2000**, *256*, 15.
(8) Elstner, M.; Jalkanen, K. J.; Knapp-Mohammady, M.; Frauenheim, T.; Suhai, S. *Chem. Phys.* **2001**, *263*, 203.
(9) Zhu, C.; Balta, B.; Aviyente, V.; Lifshitz, C. *J. Phys. Chem. A* **2000**, *104*, 7061.
(10) Strittmatter, E. F.; Williams, E. R. *J. Phys. Chem. A* **2000**, *104*, 6069.
(11) Jakli, I.; Perczel, A.; Farkas, O.; Csaszar, A. G.; Sosa, C.; Csizmadia, I. G. *J. Comput. Chem.* **2000**, *21*, 626.
(12) Han, W.-G.; Elstner, M.; Jalkanen, K. J.; Frauenheim, T.; Suhai, S. *Int. J. Quantum Chem.* **2000**, *78*, 459.
(13) Chaudhuri, P.; Canuto, S. *THEOCHEM* **2002**, *577*, 267.
(14) Benzi, C.; Improta, R.; Scalmani, G.; Barone, V. *J. Comput. Chem.* **2002**, *23*, 341.
(15) Kearley, G. J.; Johnson, M. R.; Plazanet, M.; Suard, E. *J. Chem. Phys.* **2001**, *115*, 2614.
(16) Kubelka, J.; Gangani, R. A.; Silva, D.; Keiderling, T. A. *J. Am. Chem. Soc.* **2002**, *124*, 5325.
(17) Topol, I. A.; Burt, S. K.; Deretey, E.; Tang. *J. Am. Chem. Soc.* **2001**, *123*, 6054.
(18) Improta, R.; Barone, V.; Kudin, K. N.; Scuseria, G. E. *J. Am. Chem. Soc.* **2001**, *123*, 3311.
(19) Wu, Y.-D.; Zhao, Y.-L. *J. Am. Chem. Soc.* **2001**, *123*, 5313.
(20) Wieczorek, R.; Dannenberg, J. J. Submitted for publication.
(21) Van Alsenoy, C.; Yu, C.-H.; Peeters, A.; Martin, J. M. L.; Schaefer, L. *J. Phys. Chem. A* **1998**, *102*, 2246.
(22) Dapprich, S.; Komiromi, I.; Byun, K. S.; Morokuma, K.; Frisch, M. J. *THEOCHEM* **1999**, 0166.
(23) Morokuma, K. *Philos. Trans. R. Soc. London, Ser. A* **2002**, *360*, 1149.
(24) Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902.
(25) Wieczorek, R.; Dannenberg, J. J., submitted for publication.
(26) Cui, Q.; Guo, H.; Karplus, M. *J. Chem. Phys.* **2002**, *117*, 5617.

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 core of the helix or β-strand (equivalent to a corresponding peptide containing only glycines) at the high level, with only the alkyl groups that distinguish the amino acids from each other at the medium level. The high level was performed using hybrid DFT methods at the B3LYP/D95(d,p) level, as were the pure DFT calculations (see below). This method combines Becke's 3-parameter functional,[28] with the nonlocal correlation provided by the correlation functional of Lee, Yang, and Parr.[29] 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 0.723 886 times the C−C distance. We used the AM1[24] semiempirical molecular orbital method for the ONIOM medium level. Each energy was calculated three different ways: (1) using the ONIOM procedure as described; (2) performing a single point DFT calculation at the ONIOM optimized geometry; and (3) starting with the ONIOM optimized geometry, we kept the core (calculated at the high level with ONIOM) fixed and reoptimized the side chains (originally optimized with the medium level) using DFT. We used our cluster of Intel Pentium 4 computers that are parallelized using LINDA for these calculations. The number of nodes used for each calculation varied with the sizes of the systems studied.

Hydrogen Bonds

There are two isomeric polyalanines containing 17 residues that are terminated at one end by an acetyl group and at the other by an amido group, **I** and **II**. As mentioned above, they can be interconverted by exchanging the acetyl group at one end with an H-atom at the other. The structures are depicted in Figure 1. Each α-helix contains three H-bonding chains of five amide hydrogen bonds (illustrated in Figure 2) except for the polyalanine isomer **II**, which has only 14 "normal" H-bonds. In addition, a terminal H-bonding C═O interacts with a second N−H to form an H-bond to an additional amide (see Figure 1). This additional H-bond has the topology expected for a 3_{10}-helix. The N−H···O angles for this interaction differ significantly from linearity. Table 1 and Figures 2 and 3 present the data for the "normal" hydrogen bonds at each chain starting from the acetyl end. The hydrogen bonding distances are given both as those between the O and H and the O and the N. There are two terminal H-bonds in each helix: the first H-bond in the first H-bonding chain and the last H-bond in the third chain. The data of Table 1 clearly show that these two hydrogen bonds are substantially longer (up to 0.4 Å) than the others. Only the second H-bonding chain has no terminal H-bonds. The variation in H-bond lengths within this chain are similar to those that we reported for a chain of six H-bonding formamides (containing five H-bonds).[5] However, all the H-bonds in the helices are somewhat longer, as might be expected from the steric strain inherent in the helix (but not in the formamide chains). In the second chain, the central bond is the shortest. In each of the other two chains, the long terminal bond skews the relation between H-bond distance and position in the chain so that the H-bond one position away from the center toward the end without the terminal bond becomes the shortest. Not surprisingly, the longer H-bonds in the α-helices suggest that these interactions are somewhat weaker than those previously reported for the H-bonding formamide chains.

Comparison of the individual H-bonding distances of **I**, **III**, **IV**, and **V**, which have the same pattern of termination by acetyl and NH_2 is instructive (see Figure 3). The first H-bonding chains of the helices **I**, **III**, and **IV** have a very similar pattern of H-bonding distances. This is reasonable as these helices only differ near the end that contains the NH_2 terminal H-bond. The

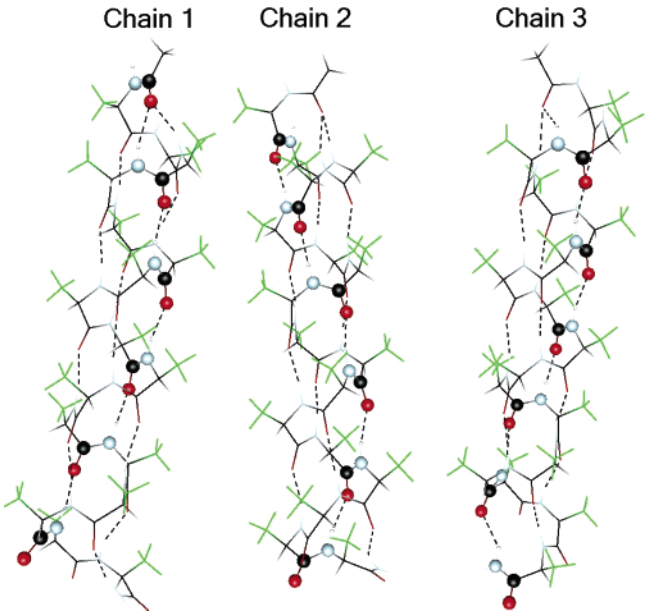

Figure 2. Structure of **I** indicating the three H-bonding chains which are highlighted separately. Note that the H-bonding chains have a helical cant of opposite direction to the backbone helix.

Table 1. H-bonding Distances (Å) for the Five Optimized Peptides[a]

	I		II		III		IV		V	
	O···H	O···N	O···H	O···N	O···H	O···N	O···H	O···N	O···H	O···N
1	2.378	3.350	2.297	3.136	2.427	3.393	2.324	3.298	2.213	3.209
2	2.010	3.012	2.024	2.973	2.020	3.023	2.008	3.009	2.047	3.055
3	2.025	3.015	2.036	3.015	2.030	3.021	2.027	3.018	2.154	3.116
4	1.995	2.995	1.943	2.945	1.985	2.985	1.989	2.989	1.966	2.967
5	1.983	2.980	1.970	2.969	1.984	2.982	1.974	2.973	2.014	3.018
6	1.955	2.953	1.960	2.954	1.963	2.962	1.962	2.961	2.011	3.018
7	1.959	2.958	1.960	2.957	1.955	2.955	1.956	2.955	1.951	2.939
8	1.967	2.963	1.959	2.955	1.950	2.948	1.965	2.960	1.954	2.950
9	1.957	2.954	1.964	2.963	1.961	2.959	1.958	2.957	1.974	2.962
10	1.951	2.945	1.972	2.968	1.952	2.949	1.955	2.949	1.960	2.967
11	1.980	2.979	1.973	2.973	1.953	2.962	1.971	2.970	2.020	2.998
12	1.973	2.965	2.033	3.026	1.965	2.949	1.968	2.963	1.980	2.979
13	2.038	3.017	1.991	2.995	2.065	3.025	2.042	3.018	2.105	3.072
14	2.031	2.973	2.258	3.235	2.049	2.989	2.030	2.972	1.986	2.933
15	2.194	3.048			2.197	3.050	2.197	3.049	2.191	3.041
average	2.026	3.007	2.348	2.804	2.030	3.010	2.022	3.003	2.035	3.015

[a] The H-bonds are numbered starting from the acetyl end. Only the "normal" H-bonds are included (see text). H-bonds 1, 4, 7, 10, and 13 are in the first, 2, 5, 8, 11, and 14 in the second, and 3, 6, 9, 12, and 15 in the third H-bonding chain (see Figure 2).

(27) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewsk, V. G.; Montgomery, J. A. J.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; J. Tomasi; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Salvador, P.; Dannenberg, J. J.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*, revision A.11; Gaussian, Inc.: Pittsburgh, PA, 1998−2001.
(28) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648.
(29) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785.

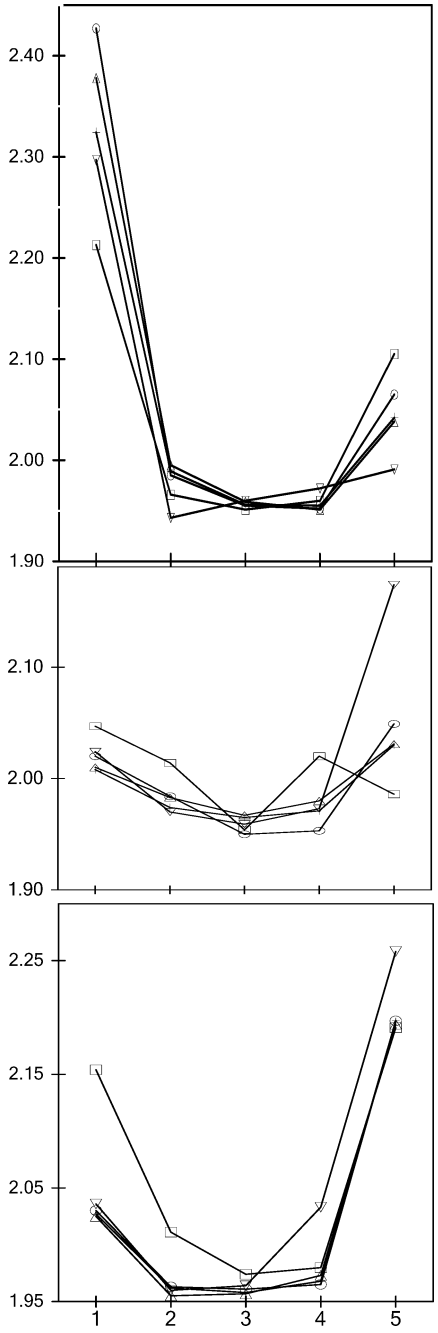

Figure 3. H-bond distances (Å) in each H-bonding chain starting from the acetyl end. Top, middle, and lower plots are for chains 1 to 3, respectively. Triangle is **I**, inverted triangle, **II**, circle, **III**, +, **IV**, and square, **V**.

corresponding chain of helix **V** differs from **I** mostly at the end nearer the terminal acetyl. The NH_2-terminal H-bond of **V** in this (first) chain is between Gly and Ala, which would not provoke a significant steric perturbation of the H-bond. In the second chain, the H-bond distances for helices **I**, **III**, and **IV** remain similar to each other. However, the second and fourth H-bonds of chain **V** (both H-bonds between Ile and Val) are significantly longer than the corresponding interactions in chains **I**, **III**, and **IV**. These differences might be due to steric strain. For the third chain, helices **I**, **III**, and **IV** differ slightly for the (fifth) NH_2-terminal H-bond. Here, the H-bond distances vary in the order **I** > **III** > **IV** > **V**. Curiously, the terminal H-bond involving the acetyl seems to shorten when amino acids with longer side chains are present (chain **III** seems to be an exception). This might indicate that this end of the helix is impeded from unraveling by the bulky side chains, which need to pass each other as the unraveling proceeds. Thus, these bulky groups might be thought of as locking this end of the helix. Further study should clarify this point.

Table 2. Stabilization of the α-helix Relative to the β-strand in (kcal/mol)[a]

	E_{ONIOM}	$E_{DFT/SP}$	$E_{DFT/reopt}$
I	32.58	33.97	30.83
II	33.76	33.59	31.05
III	31.70	32.22	29.51
IV	33.52	34.33	32.27
V	39.04	40.32	37.38

[a] The first column presents the ONIOM energies, the second, single point DFT at the optimized ONIOM geometries, and the third column, the energies after the alkyl side chains have been reoptimized using DFT (see text for explanation). The $E_{DFT/reopt}$ values are those used in the energetic discussion.

Grzesiek has reported the trans H-bond $^{13}C-^{15}N$ scalar NMR couplings for a 22 residue peptide in various mixtures of water and trifluoroethanol (TFE).[30] The magnitude of these couplings increases with decreasing H-bond length.[31] At the highest mole fraction of TFE, (which enhances α-helix formation) the couplings of the H-bonds nearest the centers of the H-bonding chains in the α-helices are the largest. These results agree with the calculations presented here.

α-Helix Stability

The stabilization energies of the α-helical structures relative to the β-strands are presented in Table 2, which contains entries for all three calculational methods described above. To simplify the discussion, we use energies obtained using DFT after the side chains (originally optimized using AM1) have been reoptimized using DFT. This is the third method mentioned above and corresponds to the third column of Table 2. The α-helices are all more stable than the corresponding β-strands by 29.5 to 37.4 kcal/mol (depending upon the structure), in qualitative agreement with Kemp's observations.[2] These energetic differences are significantly larger than those that we have previously reported for several smaller (five amino acid) peptides.[20] The latter group of peptides is too small to form α-helices. They form 3_{10} helices containing four H-bonds (two in each of two H-bonding chains) which are more stable than the corresponding β-strands by approximately 3 kcal/mol (again depending upon structure). Thus the average H-bonds in the larger α-helices reported here (2.0 to 2.5 kcal/mol) are roughly 3 times as strong as those for the smaller peptides, once again indicative of cooperative H-bonding.

The variation of stabilization of the α-helices with structure shows that they become more stable relative to the β-strands with increasing alkyl substitution. Thus, exchanging a terminal alanine for a glycine reduces the relative stability of the helix by 1.9 kcal/mol, while exchanging the two alanines nearest the NH_2-end for valines increases it by 0.9 kcal/mol. The heterogeneous peptide, **V**, which contains two glycines, four alanines, four valines, three leucines, and four isoleucines, is 6.6 kcal/

(30) Jaravine, V. A.; Alexandrescu, A. T.; Grzesiek, S. *Protein Sci.* **2001**, *10*, 943.
(31) Barfield, M.; Dingley, A. J.; Feigon, J.; Grzesiek, S. *J. Am. Chem. Soc.* **2001**, *123*, 4014.

mol more stable relative to the β-strand than the homogeneous alanine peptide, **I**. These results are again consistent with previous results on the smaller peptides, where alkylation of a single glycine to form alanine, valine, leucine or isoleucine all increased the stability of the 3_{10}-helices.[20] They are also qualitatively consistent with the difference in energies of periodic calculations of polyalanine and polyglycine.[18] Comparison of the helix stabilities of the two isomers of polyalanine, **I** and **II**, is particularly interesting. While the respective helices have similar stabilities (30.83 and 31.05 kcal/mol), they differ in the number of H-bonds in their structures. Thus, **II**, which has one fewer, must have stronger H-bonds on average since the difference in energies for the α-helical and β-strand structures are about the same for the two isomers. Unlike the other helices, in which all three of the N-terminal unsatisfied H-bonding donors are amidic, **II** has two amide and one amino unsatisfied H-donor. Thus, **II** contains one more H-bond from an amide, which is a better H-donor than an amine. Inspection of Table 1 shows that the average length of the H-bonds of **II** is slightly shorter than the corresponding value for **I**. This observation is consistent with our reports on formamide chains that indicate that the strengths of H-bonds are generally inversely proportional to their respective lengths.[4,5]

Substitution of alanine for glycine replaces an H-atom with an alkyl (methyl) group. Such substitutions appear to stabilize the H-bonds as (1) they become shorter and (2) **I** is more stable as an α-helix than **III** (which has one Gly in place of an Ala). While the precise physical cause probably needs further study, this effect appears to be of electronic rather than steric origin. We have shown that replacing a G with an A to form GAGGG stabilizes both the 3_{10}-helical and (to a lesser extent) β-strand forms.[20] Normally, one would not expect increasing the size of an alkyl group (in the present case changing Ala to Val, Leu, or Ile) to have no more than a negligible incremental effect upon such an electronic interaction. Nevertheless, the α-helical stability of **IV**, in which two Ala's in **I** are replaced by Val's, is greater than that of **I**. Furthermore, the helical stability of **V**, where many Ala's in **I** are replaced by Val's, Leu's, and Ile's, is by far the greatest of the five peptides studied. Careful examination of the helical structures indicates C−H···O H-bonding interactions to be the cause of the additional stabilization. Only Val, Leu, and Ile, among the five amino acids contained in the peptides studied, can form these interactions as only β-hydrogens (on the alkyl group) can form these interactions without causing significant strain within the alkyl side chains. Thus, the Val's in **IV** have C−H···O distances of 2.477 and 2.437 Å with the proximate C═O, while the C−H···O distances for the Val's, Leu's, and Ile's in **V** vary from 2.418 to 2.575 Å. The C−H···O distances in the β-strands are all significantly longer. Figure 4 illustrates a typical C−H···O interaction in a helix. These interactions are similar to those in the crystal of the enol of 1,3-cyclohexanedione.[32]

The average H-bond lengths in the helices follow the expected order (shorter as the helices become more stable) with the notable exception of **V**, where the average H···O distance is stlightly longer than that of **IV**. One can explain this apparent discrepancy by the effect of the CH···O interaction upon the N−H···O interaction at the same oxygen, as formation of a second H-bond generally slightly weakens the first.

(32) Turi, L.; Dannenberg, J. J. *Chem. Mater.* **1994**, *6*, 1313.

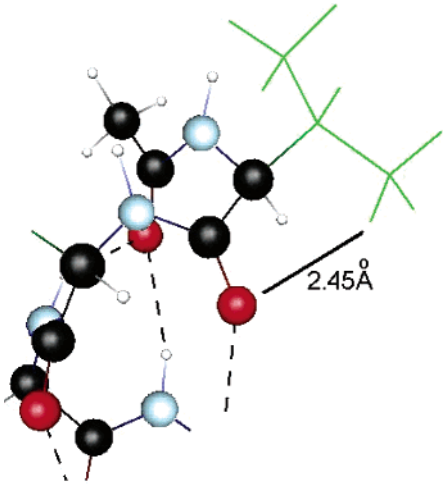

Figure 4. A detail of an α-helix that illustrates a C−H···H interaction. Note the 2.45 Å distance between a β-hydrogen on the side chain of the valine residue and the proximate C═O, which now has two H-bonding interactions.

The helical torsional angles CNCC (φ) and NCCN (ψ) fall within the range of −59.2° to −70.4° (φ) and −32.7° to −46.4° (ψ) for the helix forming residues (i.e., exclusive of the last three residues of **I**, **III**, **IV**, and **V** and the first three of **II**). For **I**−**IV**, the magnitudes of φ become smaller (less negative) upon moving from the ends to the center of the helices, while that of ψ becomes larger (more negative). The torsional angles for **V** do not follow this pattern. Rather, the torsional angles tend to change in the reverse order. We note that the starting geometry of the helical form of **V** was taken from that of optimized **I** by simply morphing the methyls into the appropriate side chains. Thus, the optimized geometry of **V** is unlikely to be a local minimum that might have another, lower energy geometry more similar to that of **I**. Clearly, the higher concentration of larger alkyl groups in **V** must cause these contrasting structural manifestations. The two most likely physical causes of this phenomenon are (1) greater steric interactions between the alkyl side chains in **V** and (2) the presence of the C−H···O interactions in **V**. Inspection of the structure of helical **V** does not reveal any obvious steric interactions between the alkyl groups of the side chains (see Figure 1). Furthermore, **V** is the most stable in the helical form, so whatever steric interactions that exist could not be very destabilizing. Thus, we suggest the C−H···O interactions to be the most likely physical cause of this observed behavior of the dihedral angle variation. However, these effects could be cumulative and subtle.

We note that empirical methods based upon common force fields should not be able to properly describe the electronic effects upon the H-bonding energies or the C−H···O interactions, even when used in combined QM/MM methods. The MM methods cannot properly polarize the QM system, nor do they properly describe C−H···O interactions. The present discussion rests on the C−H···O interactions calculated at the DFT level as we have used the energies for the systems with the side chains (originally calculated using AM1) reoptimized using DFT. However, the energy differences calculated by the other two methods do not significantly differ from the values used for discussion (see Table 2). Also, AM1 describes C−H···O interactions extremely well as noted from comparisons with high level ab initio calculations.[33]

Helical Strain

There is considerable strain inherent in the formation of the α-helix which provides a force that both weakens and lengthens the H-bonding interactions relative to an unconstrained chain of H-bonding amides. An estimate of this strain can be obtained from the difference between the interaction enthalpies of three five-H-bond formamide chains[5] (3×44.7 kcal/mol) and the difference in energies between the helical and open β-strand forms of the polyalanine (31 kcal/mol). This estimate must be viewed as somewhat of an approximation as (1) we have not calculated the vibrational contribution to the energy difference; (2) basis set superposition error (BSSE) is calculated only for the formamide chains; and (3) the zero-point vibrational corrections will be quite different as going from eighteen formamides to three chains of six creates 90 new vibrational modes (30 per chain) while forming an α-helix from a β-strand stiffens some vibrations, but creates no new ones. The enthalpies of 3_{10}-helix formation for the pentapeptides that we have reported were about 1 kcal/mol less than the energies for the formation of four H-bonds.[20] An extrapolation of this value to a 15-H-bond α-helix would indicate the enthalpy of helix formation should be reduced by about 4 kcal/mol. Estimating the effect of BSSE is somewhat more difficult as (1) techniques for calculating intramolecular BSSE have not been perfected and (2) the BSSE will diminish with increasing length of the H-bond, so the values used for the formamide chains cannot be transferred here. Nevertheless, the effect of BSSE will lower the enthalpy of helix formation a bit more. Using these estimates, one obtains a value of $(3 \times 44.7 - 31 - 4)/15 = 6.6$ kcal/mol of strain per H-bond. This value should be used with some caution until it can be confirmed due to the speculative nature of the approximations employed. Nevertheless, we can expect that the H-bonding energy must be sufficient to overcome the helical strain in order for an α-helix to be stable. Since our previous studies indicate that a single H-bond between two formamides in a dimer provides only 4.5 kcal/mol of stability, the significant cooperativity inherent in H-bonding chains must be present to overcome the strain.

Conclusions

The present results clearly illustrate several important characteristics of the relative energetics of peptide secondary structures:

(1) Alkylation of amino acids residues (i.e., converting gly to ala, val, leu or ile) favors α-helix formation over β-strand, even in the absence of any solvent effects. Thus, this is an intrinsic enthalpic property of peptides which might be accentuated by solvation and/or entropic factors. The experimental reports that indicate (for example) that α-helical structures become more favored as the proportion of TFE increases in TFE/H_2O mixtures increases[30] should be interpreted in the light of these intrinsic energetic properties.

(2) The relative H-bond lengths in α-helical structures follow the qualitative order already reported for chains of H-bonding formamides.[20] However, the H-bonds of the helices are generally longer, particularly for the terminal H-bonds at each end of the helices. These observations are consistent with the supposition that formation of the α-helical structure induces considerable steric strain for which the H-bonds compensate.

(3) The much larger energies of helix formation for the peptides discussed in this paper as compared to the five smaller peptides previously reported strongly suggest that H-bond cooperativity significantly affects helical stability. However, further study on peptides of larger and smaller size should be performed to confirm this.

(4) Since many empirical models used to study peptide structure use pairwise H-bonding interactions (which are ill-suited ro reproduce the energetics of cooperative H-bonds) to approximate H-bond energies, these methods cannot be expected to properly describe the potential surfaces of peptide systems. To the extent that empirical models might be useful, they must be reexamined in the context of H-bond cooperativity.[5]

(5) Changes in individual amino acids in complex α-helical structures can have a subtle but significant effect on the relative stabilities of α-helices and upon their H-bonding structures. Alkyl groups stabilize helices better than an H-atom (as in glycine). Those that have alkyl β-H's (Val, Leu, and Ile in this study) form stabilizing C−H···O with the nearest C═O, which increases the helical stability over that of the methyl side chain in Ala.

Acknowledgment. This work was supported in part by grants from the National Institutes of Health (S06GM60654), from the donors of the Petroleum Research Foundation administered by the American Chemical Society, and from PSC-CUNY.

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

JA035302Q

(33) Dannenberg, J. J. *THEOCHEM* **1997**, *401*, 279.