Stabilization of Helices in Glycine and Alanine Dipeptides in a Reaction Field Model of Solvent

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Abstract: We present molecular orbital calculations of the full conformational space of blocked glycine and alanine dipeptide in the presence of a reaction field representation of water. Secondary structures of right- and left-handed helices are found, in contrast to recent gas-phase results, indicating that the origin of helical stabilization in dipeptides is strictly due to environment. Limitations of the reaction field model and the various implications of stabilization due to environment are discussed.

Introduction

The origin of secondary structure in folding proteins is an active area of research. Three key questions are (1) whether particular amino acids have an intrinsic propensity to form secondary structures, (2) at what polypeptide lengths do secondary structures become stable, and (3) what are the time scales and molecular forces activating and stabilizing secondary structure formation.[1-10] We can begin to address these questions by interrogating the conformational preferences of peptides in gas and solution phases with the best theoretical means possible. The value of ab initio electronic structure methods was recognized early[11-15] on as a suitable means for evaluating biologically relevant conformational minima for peptides in the gas phase. Recent algorithmic and computer hardware advances permit these same systems to be studied at higher levels of theory,[16-19] to investigate their conformational space more fully,[16,17] and to incorporate some aspects of aqueous environment.[20-23] There is growing consensus[16-19] that reasonable levels of molecular orbital theory are robust enough to capture the relative energy ordering of the intramolecular hydrogen-bonding conformational minima of glycine and alanine dipeptide in the gas phase, although relative energies may differ from 1-2 kcal/mol depending on the theory used. We also wish to reliably model the electronic structure of these molecules in an aqueous environment, and this work reports our first exploration of solvation of small dipeptides, using the Onsager reaction field model.[24] The systematic investigation of small peptides in vacuum and solution is especially relevant for secondary structures such as helices and turns, where spatially localized interactions are more likely to dominate.

The conformational space of these molecules is described by the backbone dihedral angles ϕ and ψ.[25] For peptide chains of at least four amino acids, particular ϕ, ψ values give rise to well-defined hydrogen bond patterns of secondary structure such as the α-helix, β-sheet, and turns.[25] Whether specific secondary structures are energetically stabilized in smaller lengths such as dipeptides is more uncertain, since hydrogen bonding at the relevant values of ϕ and ψ is not possible for these short lengths. Ramachandran maps indicate that the helix, turn, and sheet regions should be energetically accessible for dipeptides, and popular empirical protein force fields[26,27] exhibit stable secondary structure minima in the gas phase. We note that the early ab initio results of Schäfer showed no stabilization of the right-hand helix, but the left-handed helix and β structures were found to be minima for alanine dipeptide.[15] We have recently completed a high-level ab initio gas-phase study of α-(formylamino)-ethanamide (GDA) and (*S*)-α-(formylamino)propanamide (ADA).[16,17] They are closely related to blocked glycine and alanine dipeptide, respectively, where the free rotor methyls of the blocked species have been replaced by hydrogens. Our ab initio results,[16,17] subsequent studies,[28,29] and molecular orbital calculations using even more sophisticated methods[18,19] indicate that there is no intrinsic propensity for glycine and alanine dipeptide to form any secondary structure in the gas phase and that any stabilization of secondary structure conformers would strictly be a function of environment for these molecules.

In this work, we apply a continuum reaction field treatment of environment as originally introduced by Onsager.[24] While

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this highly simplified representation of solvent may seem incommensurate with the high levels of theory used to optimize the electronic structure of these molecules, we provide physical arguments that the reaction field may be adequate in the helical region of ϕ, ψ space. We therefore conclude that the origin of helical structure in dipeptides may be due to environment, although further studies employing more realistic solvent models are highly desirable. We also argue that the reaction field treatment of dipeptide conformations in solution is qualitatively incorrect in other regions of ϕ, ψ space due to the absence of molecular detail; these errors will likely be sustained with more advanced reaction field treatments.[21,30-33] The origin of helical stabilization in dipeptides bears directly on the design of initiation parameters for Zimm–Bragg theory,[1,2] early folding theories,[3,9] and intrinsic helix propensities of the various amino acids investigated with simulation[8] and within de novo designed peptides.[5-7]

Methods

Our gas-phase results have been presented in detail before, and we simply summarize the methods used to obtain those results.[16,17] The small split valence 3-21G basis[34] was chosen to generate a grid in 15° increments for GDA (Figure 1a) and ADA (Figure 2a), as a means for exploring the full conformational space. Based on the HF/3-21G grids, additional full geometry optimizations were performed at both HF/3-21G and HF/6-31+G* [34] to determine all stationary points. Relative energies were also determined with MP2/6-31+G**//HF/6-31+G*.[34] An important conclusion is that shallow minima (stabilized by 1 kcal/mol or less) corresponding to secondary structure on the HF/3-21G map disappear altogether at the more reliable HF/6-31+G* level of theory for both GDA and ADA.[16,17] The gas-phase studies indicate that such small peptides do not intrinsically exhibit stable secondary structures. It is noteworthy that the protein force field CHARMM[35] exhibits the same number and type of gas-phase minima as that found with the best quantum mechanical calculations done to date; that is, there are no stable secondary structure minima on the CHARMM gas-phase surface.

The self-consistent reaction field (SCRF)[21,36,37] method has been used to advantage in describing the influence of a dielectric solvent on conformational equilibria of organic compounds. In this theory, the electric field of the solute polarizes the surrounding dielectric medium in such a way as to produce a reaction field, which in turn interacts with the charge distribution of the solute.[24] The primary approximations inherent in the theory are that the electrons of the solute are distinguishable from that of the immediate surroundings of solvent, and that specific interactions with molecular solvent are unimportant.[36] These approximations are manifested by enclosing the solute in a low-dielectric geometry, such as a sphere, and replacing the surrounding molecular solvent with an appropriately chosen value of the dielectric constant. We note that the solute charge distribution is limited to the molecular dipole moment (expanded about the center of charge, which is also the cavity origin) in its interaction with the reaction field,[37,38] and we return to this point in the next section.

We have used the Gaussian series of programs[38] to generate both gas and reaction field solvent-modified molecular orbital calculations of the full conformational space of GDA and ADA using HF/3-21G. A quantum mechanical approach[37] was used to estimate the spherical cavity radius. At each ϕ, ψ grid point, the spherical radius was approximated by calculating the electron density envelope out to 0.001 au using HF/3-21G, scaling by 1.33, and adding 0.5 Å to account for nearest approach of water molecules.[37] The HF/3-21G radii were ~3% smaller than those evaluated at HF/6-31+G* for certain stationary points discussed below, and both methods gave volumes which easily accommodated the physical size of any given conformer.

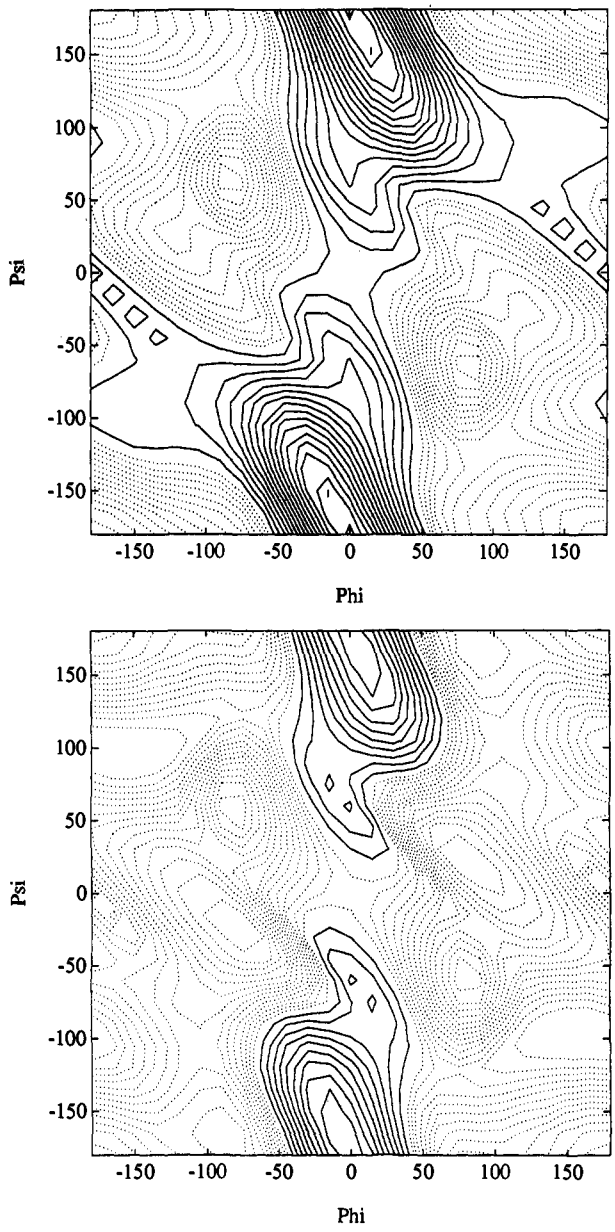

Figure 1. (a, top) The (ϕ, ψ) surface for (*S*)-2-(acetylamino)-*N*-methylethanamide. The ϕ and ψ variables are held fixed at each grid point (15° spacing), and all other degrees of freedom are relaxed. The dashed lines denote contours of 0.5 kcal/mol and extend from the zero of energy to 7.0 kcal/mol. Solid contours are drawn every 1.0 kcal/mol thereafter. Secondary structures disappear at higher levels of theory. (b, bottom) The solvent-modified (ϕ, ψ) surface generated by HF/3-21G using a reaction field model of aqueous environment. Each grid point involved the calculation of a cavity radius.[30] Helical minima are stable at higher levels of molecular orbital theory.

Results and Discussion

Figures 1 and 2 display the ϕ, ψ conformational space of GDA and ADA evaluated at HF/3-21G in vacuum and reaction field, respectively. A visual comparison of the gas-phase and solvent-modified maps indicate that the influence of a solvent dielectric of 80 results in the well-appreciated flattening of the energy

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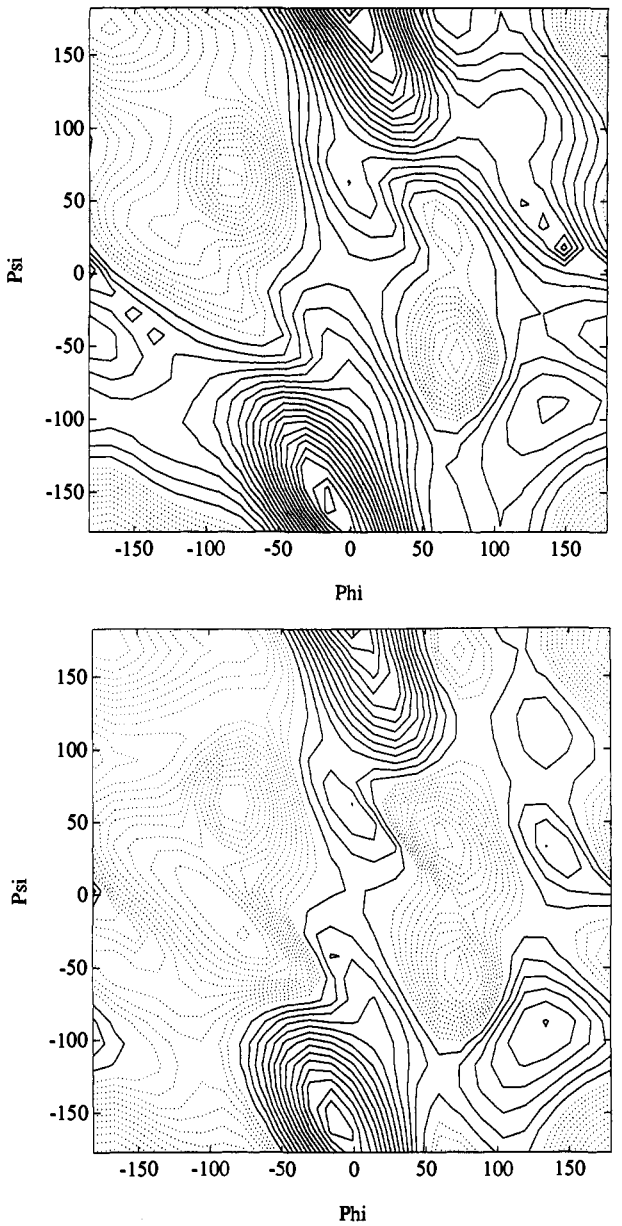

Figure 2. (a, top) The gas-phase (ϕ, ψ) surface of (*S*)-α-(formylamino)-propanamide generated by HF/3-21G. Secondary structures are unstable at higher levels of theory. (b, bottom) The solvent-modified (ϕ, ψ) surface of (*S*)-α-(formylamino)propanamide. Helical minima are also stable at higher levels of theory in ADA. See Figure 1 and text for all other details.

surface.[39] The more interesting feature is the appearance of the right-handed helix, and a deepening of the left-handed helix minimum, with inclusion of a dielectric environment. Tables 1 and 2 contain a selection of stationary points found at HF/3-21G for GDA and ADA in gas and solution phases. Based on our experience with the gas-phase maps, confirmation of results obtained at HF/3-21G is required, and we have used HF/6-31+G* to investigate the energy barriers between the helical minima and the C7 conformers. Table 3 provides the stationary points and their energies evaluated at HF/6-31+G* in the reaction field. Table 3 also contains MP2 single-point energies for GDA in the presence of a reaction field, and these calculations merely demonstrate that the effect of correlation is small. We did not find it appropriate to pursue such accuracy for ADA, since the solvent model is so crude relative to the computational cost of an MP2 reaction field calculation (on the order of an MP2 gradient[37]). The tabulated results confirm the persistence of the helical minima with better basis sets and inclusion of correlation, and provide a robust conclusion that helical structures are stabilized strictly by an Onsager reaction field representation of solvent in these small peptides.

Certainly the highly simplified treatment of solvent environment, which may seem incompatible with the sophisticated molecular orbital theories used to describe the electronic structure in these molecules, is suspect. The primary weaknesses of the reaction field model we have used are (1) truncation of the solute charge distribution at the dipole level, (2) the use of a spherical cavity and the associated uncertainty in the cavity radius, and (3) the lack of molecular water for at least the first solvation shell. Because we have evaluated a full conformational map for GDA and ADA, there will be variations in the severity of the three weaknesses depending on the values of ϕ and ψ. Since we are primarily concerned with the origin of helical secondary structure in these small peptides, we will argue on physical grounds that the helix should be net stable, while other reaction field minima will be destabilized, with a more realistic treatment of solvent.

The primary polar groups in GDA and ADA are the two peptide groups flanking the α-carbon, and we may consider their electrostatic potential to be described with a dipole centered at the midpoint of the C–N bond. We mentally divide the maps appearing in Figures 1b and 2b into ϕ, ψ quadrants of + +, + –, – +, and – – and draw two lines, from + + to – – and from – + to + –.[40] The ϕ, ψ direction corresponding to the + + to – – line approximates the positions of the intramolecular-hydrogen-bonding conformers C5 and C7(s). The charge distribution along this line is primarily due to the "dipole" of the intramolecular hydrogen bond, since the two peptide dipoles are aligned antiparallel[40] and hence nearly cancel. The reaction field representation of solvent fails spectacularly along this ϕ, ψ line because of the stabilization it affords the intramolecular hydrogen bond, whose influence should wane in a molecular model of solvent where alternative means for hydrogen bonding are made available. Stabilization due to higher order multipoles of the solute will be unlikely to overcome the $1/r$ dependence of this destabilizing interaction, and more physically satisfying cavity geometries will not capture explicit intermolecular hydrogen bonding of water in the close vicinity of the solute. Thus, more sophisticated treatments of the reaction field will also likely fail in this region of ϕ, ψ space.[21,30-33]

Along the – + to + – lines near the helical minima, however, the peptide dipoles are aligned parallel,[40] a repulsive interaction in the gas phase (and providing a likely explanation for their disappearance on the gas-phase map) but strongly stabilized in the reaction field. It is not clear how the introduction of molecular solvent should alter this picture since there are no intramolecular hydrogen bonds to disrupt. One water molecule could stabilize the aligned dipoles by contributing two hydrogens which interact with the two carbonyl oxygens, for example. A supermolecule approach to the reaction field may resolve the validity of this hypothesis, where complexed water molecules are included in the calculation as part of the solute. Nonetheless, the helical region has one of the largest and physically realistic molecular dipoles, so that the reaction field may be qualitatively correct in this region. Due to the vast reordering of energy minima expected for molecular treatments of solvent and the fact that the positions of the transition structures between the helices and C7 conformers bisect the two lines discussed above, the reaction field treatment in this region is more uncertain. Because the nonzero quadrupole is neglected in the charge distribution, further energy lowering of the transition states is expected, while the influence of molecular

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Table 1. HF/3-21G Vacuum and Reaction Field Structures and Energies for GDA

	vacuum			reaction field			
structure	ϕ (deg)	ψ (deg)	E (kcal/mol)	ϕ (deg)	ψ (deg)	E (kcal/mol)	a_0 (Å)
C7	−83.3	64.7	0.00[a]	−81.8	59.2	0.07	3.78
C5	−180.0	180.0	0.65	−180.0	180.0	0.84	3.78
β	−121.9	25.2	3.27				
helix				−75.0	−27.7	0.00[b]	3.78
PII				−85.3	−166.4	2.20	3.80
$\beta \rightarrow$ C7	−110.4	38.4	3.55				
helix $\rightarrow$ C7				−92.4	27.7	2.75	3.80
helix $\rightarrow$ PII				−104.8	−106.7	3.80	3.80
C5 $\rightarrow$ PII				−115.3	−174.0	2.70	3.80

[a] Zero of energy = −373.6487903 hartrees. [b] Zero of energy = −373.6522729 hartrees.

Table 2. HF/3-21G Vacuum and Reaction Field Structures and Energies for ADA

	vacuum			reaction field			
structure	ϕ (deg)	ψ (deg)	E (kcal/mol)	ϕ (deg)	ψ (deg)	E (kcal/mol)	a_0 (Å)
$C7_{eq}$	−84.5	67.3	0.00[a]	−83.4	63.5	0.00[b]	3.99
C5	−168.4	170.5	1.26	−170.9	172.5	1.26	4.00
$C7_{ax}$	74.1	−57.3	2.53	73.1	−53.4	2.02	3.99
l-helix	63.8	32.7	5.95	61.1	38.4	1.78	4.00
PII	67.5	−177.3	8.16	70.6	168.2	5.53	3.99
β	−128.0	29.7	3.83				
r-helix				−74.5	−28.3	1.63	3.99
$\beta \rightarrow C7_{eq}$	−116.7	42.5	4.04				
r-helix $\rightarrow C7_{eq}$				−99.4	32.0	3.51	4.01

[a] Zero of energy = −412.4747800 hartrees. [b] Zero of energy = −412.4771931 hartrees.

Table 3. Reaction Field Results for Helical Minima and Transition Structures for GDA and ADA at HF/6-31+G*, and MP2 Energies for GDA Only

	HF/6-31+G*				MP2/6-31+G**//HF/
	ϕ (deg)	ψ (deg)	E (kcal/mol)	a_0 (Å)	6-31+G* E (kcal/mol)
GDA structure					
helix	−84.5	−21.3	0.00[a]	4.00	0.00[c]
C7 $\rightarrow$ helix	−83.5	23.6	2.12	3.97	1.50
ADA structure					
r-helix	−81.3	−24.6	0.00[b]	4.10	
l-helix	65.3	36.1	2.38	4.09	
r-helix $\rightarrow C7_{eq}$	−85.4	21.3	2.27	4.09	
l-helix $\rightarrow C7_{ax}$	71.9	−3.7	4.00	4.08	

[a] Zero of energy = −375.7672683 hartrees. [b] Zero of energy = −414.8029185 hartrees. [c] Zero of energy = −376.8822327 hartrees.

water is unclear. The conclusions reached in this section await confirmation with alternative reaction field implementations[21,30-33] and inclusion of molecular solvent.[22,23]

Conclusion

Robust levels of molecular orbital theory used to evaluate the full conformational space of GDA and ADA in vacuum and in a reaction field model of solvent indicate that solvent environment is a key factor in stabilizing helical minima for small peptides with no explicit means to hydrogen bond. The molecular origin of a dipeptide helical minimum in the reaction field model is the stabilization it imparts to the aligned peptide dipoles, which is a repulsive interaction in the gas phase. We have argued that the reaction field model may be qualitatively correct in the helical region, in spite of its many well-appreciated shortcomings,[33] and that more sophisticated treatments of the reaction field or the introduction of molecular water will not alter this finding. Empirical force field calculations of a model of alanine dipeptide in molecular water support this conclusion[41,42] (where the gas-phase results are quite good in the case of ref 40), although inherent structure minima have not been found to relate those studies to the results presented here. A recently introduced semiempirical continuum solvent model,[32] more advanced than the Onsager reaction field treatment, also shows greater stabilization for the right-hand helix than the intramolecular-hydrogen-bonded conformers in alanine dipeptide. We also emphasize that any reaction field model will be qualitatively incorrect in the regions of ϕ, ψ space corresponding to the intramolecular-hydrogen-bonding conformers, since they lack a description of explicit intermolecular-hydrogen-bonding interactions. While more sophisticated reaction field models[21,30-33] should be explored for possible benefits overlooked here, our next level of study is to introduce molecular aqueous water into the calculations of the full conformational space to provide better structures and energies of the modified conformational properties of dipeptides due to solvation.

Our preliminary conclusions on solvent stabilization of helical secondary structure minima of small peptides bear on several aspects of protein folding. First, designed initiation parameters used in helix–coil transition theories[1,2] are thought to overestimate the difficulty of helix initiation.[8] Stabilization of the helix at the dipeptide level would support this view. Secondly, the validity of intrinsic helix propensity might be investigated with the reaction field model of solvent in the helical region of all twenty commonly occurring amino acids and may provide an interesting alternative to helix promotion scales derived from statistical analyses[43] and experiments on designed peptides.[5-7] Finally, the relative importance of solvent and intrinsic side-chain conformational preferences is often debated in the interpretation of experiments and theories concerning early folding events.[3,9] Interestingly, polypeptide collapse (solvent induced) and secondary structure formation (solvent induced?) occur on roughly the same time

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scale for predominantly helical proteins, while collapse precedes secondary structure in largely β-sheet proteins. This is consistent with the conformational preferences of the dipeptides investigated here. Needless to say, caution should be exercised in drawing connections between dipeptides in crude models of solvent and protein folding, and systematic extension of these studies to better solvent descriptions, longer peptide lengths, and full side-chain diversity is essential.

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