COMMENTS

Comment on "Vibrational Assignments of *trans*-*N*-Methylacetamide and Some of Its Deuterated Isotopomers from Band Decomposition of IR, Visible, and Resonance Raman Spectra"

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N-Methylacetamide, a much-studied model compound for the peptide bond, has been extensively characterized by ultraviolet resonance Raman (UVRR) spectroscopy, because of the potential of UVRR spectroscopy to probe the structure and dynamics of proteins. Recent papers on this subject by Chen et al.[1,2] have called our work[3,4] into question, and we would like to clarify the issues:

1. Enhancement Mechanism of Amide S. We have drawn attention to amide S,[3,4] a UVRR peptide band near 1390 cm^{-1}, which is not one of the classic amide modes (I−V) but is resonance enhanced in a structure-specific manner: its intensity decreases linearly with α-helical content in polypeptides and proteins.[3] This band arises from a C_α−H bending vibration as revealed by H/D substitution,[3] and we attributed its enhancement to vibrational mixing with the nearby amide III, which is enhanced via resonance with the $\pi-\pi^*$ electronic excitation.

Chen et al.[1] find this explanation to be "inaccurate because in the harmonic case normal modes of the same molecule do not mix with each other." By "vibrational mixing" we meant the sharing of internal coordinates between normal modes of similar energy, rather than the interaction of normal modes themselves. Since this commonly used shorthand is subject to misinterpretation, perhaps it should be dropped. A clear indication that amide S and amide III do share internal coordinates is the *upshift* of amide III (1313 → 1334 cm^{-1} in the case of NMA) upon C_α−H/D substitution, which downshifts amide S out of the spectral region. This coordinate sharing is important because it can explain both the diminished amide S intensity and the elevated amide III frequency of α-helical peptides, in which the C_α−H reorientation alters the kinematics and diminishes the amide S/III coordinate sharing.[3] However, the exact nature of the coordinate displacement in the excited state, which is responsible for the amide S enhancement, remains an open question. We suggested that the C−N bond displacement was responsible in view of the dramatic intensity redistribution that is produced by amide NH/D exchange: amide III and S both disappear, while amide II′, now a nearly pure C−N stretching vibration, is greatly intensified. But Chen et al. calculate a negligible C−N contribution to the potential energy distribution of amide S (labeled CCH_3 sb in their paper). They suggest that a contribution from C−C stretching is instead responsible for the intensity. We do not contest this point.

[In their response to this comment, Asher et al.[5] object to calling the mode amide S since it is not encompassed by the coordinates of the CONH group. However, the name seems appropriate to us since a band at ∼1380 cm^{-1} is a characteristic and structure-sensitive feature in the UVRR spectra of any amide[6] having a CH group attached to N, a category that includes all peptides; the designation "S" leaves the standard amide mode numbering system undisturbed.]

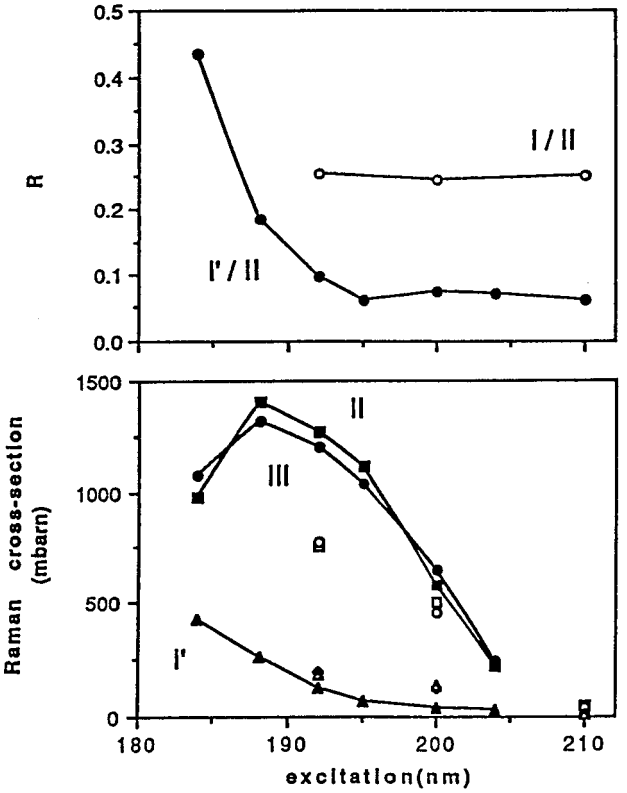

Figure 1. Aqueous NMA intensity data from Chen et al.[2] (open symbols) and Wang et al.[4] (closed symbols). Bottom: cross sections for amide I (◇), I′ (in D_2O, △, ▲), II (□, ■), and III (○, ●). Top: ratios of amide I to II (○) and amide I′ to II (●).

2. Enhancement Mechanism of Amide I. Chen et al.[1,2] also dispute our finding[4] that the amide I band, which arises mainly from the C═O stretching coordinate, is not enhanced via resonance with the first electronic transition ($\pi-\pi^*$) of aqueous NMA, at ca. 190 nm, but rather by a deeper UV transition, at ca. 165 nm. This finding was based on measured excitation profiles, reproduced in Figure 1, which show a maximum with excitation at 188 nm for amide II and III, but not for amide I. The amide I band has very low intensity, which increases continuously at wavelengths down to 184 nm, showing no local maximum or inflection. [Our measurements were actually on amide I′ of NMA-*d* in D_2O, in order to avoid interference from the near-coincident water bending mode.] The amide I′ intensities could be fit[4] to an Albrecht *A* term expression for preresonance with an electronic transition at 165 nm; an absorption band near this wavelength has been assigned to a locally excited carbonyl transition.

Chen et al. concluded instead that amide I *is* resonant with the 190 nm $\pi-\pi^*$ transition, citing their own intensity measurements.[2] Apart from quantitative discrepancies in cross-section values, possibly attributable to self-absorption corrections,[2] the two data sets are not in disagreement about the shape of the excitation profiles; the amide I or I′ intensity increases continuously with decreasing wavelength in both cases. Because their measurements did not extend below 192 nm, Chen et al. could not actually determine whether or not their excitation profiles

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reach maxima at this wavelength. They obtained Albrecht *A* term fits with $\lambda_0 \approx 185$ nm, but the fitting data were limited to $\lambda > 215$ nm, and the long extrapolation is of uncertain reliability. Mainly, they argued that amide I is resonant with the $\pi-\pi^*$ transition, because its intensity tracks amide II.[2] Their evidence for this assertion was a plot of the intensity ratio for the two bands, showing a nearly constant value at wavelengths down to 192 nm. These data are reproduced in Figure 1 and compared with our intensity ratio for amide I′ and II. Our ratio is also roughly constant at wavelengths down to 192 nm; this phenomenon merely reflects the fact that the intensities increase more or less in parallel in the preresonance region. However, the ratio increases markedly at 188 and 184 nm, where the amide II profile bends over, while the amide I′ profile does not. Our ratios differ quantitatively from those of Chen et al. because they used H_2O, not D_2O, solutions, in which amide I overlaps, and indeed interacts with,[2] the H_2O bending mode. The trends in the two data sets are entirely compatible, however, and it is evident that the amide I and II intensities do *not* track one another, when data are considered which adquately bracket the 190 nm resonance.

This issue is important because the profiles help explain a long-standing enigma of amide UVRR spectra, namely, that amide I is very weak in aqueous solution but becomes the strongest band in non-H-bonding solvents. We have argued[4] that the main effect of H bonding is to lower the energy of the C═O π^* fragment orbital, giving it a greater contribution to the amide HOMO, which therefore becomes antibonding with respect to the C═O bond. [See Figure 5 of ref 4 for a pictorial diagram of the molecular orbitals.] Since the LUMO is also C═O antibonding in character, the electronic excitation produces minimal displacement along the C═O stretching coordinate. Only a higher energy excitation, from a deeper filled orbital, produces significant enhancement of amide I. However, in the absence of H bonding the C═O π fragment orbital is dominant in the HOMO, which is therefore C═O bonding in character. Electronic excitation then produces a large displacement along the C═O bond as well as the C−N bond. [The HOMO is bonding and the LUMO antibonding with respect to the C−N bond, regardless of H bonding.] This is reflected in parallel excitation profiles for amide I, II, and III when NMA is dissolved in acetonitrile.[4]

[In their response, Asher et al.[5] continue to emphasize the apparent constancy of the preresonant amide I/II intensity ratio. In addition, they present new data, which are intended to disprove our excitation profile. In their newly reported 184 nm excited resonance Raman spectrum, they are unable to detect the amide I′ band of NMA in D_2O, but only the amide II′ band. However, the amide II′ band is very strong, much stronger than the amide II band in H_2O, and the spectrum displayed by Asher et al. is quite noisy. Consequently, the significance of amide I′ undetectability is uncertain. Asher et al. claim superior technology for their experiment (stronger laser, better detector), but our experimental design was superior in one crucial aspect: we constrained the entire light path, from laser to detector, in a purged enclosure, whereas Asher et al. purged only their laser assembly and sample compartment. They point out that absorption by O_2 "efficiently rejected the Rayleigh scattering"; no doubt, it rejected most of the Raman scattering as well. Indeed, it is possible that the 10-fold higher laser power employed by Asher et al. might have produced saturation of their Raman signal, due to excited state pumping and/or photodegradation of the sample. Our purged enclosure permitted detection of the RR signal with lower laser power. Also, we employed a sensitive photomultiplier and scanned only the amide I′ and sulfate internal standard bands, in order to minimize the signal accumulation time. We collected data at 188 as well as 184 nm; both cross sections reveal the upward trend in the amide I′ excitation profile. For all these reasons, we do not accept Asher et al.'s conclusion that our "measured spectrum was spurious".

We also reject Asher et al.'s contention that a depolarization ratio of 0.33 at 244 or 206 nm "comes very close to proving that the amide I′ enhancement is dominated by a single 186 nm amide $\pi-\pi^*$ transition". If the amide I′ intensity derives mainly from the 165 nm resonance, as we contend, then the depolarization ratio would also be close to 0.33, especially if the dipole directions of the 186 and 165 nm transitions are not very different, as seems likely. Nor are we persuaded by the very weak amide II′+I′ combination band observed by Asher et al. with 206 nm excitation. Because the amide II′ enhancement is so high, it would not take much displacement of the 190 nm excited state along the amide I′ coordinate to produce a weak combination band. We do not insist that this displacement is zero, only that it is much less than the amide I′ displacement in the 165 nm excited state.]

References and Notes

(1) Chen, X. G.; Schweitzer-Stenner, R.; Asher, S. A.; Mirkin, N. G.; Krimm, S. *J. Phys. Chem.* **1995**, *99*, 3074−3083.

(2) Chen, X. G.; Asher, S. A.; Schweitzer-Stenner, R.; Mirkin, N. G.; Krimm, S. *J. Am. Chem. Soc.* **1995**, *117*, 2884−2895.

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

(4) Wang, Y.; Purrello, R.; Georgiou, S.; Spiro, T. G. *J. Am. Chem. Soc.* **1991**, *113*, 6368−6377.

(5) Asher, S. A.; Li, P.; Chi, Z.; Chen, X. G.; Schweitzer-Stenner, R.; Mirkin, N. G.; Krimm, S. *J. Phys. Chem. A* **1997**, *101*, 3992.

(6) Jordan, T.; Spiro, T. G. *J. Raman Spectrosc.* **1994**, *25*, 537.