Effects of Intermolecular Hydrogen-Bonding Interactions on the Amide I Mode of *N*-Methylacetamide: Matrix-Isolation Infrared Studies and ab Initio Molecular Orbital Calculations

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Received: September 3, 1997; In Final Form: October 23, 1997[⊗]

Effects of intermolecular hydrogen-bonding interactions on the amide I mode of *N*-methylacetamide (NMA) are studied by matrix-isolation infrared (IR) spectroscopy and ab initio molecular orbital calculations. The wavenumbers of the amide I IR bands of NMA in Ar and N_2 matrixes with various NMA/matrix gas mixing ratios are compared with those calculated for the monomer, dimers, and trimers of *trans*-NMA and the monomer and dimer of *cis*-NMA. The band at 1708 (1706) cm^{-1} in Ar (N_2) matrixes is assigned to the amide I mode of the monomer of *trans*-NMA. The band observed at 1686 (1681) cm^{-1} in Ar (N_2) matrixes with the NMA/matrix gas mixing ratio larger than 1/500 is assigned to the amide I band due to the dimers of *trans*-NMA. The bands observed at lower wavenumbers for samples with the NMA/matrix gas mixing ratio as large as 1/100 are assigned to the amide I bands of the trimers and larger clusters of *trans*-NMA. It is likely that the band observed at 1695 (1693) cm^{-1} in the Ar (N_2) matrix arises from the dimer of *cis*-NMA, which is as stable as the *trans*-NMA dimers because of the formation of two hydrogen bonds in a cyclic form. Although there are two amide I modes in an NMA dimer and three in an NMA trimer, only one mode is strongly IR active in each species. The intrinsic amide I wavenumbers of individual peptide groups in NMA clusters, i.e., the amide I wavenumbers in the case where there is no resonant vibrational coupling between the peptide groups, are examined by calculating the amide I wavenumbers for the dimers and trimers whose constituent molecules other than the target molecule have the C═O group(s) substituted with ^{13}C and ^{18}O. It is shown that the shifts of the amide I band to lower wavenumbers induced by hydrogen bonding to the C═O group are 20−25 cm^{-1}, while those induced by hydrogen bonding to the N−H group are 15−20 cm^{-1}. These shifts are approximately in line with the changes in the C═O bond lengths and are approximately additive if both the C═O and N−H groups of a peptide group are hydrogen bonded.

1. Introduction

The amide I band is known to be especially sensitive to the secondary structures of polypeptides and proteins[1−6] and is useful in analyzing structural changes in polypeptides and proteins arising from various perturbations such as variations in temperatures and pH.[7−10] The amide I mode is affected not only by vibrational interactions between the peptide groups, which are expressed by off-diagonal terms in the force constant matrix (**F** matrix) in the coordinate system of the "amide I subspace",[5,11] but also by changes in the "intrinsic" amide I wavenumbers of individual peptide groups (i.e., the amide I wavenumbers in the case where there is no resonant vibrational coupling between the peptide groups), which are directly related with the diagonal terms of the **F** matrix. The sensitivity of the amide I band envelope to secondary structures mainly arises from electrostatic interactions between the peptide-group vibrations, recognized as the transition dipole coupling (TDC).[5,12−14] The TDC is a mechanism determining the off-diagonal terms of the **F** matrix, because it describes vibrational coupling between different peptide groups. The TDC constant is determined by the distance between the two relevant peptide groups and their relative orientation. Therefore, the **F** matrix, from which the wavenumbers and vibrational patterns of the amide I modes are calculated, depends on secondary structures.[5,11]

However, there is experimental evidence for the importance of changes in the diagonal terms in determining the amide I band envelope. For example, in the case of parvalbumin and calmodulin, the amide I components originating from the vibrations of α-helixes appear in the 1650−1640 cm^{-1} region.[15,16] One of the possible reasons for the low-wavenumber position of these components shifted from the wavenumber region where α-helix bands are ordinarily located is considered to be the formation of strong hydrogen bonds between the peptide groups in the α-helixes and solvent molecules.[15,16] The low-wavenumber β-sheet bands appearing in the 1640−1620 cm^{-1} region observed for many proteins rich in β-sheets are also considered to be affected by strong intermolecular hydrogen bonding with other peptide groups or solvent molecules.[7,17,18] It is therefore necessary to clarify quantitatively the factors significantly affecting the values of diagonal terms of the **F** matrix.

In the present study, changes in the amide I wavenumber arising from intermolecular hydrogen-bonding interactions between the peptide groups are studied experimentally and theoretically. *N*-methylacetamide (NMA), one of the simplest molecules having a peptide group, is selected as the subject

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⊗ Abstract published in *Advance ACS Abstracts,* December 15, 1997.

S1089-5647(97)02879-4 CCC: $15.00
Published on Web 01/01/1998

molecule. Experimentally, the infrared (IR) spectra of NMA in Ar and N_2 matrixes are measured in the wavenumber region of the amide I band. By changing the mixing ratio of NMA and matrix gases, the spectra of the monomer and clusters (dimer, trimer, etc.) are obtained. The amide I bands originating from clusters of NMA can be identified from the dependence of the intensities of individual bands on the NMA/matrix gas mixing ratios. Theoretically, the amide I bands of an isolated molecule of NMA and its dimers and trimers are studied by ab initio molecular orbital (MO) calculations. The intensities of the IR bands of each cluster of NMA are examined in relation to the phase relationship of the vibrations of NMA molecules constituting the cluster. Effects of intermolecular hydrogen-bonding interactions on the amide I wavenumbers are discussed on the basis of these experiments and calculations.

2. Materials and Methods

The sample of NMA purchased from the Tokyo Chemical Industry Co., Ltd. was first distilled under 4 Torr and then dried by freeze-and-thaw under vacuum several times before use. The purified NMA sample was diluted with N_2 or Ar gas to a desired mixing ratio, ranging from 1/2000 to 1/100 for NMA/N_2 and from 1/200 to 1/100 for NMA/Ar. The N_2 gas (99.999%) and Ar gas (99.9999%) were obtained from the Takachiho Chemical Industrial Co., Ltd. The mixed gas was slowly sprayed onto a CsI plate maintained under vacuum at about 20 K by using a closed-cycle He cryocooler (Osaka Oxygen CRYOMINI D). IR spectra were measured on a Fourier-transform IR spectrophotometer (JEOL JIR-100) equipped with a TGS detector.

Ab initio MO calculations were performed by using the Gaussian 92 and 94 programs[19,20] on a Hewlett-Packard workstation (Apollo 9000 series model 735) at the Research Center for Spectrochemistry of the University of Tokyo and on IBM SP2 computers at the Computer Center of the Institute for Molecular Science. All the calculations were carried out at the Hartree−Fock (HF) level. The 6-31++G** basis set was used, which contains diffuse and polarization functions, to reduce the basis set superposition error for intermolecular interactions.

The amplitudes of the amide I vibrations of constituent molecules in each normal mode of a dimer or trimer are evaluated by those of the C═O stretches. The internal coordinates of the monomer, dimers, and trimers of NMA are defined in an ordinary manner.

3. Results and Discussion

A. Observed IR Spectra in Ar and N_2 Matrixes. Observed IR spectra in the wavenumber region of the amide I band of NMA in N_2 at various concentrations are shown in Figure 1. For the sample with the NMA/N_2 mixing ratio of 1/2000, a single prominent band is observed at 1706 cm^{-1}. The wavenumber of this band is in agreement with that observed in a previous study.[21] On the low-wavenumber side of this band, several weak bands are also observed. The intensities of the latter bands increase as the NMA/N_2 mixing ratio increases. For the sample with the mixing ratio of 1/200, two bands at 1693 and 1681 cm^{-1} can be seen clearly. The asymmetric shape of the 1706 cm^{-1} band is also seen at this concentration. By changing the mixing ratio from 1/200 to 1/100, another band appears at 1663 cm^{-1}.

The observed IR spectra of NMA in Ar are shown in Figure 2. For the sample with the NMA/Ar mixing ratio of 1/200, two bands are clearly observed at 1708 and 1686 cm^{-1}. The existence of another weak band at 1695 cm^{-1} is also evident.

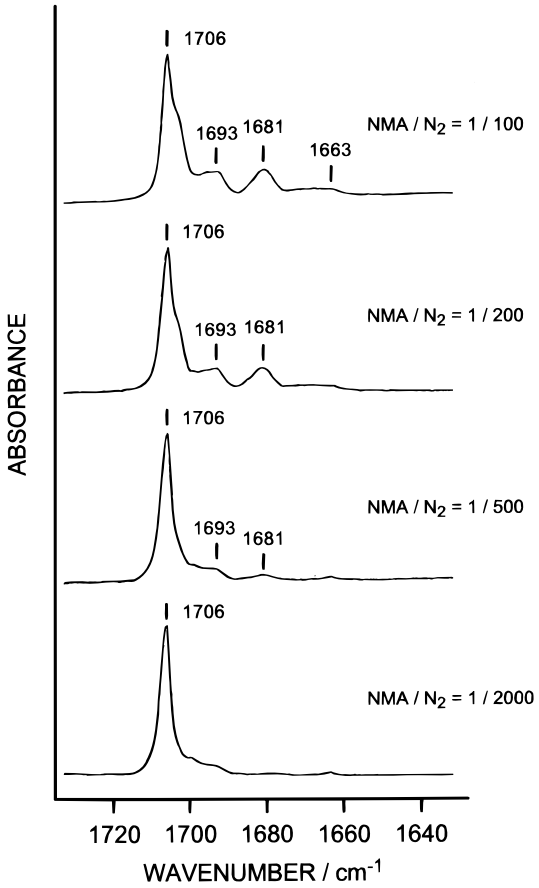

Figure 1. Observed IR bands in the amide I band region of *N*-methylacetamide in N_2 matrixes with the NMA/N_2 mixing ratio of 1/2000, 1/500, 1/200, and 1/100.

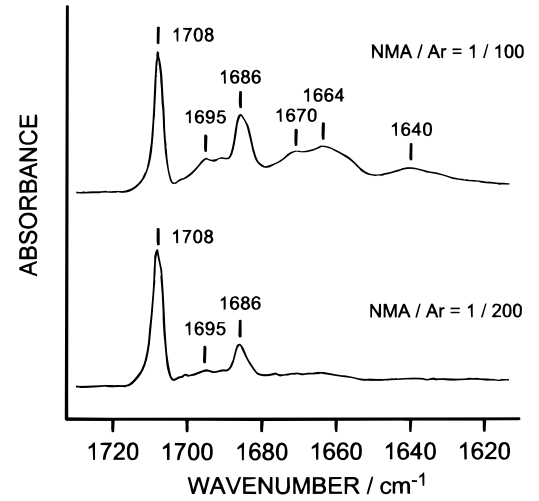

Figure 2. Observed IR bands in the amide I band region of *N*-methylacetamide in Ar matrixes with the NMA/Ar mixing ratio of 1/200 and 1/100.

By changing the mixing ratio from 1/200 to 1/100, the intensities of the 1695 and 1686 cm^{-1} bands increase. In addition, three additional bands are observed at 1670, 1664, and 1640 cm^{-1} at the mixing ratio of 1/100.

It is clear that the observed IR bands of NMA in Ar correspond to those in N_2 in the following manner: 1708 cm^{-1} (Ar) and 1706 cm^{-1} (N_2), 1695 cm^{-1} (Ar) and 1693 cm^{-1} (N_2), 1686 cm^{-1} (Ar) and 1681 cm^{-1} (N_2), 1670 and 1664 cm^{-1} (Ar) and 1663 cm^{-1} (N_2). The 1708 (1706) cm^{-1} band in Ar (N_2) is assigned to the amide I band of the monomer of NMA, since

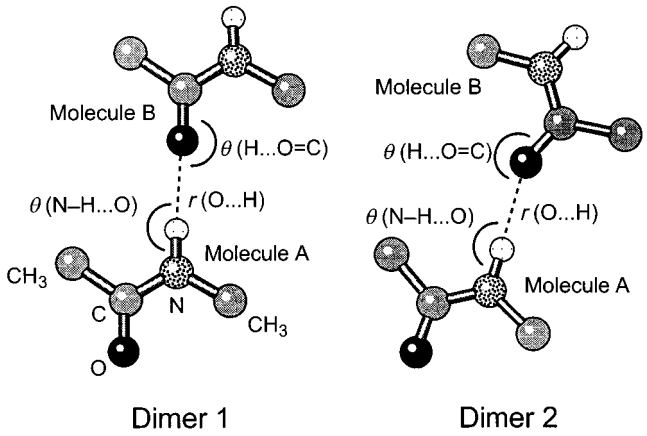

Figure 3. Structures of dimers 1 and 2 of *trans*-*N*-methylacetamide. The definitions of the structural parameters for the hydrogen bonds [r(H···O), θ(N−H···O), and θ(H···O=C)] are also shown.

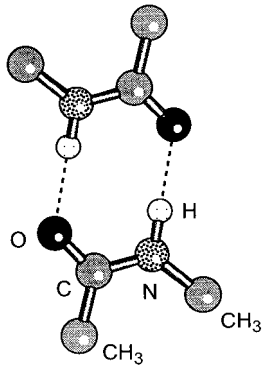

Figure 4. Structure of the dimer of *cis*-*N*-methylacetamide.

it exists in the sample of the lowest NMA/matrix gas mixing ratio and it is not enhanced by increasing the mixing ratio. Other lower-wavenumber bands originate from clusters of NMA. Among these, 1695 and 1686 (1693 and 1681) cm^{-1} bands observed in Ar (N_2) are assigned to the amide I bands of the dimers of NMA, since they are observed when the NMA/N_2 mixing ratio is 1/500, and their intensities increase as the NMA/matrix gas mixing ratio increases. The bands in the region below 1675 cm^{-1} are assigned to the amide I bands of larger clusters of NMA.

B. Calculated Structures and Hydrogen-Bond Energies. In the present study, ab initio MO calculations are carried out for the monomers and dimers of both *trans*- and *cis*-NMA as well as trimers of *trans*-NMA. As shown in Figure 3, two kinds of dimers consisting of molecules A and B are considered for *trans*-NMA; the peptide groups in dimers 1 and 2 of *trans*-NMA are in parallel (p) and antiparallel (ap) arrangements, respectively. In *trans*-NMA trimers consisting of molecules A, B, and C, NMA molecules are hydrogen bonded in the sequence A−B−C. Four kinds of trimers in the p−p, p−ap, ap−p, and ap−ap arrangements are considered. In each of the dimers and trimers of *trans*-NMA, the C═O bond of molecule A is not hydrogen bonded. As shown in Figure 4, the *cis*-NMA dimer has an eight-membered ring due to the formation of two hydrogen bonds. The purpose of the calculations for *cis*-NMA and its dimer is to check the possibility of the existence of the *cis*-NMA dimer, which has recently been suggested[22] to be more stable than a *trans*-NMA dimer because of the formation of two hydrogen bonds in a cyclic form.

The optimized structural parameters of all the species treated in this study are shown in Tables 1−4. A planar structure of C_s symmetry is assumed for each species unless otherwise stated.

TABLE 1: Optimized Structural Parameters (at the HF/6-31++G Level) of the Peptide Group(s) in the Monomer and Dimers of *trans*-*N*-Methylacetamide[a,b]**

		dimer 1 (p)		dimer 2 (ap)	
	monomer	molecule A	molecule B	molecule A	molecule B
r(C−N)	1.3495	1.3431	1.3429	1.3435	1.3426
r(C═O)	1.2043	1.2085	1.2096	1.2085	1.2099
r(N−H)	0.9914	0.9957	0.9918	0.9962	0.9918
r(C−C_a)	1.5127	1.5134	1.5106	1.5132	1.5107
r(N−C_b)	1.4483	1.4450	1.4497	1.4449	1.4500
θ(O═C−N)	122.10	122.64	121.95	122.59	121.84
θ(C−N−H)	119.31	119.78	119.08	119.75	119.10
θ(N−C−C_a)	116.52	116.21	116.91	116.20	116.93
θ(C−N−C_b)	121.58	121.30	121.94	121.24	121.91

[a] In units of angstroms (bond lengths) and degrees (bond angles). [b] The carbon atoms of the *C*-methyl and *N*-methyl groups are denoted by C_a and C_b, respectively.

The dihedral angles for the in-plane hydrogen atoms of the methyl groups are τ(H−C−C═O) = 180° and τ(H−C−N−H) = 0° for *trans*-NMA and τ(H−C−C═O) = 0° and τ(H−C−N−H) = 0° for *cis*-NMA. The structural parameters for the hydrogen bonds (Table 4) are defined in Figure 3, so that θ-(N−H···O) [θ(H···O═C)] is smaller than 180° when the relevant dihedral angle τ(C−N−H···O) [τ(H···O═C−N)] is 0°.

For *trans*-NMA and its dimers and trimers, the wavenumbers of all the modes are real for the optimized structures with C_s symmetry. In other words, the structures at the potential energy minima have planar peptide groups. For the *trans*-NMA monomer, the structure with a planar peptide group is not stable if a basis set without diffuse functions on the peptide oxygen and nitrogen atoms is used. Therefore, it may be said that the use of the 6-31++G** basis set[23] (or a similar basis set as used in ref 24) is essential for discussing the structures of NMA and its clusters. In the case of *cis*-NMA, one out-of-plane mode with an imaginary wavenumber is found for the optimized structure with a planar peptide group ["monomer (planar)" in Table 3]. The fully optimized structure of *cis*-NMA ["monomer (nonplanar)" in Table 3] has a dihedral angle τ(C_a-C−N−C_b) of about 5°. However, for the *cis*-NMA dimer, the optimized structure with planar peptide groups (C_{2h} symmetry) is stable with respect to both the in-plane and out-of-plane deformations.

As shown in Tables 1−3, the C−N and C═O bond lengths change substantially by hydrogen-bond formation but are not sensitive to the relative arrangement (parallel or antiparallel) of the peptide groups in the *trans*-NMA dimers and trimers. The effects of hydrogen bonding on the other structural parameters of the peptide group are not significant. It is noticeable that hydrogen bonding to the N−H group has significant effects on the lengths of both the C−N and C═O bonds. In a separate paper, it is shown that hydrogen bonding of a water molecule to the N−H group also induces significant changes in the C−N and C═O bond lengths.[25] Such an effect has also been pointed out by Guo and Karplus.[26] In each *trans*-NMA trimer, the molecule with two hydrogen bonds (molecule B) has a longer C═O bond and a shorter C−N bond than the other two molecules (molecules A and C), which have one hydrogen bond. The differences in the C−N and C═O bond lengths between the *cis*-NMA dimer and monomer [0.016 Å for r(C−N) and 0.012 Å for r(C═O)] are similar to those between molecule B in a *trans*-NMA trimer and the *trans*-NMA monomer [0.014 Å for r(C−N) and 0.011 Å for r(C═O)], indicating that these differences originate from the formation of hydrogen bonds to both the C═O and N−H groups.

The calculated hydrogen-bond energies (E_{H-bond}) are shown

TABLE 2: Optimized Structural Parameters (at the HF/6-31++G Level) of the Peptide Groups in the *trans-N*-Methylacetamide Trimers[a,b]**

	trimer 1 (p−p)			trimer 2 (p-ap)		
	molecule A	molecule B	molecule C	molecule A	molecule B	molecule C
r(C−N)	1.3422	1.3360	1.3416	1.3421	1.3363	1.3412
r(C═O)	1.2095	1.2145	1.2108	1.2095	1.2146	1.2112
r(N−H)	0.9968	0.9967	0.9919	0.9968	0.9972	0.9919
r(C−C_a)	1.5133	1.5110	1.5103	1.5133	1.5109	1.5104
r(N−C_b)	1.4444	1.4462	1.4499	1.4444	1.4461	1.4502
θ(O═C−N)	122.68	122.52	121.95	122.69	122.46	121.83
θ(C−N−H)	119.79	119.54	119.02	119.79	119.50	119.05
θ(N−C−C_a)	116.15	116.64	116.96	116.15	116.64	116.98
θ(C−N−C_b)	121.29	121.68	122.06	121.30	121.62	122.01
	trimer 3 (ap-p)			trimer 4 (ap-ap)		
	molecule A	molecule B	molecule C	molecule A	molecule B	molecule C
r(C−N)	1.3423	1.3356	1.3417	1.3423	1.3359	1.3412
r(C═O)	1.2096	1.2149	1.2108	1.2096	1.2150	1.2112
r(N−H)	0.9974	0.9966	0.9919	0.9974	0.9972	0.9919
r(C−C_a)	1.5133	1.5110	1.5103	1.5133	1.5110	1.5104
r(N−C_b)	1.4443	1.4466	1.4499	1.4442	1.4465	1.4502
θ(O═C−N)	122.66	122.34	121.96	122.66	122.30	121.83
θ(C−N−H)	119.77	119.55	119.01	119.77	119.52	119.05
θ(N−C−C_a)	116.14	116.65	116.96	116.14	116.65	116.98
θ(C−N−C_b)	121.25	121.59	122.07	121.25	121.54	122.01

[a] In units of angstroms (bond lengths) and degrees (bond angles). [b] The carbon atoms of the *C*-methyl and *N*-methyl groups are denoted by C_a and C_b, respectively.

TABLE 3: Optimized Structural Parameters (at the HF/6-31++G Level) of the Peptide Groups in the Monomer and Dimer of *cis-N*-Methylacetamide[a,b]**

	monomer (planar)	monomer (nonplanar)	dimer
r(C−N)	1.3555	1.3559	1.3397
r(C═O)	1.2037	1.2036	1.2159
r(N−H)	0.9949	0.9950	1.0035
r(C−C_a)	1.5122	1.5123	1.5120
r(N−C_b)	1.4462	1.4462	1.4461
θ(O═C−N)	121.22	121.20	121.78
θ(C−N−H)	114.13	114.01	115.77
θ(N−C−C_a)	116.94	116.99	117.67
θ(C−N−C_b)	127.21	127.15	126.48
τ(O═C−N−H)	0.0	−2.58	0.0
τ(C_a−C−N−H)	180.0	178.01	180.0
τ(O═C−N−C_b)	180.0	−175.87	180.0
τ(C_a−C−N−C_b)	0.0	4.71	0.0

[a] In units of Å (bond lengths) and degrees (bond angles). [b] The carbon atoms of the *C*-methyl and *N*-methyl groups are denoted by C_a and C_b, respectively.

TABLE 4: Structural Parameters for Hydrogen Bonds in Various Species[a,b]

species	molecule pair	r(H···O)/Å	θ(N−H···O)/deg	θ(H···O═C)/deg
trans-dimer 1	A−B	2.1061	183.77	181.65
trans-dimer 2	A−B	2.1040	179.67	205.42
trans-trimer 1	A−B	2.0648	181.62	174.60
	B−C	2.0658	183.24	180.61
trans-trimer 2	A−B	2.0638	181.88	175.95
	B−C	2.0649	178.86	205.23
trans-trimer 3	A−B	2.0617	178.77	205.72
	B−C	2.0674	182.12	178.14
trans-trimer 4	A−B	2.0611	178.77	207.51
	B−C	2.0655	178.45	206.03
cis-dimer	A−B	2.0112	177.93	124.52

[a] Calculated at the HF/6-31++G** level. [b] Definition of the structural parameters is shown in Figure 3.

in Table 5. The value of E_{H-bond} of each *trans*-NMA dimer is about 26 kJ mol^{-1}. A similar value of E_{H-bond} has been obtained by Aida[27] for dimer 1 of *trans*-NMA. This value is a little

TABLE 5: Total Energies and Hydrogen-Bond Energies (at the HF/6-31++G Level) of Various Species**

species	energy/hartree	hydrogen-bonding energy/kJ mol^{-1}
trans-NMA	−247.027 303 6	
trans-NMA dimer 1	−494.064 403 6	25.720
trans-NMA dimer 2	−494.064 573 4	26.166
trans-NMA trimer 1	−741.103 724 4	57.272
trans-NMA trimer 2	−741.103 920 5	57.786
trans-NMA trimer 3	−741.103 910 0	57.759
trans-NMA trimer 4	−741.104 115 1	58.297
cis-NMA (planar)	−247.023 054 2	
cis-NMA (nonplanar)	−247.023 059 9	
cis-NMA dimer	−494.064 624 0	48.613[a]

[a] 2E [*cis*-NMA (planar)] − E(*cis*-NMA dimer).

smaller than E_{H-bond} of *trans*-NMA with H_2O hydrogen bonded to the carbonyl C═O group (28 kJ mol^{-1}) but is larger than that of *trans*-NMA with H_2O hydrogen bonded to the amide N−H group (20 kJ mol^{-1}).[25] A similar result has been obtained by Dixon et al.[22] The value of E_{H-bond} of each *trans*-NMA trimer is about 58 kJ mol^{-1}, which is larger by about 6 kJ mol^{-1} than twice the value of E_{H-bond} of a *trans*-NMA dimer, indicating that formation of one hydrogen bond enhances the hydrogen-bonding energy of the other. Such a cooperativity effect has been suggested also by Guo and Karplus.[28]

By contrast, E_{H-bond} of the *cis*-NMA dimer is about 49 kJ mol^{-1}, which is smaller by 3 kJ mol^{-1} than twice the value of E_{H-bond} of a *trans*-NMA dimer. This negative cooperativity effect may be due to the strain in the structure of this species arising from the formation of two hydrogen bonds in a cyclic form. The presence of two hydrogen bonds in the *cis*-NMA dimer nonetheless makes this species as stable (in total energy) as the *trans*-NMA dimers, as pointed out by Dixon et al.[22] It is therefore necessary to examine the possible existence of this species by comparing its calculated vibrational wavenumbers with the experimental data.

C. Calculated Vibrational Wavenumbers. The calculated vibrational wavenumbers and IR intensities of the amide I modes of *trans*- and *cis*-NMA and their clusters are shown in Table 6.

TABLE 6: Calculated Wavenumbers (cm^{-1}) and Infrared Intensities (km mol^{-1}) of the Amide I Modes of Various Species[a]

species	wavenumber unscaled	wavenumber scaled	IR int.	amplitude[b] A	amplitude[b] B	amplitude[b] C
trans-monomer	1921	1710	391	1.000		
trans-dimer 1	1908	1698	17	0.824	−0.566	
	1895	1687	959	0.562	0.827	
trans-dimer 2	1907	1697	42	0.866	−0.501	
	1894	1686	933	0.494	0.869	
trans-trimer 1	1901	1692	242	0.941	−0.310	0.136
	1896	1688	110	−0.226	−0.285	0.931
	1876	1670	1248	0.247	0.907	0.341
trans-trimer 2	1901	1692	222	0.950	−0.300	0.089
	1894	1686	107	−0.188	−0.324	0.927
	1876	1670	1269	0.248	0.897	0.367
trans-trimer 3	1901	1692	256	0.956	−0.273	0.105
	1896	1688	141	−0.181	−0.281	0.943
	1875	1668	1202	0.222	0.921	0.320
trans-trimer 4	1901	1692	238	0.963	−0.263	0.065
	1894	1686	137	−0.147	−0.313	0.938
	1875	1668	1218	0.221	0.913	0.343
cis-monomer (planar)	1932	1719	589	1.000		
cis-monomer (nonplanar)	1932	1719	587	1.000		
cis-dimer	1908	1698	1295	0.707	−0.707	
	1872	1666	0	0.707	0.707	

[a] Calculated at the HF/6-31++G** level. [b] Normalized relative amplitudes of the C═O stretches in each mode.

The calculated wavenumbers are scaled by 0.89 so that the scaled wavenumber of the *trans*-NMA monomer (1710 cm^{-1}) is in agreement with the observed wavenumbers of this species in Ar (1708 cm^{-1}) and in N_2 (1706 cm^{-1}). The vibrational amplitudes of the constituent molecules in each mode are also shown in Table 6.

Although there are two amide I modes in each NMA dimer and three in each NMA trimer, the IR intensities of these modes are significantly different from each other. In each of the *trans*-NMA dimers and trimers, the lowest-wavenumber mode has an IR intensity much stronger than the other mode(s). In this lowest-wavenumber mode, all the constituent molecules vibrate in phase. Since the C═O bonds of all the molecules in each *trans*-NMA cluster are almost parallel to each other, the vectors of transition dipoles of those molecules are added with no cancellation in such an in-phase mode. In the other out-of-phase modes, contributions from the constituent molecules to the IR intensity cancel each other.

By contrast, in the *cis*-NMA dimer, one of the amide I modes (in-phase mode) is strictly IR inactive due to its C_{2h} symmetry. The out-of-phase mode is IR active, because the C═O bonds are antiparallel to each other.

The amide I modes in the dimers and trimers of NMA are delocalized due to the strong vibrational interactions between the constituent molecules. Therefore, those interactions must be lifted in order to obtain the "intrinsic" amide I wavenumbers (explained in section 1) of individual molecules. For this purpose, the amide I wavenumbers are calculated for isotopically substituted (actually nonexistent) *trans*-NMA dimers and trimers, in which the carbon and oxygen atoms of the C═O group(s) of constituent molecules other than the target molecule are substituted with ^{13}C and ^{18}O. The amide I wavenumber of such species appearing in the 1710−1670 cm^{-1} region may be regarded as the intrinsic amide I wavenumber of the unsubstituted target molecule in such species.

The results are shown in Table 7. For all the constituent molecules in the dimers and trimers, the intrinsic amide I wavenumbers are lower than that of the monomer. It is especially notable that the amide I wavenumber of the molecule with a free C═O bond in each dimer (molecule A) is shifted to an extent comparable to that of the other molecule (molecule B), which has a hydrogen-bonded C═O group. Therefore, hydrogen bonding to the N−H group has a significant effect on the amide I wavenumber. These shifts of the amide I wavenumbers are approximately in line with the changes in the C═O bond lengths, as shown in Table 7.

TABLE 7: Calculated Intrinsic Amide I Wavenumbers (cm^{-1}), C═O Bond Lengths (Å), and C═O Stretching Force Constants (mdyn $Å^{-1}$) for Peptide Groups in Various Species[a]

species	molecule	wavenumber[b] unscaled	wavenumber[b] scaled	C═O bond length	C═O stretching force const.[c]
trans-monomer		1921	1710	1.2043	11.521
trans-dimer 1	A	1904	1695	1.2085	11.228
	B	1900	1691	1.2096	11.217
trans-dimer 2	A	1904	1695	1.2085	11.225
	B	1898	1689	1.2099	11.195
trans-trimer 1	A	1900	1691	1.2095	11.158
	B	1881	1674	1.2145	10.896
	C	1894	1686	1.2108	11.141
trans-trimer 2	A	1900	1691	1.2095	11.157
	B	1881	1674	1.2146	10.892
	C	1892	1684	1.2112	11.117
trans-trimer 3	A	1900	1691	1.2096	11.154
	B	1879	1673	1.2149	10.869
	C	1894	1686	1.2108	11.140
trans-trimer 4	A	1900	1691	1.2096	11.152
	B	1879	1673	1.2150	10.864
	C	1892	1684	1.2112	11.114

[a] Calculated at the HF/6-31++G** level. [b] The carbon and oxygen atoms of the C═O group(s) other than that of the target molecule are isotopically substituted by ^{13}C and ^{18}O. [c] Scaled by $(0.89)^2$.

In each trimer, the amide I wavenumber of molecule B in the center of the hydrogen-bond chain, which has hydrogen bonds to both the C═O and N−H groups, is significantly lower than the amide I wavenumbers of molecules A and C at the ends, which have only one hydrogen bond. The amide I wavenumbers of the latter molecules are slightly lower than those of the corresponding molecules (A and B) in a dimer, probably because of the shorter lengths of the hydrogen bonds (see Table 4).

From the above results, the low-wavenumber shifts of the amide I mode induced by hydrogen bonding to the C═O group are 20−25 cm^{-1}, while those induced by hydrogen bonding to the N−H group are 15−20 cm^{-1}. The amide I wavenumber of molecule B in a trimer is explained by assuming an approximate additivity of these low-wavenumber shifts for the species having one hydrogen bond.

D. Assignments of the Observed IR Bands. From the calculated wavenumbers and IR intensities of the amide I modes of various species described above, the following assignments (summarized in Table 8) can be given to the IR bands observed in Ar (N_2). As noted in section 3.A, the band at 1708 (1706) cm^{-1} originates from the *trans*-NMA monomer. The shoulder on the low-wavenumber side of the 1706-cm^{-1} band observed in N_2 may be explained by the presence of an alternate site in the matrix. The bands at 1695 and 1686 (1693 and 1681) cm^{-1} arise from the NMA dimers. The lower-wavenumber band of these two [at 1686 (1681) cm^{-1}] is assigned to the strongly IR active modes of the dimers of *trans*-NMA, which are calculated at 1687−1686 cm^{-1}. The weakly IR active modes of the *trans*-NMA dimers calculated at 1698−1697 cm^{-1} may explain part of the IR intensities of the observed band at 1695 (1693) cm^{-1}.

TABLE 8: Assignments of the Amide I Bands of *N*-Methylacetamide Observed in N_2 and Ar Matrixes

obsvd (N_2 matrix)/cm^{-1}	obsvd (Ar matrix)/cm^{-1}	assignment
1706	1708	*trans*-monomer
1693	1695	*cis*-dimer
1681	1686	*trans*-dimers
1663	1670 }	*trans*-trimers
	1664 }	
	1640	larger complexes of *trans*-NMA

However, there are probably other source(s) giving rise to this observed band, because the intensity of this band relative to that of the 1686 (1687) cm^{-1} band varies with the matrix gas used and is stronger (particularly in N_2 matrix) than that expected from the calculated intensities of the *trans*-NMA dimers. It seems most reasonable to consider that the *cis*-NMA dimer exists in the sample with the NMA/matrix gas mixing ratio larger than 1/200, at least in the N_2 matrix. The calculated wavenumber difference between the amide I mode of the *trans*-NMA monomer and the IR active amide I mode of *cis*-NMA dimer (12 cm^{-1}) is in good agreement with the wavenumber difference between the 1708 (1706) and 1695 (1693) cm^{-1} bands observed in Ar (N_2).

As noted in section 3.A, the bands in the region below 1675 cm^{-1} are assigned to the amide I bands of the trimers and larger clusters of NMA. Among these, the bands observed at 1670 and 1664 (1663) cm^{-1} in Ar (N_2) are most reasonably assigned to the amide I bands of the *trans*-NMA trimers calculated around 1670 cm^{-1}. The reason for the appearance of two bands in Ar (at 1670 and 1664 cm^{-1}) corresponding to the *trans*-NMA trimers is not clear at present. Existence of trimers with slightly bent intermolecular hydrogen bonds and/or an alternate site in the matrix may be involved in the observed results.

Kuznetsova et al.[29] have recently carried out calculations by the semiempirical MINDO/3 method and have suggested that the wavenumber difference between the amide I bands of the *trans*-NMA dimers and trimers is very small. According to the present experiments and calculations, however, the amide I bands originating from the *trans*-NMA trimers should appear at wavenumbers different from those from the dimers.

The 1640 cm^{-1} band observed for the sample with the NMA/Ar mixing ratio of 1/100 is in the region of the amide I band of neat NMA in the liquid state.[30] Therefore, this band is considered to arise from the tetramers or larger clusters of NMA. Snyder et al.[31] have also assigned the 1633-cm^{-1} band observed for deposited amorphous samples of amides with long alkyl chains at low temperatures to clusters larger than trimers. In fact, in these amorphous samples[31] and in liquid NMA,[32] there are a few component bands in the amide I band envelopes, which are assignable to the monomer, dimers, and trimers. The wavenumbers observed in these previous studies are different from those in the present study by 10−20 cm^{-1}, an amount explainable by wavenumber shifts induced by the solvents.

4. Conclusion

It is shown in the present study that the wavenumber of the amide I band of the peptide group decreases on peptide−peptide hydrogen bonding. The shifts of the "intrinsic" amide I wavenumbers of individual peptide groups induced by hydrogen bonding to the C═O group are 20−25 cm^{-1} and those induced by hydrogen bonding to the N−H group are 15−20 cm^{-1}. Such changes in the diagonal terms of the **F** matrix, as well as the delocalization of vibrational modes induced by the off-diagonal terms, explain the features of the IR bands observed for NMA in Ar and N_2 matrixes at various concentrations. As summarized in Table 8, the IR bands of the dimers and trimers of NMA are clearly observed in these matrixes.

As explained in section 1, there are some experimental results[7,15−18] demonstrating the importance of changes in the diagonal terms of the **F** matrix in determining the amide I band envelopes of polypeptides and proteins. The quantitative information on the effects of peptide−peptide hydrogen bonding obtained in the present study, as well as on the effects of peptide−water hydrogen bonding described in a separate paper,[25] will be useful for better understanding of such features of the amide I band envelopes.

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