Amide III Mode φ, ψ Dependence in Peptides: A Vibrational Frequency Map†

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A vibrational frequency map of the amide III mode of the alanine dipeptide has been obtained at the B3LYP/6-31+G* level. It shows that, contrary to an earlier speculation by Lord, the frequency of this mode is a function of φ and ψ rather than ψ alone. We note that an analysis of the H^{α} bend mode, which independently depends on φ and ψ, together with amide III may permit determination of these two torsion angles. Preliminary results on the alanine tripeptide indicate that such studies will be needed to establish the exact relationships in polypeptide chains.

Introduction

It is now half a century since vibrational spectroscopy was recognized as a potentially powerful tool for studying conformational structure in peptides and proteins.[1] Although its implementation has gained through insights from structure-spectra correlation studies, a deeper understanding of the spectroscopic information requires that interpretations be based on accurate normal-mode analyses.[2−4] In the early stages of such studies, empirical force fields were developed for these calculations, and they have provided an increasingly secure base for spectroscopic analysis of polypeptide conformation.[3] It was in this context, for example, that it was recognized that, while not significant for nonpolar systems,[5] transition dipole coupling was very important in understanding the different amide I and amide II band splittings in α-helix and β-sheet polypeptides.[6,7] This significant interaction, whose empirically determined value[6,7] is consistent with ab initio dipole derivatives,[8,9] has provided the basis for understanding band shapes in proteins,[10] interpreting the results of femtosecond nonlinear-infrared spectra,[11] and explaining anomalous intensity distributions in isotope edited spectra.[12]

Empirical force fields for polypeptides can be improved by making them more complete and basing them on ab initio results,[13] but they still suffer from being optimized to individual chain conformations.[3] What is needed in order to adequately analyze small spectral changes arising from conformational differences is a conformation-dependent force field. This dependence can be achieved through a spectroscopically accurate potential function, and although such a so-called spectroscopically determined force field (SDFF)[14] has been developed for hydrocarbon chains[15−17] (with rms deviations of the order of 5−10 cm^{-1}), an SDFF for the polypeptide chain is still in progress. It is therefore necessary at this stage to tackle some conformational questions by appropriate level ab initio calculations on model systems.

One such conformation-spectrum correlation was suggested by Lord,[18] who proposed (on the basis of the α-helix, ∼1275 cm^{-1}, and β-sheet, ∼1230 cm^{-1}, frequencies) that the amide III frequency, which is observed as a moderately strong band in the Raman spectrum, is directly related to the ψ angle (the $C^{\alpha}C$ torsion angle) in the polypeptide chain. He recognized that "this objective is at present far from quantitative realization", in particular because "among the most serious problems to be solved [is] the role of the angle φ" (the NC^{α} torsion angle). A normal-mode analysis of the "dipeptide" *N*-acetyl-L-alanine-*N*-methylamide[19] ($CH_3-CO-NH-CH(CH_3)-CO-NH-CH_3$) had already indicated that the amide III frequency depended on both φ and ψ, but the results of this calculation could be considered limited because a common force field was used for all conformations. A low level ab initio calculation[20] did not shed additional light, primarily because only four non-hydrogen bonded conformers were examined. It is desirable to resolve this question more definitively, and we have approached this through ab initio calculations designed to produce a φ,ψ frequency map for the above dipeptide.[21] Even though such a dipeptide map may not fully represent the relationship in a polypeptide chain, it is a useful starting point for examining the underlying relationship between the amide III normal-mode frequency and the local backbone conformation. A tripeptide would provide a better model, and we also present preliminary results of such calculations.

Calculations

Density functional theory (DFT) calculations were done with GAUSSIAN 98[22] using the B3LYP functional and the 6-31+G* basis set, which we have shown leads to maximally planar peptide groups in *N*-methylacetamide (NMA).[23] We implemented the unscaled DFT calculation after ascertaining that its amide III frequencies were relatively close to those of an MP2/6-31+G* calculation with force constant scale factors transferred from NMA:[24] for example, for the dipeptide with $\varphi, \psi = -113°, 13°$, the DFT frequencies are 1275 and 1267 cm^{-1}, whereas the scaled MP2 frequencies are 1263 and 1243 cm^{-1} (unscaled MP2 frequencies are 1312 and 1296 cm^{-1}).

For the dipeptide, we chose 48 conformations that span the mostly low energy φ, ψ region of the isolated molecule map (see for example ref 25), including four minimum structures: $\beta_2(-113, 13)$, α_L (73, 17), $\alpha'(-165, -44)$, and $\alpha_R(-60, -40)$. The energy of each was minimized, without further constraints, for the chosen values of φ and ψ, and frequencies and normal modes (with their potential energy distribution, PED) were calculated. Although all but three of these structures (−90,90 and −90,60, essentially $C7_{eq}$, and 61,−30, an essential $C7_{ax}$) do not have traditional hydrogen-bonded peptide groups, such

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TABLE 1: Structures and Relative Energies of Alanine Dipeptide Conformers

φ^a	ψ^a	ΔE^b	φ^a	ψ^a	ΔE^b
−180	145	1.77	−30	145	6.86
	120	3.11		120	5.45
	90	5.17		90	6.82
	60	5.77		60	7.27
	0	7.60		30	7.14
	−90	7.42		−30	7.72
	−145	3.81		−90	12.71
	−180	1.92		−145	20.91
−134	145	0.93	0	60	10.62
	120	1.41		20	9.10
	90	2.34		−50	9.51
	60	3.04	30	30	8.70
	30	2.54	61	145	10.85
	0	3.03		90	11.75
	−90	6.64		60	7.66
	−145	4.04		30	5.43
−90	145	1.80		−30	3.05
	120	1.18		−90	3.82
	90	0.08		−120	5.04
	60	0.00	−113	13	2.29
	30	1.97	73	17	5.19
	0	2.56	−165	−44	6.14
	−50	6.08	−60	−40	4.55
	−90	8.46			
	−145	6.25			

[a] Torsion angles: $\varphi = NC^{\alpha}$, $\psi = C^{\alpha}C$. [b] Energy relative to lowest in group (−90, 60), in kcal/mol.

as are present in native or in denatured states of proteins, we believe that the underlying dependence of the amide III frequency on the φ and ψ torsion angles is revealed by these calculations on the isolated peptides. To more closely approximate a peptide group in a polypeptide chain, we also did similar calculations on some alanine tripeptide conformations, choosing φ, ψ values for the central peptide group that corresponded to examined dipeptide structures.

Results and Discussion

1. Dipeptide. The structures that were studied and their energies, relative to the lowest in the group (which at −90, 60 is close to the $C7_{eq}$ global minimum), are given in Table 1. A φ, ψ map of these energies is shown in Figure 1. In Figures 2 and 3, we present vibrational frequency φ,ψ maps of the amide III modes in amide groups 1 (CH_3−CONH−) and 2 (−CONH−CH_3) of the dipeptide, respectively.

On the basis of early normal-mode studies of NMA,[26,27] it has been widely assumed that the amide III mode in peptides is a localized vibration in the amide group, consisting essentially of CN stretch (s) and NH in-plane bend (ib) internal coordinates. However, as has been pointed out,[3,4] modes in the amide III region are complex, involving significant contributions from other coordinates such as H^{α} bend (b), CO ib, $C^{\alpha}C$ s, and NC^{α} s, the mixing being dependent on main-chain conformation and side-chain composition.[28] This is illustrated in Table 2, which shows the eigenvectors (in terms of the PED) of some of the conformations. Furthermore, if, as has been assumed because of the NH ib contribution, sensitivity to N-deuteration is taken as a criterion of amide III character, modes in the 1300−1400 cm^{-1} region (which are primarily H^{α} b) would have to be included in this category.[3,21] (In fact, some of these modes can also contain a CN s contribution,[21] which, in addition to a $C^{\alpha}C$ s contribution, could account for their resonance Raman enhancement.[29]) Such extensive delocalization in the vibrational character of amide III thus makes it highly likely that its frequency will depend on φ as well as ψ.

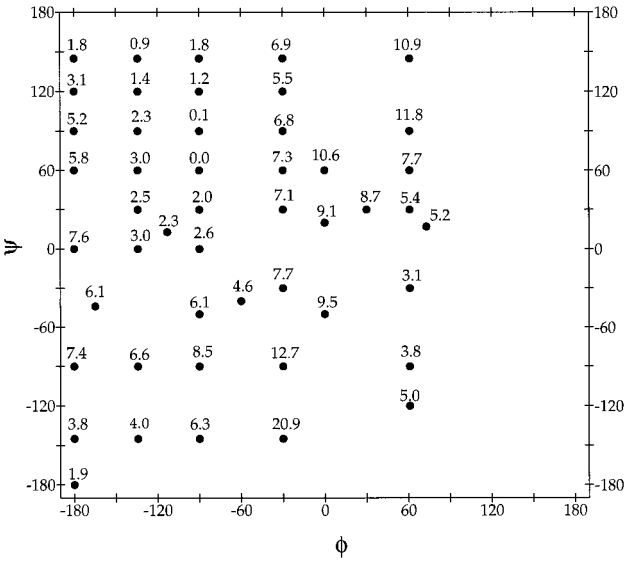

Figure 1. Conformational B3LYP/6-31+G* energy map for the alanine dipeptide. Energies are in kcal/mol relative to that of the lowest energy in the group (at −90, 60).

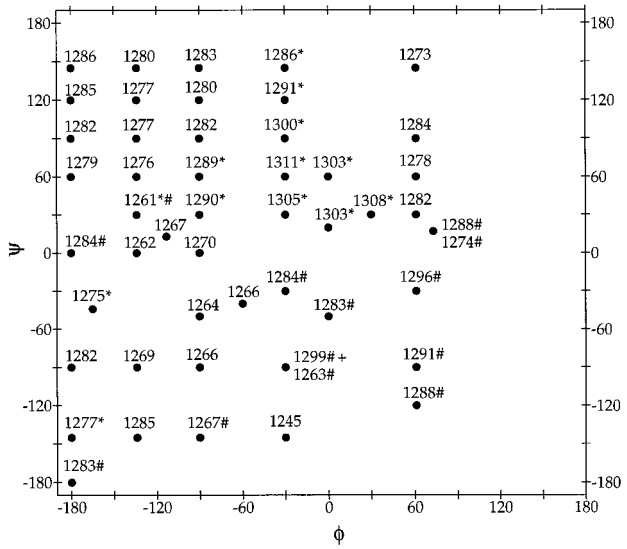

Figure 2. Conformational B3LYP/6-31+G* amide III vibrational frequency map for peptide group 1 of the alanine dipeptide. Frequencies in cm^{-1}. *: contribution to PED of H^{α} bend greater than or comparable to CN stretch; +: significant contribution of $C^{\alpha}C$ stretch; #: significant contribution from amide III of peptide group 2.

Examination of Figures 2 and 3 confirms the expectation that amide III frequencies are a function of both φ and ψ. This is particularly apparent from Figure 3, since peptide group 2, on the basis of the Lord hypothesis, should only be influenced by its adjacent ψ angle. Although the ∼45 cm^{-1} difference between α-helical and extended chain conformations is evident, it is not true that these frequencies are uniquely correlated with the respective φ, ψ: the 1292 (± 4) cm^{-1} −60, −40 (α_R) frequency can be found as far afield as −165, −44 and −30, 60 and 73, 17, whereas β-type frequencies (e.g., at −134, 145) are found at −180, 0 and −30, 120 and 61, 60. In fact, the map shows that the ψ dependence is a function of the value of φ, the trends of which are graphically illustrated by Figure 4: the amide III frequency can vary by ∼15−70 cm^{-1} depending on the value of φ. Thus, regions in proteins that are not helical or extended chain can still contribute to amide III bands in these characteristic frequency regions. The situation becomes even

TABLE 2: Amide III Modes of Some Alanine Dipeptide Conformers

φ^a	ψ^a	ν^b	potential energy distribution[c]
−134	145	1280	NH ib(27) CN s(22) CO ib(11) MC s(9) CN[2] s(6)
		1249	H^α b2(25) CN s(21) NH ib(13) NM s(12) NH[1] ib(8) CO ib(6)
−134	30	1261	NH ib(18) CN s(17) H^α b2(13) CN[2] s(12) NH[2] ib(18) CO ib(7) MC s(7)
		1271	CN s(16) NH ib(16) CN[1] s(14) NH[1] ib(8) CO ib(7) $C^\alpha C$ s(7) CO[1] ib(6)
−30	145	1286	CN s(29) H^α b1(14) NH ib(13) CO ib(12) NC^α s(11) MC s(9)
		1244	CN s(31) NM s(16) NH ib(14) H^α b2(12) CO ib(11)
−30	30	1305	CN s(25) H^α b1(13) CN[2] s(10) CO ib(10) NH ib(10) H^α b2(9) NC^α s(8) MC s(7)
		1318	H^α b1(39) CN s(23) NH ib(10) NM s(5) C^β r(5)
−60	−40	1266	CN s(20) NH ib(20) CN[2] s(10) CO ib(8) MC s(8) $C^\alpha C$ s(6) NH[2] ib(5)
		1292	$C^\alpha C$ s(18) CN s(16) NH ib(16) CN[1] s(11) CO ib(7) NC^α s(7)
73	17	1288	CN s(19) NH[2] ib(12) CN[2] s(11) NH ib(10) CO ib(7) $C^\alpha C$ s(7) NC^α s(6) MC s(6) H^α b1(5)
		1274	CN s(19) CN[1] s(15) NH ib(13) NH[1] ib(11) CO ib(8) CO[1] ib(7) $C^\alpha C$ s(7) MC s(6) NM s (5)

[a] Torsion angles: $\varphi = NC^\alpha$, $\psi = C^\alpha C$. [b] Frequency in cm^{-1}. First line: amide group 1; second line: amide group 2. Contributions from other peptide group indicated by [ ]. [c] Contributions ≥5. s = stretch, ib = in-plane bend, b1 = bend in $H^\alpha C^\alpha C^\beta$ plane, b2 = bend perpendicular to the $H^\alpha C^\alpha C^\beta$ plane, M = end methyl group carbon, r = side chain methyl group rock.

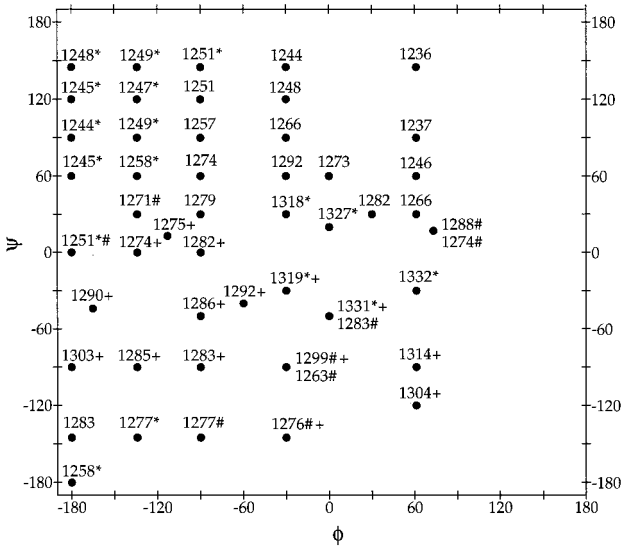

Figure 3. Conformational B3LYP/6-31+G* amide III vibrational frequency map for peptide group 2 of the alanine dipeptide. Frequencies in cm^{-1}. *: contribution to PED of H^α bend greater than or comparable to CN stretch; +: significant contribution of $C^\alpha C$ stretch; #: significant contribution from amide III of peptide group 1.

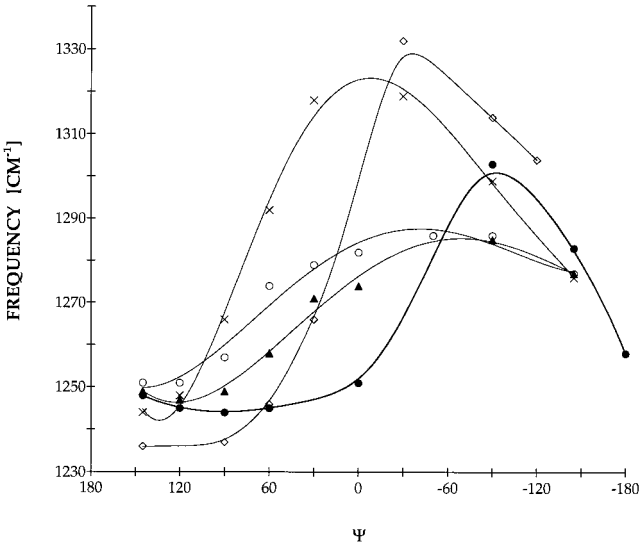

Figure 4. Dependence of amide III(2) frequency of alanine dipeptide on ψ for various values of φ. ●: −180°, ▲: −134°, ○: −90°, ×: −30°, ◇: 61°. (Curves are drawn to show frequency trends).

more complex in analyzing spectra of unfolded regions in native and denatured proteins.

Because the amide III frequency depends on both φ and ψ, it follows that these torsion angles might be determined by amide III and another mode whose frequency independently depends on φ and ψ. A likely candidate is the H^α b mode because this coordinate usually mixes with amide III[3] and the mode is found to be enhanced in the resonance Raman spectrum.[29] Although details remain to be worked out, we have preliminary evidence that such dependence is present in the dipeptide.[21] Thus, these two frequencies (in some cases together with the amide II frequency) could, at least in principle, specify the two unknown torsion angles.[21] Practical problems in such an analysis (resolution, band overlap, etc.) will have to be addressed, but the principle is sound and may be a useful basis for determining conformational structure in peptides from the vibrational spectrum.

2. Tripeptide. It might be thought that a dipeptide cannot adequately represent the situation in a polypeptide chain, since each peptide group in the dipeptide has only one of the peptide torsion angles adjacent to it, whereas in the chain each peptide group is bordered by both torsion angles. We have, therefore, done comparable vibrational calculations on the alanine tripeptide,[21] of which we present here some preliminary results.

Most tripeptide structures were chosen such that φ_2, ψ_1 coincided with φ, ψ values previously examined in dipeptides. Several other structures were chosen to coincide with minima previously located for this molecule.[30]

The results for the central peptide group show first that amide III for a given φ, ψ in the tripeptide does not correspond to values for the same φ, ψ in the dipeptide. For example, for φ_2, $\psi_1 = -134$, 30, the tripeptide frequency is 1239 cm^{-1}, whereas the dipeptide frequencies are 1271 and 1261 cm^{-1}, with both being mixed modes. Second, in some cases the amide mode is not even well-defined: for φ_2, $\psi_1 = -30$, 134 in the tripeptide, CN2 s contributes at the level of PED ≅ 10 to modes at 1278, 1256, 1238, and 1168 cm^{-1}, whereas in a comparable dipeptide (−30, 145) there are localized modes at 1286 and 1244 cm^{-1}. Finally, the ψ dependence of amide III in the tripeptide is quite different from that in the dipeptide. For example, for φ_2, $\psi_1 = -90$, 120 and −90, 60 the amide III frequencies of the central peptide group in the tripeptide are 1239 and 1239 cm^{-1}, respectively, whereas the dipeptide frequencies are 1251 and 1274 cm^{-1}, respectively. It is interesting to note that even the end peptide groups in the tripeptide do not always follow their counterparts in the dipeptide. For example, the frequency of group 1 in the −60, −40, −60, −40 tripeptide conformer is 1288 cm^{-1}, whereas that in the −60, −40 dipeptide is 1266 cm^{-1}. All of these phenomena are to be expected from the

differing interactions between delocalized modes in different peptide conformations.

Conclusions

An ab initio amide III vibrational frequency map of the alanine dipeptide shows that the frequency of this mode is distinctly a function of φ and ψ rather than just ψ alone, as had been speculated.[18] Although the detailed form of this dependence may change if the peptide groups are hydrogen bonded (rather than free, as in almost all of the present conformers), the substance of such a relationship is not expected to change. Because our analyses show that the H^{α} bend mode also depends on φ and ψ, it is possible in principle that a measurement of both of these bands could determine these two torsion angles. Preliminary studies of the alanine tripepeptide, which gives results different from that of the dipeptide, indicate that care is needed before transferring results from a dipeptide map to a polypeptide chain.

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