J. Am. Chem. Soc. **1994,** *116,* 11141−11142

N-Methylacetamide and Its Hydrogen-Bonded Water Molecules Are Vibrationally Coupled

X. G. Chen,[†] Reinhard Schweitzer-Stenner,[‡,§]
Samuel Krimm,[‡] Noemi G. Mirkin,[‡] and Sanford A. Asher[*,†]

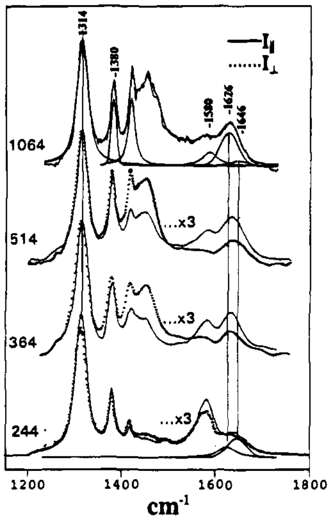
Department of Chemistry, University of Pittsburgh
Pittsburgh, Pennsylvania 15260
Biophysics Research Division, University of Michigan
Ann Arbor, Michigan 48109

Received June 13, 1994

N-Methylacetamide (NMA) is the simplest molecular model of the peptide linkage in proteins. Numerous studies have examined its ground and excited state structure and dynamics as well as its vibrational structure.[1−5] The hydrogen-bonded ground state geometry is planar,[1d,5] while the first π^* excited state of NMA may have a local minimum at a twisted geometry.[1c,4] Recent UV Raman studies[2,3] and normal coordinate analyses[1d,6] have shown that water molecules hydrogen bonded to the NH and the carbonyl of NMA alter the ground state vibrational modes and may alter the *ca.* 190 nm π^* excited state. The π^* excited state may be more delocalized than previously suspected; studies of acetylglycine and diglycine, for example, demonstrate extensive mixing between the amide and carboxylate $\pi \rightarrow \pi^*$ electronic transitions.[7]

In this work we present evidence that the hydrogen-bonded water molecules of NMA in aqueous solution are coherently vibrationally coupled to motions of the NMA nuclei and that these water molecules are intimately involved in determining the structure and dynamics of NMA. Similarly, amide hydrogen-bonded water molecules are likely to be important in determining the structure and dynamics of peptides and proteins. Our results partially explain the previous difficulties in the application of vibrational spectroscopy to monitor the conformation of peptide linkages; the coupling of water and amide motion results in a dramatic increase in the spectral complexity since the amide frequencies can become extremely sensitive to the details of the hydrogen-bonding geometry and the degree of coupling.

As indicated in Figure 1, the Raman spectra of NMA excited between the near IR and UV display numerous strong bands. Excitation in the near IR at 1064 nm results in the most complex spectrum, which shows numerous bands between 1200 and 1800 cm^{-1} including symmetric and antisymmetric deformations of the methyl groups between 1300 and 1500 cm^{-1}. Figure 1 displays a spectral deconvolution and the polarized and depolarized Raman spectra. Excitation in the UV results in a considerable simplification; those methyl bands between 1400 and 1500 cm^{-1} dramatically decrease in relative intensity, while the amide II band at 1580 cm^{-1} significantly increases in intensity. The relative intensities of the amide III band at 1314 cm^{-1} and the symmetric methyl bending motion at 1380 cm^{-1} stay fairly constant. The amide II and III modes arise mainly from a combination of in-phase and out-of-phase NH in-plane bending and CN stretching, respectively.[1d,6]



The depolarization and intensity ratios			
Amide I (H$_2$O)			Amide I' (D$_2$O)
ρ_{1626}	ρ_{1646}	R	ρ_{1629}
		0.18	
0.13	0.25	0.89	0.18
0.15	0.26	0.73	0.2
0.32	0.32	2.2	0.34

$R = I_{1646}/I_{1626}$

Figure 1. Raman spectra of 2.0 M aqueous solutions of NMA at different excitation wavelengths. Both the polarized and depolarized spectra are shown for 514, 364, and 244 nm excitation; the depolarized spectra were multiplied 3-fold and overlaid. The depolarization and intensity ratios are tabulated on the right. The 1064 nm spectra were deconvoluted to show most of the underlying bands. The 244 nm spectrum shows only the amide I deconvolution. All polarized and depolarized spectra were deconvoluted with an identical minimum set of bands. The 2.0 M concentrations are sufficiently dilute that intermolecular interactions between NMA molecules do not occur; the spectra remain identical with further dilution. The small water contribution was numerically removed.

The extremely broad amide I band, which occurs at *ca.* 1626 cm^{-1} with 1064 nm excitation (a frequency similar to that in the IR absorption measurement[1c]), decreases in relative intensity and *shifts ca.* 14 cm^{-1} to higher frequency with UV excitation. The amide I band mainly involves C−O stretching, C$^{\alpha}$−C−N deformation, and CN stretching.[1d,6] The amide I band frequency and intensity are extraordinarily sensitive to the solvent and the extent of hydrogen bonding. Amide I occurs at 1718 cm^{-1} in the gas phase but shifts to 1653 cm^{-1} in the neat liquid where the NMA molecules form a hydrogen-bonding network.[8] In aqueous solution the hydrogen bonding between the carbonyl and water causes a frequency downshift to *ca.* 1630 cm^{-1}, and the intensity decreases. Triggs and Valentini[9] found that, for concentrations above 4 M, the amide I frequency of NMA in water shows a much smaller concentration dependence than for *N,N*-dimethylacetamide. In contrast to the two amide I bands found for caprolactam and *N,N*-dimethylacetamide, they observed only one NMA amide I band. However, they required two overlapping Lorentzian bands separated by *ca.* 15 cm^{-1} to fit the amide I profile and suggested that the amide I band shape probably derives from numerous hydrogen-bonded species.

Our data clearly indicate the existence of at least two well-defined subbands at 1626 ± 2 cm^{-1} (I_1) and 1646 ± 2 cm^{-1} (I_2) which underlie the observed amide I band; these subbands are differentially enhanced at different excitation wavelengths. The underlying bands are a mixture of Gaussian and Lorentzians

---

[*] Author to whom correspondence should be addressed.

[†] University of Pittsburgh.

[‡] University of Michigan.

[§] Permanent address: GB 1 Institute of Experimental Physics, University of Bremen, 28359 Bremen, Germany.

(1) (a) Dudik, J. M.; Craig, R. J.; Asher, S. A. *J. Phys. Chem.* **1985,** *89,* 3805−3814. (b) Krimm, S.; Song, S.; Asher, S. A. *J. Am. Chem. Soc.* **1989,** *111,* 4290−4294. (c) Song, S.; Asher, S. A.; Krimm, S.; Shaw, K. D. *J. Am. Chem. Soc.* **1991,** *113,* 1155−1163. (d) Mirkin, N. G.; Krimm, S. *J. Am. Chem. Soc.* **1991,** *113,* 9742−9747.

(2) (a) Mayne, L. C.; Ziegler, L. D.; Hudson, B. *J. Phys. Chem.* **1985,** *89,* 3395−3398. (b) Hudson, B. S. *Proc. SPIE—Int. Soc. Opt. Eng.* **1991,** *1403,* 27. (c) Harhay, G. P.; Hudson, B. S. *J. Phys. Chem.* **1993,** *97,* 8158−8164.

(3) (a) Wang, Y.; Purrello, R.; Jordan, T.; Spiro, T. G. *J. Am. Chem. Soc.* **1991,** *113.* 6359−6367. (b) Wang, Y.; Purrello, R.; Georgious, S.; Spiro, T. G. *J. Am. Chem. Soc.* **1991,** *113,* 6368−6377.

(4) (a) Li, Y.; Garrell, R. L.; Houk, K. N. *J. Am. Chem. Soc.* **1991,** *113,* 5895−5896. (b) Nitzsche, L. E.; Davidson, E. R. *J. Am. Chem. Soc.* **1978,** *100,* 7201−7204.

(5) Gao, H.; Karplus, M. *J. Phys. Chem.* **1992,** *96,* 7273−7287.

(6) Mirkin, N. G.; Krimm, S. To be published.

(7) (a) Chen, X. G.; Asher, S. A. In preparation. (b) Chen, X. G.; Schweitzer-Stenner, R.; Asher, S. A.; Mirkin, N. G.; Krimm, S. *J. Phys. Chem.,* submitted for publication. (c) Chen, X. G.; Asher, S. A.; Schweitzer-Stenner, R.; Mirkin, N. G.; Krimm, S. *J. Am. Chem. Soc.,* submitted for publication.

(8) Miyazawa, T.; Shimanouchi, T.; Mizushima, S. *J. Chem. Phys.* **1956,** *24,* 408−418.

(9) Triggs, N. E.; Valentini, J. J. *J. Phys. Chem.* **1992,** *96,* 6922−6931.

0002-7863/94/1516-11141$04.50/0

with bandwidths of 45 and 48 cm^{-1}, respectively. All of the polarized and depolarized spectra at all excitation wavelengths were easily modeled in terms of these two broad components. Of course, it is possible that the amide I band contains even more than two subbands, but we have no independent data indicating this more complex situation. Excitation profile measurements clearly indicate that the higher frequency band is selectively enhanced by the 190 nm amide $\pi \rightarrow \pi^*$ transition. The depolarization ratios of these subbands differ within each spectrum and show dispersion as the excitation wavelength is varied. Figure 1 lists the excitation wavelength dependence of the depolarization ratios ($\varrho = I_\perp/I_\parallel$) and the relative intensities of these bands. The depolarization ratios of I_1 and I_2 of 0.13 and 0.25 with visible excitation indicate that a number of electronic transitions are involved in the enhancement. As excitation occurs in the UV, the depolarization ratio of I_2 goes to 0.33, which indicates the sole enhancement by the *ca.* 190 nm linearly polarized $\pi \rightarrow \pi^*$ transition, in contrast to the earlier suggestion of Wang et al.[3a] As excitation approaches the $\pi \rightarrow \pi^*$ transition, the I_1 depolarization ratio also goes to 0.33, but it becomes comparatively weak compared to I_2.

If a Raman band is predominantly resonance enhanced by a single nondegenerate dipole transition, ϱ is equal to 0.33. Amide III and the 1380 cm^{-1} symmetric C$^\alpha$-methyl bending mode exhibit $\varrho = 0.33$ at all excitation wavelengths. This suggests that the intensities of these bands are dominated by the $\pi \rightarrow \pi^*$ transition even when they are excited far off resonance. The I_2 amide I subband intensity increases as excitation occurs within the $\pi \rightarrow \pi^*$ transition, and $\varrho = 0.33$. This is similar to the behavior of the amide I′ band of CH_3-$CONDCH_3$ (NMAD), where the amide I band only shows one component at 1629 cm^{-1} (data not shown).

The two amide I subbands could derive from different conformers, from Fermi resonance interactions with overtones or combinations of other NMA modes, or from the coupling of the NMA amide I motion with that of its hydrogen-bonded water molecules. It is unlikely that the two broad subbands derive from different conformations[10] for the following reasons: We have repeated our NMA–$(H_2O)_2$ calculation[1d] for conformer III,[10] which together with conformer IV is a stable structure,[6,7b] and find identical frequencies for these conformations. More importantly, the amide I modes of both conformers have essentially identical eigenvectors; unless the excited states of these conformations differ dramatically (unlikely), they should have the same $\pi \rightarrow \pi^*$ enhancements and show identical intensity dispersions, clearly in contradiction to what we observe (Table 1). Further, the small energy difference between conformations[6] should result in very similar relative intensities, which is also in contrast to that experimentally observed.

It is unlikely that these subbands derive from Fermi resonances since there are no simple combinations of *resonance-enhanced* bands which are at the correct frequency for strong Fermi interactions (1636 cm^{-1}). Fermi resonance interactions with combinations of non-resonance-enhanced bands should not lead to two bands which show such a drastic dispersion in their relative intensities.

These two subbands probably derive from the coupling of the amide I motion with the bending modes of water molecules hydrogen bonded to the amide group. This is suggested by the disappearance of this splitting in NMAD, since the D_2O bending frequency shifts out of the amide I′ region. We expect a 7 cm^{-1} upshift of the amide I′ band of NMAD from 1629 to 1636 cm^{-1} in NMA;[6,11] this is the measured mean frequency of the two amide I subbands and indicates that some interaction between amide I and water leads to the splitting.

There is a good theoretical basis for a possible amide I–H_2O bend coupling. Such coupled modes were predicted for the *cis*-NMA–$(H_2O)_2$ system[1d] and were also found by Williams[11] in a 4-31G calculation for *trans*-NMA–$(H_2O)_3$. Williams's H_2O bend scale factors gave four components at 1626, 1629, 1637, and 1645 cm^{-1}, all of which were mostly H_2O bend. The C=O stretching motion was spread among all of these bands. However, the highest and lowest frequency bands are mainly water bending and should be only weakly enhanced. Thus, the major C=O subbands should be separated by *ca.* 8 cm^{-1}. This calculation predicts a significant contribution of C–N stretching only to the higher frequency component at 1637 cm^{-1}. This would explain the selective resonance enhancement of the higher frequency subband, since the C–N stretching coordinate is the largest contributor for vibrational enhancement by the amide $\pi \rightarrow \pi^*$ transition.[7c]

We have repeated our 4-31G* calculation[1d] of the *trans*-NMA–$(H_2O)_2$ system and find that a small change in the H_2O bend scale factor also leads to coupled modes. We have now extended these calculations to the *trans*-NMA–$(H_2O)_3$ system and have systematically varied the H_2O bend and C=O stretch scale factors, and we find significant coupling of water bending with the amide I vibration. We can calculate results similar to those of Williams,[11] but in contrast to his results we also find coupled modes where the C=O stretch is highly concentrated into two modes. For example, we calculate doublet bands at 1628 cm^{-1} [H_2O bend 2 (34), C=O stretch (31), H_2O bend 1 (18), and CCN deformation (5)] and at 1634 cm^{-1} [C=O stretch (43), H_2O bend 1 (24), H_2O bend 2 (14), C–N stretch (13), and CCN deformation (7)]. Bends 1 and 2 refer to the two H_2O molecules hydrogen bonded to the C=O, and the numbers in parentheses are the contributions to the potential energy distribution.

Our calculation shows a selective C–N stretching contribution to the higher frequency band, which explains its selective resonance enhancement. In addition, the eigenvectors show that the lower frequency band should have the largest dipole derivative, which is consistent with it having the observed, dominating IR absorbance.[1d,6] We also examined NMA in $H_2{}^{18}O$ and found only small changes compared to NMA in $H_2{}^{16}O$, as expected from our normal mode calculations.

Our calculations and those of Williams[11] do not adequately account for the observed 20 cm^{-1} separation between the I_1 and I_2 subbands; the normal mode calculations only give a 6–8 cm^{-1} splitting. We suggest two possible explanations for the smaller calculated splitting. First, the modeled NMA–$(H_2O)_3$ system may not satisfactorily account for interactions of NMA with the surrounding water solvent. The bending modes of the coupled water molecules give rise to a band of frequencies which span the amide I mode frequencies. Interactions of some of these water modes with the amide I mode could lead to a larger splitting. The existence of such water bands is supported by our results on aqueous $CD_3CONHCH_3$ (data not shown); although the amide I mode is expected to be *ca.* 10 cm^{-1} lower in frequency than the amide I mode in NMA,[6] $CD_3CONHCH_3$ still exhibits a large amide I frequency difference between IR and UV Raman excitations. This indicates the coupling to water modes for this system as well. Another possible source for an increased splitting could result from anharmonic coupling between these amide I–H_2O bending modes.[7b]

We conclude that we have found compelling experimental and theoretical evidence for the dynamic coupling of the amide I mode of NMA with the bending motions of its hydrogen-bonded water molecules. This coupled motion is likely to have biological significance in areas such as energy relaxation and vibrational energy transport since it appears that the amide group of NMA is intimately coupled to its solvent environment bath. Such strongly coupled water molecules may influence protein structure.

Acknowledgment. We gratefully acknowledge support from NIH Grant R01GM30741-12 to S.A.A. and NSF Grants MCB-9115906 and DMR-9110353 to S.K. R.S.-S. acknowledges fellowship support from the Max Kade foundation.

---

(10) Mirkin, N. G.; Krimm, S. J. *Mol. Struct.* **1991**, *242*, 143–160.
(11) Williams, R. W. *Biopolymers* **1992**, *32*, 829–847.