multiphoton ionization of all elements of the periodic system have been published[102] and "single-atom sensitivity" is claimed by the authors. Others claim a similar sensitivity also for molecules.[103] (For a review see, ref 104.) The highest sensitivity may be reached by a combination of desorption, laser ionization, and time-of-flight analysis. A gaseous probe may be gathered by freezing on a small surface and desorbed by a short laser pulse. The probe is then concentrated in a short gas pulse which can be ionized by pulsed lasers in a much more efficient way due to a favorable duty cycle. It has been possible to detect 1 part in 10^8 of a monolayer of organic molecules after freezing, laser desorption, and laser ionization.[105]

IX. Summary

In this report, the advantages and problems of multiphoton ionization and fragmentation for neutral and ion spectroscopy are explained and some information about the employment of modern time-of-flight analyzers as mass-selective ion detectors is given. As examples of mass-selective neutral spectroscopy, isotope-selective but simultaneously measured spectra of vibronic bands of benzene, toluene, xylene, and OCS are shown; positive features and problems have been discussed. As for ionized molecules, multiphoton ionization as an ion source and multiphoton dissociation spectroscopy of the cations of methyl iodide, benzene, and monofluorobenzene are presented. These examples as well as section VIII illustrate the usefulness of laser mass spectrometry (i.e., combination of resonant laser excitation and time-of-flight analysis) for the spectroscopy of neutral and ionized molecules. On the other hand, due to the simple arrangement of time-of-flight analyzers (even those with high resolution), many experimentalists should be able to build their own instruments, thus straining their budgets much less than with the laser system which is mostly used for spectroscopy anyway. If a supersonic beam is already used, the additional effort for a time-of-flight analyzer is even smaller.

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ARTICLES

Resonance Raman Spectroscopy of N-Methylacetamide: Overtones and Combinations of the C–N Stretch (Amide II') and Effect of Solvation on the C=O Stretch (Amide I) Intensity

Leland C. Mayne[†] and Bruce Hudson*

Department of Chemistry, University of Oregon, Eugene, Oregon 97403 (Received: June 11, 1990; In Final Form: October 24, 1990)

Resonance Raman spectra of N-methylacetamide (NMA) and its isotopic derivatives are reported for the vapor phase, acetonitrile, and aqueous solution with excitation wavelengths of 218, 200, and 192 nm. The 192-nm vapor-phase spectra of NMA and that of N-deuterated NMA (D–NMA), as well as that of an aqueous solution of D–NMA obtained with 200-nm excitation, exhibit identifiable overtones and combinations with considerable intensity. This intensity distribution is discussed in terms of the change of the molecular geometry along the C–N stretching coordinate upon electronic excitation. The resonance Raman spectra of NMA in the gas phase and in acetonitrile solution have considerable intensity in the amide I (C=O) stretching vibration. This mode is absent or very weak in the spectra obtained with aqueous solutions. Spectra obtained in mixtures of acetonitrile and water show that the amide I vibration decreases roughly linearly in intensity with water content. Various hypotheses concerning this dramatic effect of solvation on the resonance Raman spectrum of NMA are presented and discussed.

Introduction

The simple peptide N-methylacetamide (NMA) has long served as a model compound for understanding the spectroscopy of the peptide bond. This has been the case for the recently developed technique of ultraviolet resonance Raman spectroscopy.[1-5] These studies have illustrated several interesting points concerning the resonant excited-state and the ground-state modes of motion of this simple peptide. Isotopic effects such as N-deuteration result in very substantial changes in the resonance Raman spectra.[1] Despite rather extensive study, new aspects of the resonance

†Present address: Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA 19104.

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Raman spectra of this simple species continue to emerge.[3-5] Not only has NMA proven to be a model for the peptide bond, it has proven to be a test case for our ability to understand resonance Raman spectra of a low-symmetry molecule. In the present study we investigate the effect of solvation on the resonance Raman spectra of NMA. The spectrum of the vapor is used as reference. We also present resonance Raman spectra obtained with shorter excitation wavelengths than previously reported for NMA. These spectra show combination and overtone intensity that is characteristic of resonance-enhanced Raman spectra. The intensity of these consecutive bands is of interest in the interpretation of the resonance enhancement mechanism and is of relevance to the interpretation of the observed solvent effects.

In our earlier studies of aqueous solutions of NMA[1] we used resonance enhancement and isotopic effects to show that excitation to the $\pi\pi^*$ excited state results primarily in a change in the C–N bond length. In the N-deuterated species the effect of ^{13}C and ^{15}N substitution on the vibrational frequencies was used to show that the amide II′ vibration of D–NMA is largely C–N stretch. This is therefore the major resonance-enhanced band for aqueous solutions of NMA. There is a considerable change in the form of the normal modes of motion of the peptide bond upon replacement of deuterium by hydrogen such that in the protio form this C–N stretching motion is distributed between the amide II and III vibrations.[6] This may be viewed as being due to the similarity in the frequency of the in-plane H–N–C bending mode of motion with the C–N stretching frequency, a "resonance" that is eliminated by deuteration. Because of this distribution of the C–N stretch between two modes of motion, two enhanced bands are seen for aqueous solutions of the protio form, amide II (1316 cm^{-1}) and amide III (1581 cm^{-1}). Between these strong bands there are two weaker bands at 1382 and 1496 cm^{-1} (see Figure 2). The 1496-cm^{-1} band has been the subject of studies by other workers.[4,5] The excitation behavior, i.e., the variation of the intensity of the Raman bands as a function of excitation wavelength, has been shown to obey the form expected for enhancement by a single $\pi\pi^*$ excitation at ca. 190 nm, consistent with the observed absorption spectrum.[2]

Thus, the overall picture that has emerged of the reasonance Raman spectroscopy of NMA is very simple. There is a single relevant excited electronic state with a geometry change along a single geometric displacement, the C–N bond length. For the N-deuterated species this displacement is coincident with a normal mode of motion of the ground electronic state; for the N-protio species a linear combination of two normal modes is required to span this C–N length displacement.

The experimental observations upon which we have built our picture of the nature of resonance enhancement are unambiguous. The results of other workers[2,4,5] are in excellent agreement with our studies. The only possible cause for some concern is the absence of significant intensity in the amide I C=O stretching vibration since a description of the excited state of the peptide bond as a $\pi\pi^*$ excitation would probably involve a change in the C=O bond length on excitation and hence intensity in this band. On the other hand, there are models of the vibronic structure of the peptide absorption based on molecular orbital theory calculations of bond order changes that describe the excited-state geometry change entirely in terms of a lengthening of the C–N bond length.[7]

In the present paper we will show that the situation is not as simple as previously thought. Recent studies[3] have shown that the resonance Raman spectra of NMA observed in the vapor phase
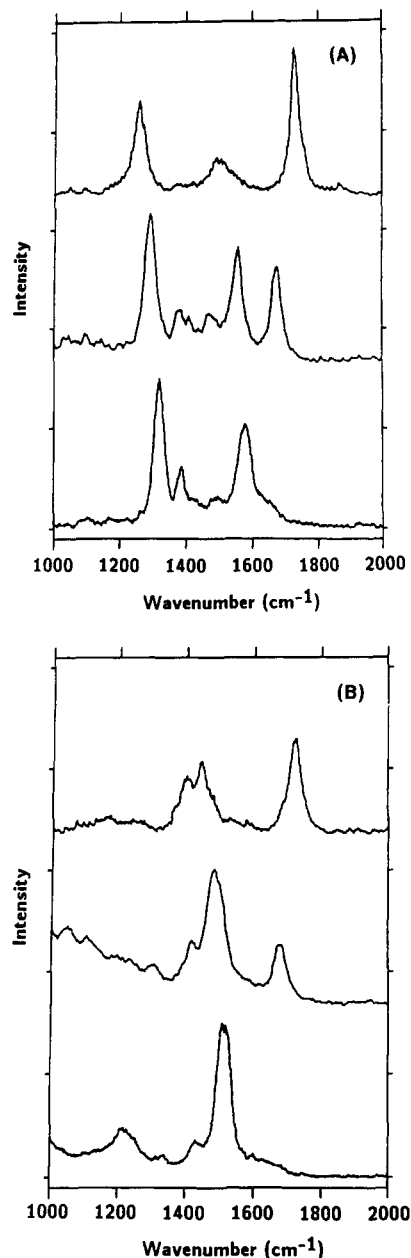

Figure 1. Resonance Raman spectra of *N*-methylacetamide (NMA, A) and N-deuterated NMA (D–NMA, B). In both cases the top trace is for the vapor phase, the middle is for a solution in deuterated acetonitrile, and the bottom is for an aqueous solution (H$_2$O or D$_2$O, respectively). The excitation wavelength for the vapor spectrum was 200 nm. For the solutions the excitation wavelength was 218 nm, and the concentration was 10 mM in each case.

and in acetonitrile solution are quite different from that observed in aqueous solution. The presentation of the nature of these differences and the discussion of possible explanations for this effect are discussed in the present paper. The use of overtone intensity data to establish the extent of the change of geometry upon electronic excitation is also presented.

Experimental Section

The experimental methods used in the present study have, for the most part, been described previously.[8-10] The basic procedure

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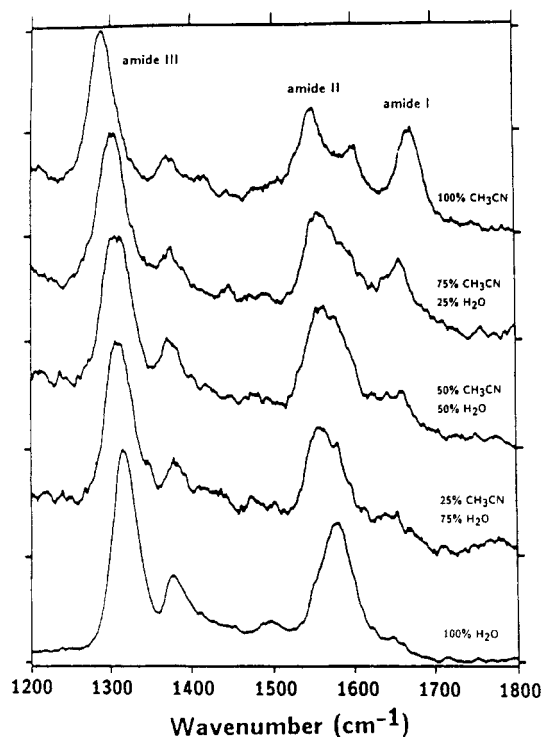
Figure 2. Resonance Raman spectra of NMA obtained with excitation at 218 nm as solutions in mixtures of water and acetonitrile. Pure acetonitrile is at the top; pure water is at the bottom. The scattering from the solvent has been subtracted.

consists of the generation of ultraviolet radiation using stimulated Raman shifting of the harmonic frequencies of a Q-switched Nd:YAG laser. The solution samples are streams circulated by a pump. The vapor samples are also streams sheathed in heated nitrogen vapor, using a specially designed high-temperature device.[11,12]

Results

As an introduction to the general nature of the resonance Raman spectra of NMA, spectra in aqueous solution and in acetonitrile of the normal and *N*-deuterio forms of NMA obtained with 218-nm excitation are shown in Figure 1. Two major points are seen from these spectra. The first is that there is a collapse of the intensity of the amide II and III bands into the single amide II′ band[19] upon N-deuteration.[1] The second is that the amide I (C=O stretching) band, clearly seen in acetonitrile solution, is not seen in the aqueous solutions. The spectra of NMA in mixtures of water and acetonitrile are shown in Figure 2. The amide I band appears to decrease in intensity continuously as water is added. There is also a smooth variation of the frequencies of the three strongly active modes as the solvent composition is changed: the amide III and amide II modes move to higher frequency as water is added while the amide I mode moves to lower frequency. The smaller peak at 1381 cm^{-1} does not change frequency with solvent composition.

The resonance Raman spectrum of N-deuterated NMA in aqueous (D_2O) solution obtained with 200-nm excitation is shown in Figure 3. Panels A and B include the spectra obtained for the isotopically labeled species with ^{13}C and ^{15}N in the peptide bond. This 200-nm excitation wavelength is near the zero–zero
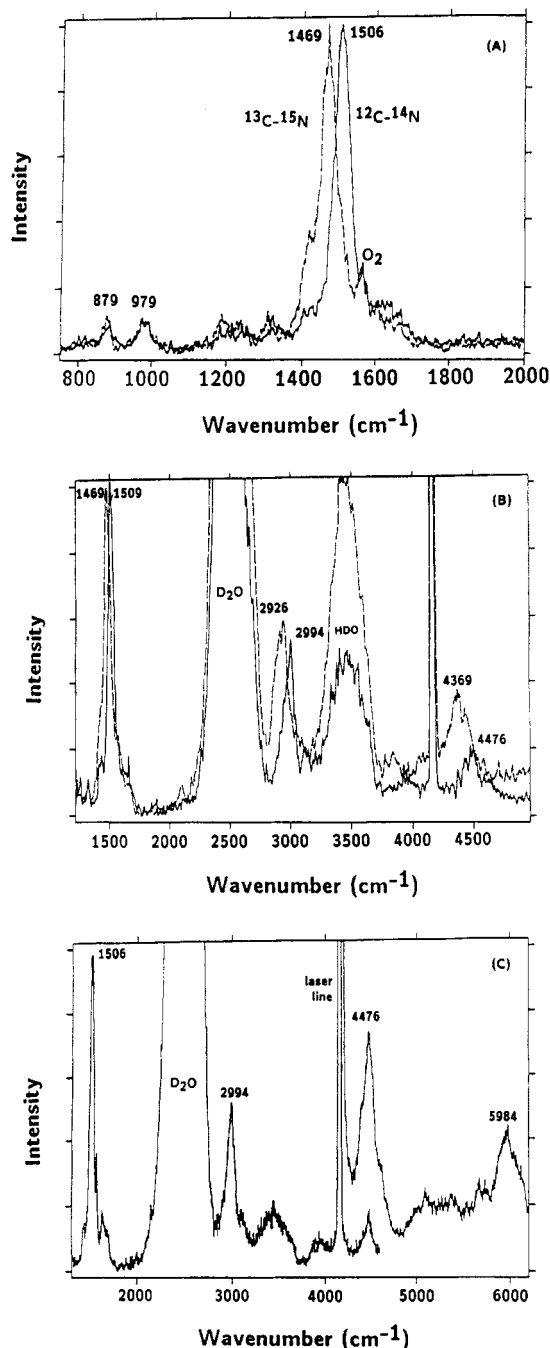
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Figure 3. Resonance Raman spectra of D–NMA in D_2O solution obtained with excitation at 200 nm. Panels A and B include spectra of ^{15}N–^{13}C-labeled NMA (shown as dashed traces). The line at 4155 cm^{-1} is due to the lower order stimulated Raman shifted line.

of the electronic absorption spectrum as estimated from the absorption spectra of Figure 4 and a simulation to be discussed below. The only fundamental vibration with appreciable intensity at this or any other far-ultraviolet excitation wavelength is the amide II′ vibration. The shift in this vibration with heavy-atom isotopic substitution has been discussed previously.[3] The two-, three-, and four-quantum transitions of this mode show appreciable intensity. There is a considerable increase in the width of the bands with increasing vibrational energy. It is also interesting to note that the ^{13}C–^{15}N-substituted NMA clearly exhibits an appreciably higher relative intensity in the overtone transitions than is exhibited by the ^{12}C–^{14}N normal isotope species.

The resonance Raman spectra of NMA vapor for both protio and deuterio forms obtained with excitation at 192 nm are shown in Figure 5. These spectra are similar to those observed for acetonitrile solution in that there is appreciable intensity in the amide I vibration. There is also considerable intensity in the first
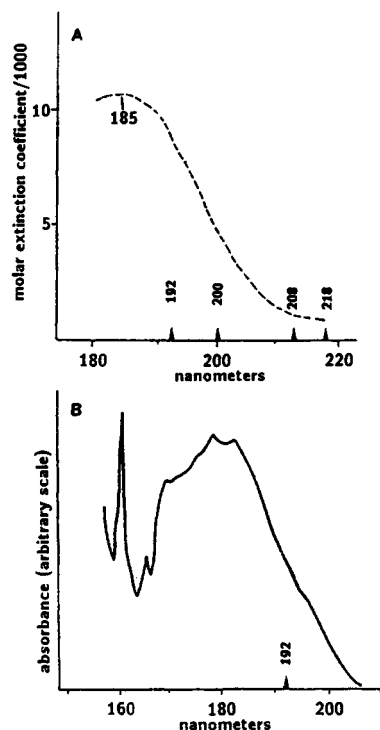
Figure 4. Electronic absorption spectra of *N*-methylacetamide in aqueous solution (A) and in the vapor phase (B). From refs 13 and 14. The wavelengths used to obtain Raman spectra are indicated.
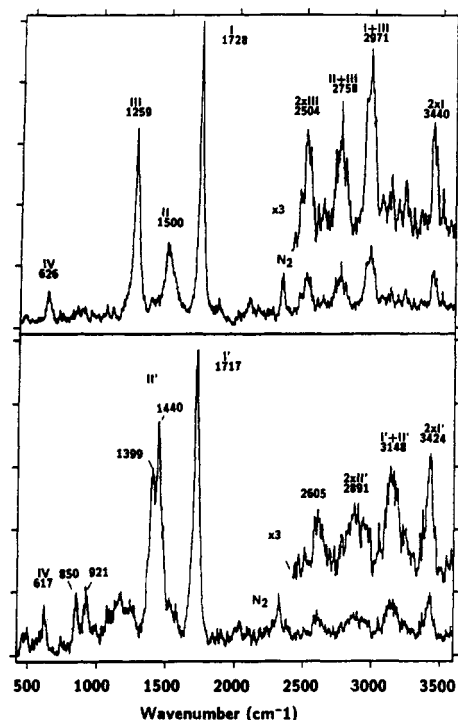

Figure 5. Resonance Raman spectra of *N*-methylacetamide (top panel, A) and *N*-deuterio-*N*-methylacetamide (bottom panel, B) in the vapor phase obtained with 192-nm excitation. The insert on the right has been expanded vertically 3-fold.

overtone region. The pattern is now more complicated than that of Figure 2 because of the activity of two or three modes and the resulting combination transitions. Combination bands can only occur in resonance Raman spectra when there is a mechanism giving rise to intensity of both of the corresponding fundamental transitions that involves the same excited electronic state. Thus, for the standard A-type (Franck–Condon) resonance Raman mechanism expected to be applicable in this case, intensity in a combination band means that a single electronic state is displaced along both of the active coordinates. The combination involving
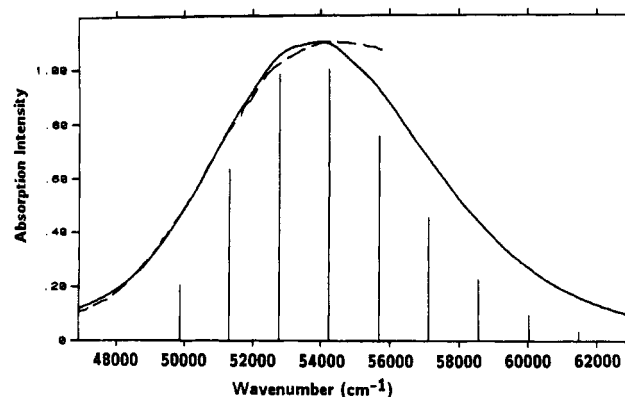

Figure 6. Simulated (solid) and experimental (dashed; from refs 13 and 14) absorption spectra of *N*-methylacetamide in aqueous solution. The simulation is based on a single vibronic progression with an interval of 1400 cm^{-1}. The positions and intensities of the vibronic components are indicated by the vertical lines. The equilibrium of the oscillator in the excited state is shifted relative to that of the ground state by 2.5 times the root-mean-square ground-state position (i.e., Δ = 2.5 in dimensionless units). Each vibronic band is assumed to a Lorentzian with a width of 1400 cm^{-1}. This line width is probably primarily homogeneous in nature (see ref 15). The origin of the transition in the simulation is at 200.3 nm. These parameters were chosen to provide the best overall fit to the rising edge of the spectrum and to have roughly the correct peak position. It was not possible to obtain an improved fit in the region to higher energy of the maximum with any other set of parameters while retaining agreement for the rising edge.

amide I' plus amide II' is particularly important since intensity in this band means that both vibrations obtain enhancement of their Raman activity from a common excited electronic state.

Discussion

We begin this discussion with the interpretation of the absorption spectrum of NMA in light of the resonance Raman spectrum observed for the deuterated form in D$_2$O. Since the resonance Raman spectrum has only one band with significant intensity in this case, the simplest interpretation of the absorption spectrum is in terms of a progression in a single mode. A calculation of the absorption spectrum for a single displaced harmonic oscillator results in an excellent fit to the observed spectrum when a displacement of 2.5 times the ground-state root-mean-square displacement is used in combination with a line width for each vibronic band of 1300–1400 cm^{-1}. The origin of the electronic transition is placed near 200 nm. The resulting absorption spectrum (Figure 6) matches the peak position of 185 nm and has the correct ratio of the intensity at the peak to the intensity at 200 nm. An excited-state frequency of 1400 cm^{-1} is used in this calculation. Given the deduction from the Raman spectrum that there is only one vibronically active mode, the absorption spectrum provides a significant constraint on the values of the parameters. This fit of the absorption spectrum results in a resonance Raman spectrum with excitation at 200 nm (very near the proposed zero–zero transition) that has relative intensities for the fundamental and v = 2, 3, and 4 overtones of 1.0/0.4/0.2/0.1, in reasonable agreement with the data of Figure 3c. This very simple model of the electronic excitation of NMA is discussed in more detail elsewhere.[15] The main point of interest here is that it is possible to construct a very simple, unambiguous model that is in good quantitative agreement with all of the available data.

We turn next to the case of the gas-phase spectrum of deuterated NMA obtained with 192-nm radiation (Figure 5b). This spectrum shows two active bands, amide II' (1399 and 1440 cm^{-1}) and amide I (1717 cm^{-1}). The splitting of the amide II' band of

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D–NMA into two components has several possible explanations. The most likely is that, due to proximity of the frequency of the amide II′ vibration and that of the methyl umbrella distortion modes, these degrees of freedom are mixed to produce two vibrations, each with significant components of C–N stretching. This is indicated by the recent calculations of Balazs[16] in which a force field determined on the basis of an ab initio calculation results in mixed bands for D–NMA at 1461, 1440, and 1392 cm^{-1}, each containing CH_3 umbrella and C–N stretching coordinates. These calculations indicate that the 1461-cm^{-1} band involves the acetyl methyl group, the 1440-cm^{-1} band involves both CH_3 groups, and the 1392-cm^{-1} band involves only the amide nitrogen CH_3 group. Our experimental results are fully consistent with this calculation if the mixing with the nitrogen methyl is retained but the mixing with the acetyl methyl is reduced or if the band at 1440 cm^{-1} consists of two overlapping transitions. Other possible explanations for this doubling include an "inversion doubling" due to a change in pyramidalization at the amide N or a Fermi resonance of the amide II′ band with a combination of lower frequency inactive modes.

The bands at higher frequency are easily assigned as due to overtone and combination transitions. The rather large width of some of these bands is consistent with the splitting of the amide II′ band into two components.

The observation of combinations involving amide I′ and amide II′ in this spectrum and the corresponding combinations of amide I with amides II and III in the protio form (Figure 5a) is particularly important because it indicates that a single electronic state contributes significant intensity to both the amide I and the other amide vibrations. Combination intensity cannot arise from distinct electronic states each contributing to only one of the vibrations. Thus, the observed combinations are consistent with the dominance of the resonance Raman spectra by a single excited electronic state.

Quantitative analysis of the intensity distribution of these Raman spectra is straightforward. This analysis shows that most (75%) of the intensity of the band at 2971 cm^{-1} is due to the I + III combination, rather than the 2 × II overtone. A detailed quantitative analysis is not warranted by the amount and quality of the data available, but it is clear from even a qualitative comparison of the overtone and combination intensity of these gas-phase spectra with the relative overtone intensities for the aqueous D–NMA of Figure 3 that each of the modes active for the gas-phase molecule has a smaller displacement than the single amide II′ mode of the aqueous deuterated NMA. The general picture that emerges is that the displacement along the amide II′ mode observed for the aqueous deuterated NMA is distributed between two (or among three) modes of vibration in the deuterated (and protonated) gas-phase species.

We now turn to possible explanations of the dramatic effect of solvent on the intensity of the amide I vibrational band. The relevant factor in loss of the amide I intensity seems to be hydrogen-bond donation by the solvent. Thus, this high-frequency C=O stretching band is also missing in a spectrum obtained for NMA in methanol solution.[11] On the other hand, considerable quantities of protic solvent are needed in order to make the amide I band disappear. This may indicate that some more general solvent property rather than specific hydrogen bonding is involved.

Several explanations of the effect of aqueous solvation on the resonance Raman spectrum of NMA have been considered.

i. The amide I C=O band is, in fact, present in the aqueous solutions but is not observed because it is very broad. This has been eliminated on the basis of infrared spectrum and Raman spectra at longer wavelengths.

ii. The amide I C=O band obtains its intensity from an electronic transition (such as an nπ* excitation) that moves to much higher energy in aqueous solution,[17] while the amide II and

III bands obtain their intensity from a different excited state such as the ππ* transition. This explanation seems to be eliminated by the gas-phase combination results. It also seems unlikely that an nπ* transition would contribute such large intensity as is observed for the amide I vibration in the vapor phase and in solution.

iii. The simplest viable explanation of the effect of hydration is that aqueous solvation changes the ground-state C=O bond length in such a way that the bond length in this environment is very similar to the value in the excited state. As a result, the enhancement of Raman activity upon resonance disappears. Examination of the known variation of amide and ester C=O bond lengths with hydrogen-bonding environment shows that this variation is comparable to or larger than the change in bond length associated with the electronic excitation as determined from the vapor-phase Raman spectrum.[11] This explanation seems a bit ad hoc, however, and it is hard to see why the shift in the ground-state equilibrium does not also occur for the excited state conserving the change in geometry and thus the intensity.

iv. Another possibility is that the nature of the electronic excited state is changed by addition of water. One way of describing such behavior is in terms of the effect of a dielectric constant change on the contributions of the major resonance structures. This is consistent with the fact that considerable water is required to obtain the observed effect. The well-known resonance description of the ground state of peptides postulates the contribution of an ionic resonance form O^-—C=N$^+$ with a weak C—O bond and a strong (and planar) C=N double bond. If this resonance form is more important in a high dielectric medium, one would expect a lower frequency for the amide I C–O stretch vibration and a higher frequency for the amide II and III bands involving the C=N stretch. (Note the continuation of the frequency trend in going from water to acetonitrile to vapor.) Similar explanations have been used to interpret infrared absorption intensity variations with solvent for the peptide bond. One might also expect a smaller change in the C–O bond length on excitation. However, this explanation would seem to imply that there should be a considerable change in the absorption spectrum of NMA upon changing the solvent from acetonitrile to water.

v. The separation of hydrogen bonding and dielectric effects is not simple in this case. Either of these effects might lead to a change in the form of motion associated with the amide I vibration in the ground electronic state. That some effect of this type occurs is indicated by the change in amide frequencies upon change of solvent. The loss of intensity in the amide III vibration upon deuteration was attributed to a change in the form of the ground-state normal modes of motion. By analogy, one could postulate that in acetonitrile solution and in the vapor phase the form of the normal modes of motion is such that both the amide II′ and amide I vibrations contain significant components of the active degree of freedom associated with electronic excitation. According to this picture, the effect of solvation by water is to change the C=O and/or C–N force constants and thus the coupling between the C–N and C=O modes of motion.

In evaluating this last possibility, it is important to keep in mind the quantitative nature of the evidence to the effect that the amide II′ vibration of D–NMA in D_2O is primarily of C–N stretching nature. The value of the shift associated with ^{13}C–^{15}N substitution is 37 cm^{-1}. The shift expected if the motion was entirely due to C–N stretching motion is 56 cm^{-1}. Thus, while it is true that the motion is largely C–N stretch in nature, there is still a significant component of some other degree(s) of freedom. What we know from the resonance Raman experiment is that, whatever the form of the atomic motion corresponding to the amide II′ vibration in D_2O solution, it is along this degree of freedom that the peptide bond suffers a displacement upon electronic excitation. This suggests that the form of this strongly active mode of motion is a C–N contraction combined with C=O expansion, i.e., an

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"antisymmetric" N—C=O stretch that is strongly weighted toward the N–C single-bond motion. The (inactive) amide I band is then primarily C=O stretch with a smaller amount of C–N component with an in-phase relative displacement. Electronic excitation is expected to select the antisymmetric form in the sense that any change in the C=O bond length will be opposite to the change in the C–N bond length.

Hydrogen bonding or the effect of a high dielectric constant is expected to decrease the C=O force constant and raise the C–N force constant. This will increase the coupling and change the resulting eigenvectors in a space spanned by the C=O and C–N stretching motions. The result will be a new distribution of intensity which will not necessarily include both modes of motion. The magnitude of this effect is very difficult to estimate although it should be amenable to theoretical treatments that include the effects of solvation.[18]

Conclusions

The major conclusions of this work are that a consistent picture

of the electronic excitation of NMA can be constructed that is in agreement with the absorption spectrum and the enhancement of Raman transitions in resonance but that this picture must be adjusted when considering this simple peptide in the vapor phase or in solution in non-hydrogen-bonding solvents relative to the particularly simple picture that appears to apply to aqueous solution. The change in geometry associated with electronic excitation appears, for the deuterated peptide in an aqueous environment, to be along a single mode of vibrational motion of the ground electronic state. The shape of the absorption spectrum, consistent with this simple picture, does not permit significant displacements along other degrees of freedom except possibly those with very low vibrational frequency. The possibilities within which readjustment of this simple picture must be made to accommodate the gas-phase and acetonitrile solution data are outlined. The most important variation is probably the change in the form of the ground electronic state normal modes with isotopic substitution and solvation.

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Ultrafast Dynamics of a Quasi-Dissociative Diatomic Molecule in Solution. 2

S.-B. Zhu and G. W. Robinson*

SubPicosecond and Quantum Radiation Laboratory, P.O. Box 4260, Texas Tech University, Lubbock, Texas 79409 (Received: July 3, 1990)

To answer the recent criticism of Keirstead and Wilson concerning our previous work, we perform a new molecular dynamics simulation of a quasi-dissociative diatomic molecule in the condensed phase. In accord with our earlier study, a noncanonical behavior of the low-mass test particle subjected to a strong systematic force is again observed. Deviations from the Maxwellian velocity distribution are found to be larger than the statistical uncertainty. This gives us confidence that the results are reliable and that they reflect the true nature of certain types of many-body systems.

I. Introduction

Recently in this journal, Keirstead and Wilson[1] have published a paper entitled "A Breakdown of Equilibrium Statistical Mechanics?" In that paper they reproduce the conditions of an earlier calculation by Zhu and Robinson.[2] They assert that the reported noncanonical behavior contradicts the predictions of classical equilibrium statistical mechanics, and by carefully examining the statistical errors, they conclude that the results in ref 2 can be attributed to poor statistics.

While Keirstead and Wilson[1] criticized the breaking of the principles of statistical mechanics, they forget that the basic assumption in statistical mechanics is no more than an assumption of the "absence of interference between phenomena", that is, the assumption of "randomness".[3] This is the foundation for understanding any stochastic phenomenon.[4] Therefore, observed deviations from canonical behavior do not necessarily violate statistical mechanics. On the contrary, such deviations may provide an example where "the development of statistical mechanics has reached the point of delving into its origin".[3]

The Maxwellian velocity distribution was initially derived for spatially homogeneous gases[5] and later was found to be suitable for describing Brownian motion[6] and a number of other physical phenomena.[7-9] In fact, over the past century, the Maxwell–Boltzmann "normal distribution" has been successfully applied to so many problems that most persons, ignoring its origin and assumptions, are accustomed to treat it as an established law, or a "by definition" equilibrium property, rather than merely a mathematical formulation.

The basic assumption for the probability distribution of a group of test particles to follow the Maxwell–Boltzmann formulation rests on the "central limit theorem", which requires that these tests particles are in contact with an infinite number of heat bath particles in the time duration of interest and further that the

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