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Vibrational Interactions in the Amide I Subspace of the Oligomers and Hydration Clusters of *N*-Methylacetamide

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The diagonal and off-diagonal vibrational interactions in the amide I subspace are examined for the oligomers and hydration clusters of *N*-methylacetamide (NMA). A method called the average partial vector method is developed for constructing the force constant matrix (**F** matrix) in the amide I subspace from that in the full Cartesian space. It is shown that the **F** matrix thus constructed can reproduce quantitatively the vibrational frequencies and vibrational patterns of the amide I modes calculated in the full Cartesian space. For the NMA oligomers consisting of three or more NMA molecules, the cooperative effect on the C═O bond length (and on the diagonal force constant of the amide I mode as well) is seen. Including the values for those oligomers, the shifts in the diagonal terms in the amide I subspace (δk_{I}) and in the C═O bond length ($\delta S_{\mathrm{C=O}}$) of the NMA oligomers and NMA−water clusters from the values of an isolated NMA molecule are approximately proportional to each other. In addition, $\delta S_{\mathrm{C=O}}$ is shown to be approximately proportional to the electric field (originating from the other molecules in the NMA oligomer or NMA−water cluster) evaluated at a specified point on the C═O bond, indicating that the origin of the cooperative effect is the enhancement of the electric field operating among the molecules. The effect of mechanical anharmonicity on δk_{I} is examined by introducing the internal−normal mixed coordinate system representation for the cubic force constants, and is shown to overestimate the variation of δk_{I}. The partial cancellation by the effect of electrical anharmonicity (dipole second derivative) is also important. For the off-diagonal terms, it is shown that the TDC model provides a good approximation. However, for the (small) coupling constants between distant peptide groups, the effect of the polarization of the intervening peptide group(s) (also called the third-body mediation and may be regarded as a dipole-induced dipole effect) is also recognized.

1. Introduction

It has been well-known that the amide I bands of polypeptides and proteins are sensitive to the secondary structures.[1−3] Employing this property, researchers have analyzed observed amide I bands of proteins to obtain information on the content of secondary structures on an empirical basis.[4−7] To obtain more detailed information from observed spectra, however, theoretical analysis on the mechanisms that generate spectral features (frequencies, intensities, and widths of the component subbands contained in the whole band) is essential. Because the amide I band is well-separated in frequency from the other bands, it is a good approximation to treat the vibrational modes contained in the amide I band as constituting a separate subspace, which is called the amide I subspace.[8] Within this picture, the off-diagonal terms represent the vibrational couplings between peptide groups, and the diagonal terms represent the intrinsic vibrational force constants of individual peptide groups (corresponding to the vibrational frequencies when all the off-diagonal couplings are switched off) and are modulated by local environment of those peptide groups.[3,8−11]

It has been pointed out that the off-diagonal couplings between distant peptide groups are well represented by the transition dipole coupling (TDC) mechanism,[1,3,8,11−14] but those between covalently bonded neighboring peptide groups contain additional contribution from the through-bond interactions.[14−16] Ab initio molecular orbital (MO) calculations have been carried out to obtain those coupling constants quantitatively,[14,15] and they have been used successfully to clarify the conformations of oligopeptides dissolved in aqueous solution.[17−21] The mechanisms that modulate the diagonal terms are more elusive. The diagonal terms of the peptide groups in polypeptides and proteins dissolved in aqueous solution are affected by peptide−peptide and peptide−water interactions. It has been pointed out that there is a good correlation between the diagonal term of the amide I mode and the C═O bond length of the peptide group.[22−24] Following the concept of the charge response kernel,[25,26] it has been suggested that the changes in the diagonal term and the C═O bond length due to the peptide−peptide and peptide−water interactions are related to the electrostatic potential operating on the atoms in the peptide group.[23] Since the coefficients representing this relation are treated as fitting parameters, however, it may be said that the mechanism that gives rise to this relation has not been fully clarified quantitatively with respect to the molecular properties of the peptide group. For the peptide−peptide interactions, it has been suggested that there is a strong cooperative effect in the interaction energy, the C═O bond length, and the vibrational frequency of the amide I mode.[27−32] Because of this cooperative effect, the vibrational frequencies and the bond lengths are modulated more significantly in a longer hydrogen-bond chain of peptide groups. There is a controversy on the origin of this cooperative effect;

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in some studies it is suggested to be purely electrostatic in origin,[29−31] but in other studies the importance of an additional mechanism is proposed.[27,28] The cooperative effect is also important for some peptide−solvent interactions.[22,33−35]

In the present study, the diagonal and off-diagonal vibrational interactions in the amide I subspace are analyzed in detail for the oligomers and hydration clusters of *N*-methylacetamide (NMA), which is a representative model compound of the peptide group. To construct the force constant matrix (**F** matrix) in the amide I subspace from that in the full Cartesian space obtained from ab initio MO calculations, a method called the average partial vector method is developed. It is shown that the **F** matrices constructed by this method can reproduce quantitatively the vibrational frequencies and the vibrational patterns of the amide I modes calculated in the full Cartesian space. The diagonal and off-diagonal terms of those **F** matrices are analyzed with emphasis on their relation with various molecular properties of the oligomers and hydration clusters to get insight into the spectrum−structure relationship and the origin of the cooperative effect.

2. Computational Procedure

Ab initio MO calculations were carried out at the Hartree−Fock (HF) level with the 6-31+G(2df,p) basis set for the following species: (1) the NMA dimers with the parallel (p) and antiparallel (ap) molecular configurations[36−38] shown in Figure 1, panels a and b, respectively, (2) the NMA trimers in four possible configurations (p−p, p−ap, ap−p, and ap−ap), (3) the NMA tetramer and pentamer in the all-ap configuration, and (4) the NMA−water clusters. For the NMA−water clusters, it is known that there are three hydrogen-bonding hydration sites,[33] as shown in Figure 1c. We took into account all the possible combinations of hydration on those sites (A, B, C, A−B, A−C, B−C, and A−B−C). Water molecules in the second hydration shell are also expected to have some effects on the amide I mode of NMA,[39] but those effects are not treated in the present study. Except for the oligomers with the parallel configuration at the free N−H end, where a small imaginary frequency mode (of ∼3*i* cm^{-1}) is calculated for the optimized planar structures, the vibrational force constants in the full Cartesian space (together with the vibrational frequencies and vibrational patterns obtained from those force constants) were calculated after the structures were fully optimized (i.e., at the potential energy minimum) for all the oligomers and hydration clusters treated in this study. No scale factor was used for the vibrational force constants and vibrational frequencies.

From the **F** matrices in the full Cartesian space obtained in the ab initio MO calculations described above, those in the amide I subspace were constructed by the average partial vector method. The detailed formulation of this method is given in the next section.

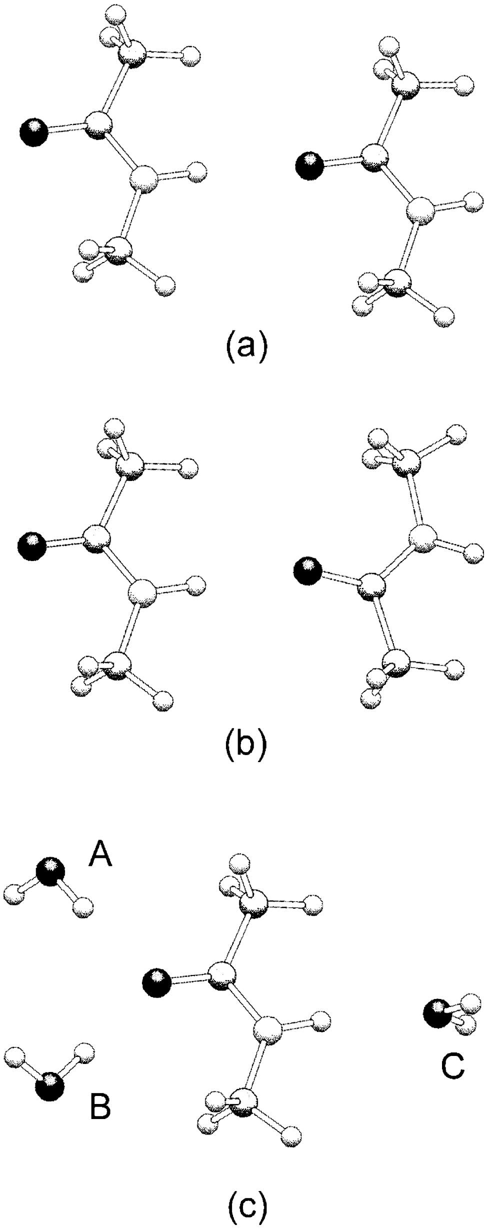

Figure 1. NMA dimers with the (a) parallel and (b) antiparallel molecular configurations and (c) NMA−water cluster. For the NMA−water cluster, the three hydrogen-bonding hydration sites (A, B, and C) are shown.

To estimate quantitatively the shifts in the diagonal terms of the **F** matrix of the amide I subspace induced by structural displacement of the NMA molecules in the oligomers and hydration clusters, the cubic (anharmonic) force constants of an isolated NMA molecule were also calculated at the HF/6-31+G(2df,p) level. It was found that, for the purpose of the present study, the best way to describe the cubic force constants is in the internal−normal mixed coordinate system representation, in which the cubic force constants are represented as the derivatives in the second order in the normal coordinate system (Q) and the first order in the internal coordinate system (S). The formula for the transformation from those in the Cartesian coordinate system is given as

$$\frac{\partial^3 V}{\partial Q_m \partial Q_n \partial S_r} = \sum_{i,j,k} \frac{\partial^3 V}{\partial x_i \partial x_j \partial x_k} \frac{\partial x_i}{\partial Q_m} \frac{\partial x_j}{\partial Q_n} \frac{\partial x_k}{\partial S_r} \tag{1}$$

where $\partial x_i/\partial Q_m$ is an element of the so-called **Lx** matrix obtained from the diagonalization of the quadratic (harmonic) **F** matrix, and $\partial x_i/\partial S_r$ is an element of the so-called **A** matrix (effectively the inverse of the so-called **B** matrix).[40,41] By taking both Q_m and Q_n in eq 1 to be the amide I normal coordinate (denoted as Q_{I}),[42] the shift in the quadratic force constant of the amide I mode (δk_{I}) induced by the displacements along S_r (denoted as δS_r) is expressed as

$$\delta k_{\mathrm{I}} = \sum_r \frac{\partial^3 V}{\partial {Q_{\mathrm{I}}}^2 \partial S_r} \delta S_r \tag{2}$$

The (nonredundant) internal coordinates of NMA were defined in a usual way.[43]

To analyze the mechanism of the structural displacements and the shifts in the diagonal elements of the **F** matrix in the amide I subspace, as well as the origin of the cooperative effect, the electric field operating on each NMA molecule from the other molecules in the same oligomer or hydration cluster was also calculated. For this purpose, the target molecule was removed, the positions of all the other molecules were fixed to those of the optimized structure of the original oligomer or hydration cluster, and the electric field was calculated at the specified positions (which will be discussed in section 4B) where the target molecule was located. This calculation was repeated by taking each molecule one by one as the target molecule.

The ab initio MO calculations described above were carried out by using the Gaussian 98 program[44] on a Fujitsu VPP5000 supercomputer at the Research Center for Computational Science of the Okazaki National Research Institutes. All the other vibrational analyses were performed on a Compaq XP1000 workstation in our laboratory by using our original programs.

3. Average Partial Vector Method

Important requirements for a method of constructing the **F** matrix in a vibrational subspace are that (1) the generated **F** matrix in the subspace can reproduce quantitatively the vibrational frequencies and vibrational patterns calculated from the original **F** matrix in the full vibrational space, and (2) the basis vectors of the subspace are sufficiently localized so that the generated **F** matrix in the subspace can be easily interpreted. This is because the elements of the constructed **F** matrix in the subspace constitute a basis for the analysis of the changes in the vibrational properties as a function of conformation, hydrogen-bonding condition, and other structural characteristics. Recently, a method called the Hessian matrix reconstruction method has been developed and applied to a few peptide systems by Cho and co-workers,[15] but it has not been fully tested concerning the first criterion mentioned above. In this section, we formulate a method called the average partial vector method that satisfies both of the above two criteria.

To construct the set of basis vectors of the vibrational subspace by this method, the normalized eigenvectors of the **F** matrix in the original mass-weighted full Cartesian space are needed. (The mass-weighted Cartesian space is based on the Cartesian coordinates weighted by the square root of atomic masses.) In the case of an oligomer consisting of *N* molecules, there are *N* eigenvectors that constitute the vibrational band in question (such as the amide I band). Those *N* eigenvectors, $\mathbf{v}_m$ ($m = 1, ..., N$) are decomposed into the contribution of each molecule as

$$\mathbf{v}_m = \mathbf{e}_{1m} + ... + \mathbf{e}_{Nm} \tag{3}$$

where only the elements for the *j*th molecule are nonzero for each $\mathbf{e}_{jm}$. Then, for each molecule *j*, the sum of $\mathbf{e}_{jm}$ over $m = 1, ..., N$ is taken. To avoid mutual cancellation in this summation and to avoid confusion in the interpretation of the off-diagonal terms in the generated **F** matrix in the vibrational subspace, the sign of each $\mathbf{e}_{jm}$ is inverted if necessary by referring to its projection onto an appropriate internal coordinate (such as the C=O stretching coordinate for the amide I mode).

$$\mathbf{s}_j' = \sum_{m=1}^{N} (\pm)\mathbf{e}_{jm} \tag{4}$$

Note that $\mathbf{v}_m$ is normalized but each $\mathbf{e}_{jm}$ is not normalized. Therefore, if a normal mode $\mathbf{v}_m$ is dominated by the contribution of a certain molecule *j*, $\mathbf{s}_j'$ has a large contribution from this normal mode because the magnitude of $\mathbf{e}_{jm}$ is large. The vectors $\mathbf{s}_j'$ thus generated are then normalized as

$$\mathbf{s}_j = \mathbf{s}_j'/|\mathbf{s}_j'| \tag{5}$$

Since only the elements for molecule *j* are nonzero for each $\mathbf{s}_j$, the set of $\mathbf{s}_j$ ($j = 1, ..., N$) is a localized and mutually orthogonal basis of the vibrational subspace. The **F** matrix in this subspace (denoted as $\mathbf{F}_{\text{sub}}$) is calculated as

$$\mathbf{F}_{\text{sub}} = \begin{pmatrix} \mathbf{s}_1^{\mathrm{T}} \\ \vdots \\ \mathbf{s}_N^{\mathrm{T}} \end{pmatrix} \mathbf{F} (\mathbf{s}_1 \ \cdots \ \mathbf{s}_N) \tag{6}$$

where **F** is the **F** matrix in the mass-weighted full Cartesian space, and the superscript T stands for taking the transpose of the vector. Since **F** is symmetric, $\mathbf{F}_{\text{sub}}$ is also symmetric.

In fact, this method may be used also for clusters made of different compounds (such as NMA−water clusters) if at most one vibrational motion per molecule contributes to the vibrational band in question, since this method is not based on the condition that the vibrational motions of all the molecules are similar to each other. Of course, the vibrational band should be well separated in frequency from the other bands so that the picture of the vibrational subspace provides a good approximation.

To apply this method to covalently bonded oligopeptides, the dividing points along the backbone should be carefully chosen. This point will be discussed in detail elsewhere.

To check the validity of this method for constructing the **F** matrix in the amide I subspace of NMA oligomers, we show the results obtained for the NMA pentamer (in the all-ap configuration) in detail. The vibrational frequencies and the vibrational patterns ($\partial S_{\text{C=O}}/\partial Q_m$, where $S_{\text{C=O}}$ is the C=O stretching internal coordinate) of the five amide I modes calculated from the original **F** matrix in the full Cartesian space and those calculated from the **F** matrix in the amide I subspace are shown in Figure 2, panels a and b, respectively. The NMA molecules are numbered along the hydrogen-bond chain, and are arranged so that NMA 1 has a free C=O bond and NMA 5 has a free N−H bond. It is seen that the two high-frequency modes are rather localized on the molecules on the edges of the hydrogen-bond chain, but the other three modes are delocalized in the central part of the oligomer. These vibrational patterns shown in Figure 2a,b are in good agreement with each other (with the root-mean-square deviation of 3.38×10^{-3} amu$^{-1/2}$). The vibrational frequencies calculated from the **F** matrix in the amide I subspace are a little lower than those calculated from the original **F** matrix. Taking the average over all the oligomers and cluster species treated in the present study, this offset value for the vibrational frequencies is -0.394 ± 0.254 cm^{-1}, corresponding to the offset value of the diagonal force constant of $(-0.887 \pm 0.572) \times 10^{-3}$ mdyn Å^{-1} amu^{-1}. This result means that, by correcting for this offset value, the **F** matrix in the amide I subspace can reproduce the vibrational frequencies with an accuracy of ± 0.254 cm^{-1} with the correct vibrational patterns.

As another check of the validity of the average partial vector method, the **F** matrix elements in the amide I subspace obtained for a ^{13}C-substituted (on the carbonyl group of NMA 1) species are compared with those of the normal species.[45] The result is shown in Table 1. As expected, the diagonal element for the

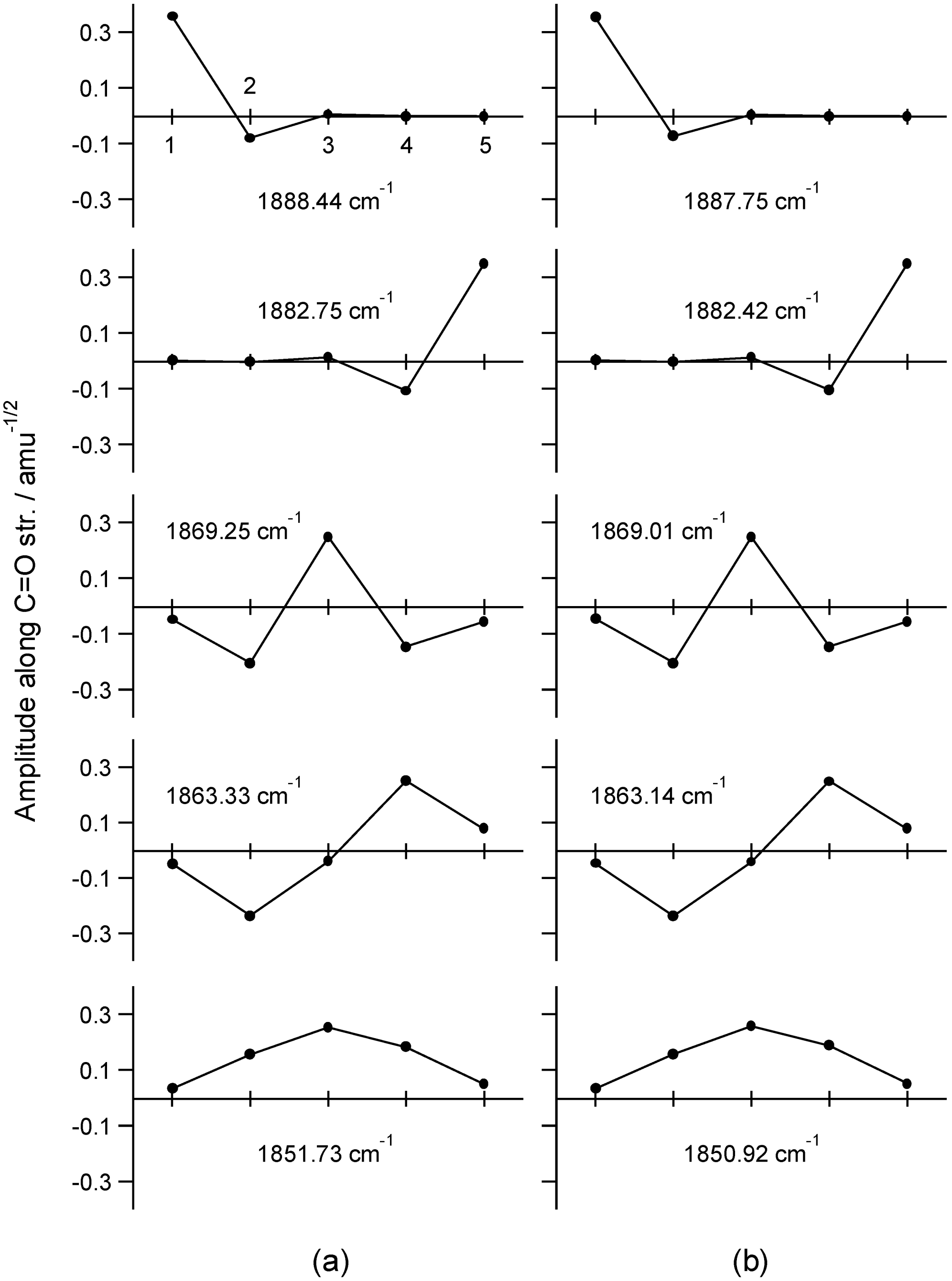

Figure 2. Vibrational frequencies and vibrational patterns (amplitudes along the C═O stretch, expressed as $\partial S_{C=O}/\partial Q_m$) of the five amide I modes of the NMA pentamer in the all-ap configuration calculated (a) from the original **F** matrix in the full Cartesian space and (b) from the **F** matrix in the amide I subspace constructed by the average partial vector method. The NMA molecules are numbered along the hydrogen-bond chain, and are arranged so that NMA 1 has a free C═O bond and NMA 5 has a free N−H bond.

^{13}C-substituted molecule gets lower significantly (by -0.0999 ± 0.0020 mdyn Å^{-1} amu^{-1} when the average is taken over all the oligomers treated in the present study), and all the other elements remain almost unchanged. This result indicates that it is possible to calculate the vibrational frequencies and vibrational patterns of the amide I modes of ^{13}C-substituted species only by changing the diagonal element of the ^{13}C-substituted molecule in the **F** matrix in the amide I subspace, so this method may be used for analyzing the amide I bands obtained in isotope-labeling measurements.[46−50]

4. Shifts in the Diagonal Force Constants in the Amide I Subspace

A. Relation with the Bond Length Changes and Mechanical Anharmonicity. The relation between the shifts in the diagonal elements of the **F** matrix in the amide I subspace (δk_I) and in the C═O bond length ($\delta S_{C=O}$) from the values of an isolated NMA molecule is shown in Figure 3. The values for the NMA oligomers and NMA−water clusters are shown in open and filled circles, respectively. The diagonal elements of the **F** matrix are corrected for the offset value (-0.887×10^{-3} mdyn Å^{-1} amu^{-1}) obtained in section 3. For the NMA−water clusters, the amide I mode of NMA and the HOH bending mode of water (without deuteration) are regarded as constituting a vibrational subspace, because the HOH bending mode of water is coupled more or less with the amide I mode of NMA,[51] and these modes are well separated in frequency from the other modes in the system. In this case, we can get the force constant of the amide I mode as one of the diagonal elements of the **F** matrix in the vibrational subspace. It is clearly seen in Figure

TABLE 1: Quadratic Force Constants of the Amide I Subspace of the *N*-Methylacetamide (NMA) Pentamer and One of Its ^{13}C-Substituted Species Calculated by the Average Partial Vector Method[a,b]

species	molecule	NMA 1	NMA 2	NMA 3	NMA 4	NMA 5
normal	NMA 1	2.0973	−0.0111	−0.0025	−0.0008	−0.0004
	NMA 2		2.0466	−0.0135	−0.0029	−0.0009
	NMA 3			2.0376	−0.0139	−0.0028
	NMA 4				2.0438	−0.0130
	NMA 5					2.0839
^{13}C (1)[c]	NMA 1	1.9976	−0.0114	−0.0024	−0.0008	−0.0003
	NMA 2		2.0465	−0.0131	−0.0028	−0.0009
	NMA 3			2.0377	−0.0137	−0.0028
	NMA 4				2.0438	−0.0129
	NMA 5					2.0839

[a] In units of mdyn Å^{-1} amu^{-1}. The original force constants (used as the uncontracted **F** matrix in eq 6) are calculated at the HF/6-31+G(2df,p) level. [b] The NMA molecules are numbered along the hydrogen-bond chain, and are arranged so that NMA 1 has a free C═O bond and NMA 5 has a free N−H bond. [c] The carbonyl carbon in one of the NMA molecules (NMA 1) is substituted with ^{13}C.

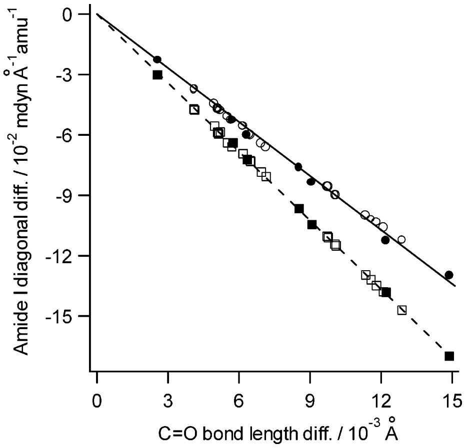

Figure 3. Relation between the shifts in the diagonal elements of the **F** matrix in the amide I subspace (corrected for the offset value of −0.887 × 10^{-3} mdyn Å^{-1} amu^{-1} obtained in section 3, denoted as δk_I) and the C═O bond length ($\delta S_{C=O}$) of the NMA oligomers and the NMA−water clusters from the values of an isolated NMA molecule: (○, ●) diagonal force constants calculated by the average partial vector method for the NMA oligomers and the NMA−water clusters, respectively; (□, ■) contribution of the mechanical anharmonicity calculated by using eq 2 for the NMA oligomers and the NMA−water clusters, respectively; (solid and broken lines) the least-squares fitted lines (constrained to pass the origin) for these points.

3 that δk_I is approximately proportional to $\delta S_{C=O}$. The gradient of the least-squares fitted line (constrained to pass the origin), which is shown in a solid line, is −8.932 mdyn Å^{-2} amu^{-1}. A similar linear relationship has been obtained for the NMA dimers and NMA−water clusters in previous studies.[22−24] The result shown in Figure 3 of the present study demonstrates that the same linear relationship holds also for NMA oligomers with longer hydrogen-bond chains, where the effect of cooperativity on the force constants and bond lengths is expected. The existence of this linear relationship suggests the following two possibilities: (1) a change in one of these quantities is a consequence of that in the other, or (2) the two quantities are controlled by the same mechanism.

To evaluate the effect of cooperativity, the C═O bond lengths of the NMA molecules in the oligomers are plotted in Figure 4. It is seen that the C═O bond length gets longer as we go toward the center of a hydrogen-bond chain and as the hydrogen-bond chain becomes longer. Even for the NMA molecules on the edges of a hydrogen-bond chain, a longer C═O bond is calculated in a longer hydrogen-bond chain. Note that, because of the linear relationship shown in Figure 3, the diagonal force constant of the amide I mode behaves in the same way (with the opposite sign). The mechanism that gives rise to this cooperative effect will be discussed in section 4B.

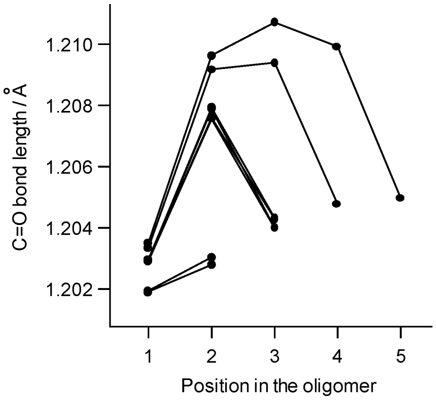

Figure 4. C═O bond lengths of the NMA molecules in the oligomers. See the caption for Figure 2 for the numbering of molecules.

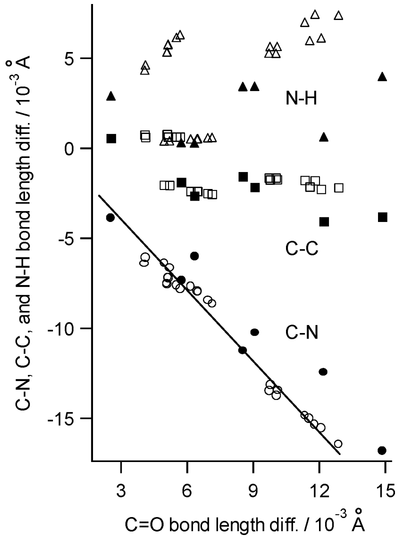

Figure 5. Relation among the changes in the C═O, C−N (amide), C−C (methyl), and N−H bond lengths calculated for the NMA oligomers (open symbols) and the NMA−water clusters (filled symbols). The solid line is obtained for the relation between the C═O and C−N bond length changes by the least-squares fitting (constrained to pass the origin) excluding the four points (filled circles) with significant deviations.

The relation among the changes in the C═O, C−N (amide), C−C (methyl), and N−H bond lengths is shown in Figure 5. The values for the NMA oligomers and NMA−water clusters are shown in open and filled symbols, respectively. It is seen that, except for those in four NMA−water clusters, the changes in the C═O and C−N bond lengths are mutually correlated. The gradient of the least-squares fitted line (calculated by excluding the four exceptional points and constrained to pass the origin), which is shown in a solid line, is −1.314. The following two points are considered to be noteworthy for this result: (1) The change in the C−N bond length is larger in magnitude than that in the C═O bond length. This result cannot be obtained solely by the displacement along the amide I normal coordinate, since the amide I normal mode is mainly made of the C═O stretch with a minor contribution from the C−N stretch. It is therefore suggested that, aside from the displacement along the amide I normal coordinate, there is additionally at least one effect that perturbs the C−N bond length, but it is

TABLE 2: Cubic Force Constants for the Amide I Normal Mode (Q_I) of *N*-Methylacetamide in the Internal−Normal Mixed Coordinate System Representation ($\partial^3 V/(\partial Q_I^2 \partial S_r)$) Calculated at the HF/6-31+G(2df,p) Level

internal coordinate (S_r)	cubic force constant ($\partial^3 V/(\partial Q_I^2 \partial S_r)$), mdyn Å^{-2} amu^{-1}
C=O stretch	−12.431
C−N (amide) stretch	−0.631
N−H stretch	−0.006
C−C (methyl) stretch	−0.650
N−C (methyl) stretch	−0.094
CCN bend	−0.012
C=O in-plane bend	−0.011
CNC bend	0.072
N−H in-plane bend	−0.116
methyl (C) symmetric stretch	−0.044
methyl (C) in-plane asymmetric stretch	−0.052
methyl (C) symmetric bend	0.031
methyl (C) in-plane asymmetric bend	−0.003
methyl (C) in-plane rock	−0.107
methyl (N) symmetric stretch	0.005
methyl (N) in-plane asymmetric stretch	0.009
methyl (N) symmetric bend	0.003
methyl (N) in-plane asymmetric bend	0.001
methyl (N) in-plane rock	−0.011

also strongly correlated with the effect that perturbs the C=O bond length. (2) The four exceptional points in the plot shown in Figure 5 are for the NMA−water clusters with a water molecule on position A in Figure 1c. This water molecule affects the C−N bond length differently from the other water molecules.

The change in the C−C bond length is also weakly correlated with that in the C=O bond length. Because the N−H bond length is perturbed by the hydrogen bonding on itself, its change seems to be uncorrelated with the changes in the other bond lengths.

To estimate quantitatively the magnitudes of δk_I induced by these structural displacements, the cubic force constants of an isolated NMA molecule are calculated and represented in the internal−normal mixed coordinate system as explained in section 2. The values of those cubic force constants are shown in Table 2. It is seen that the value for the C=O stretch is significantly larger in magnitude than the others, but those for the C−N (amide) stretch and the C−C (methyl) stretch are as large as about 5% of this and cannot be regarded as negligible. By using these values (all those listed in Table 2), we calculated the values of δk_I due to the structural displacements by using eq 2. The result is shown in open and filled squares (for the NMA oligomers and NMA−water clusters, respectively) in Figure 3. The gradient of the least-squares fitted line (constrained to pass the origin), which is shown in a broken line, is −11.385 mdyn Å^{-2} amu^{-1}, which is a little smaller in magnitude than the cubic force constant calculated for the C=O stretch ($\partial^3 V/(\partial Q_I^2 \partial S_{C=O})$ = −12.431 mdyn Å^{-2} amu^{-1}) because of the contribution from the other coordinates, such as the C−N (amide) stretch and the C−C (methyl) stretch. The difference in the gradient between the solid and broken lines in Figure 3 suggests that there is at least one additional mechanism that gives rise to a shift in the diagonal force constant of the amide I mode, but it is also strongly correlated with the structural displacement (mainly along the C=O bond).

B. Effect of Electric Field. Since NMA is a polar and hydrogen-bonding molecule, it is most reasonable to consider that the C=O bond length and the diagonal force constant of the amide I mode of an NMA molecule in NMA oligomers and NMA−water clusters are affected strongly by the electric field **E** originating from the other molecules in the system. The shift in the diagonal force constant of the amide I mode is expressed as

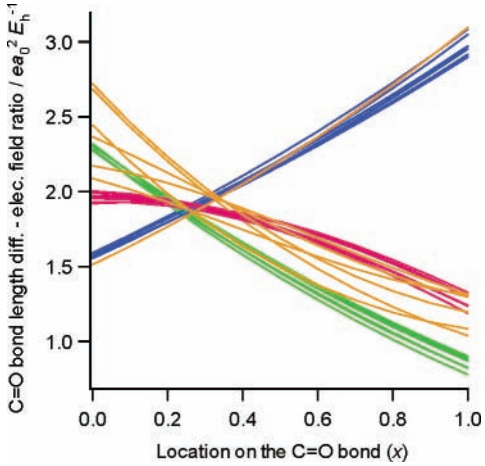

Figure 6. Plot of the ratio between the C=O bond length change and the electric field ($\delta S_{C=O}/E_{proj(C=O)}$) against the position on the C=O bond (x), where $E_{proj(C=O)}$ is the projection of the electric field evaluated at $\mathbf{r} = x\mathbf{r}_O + (1 - x)\mathbf{r}_C$ onto the direction of $\partial\boldsymbol{\mu}/\partial S_{C=O}$: (blue) NMA molecules with a free C=O bond in the oligomers; (green) NMA molecules with a free N−H bond in the oligomers; (red) the other NMA molecules in the oligomers; (orange) NMA−water clusters.

$$\delta k_I = \sum_r \frac{\partial^3 V}{\partial Q_I^2 \partial S_r} \delta S_r - \frac{\partial^2 \boldsymbol{\mu}}{\partial Q_I^2} \mathbf{E} \qquad (7)$$

where the first term (equal to the right-hand side of eq 2) is the effect of the mechanical anharmonicity and is induced by structural displacement, and the second term is the effect of the electrical anharmonicity[11,52] and is obtained as the second derivative of the dipole interaction energy with respect to Q_I. As discussed above in section 4A, the first term in eq 7 is dominated by the contribution from the C=O stretch. Since the C=O stretch has a large dipole derivative, the displacement along this coordinate ($\delta S_{C=O}$) is also considered to be strongly affected by the electric field **E**. $\delta S_{C=O}$ is approximately expressed as[53,54]

$$\delta S_{C=O} \cong \frac{1}{k_{C=O}} \frac{\partial \boldsymbol{\mu}}{\partial S_{C=O}} \mathbf{E} \qquad (8)$$

where $k_{C=O}$ is the diagonal force constant for the C=O stretching internal coordinate. As a result, in addition to the second term, the major part of the first term of eq 7 is expected to be controlled by the electric field **E**.

Since the electric field operating on an NMA molecule in NMA oligomers and NMA−water clusters is not uniform, it is important to examine where in the molecule we should evaluate the electric field. For this purpose, the ratio between $\delta S_{C=O}$ and the projection of **E** onto the direction of $\partial\boldsymbol{\mu}/\partial S_{C=O}$ (denoted as $E_{proj(C=O)}$) is calculated for the NMA molecules in all the NMA oligomers and NMA−water clusters, following the procedure described in section 2. The result obtained by evaluating the electric field at $\mathbf{r} = x\mathbf{r}_O + (1 - x)\mathbf{r}_C$ on the C=O bond is shown in Figure 6, where $\mathbf{r}_O$ and $\mathbf{r}_C$ are the positions of the carbonyl oxygen and carbon atoms, respectively. It is seen that, except for four NMA−water clusters, the value of the ratio $\delta S_{C=O}/E_{proj(C=O)}$ nearly coincides at $x \cong 0.24$. In other words, $\delta S_{C=O}$ is nearly proportional to $E_{proj(C=O)}$ evaluated at $x \cong 0.24$. Note that this plot includes all the NMA molecules in the NMA oligomers treated in the present study, ranging from dimer to pentamer, where the cooperative effect on $\delta S_{\rm C=O}$ is recognized as shown in Figure 4. The result shown in Figure 6 indicates that the origin of the cooperative effect on $\delta S_{\rm C=O}$ (and hence on the diagonal term of the **F** matrix in the amide I subspace because of the relation shown in Figure 3) is the enhancement of the electric field operating among the molecules in the oligomers. The four exceptional cases that show noticeable deviation in this plot are for the NMA−water clusters with a water molecule on position B in Figure 1c. It may be said that the effective electric field originating from this water molecule is slightly underestimated at $\mathbf{r} = x\mathbf{r}_{\rm O} + (1 - x)\mathbf{r}_{\rm C}$ with $x \cong 0.24$.

According to eq 8, the ratio $\delta S_{\rm C=O}/E_{\rm proj(C=O)}$ is equal to $(1/k_{\rm C=O})|\partial\boldsymbol{\mu}/\partial S_{\rm C=O}|$, which may be regarded as a molecular property of NMA. The values of $k_{\rm C=O}$ and $|\partial\boldsymbol{\mu}/\partial S_{\rm C=O}|$ calculated for an isolated NMA molecule at the HF/6-31+G(2df,p) level are $k_{\rm C=O} = 14.48$ mdyn Å^{-1} ($0.9300E_{\rm h}a_0^{-2}$) and $|\partial\boldsymbol{\mu}/\partial S_{\rm C=O}| = 7.470$ D Å^{-1} ($1.555e$). From these values, $(1/k_{\rm C=O})|\partial\boldsymbol{\mu}/\partial S_{\rm C=O}|$ is calculated to be $1.672ea_0^2E_{\rm h}^{-1}$, which is slightly smaller than the value of $\sim1.9ea_0^2E_{\rm h}^{-1}$ obtained for $\delta S_{\rm C=O}/E_{\rm proj(C=O)}$ at $x \cong 0.24$ in Figure 6. This result suggests that eq 8 roughly describes the response of the C═O bond length to the electric field, but the coefficient of the response should be a little larger than the value of $(1/k_{\rm C=O})|\partial\boldsymbol{\mu}/\partial S_{\rm C=O}|$ calculated for an isolated NMA molecule. The precise reason for this deviation is not clear at present.

By using the ratio of $\delta S_{\rm C=O}/E_{\rm proj(C=O)} \approx 1.9ea_0^2E_{\rm h}^{-1}$ and the gradient of −11.385 mdyn Å^{-2} amu^{-1} of the broken line in Figure 3, we obtain

$$\delta k_{\rm I} = b_{\rm eff}E_{\rm proj(C=O)} - \frac{\partial^2\boldsymbol{\mu}}{\partial {Q_{\rm I}}^2}\mathbf{E} \qquad (9)$$

with the effective coefficient of $b_{\rm eff} \approx (-4.0 \times 10^{-4})ea_0^{-1}m_{\rm e}^{-1}$. Since the dipole second derivative $\partial^2\boldsymbol{\mu}/\partial Q_{\rm I}^2$ is directed in the opposite direction as compared to the dipole (first) derivative $\partial\boldsymbol{\mu}/\partial Q_{\rm I}$ or $\partial\boldsymbol{\mu}/\partial S_{\rm C=O}$,[42] the second term in eq 9 (arising from the electrical anharmonicity) partially cancels the contribution of the first term, which is derived from the mechanical anharmonicity. The magnitude of $\partial^2\boldsymbol{\mu}/\partial Q_{\rm I}^2$ is calculated to be $(5.94 \times 10^{-5})ea_0^{-1}m_{\rm e}^{-1}$ at the HF/6-31+G(2df,p) level. Comparing this value with $b_{\rm eff}$, it is suggested that the partial cancellation by the second term is about 15%. This partly explains the difference (of ~25%) in the gradient between the solid and broken lines in Figure 3. Although the mechanism that controls the variation of the diagonal term of the **F** matrix in the amide I subspace has not been fully clarified, the present result clearly indicates that both the mechanical and electrical anharmonicities are important for the variation of this quantity.

5. Off-Diagonal Coupling

As described in section 1, it has been pointed out in previous studies[1,3,8,11−14] that the off-diagonal coupling between peptide groups that are not connected directly by a covalent bond is well represented by the TDC mechanism. According to this mechanism, the coupling between the amide I vibrations of the mth and nth peptide groups is expressed as

$$\frac{\partial^2 V}{\partial Q_{{\rm I},m}\partial Q_{{\rm I},n}} = -\frac{\partial\boldsymbol{\mu}_m}{\partial Q_{{\rm I},m}}\mathbf{T}_{mn}\frac{\partial\boldsymbol{\mu}_n}{\partial Q_{{\rm I},n}} \qquad (10)$$

where $Q_{{\rm I},m}$ stands for the amide I vibration of the mth peptide group and $\mathbf{T}_{mn}$ is the dipole interaction tensor between the mth and nth peptide groups. The off-diagonal coupling constants in the **F** matrix in the amide I subspace constructed by the average partial vector method are compared with those according to eq 10 for the NMA oligomers and shown in open squares in Figure 7. (In calculating the latter, the dipole derivative $\partial\boldsymbol{\mu}_m/\partial Q_{{\rm I},m}$ is assumed to be the same as that of an isolated NMA molecule and is located at $\mathbf{r} = x\mathbf{r}_{\rm O} + (1 - x)\mathbf{r}_{\rm C}$ with $x = 0.24$ on each NMA molecule.) It is seen that the couplings between hydrogen-bonded neighboring peptide groups are well represented by the TDC mechanism. However, the TDC mechanism underestimates to some extent the coupling constants between more distant peptide groups.

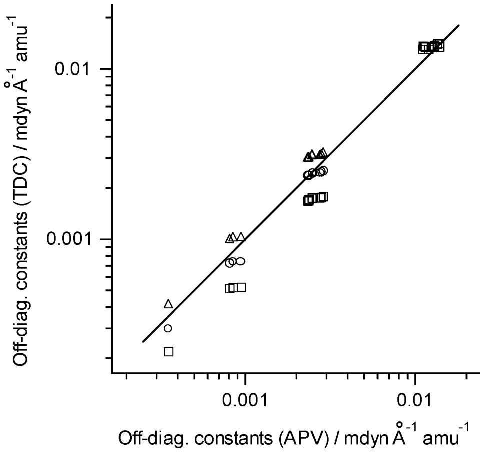

Figure 7. Comparison of the off-diagonal elements of the **F** matrix in the amide I subspace of the NMA oligomers calculated by the average partial vector (APV) method and by the TDC mechanism (log−log plot): (□) simple TDC mechanism (eq 10); (△) including the enhancement due to the polarization of the intervening peptide group(s) with the molecular polarizability of NMA being taken as $\boldsymbol{\alpha}_j$ in eq 11; (○) the same as the triangles, but with the trace part of the molecular polarizability of formamide being taken as $\boldsymbol{\alpha}_j$ in eq 11.

One possibility that is considered to be the reason for this deviation is that the enhancement due to the polarization of the intervening NMA molecule(s) is not taken into account in eq 10. This enhancement is expressed as

$$\Delta\frac{\partial^2 V}{\partial Q_{{\rm I},m}\partial Q_{{\rm I},n}} = -\frac{\partial\boldsymbol{\mu}_m}{\partial Q_{{\rm I},m}}\mathbf{T}_{mj}\boldsymbol{\alpha}_j \ldots \mathbf{T}_{kn}\frac{\partial\boldsymbol{\mu}_n}{\partial Q_{{\rm I},n}} \qquad (11)$$

where $\boldsymbol{\alpha}_j$ is the polarizability tensor of the jth molecule. This enhancement mechanism is called the third body mediation in ref 55 and may also be regarded as a dipole-induced dipole (DID) mechanism.[56,57]

The off-diagonal coupling constants calculated by taking into account the enhancement according to eq 11 with the molecular polarizability of NMA used as $\boldsymbol{\alpha}_j$ are shown in open triangles in Figure 7. Now it is seen that the coupling constants are slightly overestimated. The use of the molecular polarizability of NMA as it is may not be appropriate for describing the polarization of the intervening peptide group(s) in a hydrogen-bond chain of peptide groups, because the polarization of the methyl groups is also included in it. When the trace part of the molecular polarizability of formamide is used as $\boldsymbol{\alpha}_j$ in eq 11 as an approximation, the result shown in open circles in Figure 7 is obtained, indicating good agreement between the model (eqs 10 and 11) and the ab initio MO calculations.

The above result suggests that the TDC model provides a good approximation for the off-diagonal coupling of the amide I vibrations in NMA oligomers, but the effect of the intervening peptide group(s) is also recognized for the (small) coupling between distant peptide groups.

6. Summary

The main conclusions obtained in the present study may be summarized as follows. (1) The average partial vector method is developed for constructing the **F** matrix in a vibrational subspace. It is shown that the **F** matrix for the amide I subspace of NMA oligomers calculated by this method can reproduce the vibrational frequencies (by correcting for the small offset value) and the vibrational patterns calculated from the original **F** matrix in the full Cartesian space. Therefore, this method can be used for examining the changes in the diagonal and off-diagonal terms in the amide I subspace in detail. (2) The shifts in the diagonal elements of the **F** matrix in the amide I subspace (δk_{I}) and in the C=O bond length ($\delta S_{\mathrm{C=O}}$) of the NMA oligomers and the NMA−water clusters from the values of an isolated NMA molecule are approximately proportional to each other. This linear relation includes the points for the NMA oligomers consisting of three or more NMA molecules, for which the cooperative effect on the force constants and bond lengths is seen. (3) The effect of mechanical anharmonicity (cubic force constants and structural displacements) overestimates the gradient of this linear relation. The partial cancellation by the effect of electrical anharmonicity (dipole second derivative) is also important. (4) $\delta S_{\mathrm{C=O}}$ of the NMA molecules in the NMA oligomers and NMA−water clusters is approximately proportional to the electric field (projected onto the direction of $\partial\boldsymbol{\mu}/\partial S_{\mathrm{C=O}}$) evaluated at $\mathbf{r} = x\mathbf{r}_{\mathrm{O}} + (1 - x)\mathbf{r}_{\mathrm{C}}$ with $x = 0.24$. In this case also, the NMA oligomers consisting of three or more NMA molecules are included, for which the cooperative effect is seen. Therefore, the origin of the cooperative effect on $\delta S_{\mathrm{C=O}}$ (and hence on δk_{I} because of the linear relation between δk_{I} and $\delta S_{\mathrm{C=O}}$) is the enhancement of the electric field operating among the molecules in the oligomers. (5) The TDC model provides a good approximation for the off-diagonal coupling of the amide I vibrations in NMA oligomers. However, the effect of the polarization of the intervening peptide group(s) is also recognized for the (small) coupling between distant peptide groups.

The results obtained in the present study clearly show that the electrostatic effect is important for the diagonal and off-diagonal force constants in the amide I subspace, but it is also clear at the same time that it is not totally described by the interactions between neighboring (directly hydrogen-bonded) peptide groups because of the existence of the cooperative effect among the peptide groups in a hydrogen-bond chain. It is considered that this point should be carefully taken into account for correct description of the vibrational motions that give rise to the amide I band profiles of polypeptides and proteins.

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