Amide V overtone assignment of a conformation-sensitive band in the UV resonance Raman spectra of peptides and proteins

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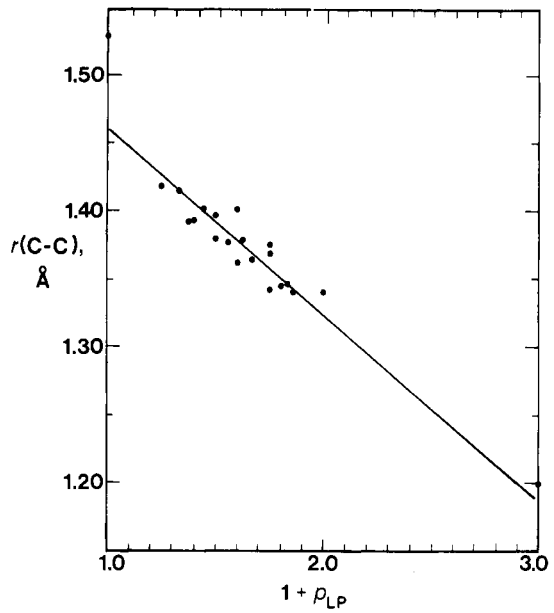

Figure 5. Experimental data for the C–C bond distances, r(C–C) in angstroms plotted as a function of the σ-bond order plus Pauling π-bond order $1 + p_{LP}$. The straight line conforms to the linear least-squares fit r(C–C) = $-0.136(1 + p_{LP}) + 1.597$ Å. The standard deviation in r(C–C) is 0.019 Å, and the correlation coefficient $r^2 = 0.8762$.

with any of the SCF results. In comparison, the correlation of Pauling bond order with bond length, which is plotted in Figure 5, is not as good even though this has been considered to be one of the better uses of this type of bond order.

On purely empirical grounds the orthobenzylic coupling constants emerge as a valuable experimental method for investigations of bonding in conjugated systems, particularly in view of the fact that unlike vicinal (cis) H–H coupling constants, which are a very well investigated parameter,[31,32] J_{ob} exhibit very little dependence[4] on other structural variables such as ring size and the presence of substituents on the aromatic rings.

Experimental Section

Spectral Data. All experimental NMR data in Table I were obtained on a Bruker Instruments WM-400 spectrometer using 5-mm sample tubes with approximately 5% w/v solutions in chloroform-*d* unless otherwise stated. Digital resolution was at least 0.05 Hz. All spectra were interpreted as first-order, and orthobenzylic coupling constants were assumed to be negative.[4] Details are given in the supplementary material.

Syntheses. The details of the syntheses of 2-methyltriphenylene (**10**), 3-methylchrysene (**11**), 6-methylchrysene (**12**), 1-methylpyrene (**13**), 2-methylpyrene (**14**), 4-methylpyrene (**15**), 6-methylbenzanthracene (**16**), and 6-methyl[3,4]benzphenanthrene (**17**) are given in the supplementary material.

Supplementary Material Available: Descriptions of experimental procedures, syntheses of compounds, and spectral analyses (16 pages). Ordering information is given on any current masthead page.

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Amide V Overtone Assignment of a Conformation-Sensitive Band in the UV Resonance Raman Spectra of Peptides and Proteins

Samuel Krimm,*,† Sunho Song,‡ and Sanford A. Asher‡

Contribution from the Biophysics Research Division, University of Michigan, Ann Arbor, Michigan 48109, and Department of Chemistry, University of Pittsburgh, Pittsburgh, Pennsylvania 15260. *Received October 31, 1988*

Abstract: A band in the 1300–1500-cm^{-1} region has been observed to be enhanced in the UV resonance Raman spectra of peptides and proteins. We show, on the basis of normal-mode analysis of experimental data from *N*-methylacetamide (NMA) and several conformations of poly(L-glutamic acid), that this band can be definitively assigned to the overtone of the amide V mode. The results of $^{13}C^{15}N$ isotopic substitution on some NMA analogues support this assignment. The sensitivity of this band to polypeptide chain conformation can make it a new sensitive probe of secondary structure in proteins.

We recently presented[1,2] experimental evidence that a band in the ~1400-cm^{-1} region of the UV resonance Raman spectra of aqueous solutions of peptides and polypeptides is associated with the amide π–π^* electronic transition of the peptide group and that its vibrational mode, because of the disappearance of the band on deuteration, must have an NH component. Although not seen in preresonance spectra,[3,4] such a band has been observed in UV resonance spectra of *N*-methylacetamide[5,6] (NMA; although at the higher frequency of 1496 cm^{-1}), in polypeptides,[4,7] and in proteins.[8-10] Previous to our study,[1] it had not been conclusively assigned, having been attributed to CH_3 antisymmetric bend in NMA,[3,5] to CH_2 wag or twist in poly(L-lysine) (PLL)[7] and CH_2 bend in cytochrome c,[9] to COO^- symmetric stretch in ionized

† University of Michigan.
‡ University of Pittsburgh.

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poly(L-glutamic acid) (PGA)[4] and tropomyosin,[10] to a "coupled vibration of the –NH–CH–CO group" in ionized PLL,[4] and to the photoinduced cis-amide group in NMA.[11]

In the present paper we show how this band can be definitively assigned to the overtone of amide V, a mode containing CN torsion plus NH out-of-plane bend. This assignment is based on reliable normal-mode analyses of peptides and polypeptides[12] that account in detail for the conformation dependence of the amide V frequency. It is further supported by isotopic substitution studies that we report on some NMA analogues. Since this mode, while usually strong in the infrared, is weak in the normal Raman spectrum, the ability to identify it through its strong overtone in the UV resonance Raman spectrum provides important new conformational information on such molecules, especially in aqueous solution. As we have noted in a preliminary communication,[1] this band can be a new sensitive probe of secondary structure in proteins.

Experimental Section

Amino acid dimers were obtained from Sigma Chemical Co. Sodium acetate, glutaric acid, and butyric acid, all of the highest purity available, were obtained from Aldrich Chemical Co. [1-^{13}C]Glycine and [^{15}N]-glycine of 99% isotope purity were obtained from Cambridge Isotope Laboratory. Carbobenzoxy chloride and *p*-nitrophenol were obtained from Aldrich Chemical Co.

Carbobenzoxy[1-^{13}C]glycyl[^{15}N]glycine (I) was synthesized via the carbobenzoxy[1-^{13}C]glycine-*p*-nitrophenyl ester[13] according to the procedures of Swenson and Koob.[14] Glycylglycine with a $^{13}C^{15}N$-labeled amide group, [$^{13}C^{15}N$]DGL, was prepared by catalytic hydrogenation of I over palladium in 1:1 methanol–water at atmospheric pressure. Glycylglycine methyl ester hydrochloride, DGL-OMe, and its isotopic analogue [$^{13}C^{15}N$]DGL-OMe were prepared from DGL and [$^{13}C^{15}N$]DGL, respectively, by the method of Boreham et al.[15]

Raman spectra were obtained by using an instrument based on a Nd:YAG pumped dye laser with doubling and mixing crystals, a triple monochromator, and a Reticon detector.[16] Infrared spectra were measured on a Mattson Cygnus 100 FT-IR. Raman cross sections were calculated from measured peak-height ratios, corrected for spectrometer throughput, between the Raman band of the molecule and the 932-cm^{-1} band of the perchlorate ion added as an internal intensity standard.[5]

Normal-Mode Assignment of Amide V Overtone

As first pointed out by Hirakawa and Tsuboi,[17,18] the normal modes that are expected to undergo resonance Raman enhancement are those involving atomic displacements that distort the ground electronic-state geometry toward that of the resonant-excited electronic state. Since the amide II (NH in-plane bend plus CN stretch) and amide III (CN stretch plus NH in-plane bend) modes of NMA[5,6] and the amide II′ (essentially CN stretch) mode of N-deuterated NMA[6] are significantly enhanced in the UV region, it has been inferred[6] that the geometry change on excitation is dominated by a change in the CN bond length. We will consider below the mechanism for the enhancement of the overtone of amide V. First we discuss the arguments, based on normal-mode analyses and isotopic substitution, for the overtone assignment.

***N*-Methylacetamide.** It is useful to examine in detail those bands of aqueous NMA that are enhanced as the excitation wavelength decreases from the visible region to 220 nm.[5] These are listed in Table I, together with the calculated frequencies and detailed potential energy distributions (PED) from several all-atom normal-mode calculations.[3,19-21]

Table I. Enhanced UV Resonance Raman Bands of Aqueous *N*-Methylacetamide and Calculated Frequencies and Normal Modes

ν(obsd)[a]	ν(calcd)[b]	potential energy distribution[c]
1639	1666	CO s (67), CCN d (10)
	1661	CO s (70), CN s (16)
	1706	CO s (86), CCN d (9)
	1655	CO s (70), CCN d (13), NH ib (13)
1581	1568	NH ib (48), CN s (38)
	1557	NH ib (71), CN s (24)
	1516	NH ib (48), CN s (31), C(N)H$_3$ r (11)
	1570	NH ib (41), CN s (41), C(N)H$_3$ sb (7)
1496	1496[d]	741: NH ob (73), CN t (17), CO ob (10) (ref 19)
		589: CN t (39), NH ob (37), (ref 20)
1316	1305	NH ib (36), CN s (20), CO ib (13), CC s (12)
	1293	CN s (27), CC s (23)
	1299	CN s (25), CO ib (22), NH ib (19)
	1312	CN s (29), NH ib (25), CC s (15)
1162	1173	C(N)H$_3$ r (51), NC s (13), CNC d (10)
	1169	C(N)H$_3$ r (51), NC s (26)
	1154	C(N)H$_3$ r (61), NH ib (18), NC s (9)
	1153	C(N)H$_3$ r (55), NC s (10), CO s (10)
881	875	CN s (18), CC s (18), CO s (17), C(N)H$_3$ r (15), CCN d (13)
	893	CC s (23), C(N)H$_3$ r (19)
	863	CN s (35), CC s (14), C(N)H$_3$ r (13)
	881	CN s (25), C(N)H$_3$ r (20), CC s (15)
628	628	CO ib (44), CC s (26), CNC d (11)
	629	CO ib (37), CC s (26)
	637	CO ib (43), CC s (32), CNC d (6)
	629	CO ib (35), CC s (22), CNC d (12)

[a] Reference 5 (in cm^{-1}). [b] The four values are from ref 19 (an empirical refinement to liquid-state frequencies), 3 (as in ref 19), 20 (an empirical refinement to N_2-matrix-isolated frequencies), and 21 (an ab initio calculation of in-plane modes of an isolated molecule), respectively (in cm^{-1}). [c] s = stretch, d = deformation, r = rock, sb = symmetric bend, ib = in-plane bend, ob = out-of-plane bend. [d] Based on overtone of amide V, measured at 748 cm^{-1} in the infrared spectrum of aqueous NMA.[1]

Enhancements occurring in the amide I, II, and III modes, at 1639, 1581, and 1316 cm^{-1}, respectively, have been well recognized, as has the association of the amide II and III enhancements with a change in the CN bond length on excitation. The latter also explains the enhancement of the 881-cm^{-1} skeletal mode. It is interesting that bands at 1162 and 628 cm^{-1} are also enhanced, although their main displacement coordinates related to the amide group are NC stretch (s) (and possibly CO s or NH in-plane bend (ib)) for the former and CO ib and CC s for the latter. This probably indicates that significant geometrical changes in the amide group of NMA other than just in the CN bond accompany this excitation.

None of the normal-mode analyses support the assignment of the 1496-cm^{-1} band to a fundamental of the amide group. Calculated bands near this region,[3,19-21] but not as high, are uniformly assignable to bending modes of the CH_3 groups, which are not expected to be enhanced by the preresonant excitation of the amide π–π^* transition. Nor can the 1496-cm^{-1} band be reasonably assigned to a cis-amide group, despite the suggestion[11] that this band could correspond to the calculated 1487-cm^{-1} amide II mode (observed at 1485 cm^{-1}) of *cis*-NMA.[20] The latter studies were on NMA molecules (mostly trans but some cis) isolated in a N_2 matrix. This is evident from the non-hydrogen-bonded calculated (and observed) frequencies:[20] amide I, 1706 (1707); amide II, 1516 (1511); amide V, 589 (619) cm^{-1}. Since hydrogen bonding of *trans*-NMA to H_2O results in an observed increase in amide II of 1581 – 1511 = 70 cm^{-1}, the predicted location of amide II of hydrogen-bonded *cis*-NMA should be near 1485 + 70 = 1555 cm^{-1}. There is no evidence of such a band in the

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Table II. Enhanced UV Resonance Raman Bands of Poly(L-glutamic acid) and Calculated Frequencies and Normal Modes

ν(obsd)[a]	ν(calcd)[b]	potential energy distributions[c]
		β-Poly(L-glutamic acid)
1648	1649[d]	CO s (74), CN s (18)
1548	1550	NH ib (58), CN s (18)
1445	1462	2 × 731: CN t (50), NH ob (20)
	1452	2 × 726: CN t (36), NH ob (15)
	1436	2 × 718: CN t (34), NH ob (31), CO ob (11)
	1426	2 × 713: CN t (42), NH ob (31)
1393	1408	2 × 704: CN t (35), NH ob (15)
1338(?)	1342	2 × 671: CN t (25), CO ib (21), CO ob (13) α-PGA
1279		α-PGA
1248	1249	H^{α} b (22), NC^{α} s (18), CN s (15), CH_2 tw (15), NH ib (11)
		α-Poly(L-glutamic acid)
1651	1656	CO s (82), CN s (11), $C^{\alpha}CN$ d (10)
1552	1537	NH ib (46), CN s (33), CO ib (11), $C^{\alpha}C$ s (10)
1338	1356	2 × 678: CN t (19), $NC^{\alpha}C$ d (12), NH ob (11) [670][e]
1300	1286	2 × 643: CN t (77), NH ob (44), NH···O ib (14)
1279	1263	NH ib (37), H^{α} b (23), CN s (10), CO ib (10)
1241	1252	2 × 626: CN t (42), NH ob (17) [618][e]
		Ionized Poly(L-glutamic acid)[f]
1664	1675	CO s (71), CN s (18), $C^{\alpha}CN$ d (11)
1564	1572	NH ib (57), CN s (20), $C^{\alpha}C$ s (10)
1397	1384	2 × 692: CN t (31), CO ib (21), NH ob (13)
1320	1304	2 × 657: CN t (54), CO ob (19), NH ob (19), NH···O t (10)
1257	1271	CH_2 w (39), CN s (14), NH ib (11), NC^{α} s (10)

[a] Reference 1 (in cm^{-1}). [b] β-PGA, ref 24; α-PGA, ref 25; ionized PGA, ref 26 plus unpublished calculations (in cm^{-1}). [c] s = stretch, w = wag, t = torsion, ib = in-plane bend, ob = out-of-plane bend, tw = twist. [d] Average of calculated (which agree with solid state observed) frequencies of 1668 (1666) and 1630 (1624) cm^{-1}, splitting being due to transition dipole coupling.[28,29] [e] Observed amide V bands.[25] [f] Calculations by using polyglycine II force field[34] (ref 26 plus unpublished calculations).

220-nm excited spectrum,[5] although it might be overlapped by the strong 1581-cm^{-1} band. In any case, there is no sound basis for assigning the 1496-cm^{-1} band to a fundamental of the cis-amide group.

On the other hand, an assignment of this band to the overtone of amide V is consistent with the location of this fundamental. Amide V is found at 619 cm^{-1} in N_2-matrix-isolated *trans*-NMA,[20] at 725 cm^{-1} in neat NMA,[22] and at 780 cm^{-1} in crystalline NMA.[23] It is thus very sensitive to the environment of the molecule. We have recently observed[1] that in aqueous solution this mode occurs at 748 cm^{-1}, an assignment confirmed by its disappearance on N deuteration. The 1496-cm^{-1} band, at twice this frequency, is therefore a strong candidate for an overtone assignment (Fermi resonance with amide II, established in crystalline NMA,[23] does not seem to be a dominating factor). It also seems to disappear on N deuteration,[6] although this is difficult to be sure of because of the strong amide II′ band near 1500 cm^{-1}. Incidentally, we observe a band at 2948 cm^{-1}, using 218-nm excitation, that can be assigned to the fourth harmonic of amide V (no third harmonic band is seen).

Thus, a very strong case can be made for assigning the enhanced 1496-cm^{-1} band in the UV resonance Raman spectrum of aqueous NMA to the overtone of amide V.

Poly(L-glutamic acid). Experimental studies[1,2] clearly show that the frequencies of UV resonance-enhanced bands of PGA in the region near 1400 cm^{-1} are dependent on the conformation of the polypeptide chain. An analysis of the well-characterized normal modes of these structures[24-26] (see Table II) demonstrates that these frequency changes follow exactly from the variations in amide V frequencies, thus providing compelling support for the overtone assignment.

The resonance-enhanced bands of β-PGA are listed in Table II, together with the frequencies and PEDs calculated for an infinite β-sheet structure.[24] The force field was transferred without change from that refined for β-poly(L-alanine) (PLA),[27] with transition dipole coupling[28,29] included for the amide I and II modes. All calculated modes with a CN torsion (t) plus NH out-of-plane bend (ob) contribution (i.e., amide V modes) are listed. It can be seen that there is good agreement between observed and calculated frequencies for the amide I, II, and III modes at 1648, 1548, and 1248 cm^{-1}, respectively. The weak 1279-cm^{-1} band and possibly the very weak contribution near 1338 cm^{-1} are probably due to the presence of some α-PGA.[2] No fundamental modes related to peptide group vibrations are expected in the 1500–1350-cm^{-1} region;[24] the observed (and calculated) frequencies in this region are mainly side-chain CH_2 bend (b) and wag (w) modes mixed with some side-chain CC s and are not expected to be resonance enhanced. On the other hand, overtones of all the calculated amide V modes have counterparts in the resonance Raman spectrum, with reasonable frequency agreement (in distinction to α-PGA, no bands assignable to amide V are observed in the infrared spectrum of β-PGA).

The calculated modes for α-PGA[25] (see Table II) are based on a force field transferred without change from that of α-PLA.[30] Again, the observed amide I, II, and III modes at 1651, 1552, and 1279 cm^{-1}, respectively, are reasonably well reproduced, considering that no force constant refinement was done. For the other bands in the 1400–1200-cm^{-1} region, the assignments to overtones of amide V modes are quite convincing, for two reasons. First, the frequency agreement with observed amide V infrared bands[25] at 670 cm^{-1} (overtone predicted at 1340 cm^{-1}, compared to observed band center of gravity at 1338 cm^{-1}) and 618 cm^{-1} (overtone predicted at 1236 cm^{-1}, compared to observed band at 1241 cm^{-1}) is very good. Second, because the hydrogen bond in α-PGA is weaker than that in β-PGA,[12] the amide V frequencies of the former are lower than those of the latter, and this is mirrored in the lower expected and observed overtone frequencies.

Ionized PGA has been referred to as having a "random coil" conformation, but CD studies[31] and conformational energy calculations[32,33] as well as recent normal-mode analyses[26] provide strong support for the earlier proposal[31] that the local chain structure is not "random" but exhibits a preference for conformations corresponding to a helix with ~2.5 residues/turn. This structure was the basis for normal-mode calculations (ref 26 and unpublished results by P. K. Sengupta and S. Krimm), using β-PLA,[27] α-PLA,[30] and 3_1-polyglycine II[34] force fields. The results for the latter case are shown in Table II (the frequencies calculated by using the other force fields are slightly different, but the conclusions are not thereby altered). It is evident that the amide I, II, and III modes are well accounted for and that the other enhanced bands, at 1397 and 1320 cm^{-1}, are again explainable as overtones of the expected amide V modes.

Diketopiperazine. While the experimental results on a number

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Table III. Enhanced UV Resonance Raman Bands of Aqueous Diketopiperazine and Calculated Frequencies and Normal Modes

ν(obsd)[a]	ν(calcd)[b]	potential energy distribution[c]
1676	1657[d]	Rs1 (28), CO s (26), NH ib (16), CH_2 w (15), NC^{α} s (12)
1533	1521	Rs2 (43), NH ib (22), CH_2 b (11), CO ib (10)
1321	1313	CH_2 w (56), CO s (19)
1153	1148	NC^{α} s (44), CO s (19)
806	795	Rs1 (58), NC^{α} s (14), Rd2 (11)

[a] Reference 2 (in cm^{-1}). [b] Reference 36 (in cm^{-1}), for crystalline diketopiperazine. [c] s = stretch, b = bend, w = wag, ib = in-plane bend, Rs1 = ring stretch 1 = 0.71 (CN s + $C^{\alpha}C$ s), Rs2 = ring stretch 2 = 0.71 (CN s - $C^{\alpha}C$ s), Rd2 = ring deformation 2 (see ref 36). [d] In crystalline DKP this mode is in Fermi resonance with 2 × 832, the latter having a PED of NH out-of-plane bend (ob) (87), this symmetry coordinate being defined as 0.88 NH ob - 0.40 $C^{\alpha}C$ t + 0.20 CN t - 0.16 NC^{α} t.

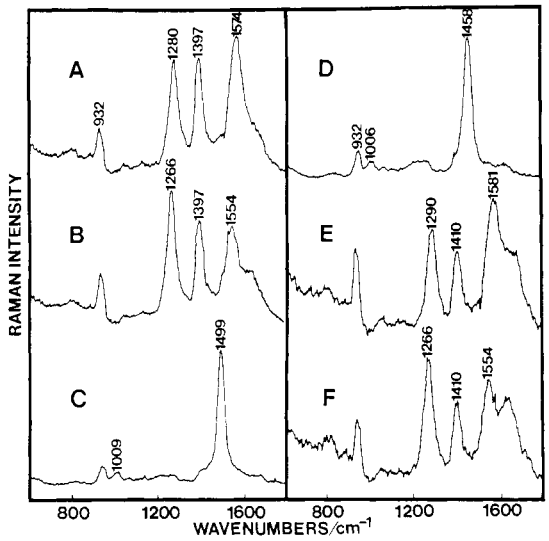

Figure 1. Resonance Raman spectra of (A) 23.1 mM glycylglycine in H_2O at pH 5.6, (B) 23.1 mM [1-^{13}C]glycyl[^{15}N]glycine in H_2O at pH 5.6, (C) 35.0 mM glycylglycine in D_2O at pD 5.6, (D) 22.4 mM [1-^{13}C]glycyl[^{15}N]glycine in D_2O at pD 5.6, (E) 55.8 mM glycylglycine methyl ester hydrochloride in H_2O at pH 7.0, and (F) 53.9 mM [1-^{13}C]glycyl[^{15}N]glycine in H_2O at pH 7.0. All spectra were obtained at 218-nm excitation with 0.2 M $NaClO_4$.

of NMA analogues and PLL[2] are consistent with the amide V overtone assignment, normal-mode analyses or infrared studies remain to be done for these molecules. However, such studies have been done for diketopiperazine (DKP),[35,36] and since the resonance-enhanced Raman spectrum of this molecule differs from those considered above,[2] it is instructive to see whether a similar understanding of its spectrum follows from knowledge of its normal modes.[36]

The observed enhanced Raman bands for DKP[2] are given in Table III together with the calculated Raman-active frequencies and normal-mode PEDs.[36] All of these bands can be assigned to fundamentals that involve more or less distortion of the cis-amide group through CO s and/or CN s displacements. Even the 1321-cm^{-1} band is enhanced, although it is mostly CH_2 w with some CO s. A similar, though smaller, enhancement is seen for a band at 1153 cm^{-1}, which is assignable to a NC^{α} s + CO s mode.[36] Since there is strong evidence[35] that the overtone of a mode (at 832 cm^{-1}) involving CN t + NH ob is in Fermi resonance with the CO s mode, it is not certain at present whether the observed 1676-cm^{-1} band should be assigned to the CO s fundamental or to this overtone. Nevertheless, it is important to note that, although different from the trans-amide group frequencies, the spectrum of DKP is very well accounted for by the normal-mode analysis.[36] This provides additional confidence in the overtone assignments for the trans-amide group, since these were based on comparable normal-mode analyses.

(35) Cheam, T. C.; Krimm, S. *Spectrochim. Acta* **1984**, *40A*, 481–501.
(36) Cheam, T. C.; Krimm, S. *Spectrochim. Acta* **1984**, *40A*, 503–517.

Table IV. Enhanced UV Resonance Raman Bands (in cm^{-1}) and Their Assignments of Aqueous Glycylglycine, Its Methyl Ester Hydrochloride, and Isotopic ND and $^{13}C^{15}N$ Derivatives

DGL		[$^{13}C^{15}N$]-DGL		DGL-OMe	[$^{13}C^{15}N$]DGL-OMe	amide mode assgnmt
NH	ND	NH	ND			
	1009		1006			III′
1280		1268		1290	1266	III
1397		1397		1410	1410	2 × V
	1499		1458			II′
1574		1554		1581	1554	II
2556		2526				2 × III
2673		2657				III + 2 × V
2784		2783				4 × V
2845		2816				II + III
2974		2942				II + 2 × V
	2977		2897			2 × II′

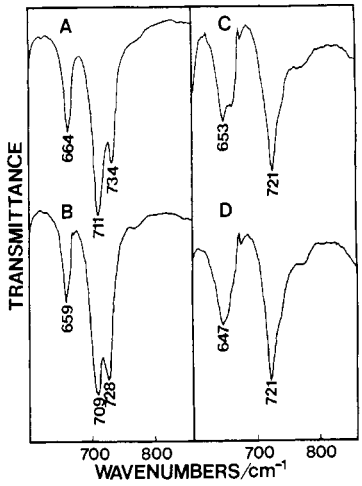

Figure 2. Infrared spectra of solid-state (A) glycylglycine, (B) [1-^{13}C]glycyl[^{15}N]glycine, (C) glycylglycine methyl ester, and (D) [1-^{13}C]glycyl[^{15}N]glycine methyl ester.

Effect of Isotopic Substitution on Amide V Overtone

In addition to the compelling conclusions from normal-mode analyses, experimental data on isotopic derivatives are consistent with the amide V overtone assignment of the ~1400-cm^{-1} UV resonance-enhanced Raman band. As seen in Figure 1, aqueous DGL exhibits a resonance-enhanced band (with 218-nm excitation) at 1397 cm^{-1} that, similar to NMA,[1] disappears on N deuteration, consistent with its assignment to 2 × amide V. On $^{13}C^{15}N$ substitution, this band remains unchanged although, as expected, the amide II (at 1574 cm^{-1}) and amide III (at 1280 cm^{-1}) modes shift down (since they both contain significant CN s contributions[12]). (Band assignments for these and various observed combination bands are given in Table IV.) Similar behavior is observed for DGL-OMe (see Figure 1), where the 1410-cm^{-1} band is unchanged on isotopic substitution. If these bands originate from 2 × amide V, we should expect that the fundamental itself would be essentially unaffected by isotopic substitution. The infrared spectra of solid-state samples of these compounds (Figure 2) show that this is indeed the case: for DGL the amide V mode undergoes only a small shift, from 711 to 709 cm^{-1}, whereas for DGL-OMe this band remains at 721 cm^{-1} on isotopic substitution. [These frequencies are, of course, not expected to be exactly half the solution overtone frequencies since the molecules are in different states for the two samples, with different strengths of hydrogen bonds (cf. the similar situation for NMA[1]) and therefore different amide V frequencies.] The absence of any significant shift in the amide V frequency on $^{13}C^{15}N$ substitution is not surprising, since such substitution is not expected, in first order, to perturb the CN t and NH ob contributions

Table V. Raman Frequencies, Cross Sections (σ_{218}), and Preresonant State Frequencies (ν_e) for Amide V Overtone Bands and for Carboxylate Ions

molecule[a]	freq[b]	σ_{218}[c]	ν_e[d]
DGL, pH 5.6	1397	15.3	197
[$^{13}C^{15}N$]DGL, pH 5.6	1397	13.2	
DGL-OMe, pH 7	1410	2.4	
[$^{13}C^{15}N$]DGL-OMe, pH 7	1410	3.5	
DLG, pH 2	1411	5.7	196
DLG, pH 4.5	1407	18.6	198
DLG, pH 11	1407	12.1	196
DLL, pH 2	1404	5.7	196
DLL, pH 4	1397	12.7	197
DLL, pH 11	1393	8.7	199
PGA, α-helix	1328	2.7	196
PGA, β-sheet	1445	4.4	
	1393		
PGA, disordered	1397	8.3	194
PLL, α-helix	1348	2.5	194
PLL, β-sheet	1400	8.3	201
	1359		
PLL, disorderedd	1386	6.0	198
Na acetate, pH 11	1420	0.56	173
	1330	0.17	174
butyric acid, pH 10	1420	0.84	
glutaric acid, pH 10	1420	0.84	

[a] DGL = glycylglycine, DGL-OMe = glycylglycine methyl ester hydrochloride, DLG = α-L-glutamyl-α-L-glutamic acid, DLL = α-L-lysyl-α-lysine, PGA = poly(L-glutamic acid), PLL = poly(L-lysine). [b] In cm^{-1}. [c] In mbarn/molecule steradian. [d] In nm.

to this mode. Thus, the results of isotopic substitutions on DGL and DGL-OMe support the amide V overtone assignment of the ~1400-cm^{-1} band.

The cross section, σ_{218}, and frequency of the preresonant state, ν_e, determined as previously[5] by an Albrecht *A*-term fit, are given in Table V for DGL and DGL-OMe, together with values we have obtained for other NMA analogues, for polypeptides,[2] and for various carboxylate ions. While the peptide cross sections depend on the charge or conformational state of the molecule, they are all significantly larger than those measured for the carboxylate derivatives, whose preresonant state occurs at a much shorter wavelength. This clearly indicates that the band under discussion in peptide systems cannot be a COO^- mode. Further, the putative amide V overtone shows the same intensity dispersion as that for the amide II and III bands,[2] with a similar calculated ν_e of ca. 196 nm associated with the peptide π–π^* transition.

Discussion

We see, therefore, that several kinds of evidence support the proposal that the overtone of amide V is strongly enhanced as the exciting wavelength approaches resonance with the π–π^* transition of the peptide group: (1) the enhanced band at 1496 cm^{-1} in NMA is at twice the frequency of the fundamental, found at 748 cm^{-1} in aqueous NMA;[1] (2) the vibrational frequency pattern of such enhanced bands for different conformations of PGA[1,2] follows that of the well-assigned amide V fundamentals for these conformations;[24-26] (3) the effects of $^{13}C^{15}N$ isotopic substitution on the frequencies of these enhanced bands closely follow those on the amide V frequencies.

The most likely explanation for the significant resonance enhancement of the overtone of amide V is that, similar to the case of ethylene,[37] the π^* state of the peptide group is twisted to a nonplanar configuration. The partial double-bond character of the CN bond of the planar peptide group in the ground electronic state is thus altered, leading to a lengthening of this bond. Since normal modes that contain a CN s component, such as amide II and amide III, distort the ground state toward the excited state, they will have large Franck–Condon factors and are expected to be enhanced as resonance is approached. Amide V has a large CN t component, which distorts the peptide group from planarity. In an idealized C_{2v} symmetry of the planar peptide group, the first derivative of the polarizability with respect to CN t would be zero. However, the second derivative is nonzero at the equilibrium geometry. An alternative view is that this fundamental has A_2 symmetry in the C_{2v} point group and cannot be a source of Franck–Condon enhancement. The even overtones, in contrast, are symmetric vibrations and can give rise to Franck–Condon enhancement. In fact, we see 4 × amide V at 2948 cm^{-1} in NMA and at 2784 cm^{-1} in DGL and [$^{13}C^{15}N$]DGL. We also see combinations of amide II and III with 2 × amide V but never observe any mode containing a contribution from an odd harmonic. A similar situation is found for the ν_4 torsion of ethylene.[37]

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Registry No. PGA (homopolymer), 25513-46-6; PGA (SRU), 24991-23-9; NMA, 79-16-3; DKP, 106-57-0; DGL, 556-50-3; [$^{13}C^{15}N$]DGL, 88815-60-5; DGL-OMe·HCl, 2776-60-5; [$^{13}C^{15}N$]DGL-OMe·HCl, 120265-10-3; Cbz-[$^{13}C^{15}N$]DGL, 88815-59-2; Cbz-[^{13}C]-Gly-ONp, 88815-58-1; H-[^{15}N]Gly-OH, 7299-33-4.

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