# Uncoupled Adjacent Amide Vibrations in Small Peptides

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Vibrational spectroscopy is a classical technique used to examine molecular structure and dynamics.[1] The major challenge often is assigning vibrational modes and interpreting their frequencies and intensities in terms of the molecular coordinates. This is often simplified if group vibrations occur which are associated with molecular coordinates of interest.[2]

Functional group vibrations within polymers can couple if they are located close to one another to give collective polymer vibrations. The observation of coupled vibrations should be more common in IR spectroscopy because IR selects for vibrations with large dipole moment changes. Vibrations with large dipole moment changes can couple through space by transition dipole coupling. In contrast, resonance Raman spectroscopy (RR) selects through criteria independent of dipole moment changes. Thus, vibrations observed by RR are less likely to be coupled.

Here we examine coupling between amide vibrations in tripeptides and in derivatives with adjacent amide groups and ask whether the vibrations observed by RR result from vibrations localized within individual amide peptide bonds, or whether these vibrations are delocalized and result from coupled motion of adjacent amide peptide bonds. This work is part of a research program where we are developing UVRR for studying biological structure and function.[3] We recently demonstrated that RR excitation within the amide $\pi \rightarrow \pi^*$ transitions enhanced amide R bands.[4] The resulting amide RR spectra quantitatively determine peptide and protein secondary structure.[5,6] In addition, we have used UVRR to probe the first steps in the folding and unfolding of peptides due to ns temperature jumps.[7,8]

We determined the extent of vibrational coupling between amide groups by measuring the RR spectra of linked amides. We compared the spectra of natural isotopomers to those formed by exchanging the labile N−H groups by N−D in $D_2O$. We then measured these derivatives in a mixed $H_2O/D_2O$ solution, where N−H groups were only partially deuterated. Replacement of NH by ND dramatically alters the normal mode, since N−H motion no longer couples to C−N motion. The hypothesis follows: if normal modes of the linked amides couple, deuteration of an amide would perturb the frequencies and RR cross sections of the linked nondeuterated amide. Thus, UVRR could not be modeled as a sum of pure deuterated and nondeuterated derivatives.[9]

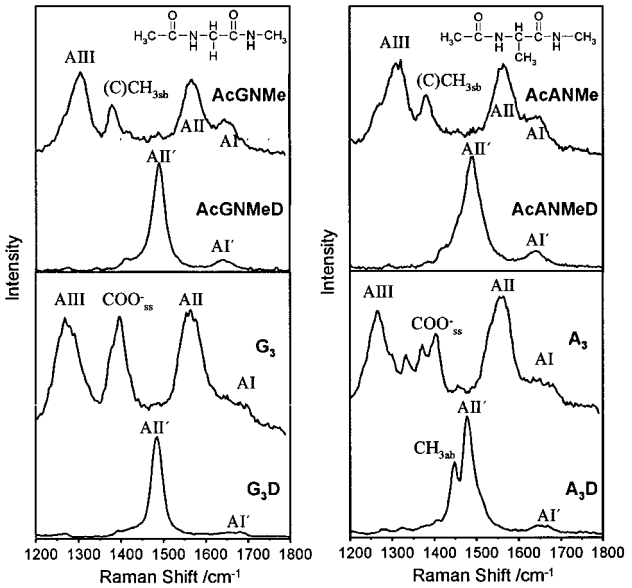


**Figure 1.** 206.5-nm excited UVRR in $H_2O$ and $D_2O$ of *N*-acetyl-*N*′-methylglycinamide (1.5 mM, pH/pD ∼ 5, AcGNMe), *N*-acetyl-*N*′-methylalanylamide (3.0 mM, pH/pD ∼ 5, AcANMe), triglycine (1.5 mM, pH/pD ∼ 5, $G_3$), and trialanine (1.5 mM, pH/pD ∼ 5, $A_3$).

Figure 1 shows the RR spectra of the linked amide derivatives *N*-acetyl-*N*′-methylglycinamide (AcGNMe), *N*-acetyl-*N*′-methyl-L-alanylamide (AcANMe), triglycine ($G_3$), and trialanine ($A_3$) in water and pure $D_2O$. We observe a more complex spectrum in $H_2O$, where the linked amides give overlapping AmI, AmII, and AmIII bands. The AmII and AmIII bands are characteristically described as involving coupled C−N stretching and N−H in-plane bending. However, as noted by others, the AmIII vibration has a more complex composition which depends on the exact molecular structure.[10−12]

The spectra considerably simplify in $D_2O$ because the AmII and III modes disappear and are replaced by very intense AmII′ modes, which are mainly C−N stretching.[13] The AmI′ modes of the deuterated derivatives shift relative to those of the hydrogenated derivative. Thus, the N−H spectra and N−D spectra differ dramatically.

AcGNMe, which is the simplest model peptide containing two adjacent amide groups, shows a spectrum similar to *N*-methylacetamide (NMA).[13] The AmIII, AmII, and AmI bands occur at ∼1305, ∼1573, and ∼1640 $cm^{-1}$. Obviously, bands from the two amide groups overlap. The AIII low-frequency shoulder (1255 $cm^{-1}$) originates from a $CH_{2tw}$ mode, which also contains $CN_s$.[14] The ∼1380 $cm^{-1}$ band is mainly due to $(C)CH_3$ sb.[13] The ND derivative of AcGNMe (AcGNMeD) shows a spectrum identical to that of NMAD. It is dominated by the 1490 $cm^{-1}$ AmII′ band, with a weaker ∼1640 $cm^{-1}$ AmI′ band. The spectra of AcGNMe and AcGNMeD are almost identical to those of AcANMe and AcANMeD.

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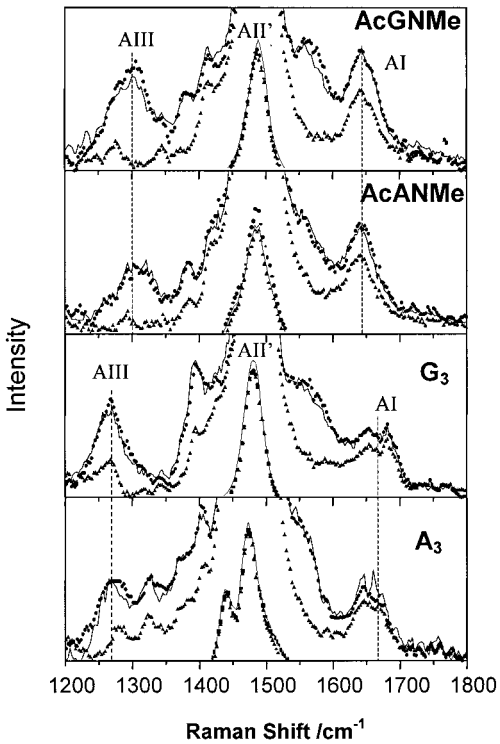


**Figure 2.** Comparison between AcGNMe, AcANMe, triglycine ($G_3$), and trialanine ($A_3$) measured in a 80%/20% $D_2O/H_2O$ mixture (solid lines) and calculated spectra (dots, see text for details). Also shown are spectra in pure $D_2O$ (triangles, scaled to 80% of their original intensities). The calculated spectra model the measured spectra in the $D_2O/H_2O$ mixture almost perfectly.

The RR spectra of $G_3$ and $A_3$ at neutral pH show additional strong bands at ~1400 $cm^{-1}$ from enhancement of symmetric $COO^-$ stretching by a charge-transfer band.[15,16] $G_3$ also shows overlapped AmIII and AmII bands at 1274 and 1560 $cm^{-1}$. In contrast, AmI is broad, suggesting a frequency difference between the two linked AmI vibrations. In $G_3D$ the strong AII′ band occurs at 1485 $cm^{-1}$, while the AI′ region remains broad. The shoulder at ~1400 $cm^{-1}$ derives from $COO^-$ symmetric stretching.

The $A_3$ spectrum shows AmIII, AmII, and AmI bands at 1265, 1560, and ~1660 $cm^{-1}$. The 1335 $cm^{-1}$ band does not occur in $A_3D$. We assign this band to a mixture of $C_\alpha H$ bending, CN stretching, and CO in-plane bending of the central amide bond.[17] Deuteration eliminates the CN contribution and thus the resonance enhancement. The 1370 $cm^{-1}$ band is due to N-terminal $C_\alpha H$ bending.[12,13]

The spectrum of $A_3D$ is dominated by a doublet at 1450 and 1480 $cm^{-1}$, while the AmI′ occurs at 1660 $cm^{-1}$. Lee and Krimm's[12] deuterated α-poly(L-alanine) study indicates strong mixing between C−N stretching and $CH_3$ asymmetric bending, which leads to the observed doublet. The strong enhancement results from the C−N stretching contribution to the vibration.[12,13]

Figure 2 compares measured spectra of AcGNMe, AcANMe, $G_3$, and $A_3$ in a 20%/80% $H_2O/D_2O$ solution to modeled spectra. These modeled spectra are calculated as the sum of the derivatives in pure $H_2O$ and $D_2O$. In 20%/80% $H_2O/D_2O$ solution, 64% will have both amides deuterated at the NH, while 32% will have only one NH deuterated, while 4% have both NH deuterated. The overlap between the calculated and measured spectra demonstrates the lack of coupling between adjacent amides.

The magnitude of vibrational coupling can depend sensitively on the detailed molecular geometry. For example, normal mode calculations for three different geometries of gas-phase alanine dipeptide demonstrated different couplings for different geometries.[12b] For example, no AmI coupling occurred where the carbonyls were perpendicular to one another. The AmII and AmIII coupling also depended on geometry. Thus, one interpretation of our results is that the four derivatives studied here possess geometries *in water* where fortuitously no interpeptide coupling occurs for the AmII, AmII′, and AmIII bands.

This finding is in accord with a recent normal coordinate calculation on AcANMe by Han et al.[17] They investigated AcANMe−$(H_2O)_4$ within an Onsager continuum and found that in water the molecule prefers a PII structure, with dihedral angles in the $\beta$-sheet region of the Ramachandran plot. An α-helical-like conformation was found at 2.5 kcal/mol higher energy. The normal mode calculation for PII reveals localized amide III and II modes with slightly different frequencies for the two peptide groups. In contrast, AmI shows significant mixing.

Alternatively, these amide derivatives in water may populate numerous conformations, some of which have Ramachandran $\Psi$, $\phi$ angles similar to α-helix and $\beta$-sheet conformations. This may occur despite significant conformational energy differences, due to compensating volume and entropy differences. Thus, our results may indicate that AII and AIII vibrations observed in α-helical, $\beta$-sheet, and disordered peptides are also uncoupled.

Our result, that AmII and AmIII are localized within each amide, does not conflict with recent IR absorption observations that AmI vibrations couple over 3−4 peptide bonds.[18] As discussed above, the large AmI dipole moment allows it to interact through transition dipole coupling. Further, a normal mode study of the Ala 7A derivative shows a case where the AmI and AmIII couple while the AmII is not coupled.[12b]

If amide groups independently contribute, then the spectra would simply be the sum of spectra of the individual peptides. The AmIII, AmII, and $C_\alpha H$ Raman spectra of the different secondary structure motifs are thought to depend mainly on the $\Psi$ and $\varphi$ angles of the amide bond.[19,20] Thus, the RR spectra would be related to the number of amides in each secondary structure. Thus, the observed RR spectra should be more linear in the number of "α-helix" conformation residues than CD, which appears to require a certain persistence length to give rise to α-helical CD spectra.[21] Thus, RR would find more α-helix content than does CD, which is consistent with our recent observations.[7,8]

It is, however, possible that amide RR undercounts the number of "α-helix" peptide bonds, since excitonic interactions that lead to hypochromism[22] decrease RR cross sections. Thus, the RR intensities of "α-helix" amide bonds in long runs would decrease compared to shorter runs. Thus, the biases for CD and RR may be diametrically opposed. CD selects against short runs, and RR selects against large runs.

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**Note Added after ASAP:** The uncorrected version of this paper was inadvertently posted ASAP August 1, 2000; the corrected version was posted August 29, 2000.

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