# Dependence of the Peptide Amide III Vibration on the Φ Dihedral Angle

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Peptide and protein UV Raman spectra excited at ∼200 nm show strong enhancement of the peptide backbone amide I, II, and III and (C)$C_\alpha$−H vibrations.[1−4] We recently demonstrated that these spectra could be analyzed to quantitatively determine secondary structure by modeling the measured spectra with a linear combination of basis spectra of the different secondary structure motifs.[4] These Raman studies also demonstrated that the amide III vibration is the most sensitive to peptide conformation; for example, the amide III frequency shifts from 1235 $cm^{-1}$ in the $\beta$-sheet to ∼1300 $cm^{-1}$ in the α-helix conformation.[1−4]

Since our recent investigations of the UV Raman spectra of peptides indicate that the vibrations of individual peptide bonds are in general uncoupled,[5] it may be possible to approximate protein Raman spectra as the linear sum of the spectra of individual peptide bonds. To test this possibility we need to know the dependence of the individual peptide bond Raman spectra on local secondary structure, as well as on their side chain identity and geometry.

Our recent observation, that the Raman basis spectra of oligopeptides have bandwidths significantly narrower than those observed for proteins, may simply result from a side-chain dependence of the amide Raman band frequencies or from a narrow distribution of peptide band geometry.[6] As previously suggested this dependence could result from either an involvement of side-chain atom motion to the amide III vibrational normal coordinate or a side-chain dependence of the amide bond conformation.[7]

Some of the earliest studies of amide vibrations empirically demonstrated a strong amide III frequency dependence on the Ramachandran Ψ angle.[8] In addition, the experimental results of Williams et al.[9,10]and theoretical calculations showed an amide III frequency dependence on the Ramachandran Φ angle. They calculated the amide I and III potential energy distributions and the vibrational frequencies for the $\beta$-sheet and α-helix conformations of dipeptides. For these conformations, they found no systematic dependence of the frequencies and potential energy distribution on side-chain molecular weight. Instead, they found that the amide III frequency shifts resulted from side-chain conformational preferences for particular dihedral angles. Their calculations demonstrated a Φ angle dependence for the magnitude of the contribution of N−H bending and methine proton bending to the amide III vibrational potential energy distribution.

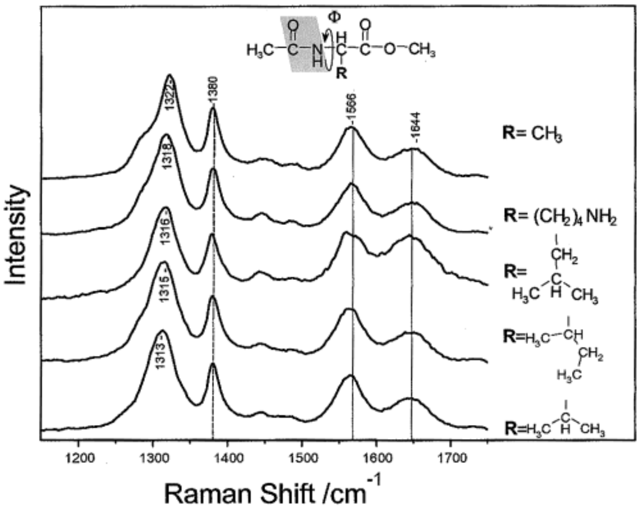

**Figure 1.** 204 nm excited UV RR spectra of peptides (10 mM, pH 6). The instrumentation is described in ref 6. The samples were measured in a temperature-controlled free-surface flowing stream. An accumulation time of 25 min was used for each spectrum. Spectral resolution was 8 $cm^{-1}$.

To further investigate the Φ angular dependence of the amide III frequency, we have examined the UV resonance Raman spectra of model dipeptides with side chains which are known to have a span of Φ angle preferences. These Φ angle preferences were calculated from Serrano's[11] recent NMR study of the side-chain Φ angle preference in proteins. It should be pointed out that the Φ angle conformational preferences are mainly determined by the side-chain identity and are independent of whether the side chain occurs in a random coil or in an ordered region of the protein.[11] We calculated the average side-chain Φ angle from Serrano's tables by weighting the different Φ angles by their relative probability of occurrence.

We measured the UV Raman spectra of a series of acetylated amino acid esters to prevent obfuscation by a spectral dependence on charged penultimate groups. Figure 1 shows the UV Raman spectra of the amide III bands of different side-chain derivatives Ac-X-$OCH_3$, where X is ala, lys, leu, ile, and val. The amide III frequency in this sequence monotonically downshifts from 1322 to 1313 $cm^{-1}$. These derivatives should show only a Φ dihedral angle conformational dependence because the amide is not linked to a second amide group, and the penultimate $CH_3$ group is 3-fold symmetric with respect to rotation around the $CH_3$−C(O) bond.

As suggested by William et al.,[9] the amide III frequency does not depend on the side-chain mass, but linearly depends on the side-chain preferences for particular Φ angles[11−14] (Figure 2, diamonds). Ac-ala-$OCH_3$, which has a preference for the least negative Φ angle (−78°), has the highest amide III frequency, while Ac-val-$OCH_3$, which has a preference for the most negative Φ angle (−96°, and prefers the $\beta$-structure[11−14]), shows the lowest amide III frequency. The other side-chain data fall between that of ala and val.

To support these empirical results, which show a Φ angle dependence of the amide III frequency, and to verify that this dependence does not result from mixing in of side chain atomic motion into the amide III vibration, we applied Gaussian 98[15] density functional theory (DFT) calculations at the B3YLP 6-31G-

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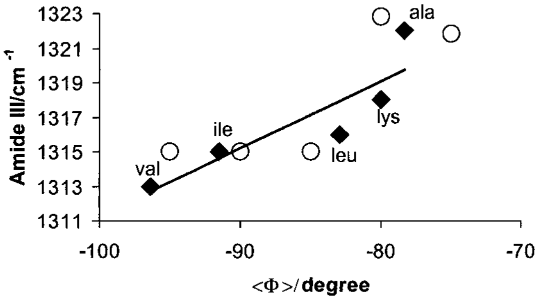

**Figure 2.** Side-chain dependence of the amide III frequency for Ac-X-$OCH_3$ (X = ala, lys, leu, ile, val) on the side-chain average $\langle\Phi\rangle$ angle propensity: experimental (diamonds) and theoretical (circles) data. $\langle\Phi\rangle$ values are calculated as $\langle\Phi\rangle = \sum\Phi_i \times (n_i/N)$, where $n_i/N$ is the probability for each amino acid to adopt angle $\Phi_i$. $N$, $\Phi_i$, and $n_i$ are taken from ref 11.

(d) level to *N*-acetyl-alanine-methyl ester *in vacuo*. Energy minimization yields a structure with the dihedral angle $\Phi = -92°$. We constrained this structure to have the following set of $\Phi$-values: −75°, −80°, −85°, −90°, and −95°. Energy minimization and a vibrational analysis were performed on each of these constrained structures. Also, a scaling factor of 0.974 was used to adjust the calculated amide III frequencies. The calculated data in Figure 2 (circles) predict a moderate 8 $cm^{-1}$ increase in the amide III frequency as the $\Phi$ angle increases from $-95° < \Phi < -75°$. These results overlap well the UV Raman data, which correlate the amide III frequency to the $\Phi$ angle proclivity.[16]

Further B3YLP/6-31G(d) theoretical calculations were carried out on ala-ala to further examine the dependence of the amide III vibration on the $\Phi$ dihedral angle. This larger peptide was studied because it contains a (C)$C_\alpha$ hydrogen and both $\Phi$ and $\Psi$ dihedral angles. The ala-ala structure was optimized to obtain the lowest energy conformation. The $\Psi$ dihedral angle was fixed at this lowest energy conformation of $\Psi = -21°$, and the $\Phi$ dihedral angle was varied (−75°, −80°, −85°, −90°, −95°). These constrained conformers were then reoptimized, and a vibrational analysis was done for each. Figure 3 shows that the amide III frequency linearly decreases by 3 $cm^{-1}$ as the $\Phi$ dihedral angle decreases from −75° to −95°. This amide III frequency dependence is similar, but smaller than the putative observed frequency dependence for Ac-X-$OCH_3$ and our calculations for Ac-ala-$OCH_3$. The decreased frequency dependence for ala-ala may signal that the $\Phi$ angle frequency dependence also has a $\Psi$ angle dependence. We also examined the $\Phi$ angle frequency dependence of the $C_\alpha$−H bending mode, which is highly structurally sensitive due to its large $\Psi$ angle dependence.[18] In ala-ala the (C)$C_\alpha$−H mode frequency shows only a slight $\Phi$ dihedral angle dependence (~1 $cm^{-1}$) between −75° and −95°.

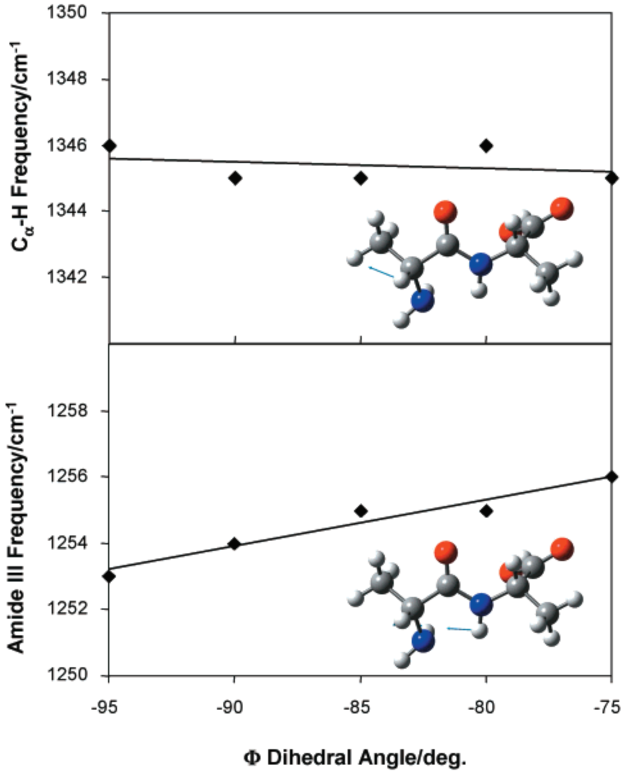

**Figure 3.** The calculated dependence of amide III and $C_\alpha$−H on the $\Phi$ dihedral angle for ala-ala. The $\Psi$ dihedral angle is fixed at −21°. The atomic displacement vectors for the major displacements are displayed for the $\Phi = -85°$ conformer.

**Conclusions.** Our experimental measurements and theoretical modeling indicate that the frequency dependence of the amide III vibrations of peptides with different side chains is a result of the side chain dependence of the peptide $\Phi$ dihedral angle. We find a modest dependence of the amide III frequencies on the span of allowed $\Phi$ dihedral angles. The amide III vibrational frequency shows a much larger $\Psi$ angle dependence. These results add to the fundamental knowledge base, which we expect will allow us in the future to interpret amide Raman spectra in terms of the amide $\Psi$ and $\Phi$ peptide conformational geometry.

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