

Direct Calculation of *trans*-Hydrogen-Bond ^{13}C - ^{15}N 3-Bond J-Couplings in Entire Polyalanine α -Helices. A Density Functional Theory Study

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Received: July 24, 2006; In Final Form: December 26, 2006

We present the first *trans*-H-bond ^{13}C - ^{15}N 3-bond J couplings calculated from entire neutral and protonated α -helical polyalanines. The neutral helices considered are those of the capped peptides, acetyl(Ala)_NNH₂, where $N = 8, 16, 17$, and 18 , while the protonated peptides are the uncapped (Ala)₁₇ protonated at three different positions. The calculated J values correlate well with O...H distances and somewhat less well with N...O distances, particularly if the terminal H-bonds are eliminated from the correlation. The J values calculated using the entire helix are about 6% lower in magnitude than those recently reported for H-bonding chains whose geometries were extracted from the same helices. Aqueous solvation favors protonation of the α -helix on the terminal COOH. Experimental measurements of the *trans*-H-bond ^{13}C - ^{15}N 3-bond J couplings in acidic solution should be interpreted with this context.

Introduction

The first report of *trans*-hydrogen bond J coupling was in nucleic acids.¹ Since then, this technique has been used to measure the ^{13}C - ^{15}N scalar ^3hJ couplings that provides direct information on the C=O...H-N H-bonding interactions in peptides and proteins.^{2–13} The utility of this spectroscopic technique has been greatly enhanced by comparison to theoretical calculations of these J values, which have been calculated as a function of local geometry about the H-bonds in question. Early calculations were based exclusively upon the Fermi contact (FC) term and used restricted geometric models containing only one H bond.^{14–16} The local geometry was generally based upon the experimental positions of the heavy atoms (C, N, O) with optimization of the hydrogens within the otherwise constrained structures. Calculations on couplings within large peptides were simplified by extracting formamide dimers from the larger structures, terminating their valences with hydrogens, and optimizing the positions of the hydrogens. Bagno also used amino acids terminated with acetyl groups.¹⁴ The locations of the H-bonding hydrogens were also determined by geometric optimization as their positions are not accurately given by the crystallographic data used. These approximations have been justified by earlier reports.¹⁷

Recent experimental^{11,12} and theoretical¹⁸ reports have indicated that proximate H-bonds within delocalized H-bonding chains have a substantial influence upon the *trans*-H-bond Js of their neighbors. The recent theoretical report¹⁸ was based upon calculations of all four contributions to the coupling constants discussed by Ramsey,¹⁹ not just the Fermi-contact term. It used formamide chains whose geometries were adapted from the optimized structures²⁰ of acetyl(ala)₁₇NH₂ as models

for the *trans*-H-bond couplings for this capped peptide. At the time those calculations were performed, program and computer limitations rendered calculations on the entire structure of the peptide impractical if not impossible. Recent improvements in the Gaussian 03²¹ program have overcome these obstacles.

Many reports of calculated NMR coupling constants on more traditional systems have been reviewed,²² most recently by Contreras et al.²³ Coupling constants are among the most difficult molecular observables to accurately calculate using molecular orbital theory. Often, even large basis sets do not lead to satisfactory convergence to consistent or observed results.²⁴ However, the ^{13}C - ^{15}N 3-bond scalar J couplings (as well as three other *trans*-H-bond Js) are not so sensitive to basis set variation²⁵ as some other Js previously studied by similar methods.²⁴ This is consistent with the relative success of the calculations previously reported.

In this paper we present *trans*-H-bond ^{13}C - ^{15}N scalar ^3hJ couplings that were calculated using the *entire structures* of several optimized α -helical peptides. The peptides chosen for this study are (1) the same acetyl(ala)₁₇NH₂ that was modeled in the previous study, as this provides a comparison of the values calculated on the entire peptide with those of the reduced models, (2) two related structures with one more and one fewer alanine, and (3) three protonated tautomers of the acetyl-(ala)₁₇NH₂ whose optimized structures have been previously reported.²⁶ While angular dependence of these couplings have been reported,^{16,18,27} we have concentrated on the dependence with H-bond length as the angles show little variation within the helical structures.

Calculational Details

All calculations were performed using the same procedures used in our previous work.¹⁸ We used the Gaussian 03 suite of programs²¹ and the B3LYP functional. This method combines Becke's 3-parameter functional,²⁸ with the nonlocal correlation provided by the correlation functional of Lee, Yang, and Parr.²⁹

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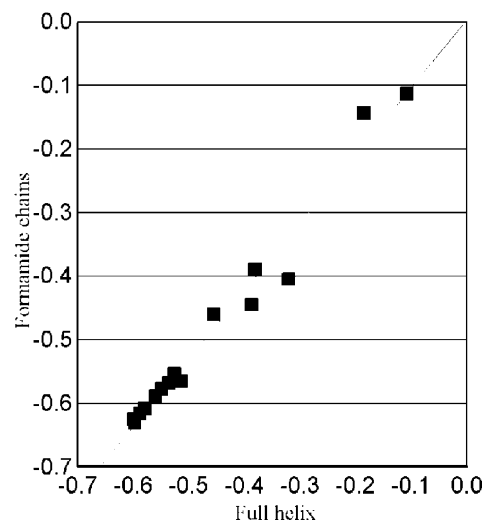
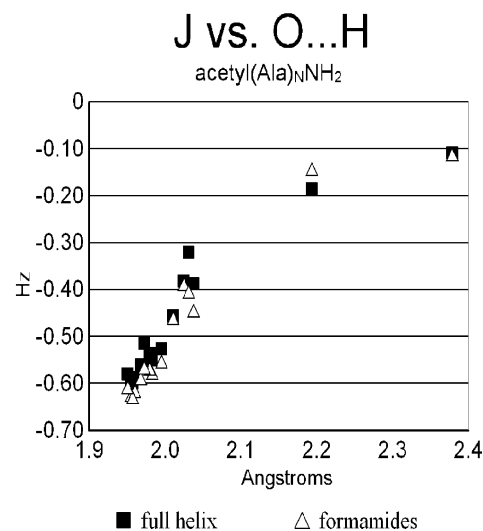
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TABLE 1: Calculated *trans*-H-bond ^{13}C – ^{15}N Scalar 3hJ Couplings Using the Complete Acetyl(ala) $_N$ NH $_2$ Helices (Values for $N = 17$ are Compared with the Formamide Chain Model of Ref 18)

H-Bond	chain	OH	J (Hz)	ref 18	ΔJ (Hz)	ratio
$N = 18$						
1	A	2.376	−0.107			
2	B	2.016	−0.450			
3	C	2.024	−0.384			
4	A	1.983	−0.550			
5	B	1.971	−0.571			
6	C	1.965	−0.588			
7	A	1.951	−0.604			
8	B	1.948	−0.613			
9	C	1.958	−0.581			
10	A	1.957	−0.594			
11	B	1.954	−0.577			
12	C	1.975	−0.537			
13	A	1.966	−0.535			
14	B	2.040	−0.386			
15	C	2.026	−0.329			
16	A	2.181	−0.199			
$N = 17$						
1	A	2.379	−0.109	−0.112	−0.003	1.032
2	B	2.010	−0.456	−0.460	−0.004	1.009
3	C	2.024	−0.381	−0.389	−0.007	1.020
4	A	1.995	−0.527	−0.554	−0.027	1.052
5	B	1.983	−0.550	−0.578	−0.028	1.050
6	C	1.955	−0.600	−0.625	−0.024	1.041
7	A	1.960	−0.588	−0.616	−0.028	1.048
8	B	1.968	−0.560	−0.589	−0.028	1.051
9	C	1.957	−0.598	−0.630	−0.032	1.053
10	A	1.950	−0.580	−0.608	−0.029	1.049
11	B	1.980	−0.537	−0.568	−0.032	1.059
12	C	1.973	−0.514	−0.566	−0.052	1.100
13	A	2.037	−0.388	−0.445	−0.057	1.148
14	B	2.031	−0.321	−0.405	−0.084	1.260
15	C	2.194	−0.185	−0.143	0.042	0.773
average					−0.026	1.050
					−0.033	1.072
$N = 16$						
1	A	2.349	−0.121			
2	B	2.017	−0.442			
3	C	2.040	−0.357			
4	A	1.989	−0.538			
5	B	1.987	−0.535			
6	C	1.971	−0.567			
7	A	1.963	−0.572			
8	B	1.969	−0.642			
9	C	1.966	−0.555			
10	A	1.975	−0.544			
11	B	1.957	−0.569			
12	C	2.058	−0.326			
13	A	2.041	−0.289			
14	B	2.203	−0.180			
$N = 8$						
1	A	2.371	−0.105			
2	B	2.033	−0.422			
3	C	2.121	−0.171			
4	A	2.137	−0.248			
5	B	2.080	−0.241			
6	C	2.280	−0.137			

As we previously noted no difference between (unrestricted) UB3LYP and (restricted) B3LYP calculations for several examples, we used B3LYP alone. All calculations used the D95-(d,p) basis set, which we have previously used in other studies of peptide and peptide-like H-bonds. We determined this basis set to be adequate for this purpose elsewhere,²⁵ as it predicted *trans*-H-bond ^{13}C – ^{15}N 3-bond scalar J couplings that were similar to those predicted by other similar and larger basis sets. The geometries of the species calculated are taken from previously optimized α -helical structures,^{20,26} as specified below. The coupling constants were calculated using the “NMR =

**Figure 1.** Values of *trans*-H-bond ^{13}C – ^{15}N scalar 3hJ couplings calculated from the full acetyl(ala) $_{17}$ NH $_2$ helix (this work) with those reported for the models made from the formamide chains (ref 18). The line represents a least-square linear fit with $y = 1.06x + 0.00$ and $r = 0.99$.**Figure 2.** Variation of calculated of *trans*-H-bond ^{13}C – ^{15}N scalar 3hJ couplings with O...H distance for the α -helices with $N = 8$ and 16 – 18 . Data from the current work is compared with that based upon the formamide chain model of ref 18.

spin–spin” option in Gaussian 03. This option calculates J values including contributions from the spin-dipole (SD) and both diamagnetic (DSO) and paramagnetic (PSO) spin–orbit terms in addition to the Fermi contact (FC) term.^{19,30–32}

While the releases of the Gaussian 03 program prior to revision C01 allowed calculation of the J values using all four contributions noted above, the program calculated all possible J values for the system under study. Our previous report¹⁸ was restricted to the interaction of eight or fewer formamides due to the limitations of our computers. Beginning with revision C01, one can specify which nuclei are of interest and restrict the coupling constant calculations to only these. Even with this revision, the calculation of all the *trans*-H-bond ^{13}C – ^{15}N 3-bond scalar J couplings in peptides as large as those reported here would be beyond our computer capabilities. To circumvent this problem, we restricted our calculations to one of the three H-bonding chains of the helix for each calculation. Thus, the data reported for each α -helix is a composite of results from three separate DFT calculations.

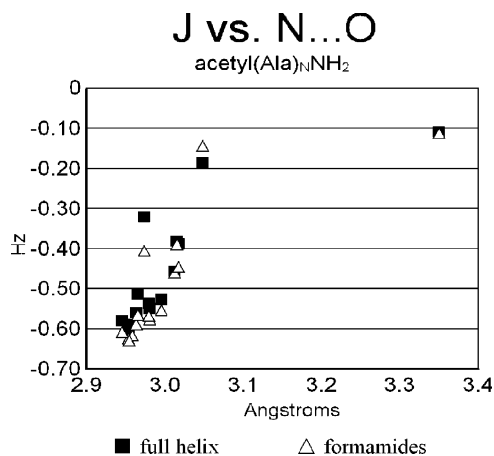


Figure 3. Comparison of calculated *trans*-H-bond ^{13}C – ^{15}N scalar 3hJ couplings with $\text{N}\cdots\text{O}$ distance using the full acetyl(ala) $_N\text{NH}_2$ helix (this work) and the formamide chain model of ref 18.

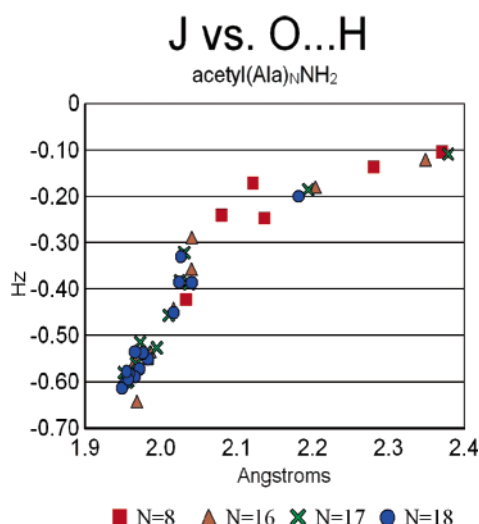


Figure 4. Variation of calculated *trans*-H-bond ^{13}C – ^{15}N scalar 3hJ couplings with $\text{O}\cdots\text{H}$ distance for the α -helices with $N = 8$ and 16 – 18 .

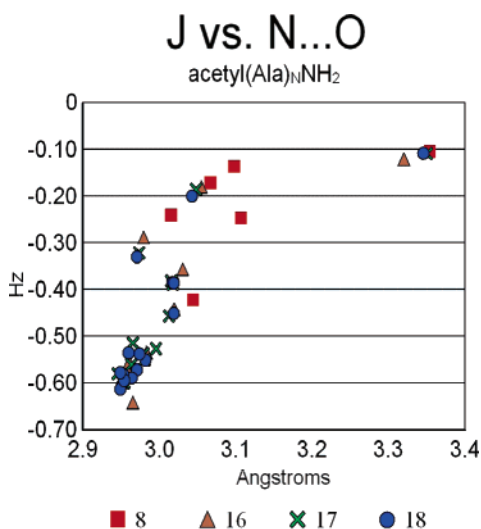


Figure 5. Variation of calculated of *trans*-H-bond ^{13}C – ^{15}N scalar 3hJ couplings with $\text{N}\cdots\text{O}$ distance for the α -helices with $N = 8$ and 16 – 18 .

The solvation energies of the helices were calculated using the SM5.2 method of Cramer and Truhlar³³ as coded in the

Ampac 8.15 program.³⁴ These calculations used the AM1³⁵ Hamiltonian at the fixed geometries that correspond to the DFT optimized structures previously reported.

Results and Discussion

Neutral Helices. We used the previously reported²⁰ completely optimized geometries of α -helical acetyl(ala) $_N\text{NH}_2$ ($N = 8, 16$ – 18) as the starting points for these studies. We chose these values of N as $N = 8$ corresponds to the smallest stable α -helix that we found to form, while $N = 16$ – 18 are representative of larger, more stable, α -helices that have (a) the same number of H-bonds in each of the three H-bonding chains ($N = 17$), (b) two chains with one more H-bond than the third ($N = 16$), and (c) two chains with one fewer H-bond than the third ($N = 18$). Table 1 presents the calculated *trans*-H-bond ^{13}C – ^{15}N 3-bond scalar J couplings for these three helices and compares them with the geometric parameters of the optimized structures. We have included the previously reported¹⁸ couplings for acetyl(ala) $_{17}\text{NH}_2$, which were based upon formamide chains constrained to the geometries that correspond to the optimized helical structure (except for the C–H and N–H bond lengths at the positions where the peptide bonds were cut). As seen from Figures 1 and 2, the J values calculated from the formamide chains are about 6% larger in magnitude (i.e., more negative) than those calculated directly from the α -helix. Figure 3 compares the dependence of J with $\text{N}\cdots\text{O}$ distance in the helix and chains.

The average value of J for this helix is -0.46 Hz. This value is consistent with a report of the J values for the α -helical portion of ubiquitin as a function of temperature that indicated that the J values become more negative as the temperature decreases. The largest average J reported was slightly less than -0.4 Hz at 5°C , the lowest reported temperature.⁸ Presumably the average J would continue to increase in magnitude if the temperature could be lowered further. Markwick et al. have reported that a better fit of calculated J values could be obtained by combining molecular dynamics with DFT calculations of the J values.³⁶ However, their calculations used only the FC terms and did not account for the effect of proximate H-bonds. Inclusion of the three other Ramsey terms and the effect of proximate H-bonds both would tend to increase the magnitudes of the calculated J values.^{11,12,25}

Figures 4 and 5 show the variations of the calculated J values with $\text{O}\cdots\text{H}$ and $\text{H}, \text{N}\cdots\text{O}$ distances for all four α -helices. The correlation with the $\text{O}\cdots\text{H}$ is clearly superior to that with the $\text{N}\cdots\text{O}$ distances; however, the $\text{O}\cdots\text{H}$ distances cannot be easily obtained from protein crystallographic data. The relation between J and $\text{O}\cdots\text{H}$ distances under 2.1 Å has a steeper slope than that with $\text{O}\cdots\text{H}$ greater than 2.1 Å.

Protonated Helices. The NMR spectra of α -helices in solution often are measured under acidic conditions as the helices appear to be more stable at low pH. For example, Grzesiek^{3,37} reported couplings measured at pH 4.5 and 2, Kemp³⁸ has measured helicities at pH 1–8, while Yamazaki et al. have shown that the polyalanine part of a block copolypeptide, Glu $_{12}$ –Ala $_{12}$ become more stable at lower pHs and are fully extended at pH < 4 .³⁹

We have recently shown that α -helices are more easily protonated on any one of the three C=Os nearest the C-terminus than on the terminal NH_2 ,²⁶ which agrees with a suggestion made earlier.⁴⁰ Protonation on these C=Os becomes more facile

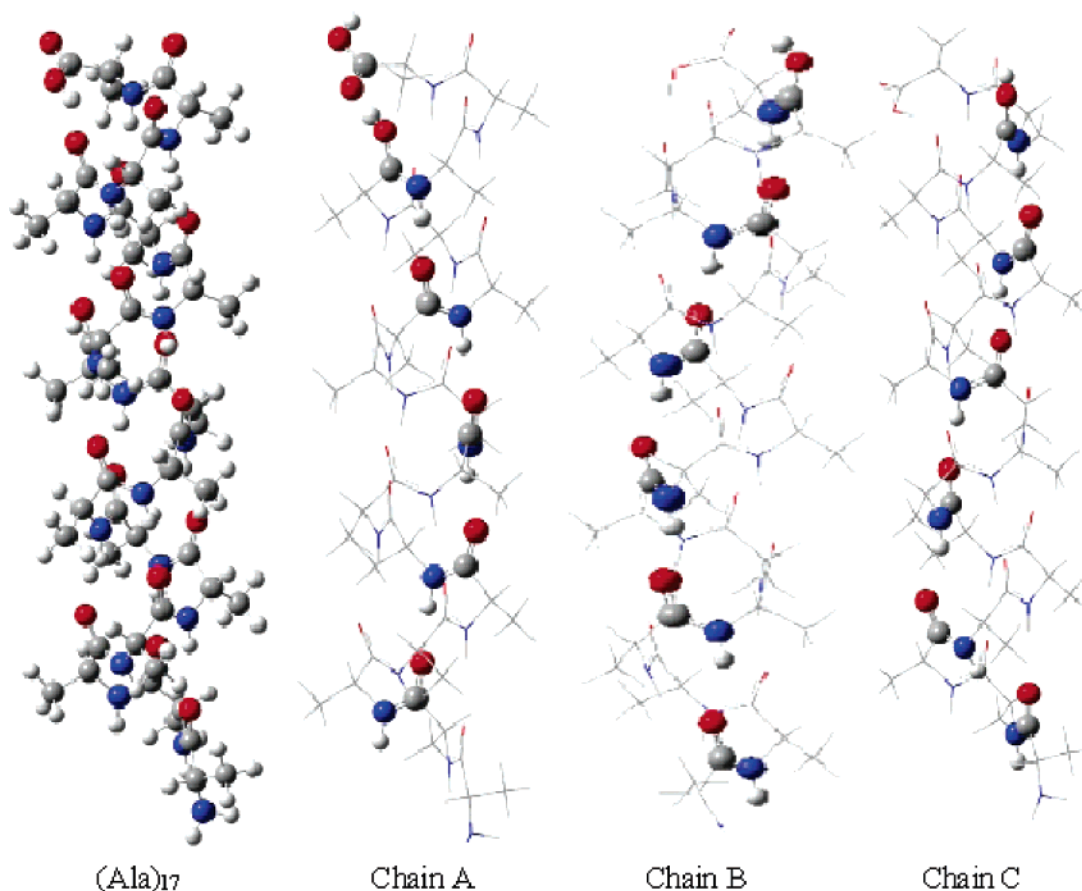


Figure 6. The (Ala)₁₇ α -helix unprotonated and protonated on each H-bonding chains (emphasized in the graphic). The helices are oriented with the C-terminus pointing up. The protonated C=O is the uppermost of each of the protonated chains.

as the chain of amide H-bonds becomes longer, while protonation on one of the C=Os that does not act as an acceptor for a helical H-bond, we thought it likely that their ^{13}C – ^{15}N 3J couplings would be affected by such protonation. Therefore, we calculated these J values for the three cases of uncapped (Ala)₁₇ protonated on each of the three H-bonding chains as defined in Figure 6. We used the optimized geometries previously reported.²⁶ Table 2 and Figures 7 and 8 present the effects of protonation on each of the three H-bonding chains in this α -helix. The calculated J values correlate extremely well with the O \cdots H distances, especially if one eliminates the terminal H-bonds of each chain from the correlation. The one case (protonation on Chain B) where the dependence of J with the terminal O–H distance J fits the correlation occurs when the protonated chain contains the terminal H-bond.

Since the H-bonds become stronger and shorter upon protonation on one of the C=Os that does not act as an acceptor for a helical H-bond, we thought it likely that their ^{13}C – ^{15}N 3J couplings would be affected by such protonation. Therefore, we calculated these J values for the three cases of uncapped (Ala)₁₇ protonated on each of the three H-bonding chains as defined in Figure 6. We used the optimized geometries previously reported.²⁶ Table 2 and Figures 7 and 8 present the effects of protonation on each of the three H-bonding chains in this α -helix. The calculated J values correlate extremely well with the O \cdots H distances, especially if one eliminates the terminal H-bonds of each chain from the correlation. The one case (protonation on Chain B) where the dependence of J with the terminal O–H distance J fits the correlation occurs when the protonated chain contains the terminal H-bond.

In order to correlate the calculations with experimental measurements of the J values, one would need to fit a weighted average of the three protonated and the unprotonated J values for each H-bond with experimental J values measured at different pHs. Since protonation would both increase the magnitude of the J values and make the helix more stable relative to dynamical unraveling, obtaining such correlations may seem to be a daunting task. Nevertheless, the data we present here should provide a reference for those NMR spectroscopists who wish to undertake it.

The gas-phase proton affinities of the three C=Os that correspond to the three protonated H-bonding chains are quite similar, 276.5, 278.0, and 279.0 kcal/mol, respectively, for

protonation on chains A, B, and C.²⁶ These values are in ΔE rather than ΔH , as they are not corrected for vibrations. In particular, the additional three vibrations created when a proton becomes attached would lower these values (for example, the ΔE value for protonating water of 176.5 becomes a ΔH of 168.3 kcal/mol upon vibrational correction). Nevertheless, the relative gas-phase proton affinities in ΔH should be correct if one makes the reasonable assumption that the vibrational corrections for protonation at each position are equivalent. However, in aqueous solution, the relative basicities of these positions might be quite different. To obtain the basicities in solution, we calculated the solvation free energies for each of the protonated helices using the SM5.2 method of Cramer and Truhlar.³³ These were calculated using single-point AM1 calculations with structures fixed at the DFT-optimized geometries, as described above (see data in Table 3). The solvation free energies of the three protonated helices differ considerably.

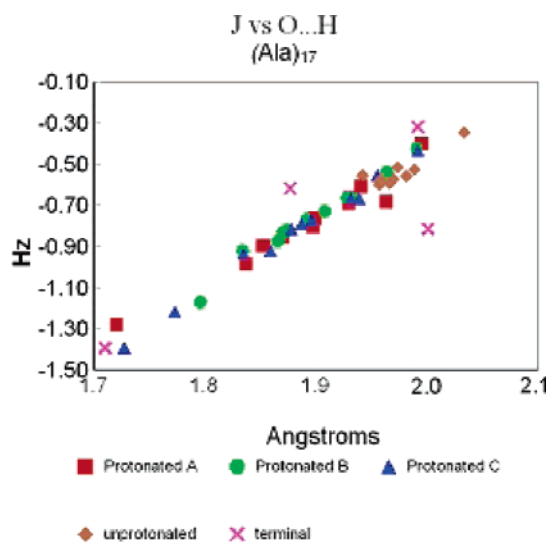
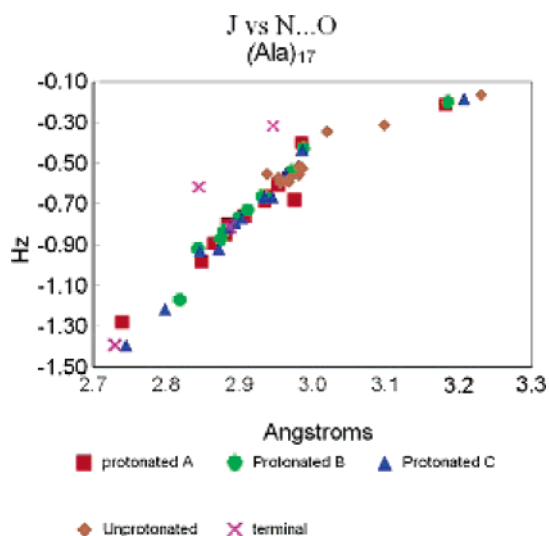
Aqueous solvation is greatest for protonation on chain A (57.2 kcal/mol). In this case, the protonated COOH group is exposed to the solvent. Aqueous solution stabilized the structures protonated on chains B and C by 28.8 and 48.9 kcal/mol respectively. For the least-well-solvated structure, protonated on chain B, both oxygens of the COOH are involved in internal H-bonds, rendering them less available to the solvent. Combining the gas-phase protonation energies (uncorrected for vibrations) with the solution free energies suggests protonation on chain A to be favored by 5.8 kcal/mol. Here again, the validity of the relative solvated energies obtained in this manner depends upon the cancellation of errors in the combination of relative proton affinities (either in ΔE or ΔH) and the ΔG values of solvation calculated for protonation at the three sites. The

TABLE 2: Calculated *trans*-H-bond ^{13}C – ^{15}N Scalar 3hJ Couplings (Hz) and Geometrical Parameters (Å) for Each H-Bond^a

HB no.	chain	J	O...H	C...N	N...O
Protonated on Chain A					
3 ₁₀	3 ₁₀	−0.004	2.094	3.839	2.981
1	A	−0.214	2.194	4.371	3.182
2	B	−0.609	1.941	4.115	2.953
3	C	−0.401	1.995	4.053	2.985
4	A	−0.691	1.930	4.103	2.935
5	B	−0.665	1.931	4.106	2.935
6	C	−0.763	1.899	4.081	2.906
7	A	−0.802	1.897	4.054	2.883
8	B	−0.854	1.870	4.059	2.881
9	C	−0.896	1.852	4.040	2.864
10	A	−0.984	1.836	4.026	2.848
11	B	−1.280	1.719	3.919	2.739
12	C	−0.682	1.963	4.171	2.975
13	A	−0.618	1.877	3.999	2.844
avg		−0.728			
Protonated on Chain B					
3 ₁₀	3 ₁₀	−0.006	2.099	3.845	2.982
1	A	−0.203	2.203	4.372	3.185
2	B	−0.541	1.964	4.131	2.971
3	C	−0.427	1.991	4.069	2.987
4	A	−0.670	1.933	4.101	2.937
5	B	−0.668	1.928	4.100	2.931
6	C	−0.729	1.908	4.084	2.910
7	A	−0.769	1.892	4.072	2.899
8	B	−0.822	1.874	4.056	2.882
9	C	−0.835	1.870	4.052	2.878
10	A	−0.921	1.834	4.019	2.843
11	B	−0.875	1.866	4.059	2.874
12	C	−1.171	1.795	4.002	2.818
13	A	−1.392	1.709	3.906	2.729
avg		−0.771			
Protonated on Chain C					
3 ₁₀	3 ₁₀	−0.009	2.091	3.842	2.976
1	A	−0.185	2.224	4.393	3.207
2	B	−0.554	1.955	4.122	2.962
3	C	−0.432	1.992	4.071	2.985
4	A	−0.666	1.939	4.114	2.944
5	B	−0.667	1.931	4.105	2.934
6	C	−0.768	1.896	4.079	2.902
7	A	−0.813	1.877	4.060	2.886
8	B	−0.790	1.887	4.066	2.893
9	C	−0.932	1.835	4.022	2.845
10	A	−0.921	1.859	4.056	2.871
11	B	−1.217	1.772	3.971	2.797
12	C	−1.394	1.726	3.929	2.744
13	A	−0.813	2.001	4.093	2.887
avg		−0.781			
Unprotonated					
3 ₁₀	3 ₁₀	−0.008	2.089	3.844	2.984
1	A	−0.166	2.249	4.414	3.231
2	B	−0.516	1.974	4.137	2.981
3	C	−0.346	2.034	4.086	3.020
4	A	−0.558	1.981	4.143	2.981
5	B	−0.525	1.989	4.149	2.986
6	C	−0.598	1.957	4.124	2.956
7	A	−0.583	1.957	4.117	2.954
8	B	−0.570	1.970	4.134	2.969
9	C	−0.571	1.959	4.113	2.953
10	A	−0.592	1.967	4.134	2.967
11	B	−0.553	1.942	4.086	2.938
12	C	−0.314	2.121	4.248	3.098
13	A	−0.317	1.992	4.091	2.946
avg		−0.478			

^a The 3₁₀ interaction is for the bifurcated terminal H-bond. The H-bonds are numbered starting with the N-terminus. The H-bonding chain for each H-bond is indicated. The averages exclude the 3₁₀ H-bonds.

relative ΔG values of solvation will be correct under the reasonable assumption that the ΔS values of both protonation

**Figure 7.** Variation of calculated *trans*-H-bond ^{13}C – ^{15}N scalar 3hJ couplings with O...H distance using the full protonated and unprotonated (ala)₁₇ helices.**Figure 8.** Variation of calculated *trans*-H-bond ^{13}C – ^{15}N scalar 3hJ couplings with N...O distance using the full protonated and unprotonated (ala)₁₇ helices.**TABLE 3: Proton Affinities, PA (in ΔE , Uncorrected for Vibrations), in Gas Phase and Aqueous Solution (kcal/mol) (Only Relative PAs should be Considered to be Reasonably Accurate)**

species	PA (gas) ^a	ΔG solv	PA (aq)	PA (aq) rel
unprotonated		−66.6		
protonated A	276.5	−57.2	267.0	0.0
protonated B	278.0	−28.8	240.2	−26.8
protonated C	279.0	−48.9	261.2	−5.8
water		−6.6		
H ₃ O ⁺	176.5	−99.7	269.6	

^a From ref 26.

and solvation are equivalent for protonation at each site of the helix. Thus, the solvation calculations predict that only protonation on chain A should occur in aqueous solution.

The extent of protonation requires a more difficult calculation. The aqueous solvation free energy for the neutral helix (−66.6 kcal/mol) calculated in the same manner is 9.5 kcal/mol greater in magnitude than that for the best solvated protonated species (on chain A). While this might seem surprising, the large dipole moment of the neutral helix (65.6 Debye) is greatly diminished

(to 23.6, 20.2, and 21.9 Debye at chains A, B, and C, respectively) by protonation on its negative end of the neutral helix. We can calculate a "proton affinity" in aqueous solution by correcting the gas-phase proton affinity by the difference in solvation energies between the neutral and protonated species. By inclusion of these differences in solvation energy, the aqueous proton affinity for chain A is reduced to 267.0 kcal/mol. Calculation of the proton affinity of aqueous water in the same manner leads to a proton affinity of 269.6 kcal/mol, only slightly greater than that for the protonated helix. Since the proton affinities appear to be reasonably similar, one expects a change in the equilibrium between protonated and unprotonated helix within the normally accessible pH range. However, while we assumed the ΔS values of both protonation and solvation to be equivalent for protonation at each site of the helix, we cannot reasonably assume that these ΔS values would also be equivalent for water, making a pK_a for the conjugate acid of the helix difficult to estimate. Systematic experimental measures of *trans*-H-bond ^{13}C – ^{15}N 3-bond J couplings at varying pH would be useful to verify our suggestions.

Conclusions

The *trans*-H-bond ^{13}C – ^{15}N 3-bond J couplings calculated from entire optimized α -helices differ from those calculated from the H-bonding chains extracted from these same helices by about 6% on average. As both experimental measurements and theoretical calculations have previously shown that the J values calculated from the chains differ substantially from those of individual dimers extracted from the same helices, the calculated *trans*-H-bond ^{13}C – ^{15}N 3-bond J couplings from amide dimers extracted from protein structures should be interpreted with appropriate caution.

The *trans*-H-bond ^{13}C – ^{15}N 3-bond J couplings substantially increase upon protonation of the helices. In accord with previous reports for unprotonated α -helices,^{16,18} correlations of *trans*-H-bond ^{13}C – ^{15}N 3-bond J couplings with O \cdots H distances within the helices are good if the terminal H-bonds are eliminated from the data. Similar correlations with N \cdots O distances are of somewhat lower quality. While the proton affinities of the α -helix of (Ala)₁₇ at each of the C=Os that terminate the H-bonding chains are very similar, the protonation at the COOH becomes favored in aqueous solution.

Acknowledgment. This work was supported in part by grants from the donors of the Petroleum Research Foundation administered by the American Chemical Society and from PSC/CUNY. J.J.D. thanks DURSI of the Generalitat de Catalunya for a visiting professorship at the Universitat de Girona.

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