Local Amide I Mode Frequencies and Coupling Constants in Polypeptides

Jun-Ho Choi, Sihyun Ham, and Minhaeng Cho*

Department of Chemistry and Center for Multidimensional Spectroscopy, Division of Chemistry and Molecular Engineering, Korea University, Seoul 136-701, Korea

Received: April 1, 2003; In Final Form: June 11, 2003

Ab initio calculations of the amide I modes of right-handed α-helical, 3_{10}-helical, left-handed α-helical, π-helical, parallel β-sheet, antiparallel β-sheet, and fully extended β-sheet polypeptide conformations with two to five peptide bonds were performed to investigate the site dependencies of the local amide I mode frequencies and vibrational coupling constants between neighboring peptides. A Hessian matrix reconstruction method is used to obtain these quantities from the ab initio-calculated amide I normal modes. The local amide I mode frequencies of the peptides in the inner region of a given helical polypeptide are significantly larger than those of terminal peptides, whereas the local amide I mode frequencies of β-sheet polypeptides are not site-dependent. The amide I vibrational coupling constants are not sensitive to the length of the polypeptide, but they are found to be strongly dependent on the three-dimensional conformation of the polypeptides. An empirical model for predicting diagonal amide I mode frequency shift is used to theoretically describe the site dependence of the local amide I mode frequency.

I. Introduction

Interpeptide interaction between any two local amide I vibrations has been extensively studied to understand the structure-spectrum correlation of polypeptide. In particular, it was shown that the amide I IR band shape and location can provide direct information on the secondary structure of an unknown polypeptide.[1−15] Recently, as demonstrated by Hamm and co-workers, a two-dimensional pump−probe spectroscopy can be effectively used to measure the vibrational coupling constant between two nearest-neighboring peptides of trialanine in solutions.[16] Thus, if there exists a quantitatively reliable Ramanchandran map of the coupling constant, one can almost determine the three-dimensional (3D) structure of the tripeptide in solution. Also, Schweitzer-Stenner and co-workers developed an interesting algorithm that can be used to determine the dihedral angles between the two peptides bonds in a tripeptide ($N = 2$) by using the spectrally decomposed isotropic Raman spectrum.[17−19] Their method critically relied on the accuracy of the spectral resolution of the two normal mode components as well as their intensities and was successfully applied to the determination of the 3D structure of an alanine-based tripeptide.

However, to develop a practical method for simulating amide I band envelopes of proteins, it is desirable to consider the amide I normal modes in the approximate N-dimensional subspace constructed by the N local amide I coordinates. In the approximate amide I subspace, a single vibrational degree of freedom (an oscillator) is assigned to each peptide group so that the corresponding Hessian matrix is written by

$$H_{jk} = (\partial^2 V/\partial Q_j \partial Q_k) \tag{1}$$

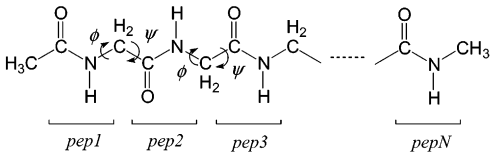
where Q_j denotes the jth local amide I coordinate and V is the total potential function. Diagonalizing the above Hessian matrix, one can obtain the eigenvectors and eigenvalues of the N amide I normal modes.

The diagonal force constants of the **H**-matrix represent the intrinsic vibrational frequencies of the local amide I modes. Torii and Tasumi used 1.605 mdyn $Å^{-1}$ amu^{-1} for the diagonal force constants of all peptide groups in several polypeptides.[20] Note that the above force constant corresponds to 1650 cm^{-1}. There are however various factors modulating the diagonal force constants. For example, the hydrogen-bonding interactions with either other peptide groups or solvent molecules can make the diagonal force constant decrease. As has been shown before, the low-frequency α-helix and β-sheet amide I bands was assigned to the strong intermolecular hydrogen bonding. Another possible source of diagonal force constant shift is from the dielectric effect. The surrounding medium, either protein matrix or polar solvent, consists of polarizable molecules or groups so that the Onsager's reaction field can induce an electronic polarization of each peptide group. These two mechanisms, that is, hydrogen-bonding and dielectric effects, have been extensively studied by using a variety of ab initio molecular orbital calculations.[21−32] Despite that a number of empirical rules were developed and successfully used to interpret experimental observations, there does not exist a simple but systematic theoretical model predicting the diagonal force constant of each peptide in a given polypeptide. This might also be due to the complex character of the amide I mode vibrations.[29] Recently, we have developed a novel approach to this problem and used to explain the diagonal force constant shifts in several model dipeptides[33] and tripeptides.[34]

The vibrational couplings between local amide I modes have been known to strongly affect the frequency shift and splitting patterns of amide I normal modes. To theoretically describe this vibrational coupling mechanism, Krimm and co-workers developed the transition dipole coupling (TDC) model, which was based on the assumption that the interpeptide interaction can be successfully described by using the dipole−dipole interaction.[35−37] This electrostatic interaction was considered to be more important than through-bond interactions.[20] However, the discrepancy between the TDC theory and the ab initio calcula-

* To whom correspondence should be addressed. E-mail: mcho@korea.ac.kr.

10.1021/jp034835i CCC: $25.00
Published on Web 08/06/2003



	ϕ	ψ
RHH	-57	-47
3_{10}H	-49	-26
LHH	60	60
πH	-57	-70
PB	-119	113
APB	-139	135
FEB	180	180

Figure 1. Seven representative conformations of polypeptides and the corresponding dihedral angles.

tion has been reported,[38] and it was noted that an appropriate description of the diagonal force constant of the local amide I mode is critical in the quantitative prediction of the frequency splitting and shift of the amide I normal-mode frequencies. In the theoretical studies of the amide I vibrations in a model tripeptide, we also found that the amide I normal-mode frequencies are mainly determined by the diagonal force constants, not by the off-diagonal coupling terms.[34] In the present paper, in section II we will present systematic ab initio calculation results of seven different conformational polypeptides with two to five peptide groups. Both the diagonal and off-diagonal Hessian matrix elements are obtained. In section III, an extrapolation method will be used to theoretically study the site dependence of the local amide I mode frequency. Finally, the main results will be summarized in section IV.

II. Ab Initio Vibrational Analyses

We carried out ab initio geometry optimizations and vibrational analyses of polypeptides containing two to five peptide groups. The seven representative conformations, for example, RHH (right-handed α-helix), 3_{10}H (3_{10}-helix), LHH (left-handed α-helix), πH (π-helix), PB (parallel β-sheet), APB (antiparallel β-sheet), and FEB (fully extended β-sheet), were chosen to study vibrational coupling between peptides and local amide I mode frequencies (see Figure 1). Ab initio vibrational analysis of a given polypeptide with N peptide bonds provides eigenvectors and frequencies of the N amide I normal modes. For short polypeptides, one can directly use a variety of ab initio calculation methods to obtain relevant vibrational properties used to interpret experimental results. However, it is prohibitively difficult to carry out a complete ab initio vibrational analysis as the number of peptide groups increases. Thus, it is necessary to develop a theoretical model that can be used to quantitatively describe inter-peptide-interaction-induced vibrational couplings and local amide I mode frequency shifts. To achieve this goal, one should have either experimental or ab initio-calculated off-diagonal and diagonal Hessian matrix elements, eq 1, of a given polypeptide as a set of reference data. Recently, we developed the so-called Hessian matrix reconstruction method that can be used to transform the ab initio-calculated eigenvectors and eigenvalues of the amide I normal modes to the corresponding Hessian matrix in the *subspace* spanned by the N local amide I coordinates.[34] We will apply this method to a variety of polypeptides in Figure 1.

All ab initio molecular orbital calculations were performed with the Gaussian 98 program.[39] Geometry optimization and vibrational frequency analysis were carried out at the RHF/6-311++G** level. Vibrational frequencies were, as usual, corrected by multiplying a single scaling factor of 0.893.[40] Ab initio-calculated normal-mode frequencies of polypeptides in Figure 1 are summarized in Table 1.

TABLE 1. Ab Initio (HF/6-311++G) Calculated Amide I Normal-Mode Frequencies in cm^{-1} [a]**

N	RHH	3_{10}H	LHH	πH	PB	APB	FEB
2	1725.6	1712.4	1729.1	1732.0	1699.6	1695.4	1700.0
	1736.7	1719.0	1748.2	1755.5	1708.7	1707.8	1713.2
3	1717.1	1694.2	1729.2	1733.0	1695.0	1689.4	1691.7
	1728.8	1703.7	1740.8	1744.5	1706.7	1703.8	1707.6
	1741.7	1727.3	1758.2	1769.4	1713.1	1711.2	1714.7
4	1709.4	1693.4	1718.4	1725.2	1696.8	1688.9	1688.9
	1713.9	1699.6	1722.0	1731.4	1700.9	1695.9	1695.5
	1732.6	1705.2	1746.9	1753.7	1708.0	1705.4	1705.5
	1744.6	1716.6	1763.1	1773.0	1712.8	1711.1	1713.4
5	1702.2	1686.9	1711.7	1710.9	1697.4	1688.8	1688.3
	1708.5	1694.7	1715.9	1720.6	1697.9	1691.4	1691.8
	1719.0	1699.0	1731.3	1741.4	1705.1	1700.6	1700.0
	1732.8	1703.5	1744.8	1759.2	1709.7	1707.0	1707.9
	1743.5	1714.6	1761.9	1774.0	1713.9	1711.8	1713.8

[a] A scaling factor of 0.893 is multiplied to the ab initio-calculated frequencies. RHH, 3_{10}H, LHH, πH, PB, APB, and FEB correspond to right-handed α-helix, 3_{10}-helix, left-handed α-helix, π-helix, parallel β-sheet, antiparallel β-sheet, and fully extended β-sheet conformations, respectively.

A. Hessian Matrix Reconstruction Method. To obtain **H**-matrix elements from the ab initio-calculated eigenvectors and eigenvalues of the amide I normal modes, we used the carbonyl coordinate displacement (CCD) method, which was proposed and tested for a variety of model tripeptides.[34] For the sake of completeness, we will briefly outline this CCD method here.

A given amide I normal coordinate, q_j, is assumed to be written as a linear combination of the N local amide I coordinates, Q_α, as

$$q_j = \sum_{\alpha=1}^{N} Q_\alpha U_{\alpha j} \tag{2}$$

where the expansion coefficient $U_{\alpha j}$ is the eigenvector elements associated with the corresponding Hessian matrix in eq 1, which has not been determined yet. It should be emphasized again that the ab initio-calculated eigenvector elements of the polypeptide are not identical to $U_{\alpha j}$ because the definition of the q_j coordinate in eq 2 is valid in the amide I subspace, not in the atomic Cartesian coordinate system.

The CCD method is based on the measurements of the N C═O bond length changes when the polypeptide structure is distorted by using the corresponding ab initio-calculated eigenvector elements in the atomic Cartesian coordinate system. Suppose that the N C═O bond lengths of the geometry-optimized polypeptide are r_1^0, r_2^0, and r_N^0. Then, by using the ab initio-calculated eigenvector of the jth amide I normal mode, the molecular structure can be properly distorted and the N C═O bond lengths r_{1j}, r_{2j}, ..., and r_{Nj} are measured. Then, the difference between $r_{\alpha j}$ and r_α^0 is assumed to be directly proportional to $U_{\alpha j}$ as

$$U_{\alpha j} \propto r_{\alpha j} - r_\alpha^{\,0} \tag{3}$$

The next step is to normalize the eigenvector, $\{U_{\alpha j}\}$. Then, not only the magnitude but also the phase (sign) of $U_{\alpha j}$ are simultaneously determined. Although each of the N $\{U_{\alpha j}\}$

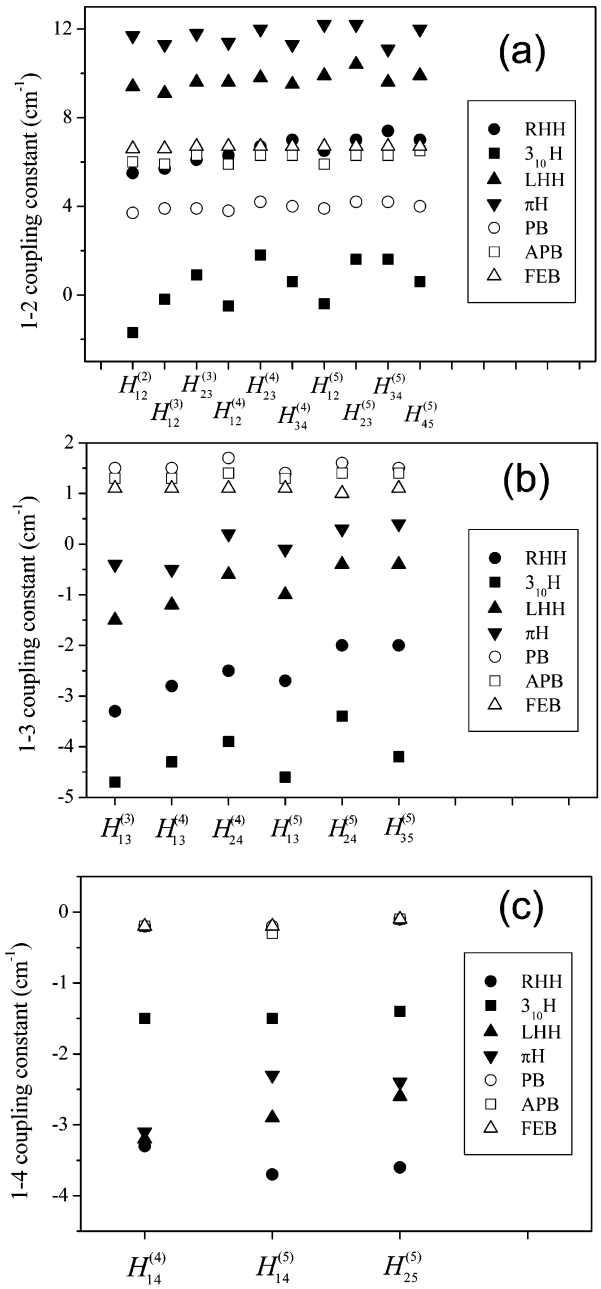


Figure 2. Vibrational coupling constants between two local amide I modes. $\langle H_{j,j+1}^{(N)} \rangle$ is the off-diagonal coupling force constant (in cm^{-1}) between the jth and $(j + k)$th peptides of polypeptides containing N peptide groups. 1−2 coupling constant is, for example, that between two nearest-neighboring peptides.

eigenvectors was properly normalized, they might not be orthogonal to each other. However, we found that quantitative errors induced by these imperfect orthogonality relations are negligibly small. Using the thus determined **U**-matrix, one can reconstruct the corresponding Hessian matrix **H** as

$$\mathbf{H} = \mathbf{U}^{-1}\mathbf{\Lambda}\mathbf{U} \tag{4}$$

where $\mathbf{\Lambda}$ is the eigenvalue matrix obtained from the ab initio vibrational analysis and the N diagonal matrix elements of $\mathbf{\Lambda}$ are the ab initio-calculated force constants of the N amide I normal modes of a given polypeptide.

B. Vibrational Coupling Constants. For polypeptides in Figure 1, the calculated off-diagonal Hessian matrix elements in cm^{-1} are plotted in Figure 2, where $H_{jk}^{(N)}$ denotes the $[j,k]$ element of the **H**-matrix of the polypeptide containing N peptide bonds. The coupling constants between any two nearest-neighboring peptides, denoted as $H_{j,j+1}^{(N)}$ for $j = 1$ to $N - 1$, are plotted in Figure 2a and found to be strongly dependent on the 3D conformation but not on the number N of the peptide groups. The average 1−2 coupling constants for the seven representative conformations are 6.5, 0.4, 9.7, 11.7, 4.0, 6.2, and 6.7 cm^{-1} for RHH, 3_{10}H, LHH, πH, PB, APB, and FEB, respectively. The 1−2 coupling constant of the πH is found to be the largest among those of seven conformations. Although the fluctuation amplitude of $H_{j,j+1}^{(N)}$ values of the 3_{10}H is slightly larger than the other conformations, the 1−2 coupling constant of the 3_{10}H is comparatively small. This observation is consistent with the previous result in refs 33 and 34.

TABLE 2. Averaged Coupling Force Constants[a]

	RHH	3_{10}H	LHH	πH	PB	APB	FEB
$\langle H_{j,j+1}^{(N)} \rangle$	6.5	0.4	9.7	11.7	4.0	6.2	6.7
$\langle H_{j,j+1}^{\mathrm{TDC}} \rangle$	6.0	3.9	7.8	9.1	1.3	0.5	−0.5
$\langle H_{j,j+2}^{(N)} \rangle$	−2.5	−4.2	−0.8	0.0	1.5	1.3	1.1
$\langle H_{j,j+2}^{\mathrm{TDC}} \rangle$	−1.7	−3.1	−0.2	0.3	1.4	1.1	0.8
$\langle H_{j,j+3}^{(N)} \rangle$	−3.5	−1.5	−2.9	−2.6	−0.2	−0.2	−0.1
$\langle H_{j,j+3}^{\mathrm{TDC}} \rangle$	−4.3	−1.6	−3.9	−3.1	−0.3	−0.3	−0.3

[a] $\langle H_{j,j+k}^{(N)} \rangle$ and $\langle H_{j,j+k}^{\mathrm{TDC}} \rangle$ are those between the jth peptide group and the $(j + k)$th peptide group and were obtained from the reconstructed Hessian matrices and from the TDC model, respectively.

The average 1−2 coupling constants, that is, $\langle H_{j,j+1}^{(N)} \rangle$, are found to be quantitatively similar to those values of seven dipeptide ($N = 2$) conformations. This observation is in practice highly important because it suggests that one can use the coupling force constant calculated by using a model dipeptide, such as glycine dipeptide analogue (see Figure 3c,d) in ref 33 for the entire Ramachandran surface of the 1−2 coupling constant), to determine the nearest-neighbor coupling constants required in the calculation of the amide I IR band of any arbitrary protein.

In Table 2, the average 1−2 coupling constants are compared with those calculated by using the TDC theory (see the second row in Table 2). Here, the magnitude of the transition dipole is assumed to be 2.73 D Å^{-1} amu$^{-1/2}$, its orientation is 10° away from the C═O axis, and the origin is located at 0.868 Å from the carbonyl carbon atom.[38,41] Overall, the TDC model with transition dipole vector and location determined by Torii et al. is found to be acceptable for the three helical conformations, for example, RHH, LHH, and πH, whereas it significantly underestimates those of PB, APB, and FEB polypeptides and overestimates that of the 3_{10}H. As found by us[42] and by other workers,[38,43] a single set of TDC parameters is not enough to describe the coupling constant between two nearest-neighboring peptides. Furthermore, mechanical coupling (other than through-space electrostatic interaction) cannot be taken into account by this TDC model. We have found that the 1−2 interpeptide vibrational coupling is intrinsically complicated so that any attempt to resolve this issue by slightly modifying TDC-like theory might not be successful.

The 1−3 coupling constants, $H_{j,j+2}^{(N)}$ for $j = 1$ to $N - 2$, are plotted in Figure 2b. As expected, they are typically smaller than the 1−2 coupling constants, except for the 3_{10}H. Except for the RHH and 3_{10}H, $H_{j,j+2}^{(N)}$ are about ±1 cm^{-1}.

The 1−4 coupling constants of PB, APB, and FEB are negligibly smaller than the 1−2 and 1−3 coupling constants (see Figure 2c). Although the 1−4 coupling constants of the

TABLE 3. Local Amide I Mode Frequencies, i.e., Diagonal Elements of the Reconstructed Hessian Matrix for Polypeptides with *N* Varying from 2 to 5; *j* is the Site Index in a Given Polypeptide

N	*j*	RHH	3_{10}H	LHH	πH	PB	APB	FEB
2	1	1730.7	1718.5	1736.6	1742.1	1701.4	1699.8	1706.1
	2	1731.6	1712.9	1740.7	1745.4	1706.9	1703.4	1707.1
3	1	1721.1	1700.1	1737.8	1742.0	1706.8	1704.0	1707.9
	2	1738.0	1727.3	1749.6	1759.0	1697.0	1693.7	1696.5
	3	1728.5	1697.8	1740.8	1746.0	1711.0	1706.7	1709.6
4	1	1712.3	1700.5	1722.7	1730.2	1705.6	1702.5	1698.5
	2	1731.4	1703.9	1749.9	1757.2	1701.9	1696.9	1697.9
	3	1740.0	1715.2	1752.9	1758.7	1701.1	1696.5	1698.4
	4	1716.8	1695.2	1724.9	1737.2	1709.9	1705.4	1708.5
5	1	1705.2	1697.3	1717.2	1715.9	1706.1	1702.5	1698.6
	2	1722.1	1703.0	1734.6	1751.0	1701.3	1696.2	1697.1
	3	1737.8	1692.5	1753.7	1759.3	1706.1	1699.8	1700.0
	4	1729.2	1713.3	1739.7	1755.6	1700.4	1695.8	1697.5
	5	1711.8	1692.5	1720.4	1724.3	1710.2	1705.4	1708.6

3_{10}H are as small as about -1.5 cm^{-1}, those of the RHH and LHH conformations are large and even comparable to the 1−3 coupling constants since in these cases the pep1 and the pep4 form a direct hydrogen bond. The average 1−3 and 1−4 coupling constants are compared with those predicted by the TDC theory in Table 2, and it is found that the TDC model works very well for such a long-distance interpeptide vibrational coupling.

Overall, it is believed that the Hessian matrix reconstruction method used in the present paper can provide consistent and quantitatively reliable information on the magnitudes and signs of the off-diagonal coupling constants between any two local amide I modes.

C. Diagonal Force Constants and 3D Structures of Polypeptides. We next consider diagonal force constants obtained by using the Hessian matrix reconstruction method. In Table 3, the calculated local amide I mode frequencies are summarized. Recently, we found that the C═O bond lengths of the peptide group in 96 different *N*-methylacetamide (NMA)−water complexes with one to five water molecules surrounding the NMA are linearly proportional to the amide I mode frequencies.[32] The same linear relationship was also observed for model dipeptide and NMA dimer.[33] Now, 98 local amide I mode frequencies in Table 3 obtained from the reconstructed Hessian matrixes are plotted with respect to the corresponding C═O bond lengths measured from the geometry-optimized structures (see Figure 3). The linear relationship is found to be excellent and the slope is estimated to be -4460 cm^{-1}/Å, which is close to that (-4390 cm^{-1}/Å) of model dipeptide and NMA dimer systems.[33] In Figure 3, the vertical dotted line is drawn to indicate the C═O bond length of the gas-phase NMA. The C═O bond lengths of peptide groups in β-sheet polypeptides are slightly larger than that of the NMA, whereas those of helical polypeptides are comparatively small. The same pattern, though the relationship between C═O bond length and the local amide I mode frequency was not explicitly discussed, was also observed by Torii and Tasumi[25] as well as by Huelsekopt and Ludwig[44] in their NMR and IR spectroscopic studies of NMA crystal and clusters.

In Figure 4, the local amide I mode frequencies are plotted with respect to its location in a given polypeptide. The site dependence of the local amide I mode frequency is notably large when a polypeptide is in one of the four helical conformations, whereas the local amide I mode frequency is rather insensitive to the exact position of the peptide group in a given β-sheet conformation. Also, it is interesting to note that, in the cases of RHH, LHH, and πH, the local amide I mode frequencies of the two terminal peptide groups are smaller than those in the inner region. Unlike the vibrational coupling constants (off-diagonal Hessian matrix elements), the local amide I mode frequency is strongly dependent on the chain length, indicating that only the diagonal Hessian matrix elements are influenced by surrounding peptide groups for a fixed polypeptide conformation.

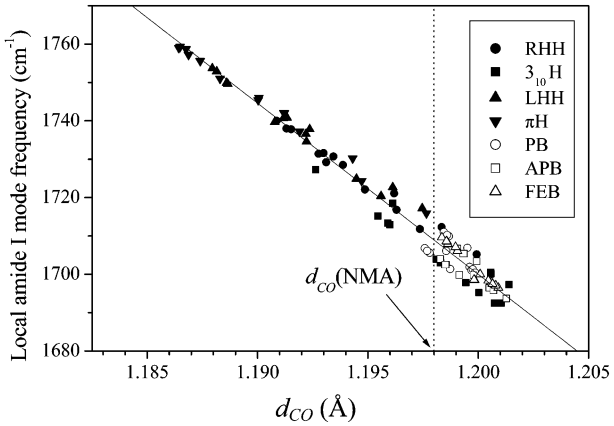


Figure 3. Local amide I mode frequencies (diagonal Hessian matrix elements in cm^{-1}) are plotted with respect to the ab initio-calculated C═O bond lengths. For the sake of comparison, the C═O bond length of the NMA, ab initio-calculated at the level of HF/6-311++G**, is also shown in this figure.

Due to the lack of a method directly calculating these diagonal force constants by using ab initio calculation methods before, there were no reference data that can be used to develop a proper theoretical model quantitatively describing diagonal force constant shifts in polypeptides. One of the previous attempts to quantitatively predict these diagonal force constant shifts was recently proposed by Mendelsohn and co-workers.[45] They added two contributions, which are associated with hydrogen- and valence-bond interpeptide interactions, to the force field. However, even though their method was found to be useful for a few specific cases, this ad hoc correction method might not be reliable. To be more specific, let us consider RHH polypeptides (see the top-left panel in Figure 4). The local amide I mode frequency of the pep1 in the case of $N = 2$ is 1731 cm^{-1} and its value for $N = 3$ decreases down to 1721 cm^{-1}. Noting that the pep1 does not make any intramolecular hydrogen bond with other peptide groups in the RHH ($N = 3$) polypeptide and that the valence-bond interaction effect, if there is, should be the same in these two cases, that is, RHH ($N = 2$) and RHH ($N = 3$), the strong red shift of the local amide I mode frequency of the terminal peptide group, pep1, cannot be explained by using the Mendelsohn's empirical correction formula. One can find a number of cases where other empirical rules reported and used before are not applicable to describing diagonal force constants of local amide I modes in polypeptides considered in the present paper. Thus, it is strongly desirable to develop a more quantitatively reliable theory that is capable of describing both the *3D conformation and site dependencies of the local amide I mode frequency*.

III. Extrapolation Method

To quantitatively describe the diagonal force constant shift when the target peptide group is surrounded by either polar solvent molecules such as water and methanol or other peptide groups, we have developed an extrapolation method as discussed in refs 32−34. The key idea is that the electrostatic interaction between the target peptide and solvent (including both real

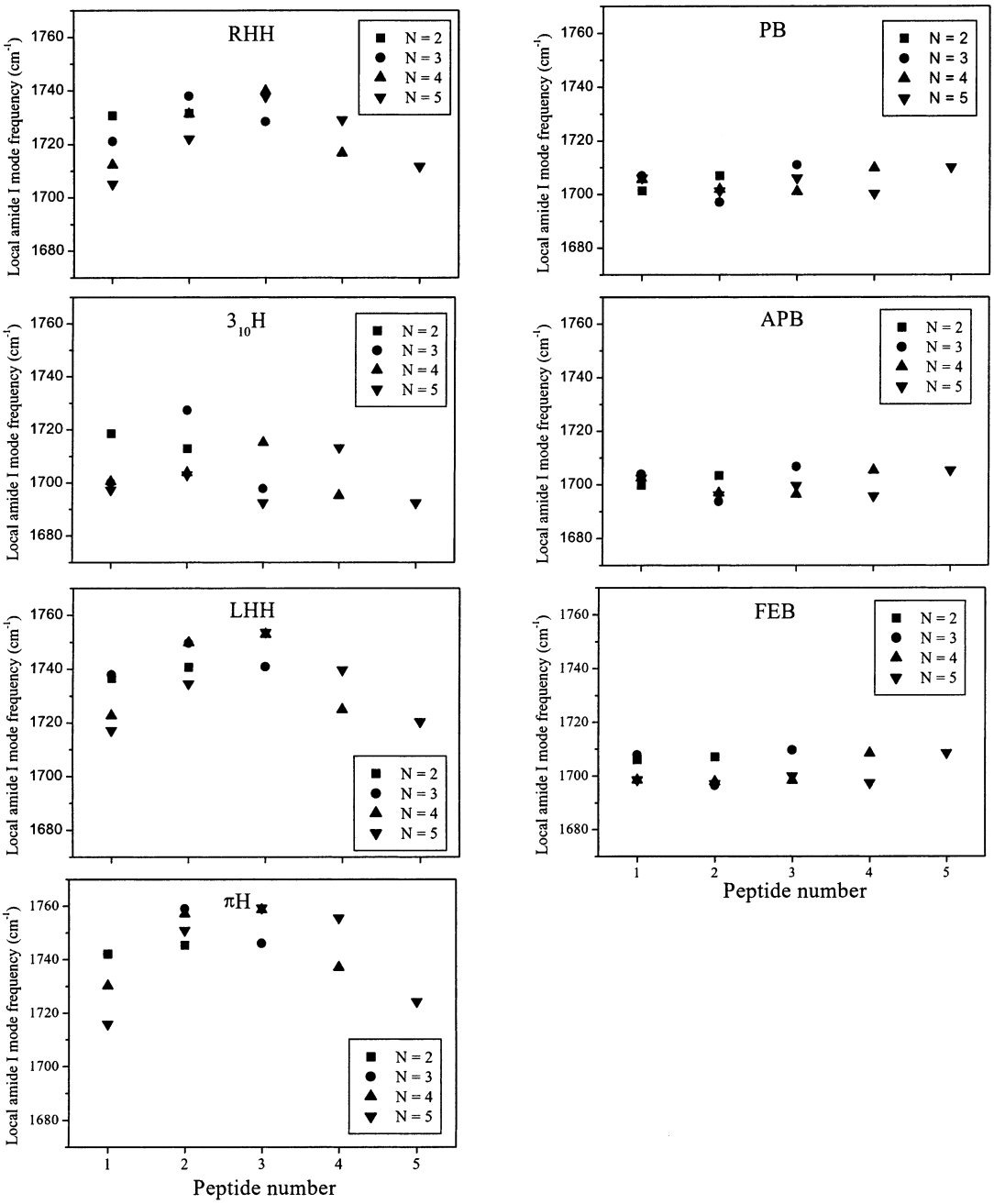


Figure 4. Local amide I mode frequencies (diagonal Hessian matrix elements in cm^{-1}).

solvent molecules and other intramolecular polar groups) induces both electronic as well as molecular structure distortions of the peptide. More specifically, if the solvent group attractively interacts with the peptide, for example, when a water molecule forms a hydrogen bond with the oxygen atom of the amide carbonyl group, the C═O bond length increases and simultaneously the amide I mode force constant (frequency) decreases. One of the most popular models describing this hydrogen-bond-induced frequency red shift is

$$\delta\tilde{\nu}_1 = -\alpha_{\mathrm{Hyd}}\{2.6 - r(\mathrm{O}\cdots\mathrm{H})\} \qquad (5)$$

where $\delta\tilde{\nu}_1$ is the amide I mode frequency shift and r(O···H) is the hydrogen-bond distance.[46,47] Typically, the proportionality constant α_{Hyd} was assumed to be 30 cm^{-1}. However, we found that this empirical formula is not accurate enough to quantitatively predict the amide I mode frequency shift of the NMA in liquid water.

To overcome this limitation, we developed a four-site model, where the four sites are O(═C), C(═O), N, and H(−N) atoms of a peptide group. Although a detailed theoretical description was presented in ref 34, for the sake of completeness we briefly outline this theory here. First of all, the interpeptide interaction potential is Taylor-expanded with respect to the N local amide I coordinates up to the second-order terms as

$$V_1 \cong V_1^0 + \sum_{m=1}^{N} V_m^{(1)} Q_m + \frac{1}{2}\sum_{m=1}^{N}\sum_{n=1}^{N} V_m^{(2)} Q_m Q_n \qquad (6)$$

where $V_m^{(1)} = (\partial V_1/\partial Q_m)_0$ and $V_{mn}^{(2)} = (\partial^2 V_1/\partial Q_m \partial Q_n)_0$. Using the charge−charge interaction potential and ignoring the Q_m and Q_n dependencies of $1/r_{j(m)k(n)}$ where $r_{j(m)k(n)}$ is the distance between the jth site of the mth peptide and the kth site of the nth peptide, we found that the linear force term, $-V_m^{(1)}$, exerting onto the mth local amide I vibration, can be written as

$$V_m^{(1)} = \sum_{j(m)} (\partial c_{j(m)}/\partial Q_m)_0^{\text{eff}} \phi_{j(m)} \quad (7)$$

where $\phi_{j(m)}$ is the electrostatic potential field at the jth site of the mth peptide and is given as

$$\phi_{j(m)} = \frac{1}{4\pi\epsilon_0} \sum_{n\neq m}^{N} \sum_{k(n)} \frac{c_{k(n)}}{r_{j(m),k(n)}} \quad (8)$$

Here, $c_{k(n)}$ is the partial charge of the kth site of the nth peptide.

Note that the electrostatic potential field $\phi_{j(m)}$ in eq 8 is created by the partial charges of surrounding peptide groups. Now, due to the non-zero linear force terms, $V_m^{(1)}$, in the total potential function, the equilibrium structures of each N peptides differ from an isolated peptide. More specifically, new equilibrium coordinates in a given conformation are shifted by, for $m = 1$ to N, $\delta Q_m \cong -V_m^{(1)}/k_m^0$, where k_m^0 is the amide I mode force constant of an isolated peptide bond. Then, taking into account these structural changes and potential anharmonicities of local amide I vibrations, one could find that the shifted diagonal force constants are K_m for $m = 1$ to N. Consequently, the mth local amide I mode frequency of a given polypeptide can be recast in the form, for $m = 1$ to N,

$$\tilde{\nu}_m = \tilde{\nu}_0 + \sum_{j=1}^{4} l_{j(m)} \phi_{j(m)} \quad (9)$$

where

$$l_{j(m)} = \frac{g_{\text{I}}}{4\pi c M_{\text{I}}^2 (\omega_{\text{I}}^0)^3} (\partial c_{j(m)}/\partial Q_m)_0^{\text{eff}} \quad (10)$$

To quantitatively calculate $\tilde{\nu}_m$ values, one should determine not only l_j values that are associated with effective transition charges but also partial charges of the four sites, that is, O(═C), C(═O), N, and H(−N). The four l_j values were obtained by carrying out a multivariate least-squares regression analysis for 96 *N*-methylacetamide−$n$$D_2O$ complexes with the same equation, $\nu_{\text{I}} = \tilde{\nu}_{\text{I}}^0 + \sum_{j=1}^{4} l_j \phi_j$ where $\tilde{\nu}_{\text{I}}^0$ is 1707 cm^{-1}, that is, the amide I mode frequency of a gas-phase NMA molecule, and ϕ_j is the electrostatic field, at the ath site of the NMA, created by the partial charges of surrounding water molecules. Here, the CHELPG partial charges of the deuterium and oxygen atoms of D_2O were calculated to be 0.412 and −0.824, respectively, at the HF/6-311++G** level. Thus determined l_j values are $l_{\text{O}} = -0.00554$, $l_{\text{C}} = 0.0016$, $l_{\text{N}} = 0.00479$, and $l_{\text{H}} = -0.00086$.[34] Here, the dimension of l_j is a fraction of the electronic charge. Since the ratio of the oxygen partial charge to the deuterium partial charge is −2 in a water molecule, any change of water partial charges that depend on the level and method of ab initio calculations would not make any difference in the relative magnitudes of each l_j other than a single scaling factor multiplied to all l_j's. Thus, the quantitative reliability of this extrapolation method used in the present paper is not sensitively dependent on the actual method of partial charge calculation. Now, using these l_j values, we determined the partial charges of the four sites of a given peptide group. This was performed by using ab initio-calculated local amide I mode frequencies of various di- and tripeptide conformations as a reference data set, and the four partial charges were found to be $c_{\text{O}} = -0.871$, $c_{\text{C}} = 0.419$, $c_{\text{N}} = 0.793$, and $c_{\text{H}} = -0.341$. Again, the dimension of partial charges, c_j, is a fraction of the electronic charge. The dimension of the electrostatic potential field should be converted into cm^{-1}/e.

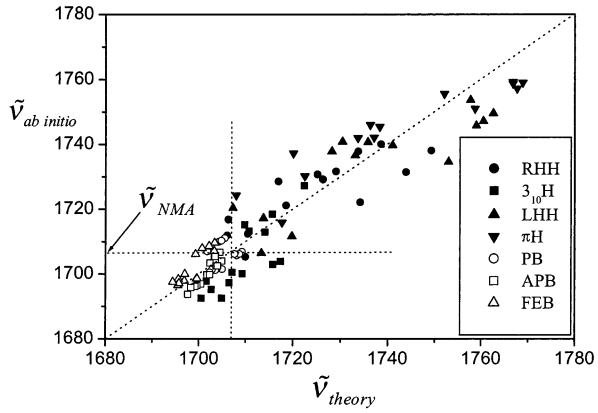


Figure 5. Numerically calculated local amide I mode frequencies by using eq 7 with parameters (l_a's and partial charges of the four sites for each peptide group) given in ref 34.

Once l_j and c_j (for $j = 1-4$) values are determined as above, the local amide I mode frequency of the jth peptide bond in a polypeptide with N peptides can be calculated by using eqs 8 and 9. The summation over n in eq 8 for the electrostatic potential is over all $N - 1$ peptides except for the mth one. In Figure 5, the local amide I mode frequencies calculated by using the above extrapolation method, eqs 9 with 8, are compared with those obtained from the reconstructed Hessian matrixes. The quantitative agreement is acceptable. In summary, for a polypeptide with an arbitrarily large N, the amide I Hessian matrix can be determined by using the following procedure: (i) $H_{j,j\pm1}^{(N)}$ are determined by using a model dipeptide as well as using the Hessian matrix reconstruction method discussed in the present paper, (ii) $H_{j,j\pm k}^{(N)}$ (for $k > 1$) are calculated by using the TDC theory, and (iii) $H_{j,j}^{(N)}$ are determined by using eqs 9 with 8. Currently, we are applying this extrapolation method to investigate the solvation and conformational fluctuation effects on the diagonal and off-diagonal amide I Hessian matrix elements of proteins, to estimate coherence lengths of amide I excitonic states of various polypeptides, and to numerically calculate amide I IR bands as well as two-dimensional vibrational spectra of short polypeptides like acetylproline, which was extensively studied by Zanni, Hochstrasser, and coworkers.[48,49]

IV. Summary

Ab initio vibrational analyses of a variety of polypeptides in seven different 3D conformations were performed. To obtain the diagonal and off-diagonal Hessian matrix elements in the local amide I subspace, we used the Hessian matrix reconstruction method. Although this method is based on the measurements of only the C═O bond length displacements for a given amide I *normal* mode, we found that it is quantitatively reliable and can provide useful information on the interpeptide interactions in polypeptides. The local amide I mode frequency was found to be strongly dependent on the location of the peptide bond in a given polypeptide as well as the chain length. In particular, the local amide I mode frequency of the peptide bond located in the inner region of a helical polypeptide is a few tens of wavenumber larger than those of terminal peptides. However, the local amide I mode frequency when a polypeptide forms one of the β-sheet conformations was found to be weakly dependent on its site. Second, we considered the vibrational coupling constants between two local amide I modes. The 1−2 coupling constants are strongly dependent on the 3D conformation but not on the chain length. The fact that these 1−2, 1−3,

and 1−4 coupling constants do not depend on the length of the polypeptide suggests that one can use a model dipeptide to calculate the 1−2 coupling constant for an arbitrary polypeptide conformation (other than the seven considered in the present paper). An empirical model discussed in ref 34 was used to quantitatively predict the local amide I mode frequencies and it was found to be useful. The largest molecule considered in the present paper was a pentapeptide. Currently, we are carrying out a systematic investigation on the chain length dependence of amide I mode frequencies for polypeptides having up to a few tens of peptide residues. Once these theoretical methods are confirmed to be useful and reliable, it will be possible to numerically calculate 1D and 2D vibrational spectra of proteins.

Acknowledgment. This work was supported by the Creative Research Initiatives Program of KISTEP (MOST, Korea) and by a Korea University Grant.

References and Notes

(1) Surewicz, W. K.; Mantsch, H. H. In *Spectroscopic Methods for Determining Protein Structure in Solution*; Havel, H. A., Ed.; VCH: New York, 1996; p 135.

(2) Arrondo J. L. R.; Goni, F. M. *Prog. Biophys. Mol. Biol.* **1999**, *72*, 367.

(3) *Infrared and Raman Spectroscopy of Biological Materials*; Gremlich, H.-U., Yan, B., Eds.; Marcel Dekker: New York, 2000.

(4) Byler, D. M.; Susi, H. *Biopolymer* **1986**, *25*, 469.

(5) Susi, H.; Byler, D. M. *Biochem. Biophys. Res. Commun.* **1983**, *115*, 391.

(6) Surewicz, W. K.; Mantsch, H. H. *Biochim. Biophys. Acta* **1988**, *952*, 115.

(7) Jackson, M.; Haris, P. I.; Chapman, D. *J. Mol. Struct.* **1989**, *214*, 329.

(8) Mantsch, H. H.; Casal, H. L.; Jones, R. N. In *Spectroscopy of Biological Systems, Advances in Spectroscopy, Vol. 13*; Clark R. J. H., Hester, R. E., Eds.; Wiley, New York, 1986; p 1.

(9) Mantsch, H. H.; Surewics, W. K.; Chapman, D. *Biochemistry* **1993**, *32*, 389.

(10) Prestrelski, S. J.; Byler, D. M.; Thompson, M. P. *Biochemistry* **1991**, *30*, 8797.

(11) Prestrelski, S. J.; Byler, D. M.; Thompson, M. P. *Int. J. Pept. Protein Res.* **1991**, *37*, 508.

(12) Torii, H.; Tasumi, M. In *Infrared Spectroscopy of Biomolecules*; Wiley-Liss: New York, 1996; p 1.

(13) Krimm, S.; Bandekar, J. *Adv. Protein Chem.* **1986**, *38*, 181. Krimm, S.; Abe, Y. *Proc. Natl. Acad. Sci.* **1972**, *69*, 2788. Moore, W. H.; Krimm, S. *Proc. Natl. Acad. Sci.* **1975**, *72*, 4933.

(14) Chen, X. G.; Asher, S. A.; Schweitzer-Stenner, R.; Mirkin, N. G.; Krimm, S. *J. Am. Chem. Soc.* **1995**, *117*, 2884. Chi, Z.; Chen, X. G.; Holtz, J. S. W.; Asher, S. A. *Biochemistry* **1998**, *37*, 2854.

(15) Moore, W. H.; Krimm, S. *Biopolymers* **1976**, *15*, 2465. Dwivedi, A. M.; Krimm, S. *Macromolecules* **1982**, *15*, 186; **1983**, *16*, 340.

(16) Woutersen, S.; Hamm, P. *J. Chem. Phys.* **2001**, *114*, 2727.

(17) Schweitzer-Stenner, R.; Eker, F.; Huang, Q.; Gribenow, K. *J. Am. Chem. Soc.* **2001**, *123*, 9628.

(18) Schweitzer-Stenner, R. *Biophys. J.* **2002**, *83*, 523.

(19) Eker, F.; Cao, X.; Nafie, L.; Huang, Q.; Schweitzer-Stenner, R. *J. Phys. Chem. B* **2003**, *107*, 358.

(20) Torii, H.; Tasumi, M. *J. Chem. Phys.* **1992**, *96*, 3379.

(21) Mirkin, N.; Krimm, S. *J. Am. Chem. Soc.* **1991**, *113*, 9742.

(22) Markham, L. M.; Hudson, B. S. *J. Phys. Chem.* **1996**, *100*, 2731.

(23) Torii, H.; Tatsumi, T.; Kanazawa, T.; Tasumi, M. *J. Phys. Chem. B* **1998**, *102*, 309.

(24) Torii, H.; Tasumi, M. *Int. J. Quantum Chem.* **1998**, *70*, 241.

(25) Torii, H.; Tatsumi, T.; Tasumi, M. *J. Raman Spectrosc.* **1998**, *29*, 537.

(26) Kearley, G. J.; Johnson, M. R.; Plazanet, M.; Suard, E. *J. Chem. Phys.* **2001**, *115*, 2614.

(27) Herrebout, W. A.; Clou, K.; Desseyn, H. O. *J. Phys. Chem. A* **2001**, *105*, 4865.

(28) Wang, Y.; Purello, R.; Georgiu, S.; Spiro, T. G. *J. Am. Chem. Soc.* **1991**, *113*, 6368.

(29) Chen, X. G.; Schweitzer-Stenner, R.; Krimm, S.; Mirkin, N. G.; Asher, S. A. *J. Am. Chem. Soc.* **1994**, *116*, 11141. Chen, X. G.; Schweitzer-Stenner, R.; Asher, S. A.; Mirkin, N. G.; Krimm, S. *J. Phys. Chem.* **1995**, *99*, 3074.

(30) Han, W.-G.; Suhai, S. *J. Phys. Chem.* **1996**, *100*, 3942.

(31) Kubelka, J.; Keiderling, T. A. *J. Phys. Chem.* **2001**, *105*, 10922.

(32) Ham, S.; Kim, J.-H.; Lee, H.; Cho, M. *J. Chem. Phys.* **2002**, *118*, 3491.

(33) Ham, S.; Cho, M. *J. Chem. Phys.* **2003**, *118*, 6915.

(34) Ham, S.; Cha, S.; Choi, J.-H.; Cho, M. *J. Chem. Phys.* **2003**, *119*, 1451.

(35) Krimm, S.; Abe, Y. *Proc. Natl. Acad. Sci.* **1972**, *69*, 2788.

(36) Moore, W. H.; Krimm, S. *Proc. Natl. Acad. Sci.* **1975**, *72*, 4933.

(37) Krimm, S.; Bandekar, J. *Adv. Protein Chem.* **1986**, *38*, 181.

(38) Torii, H.; Tasumi, M. *J. Raman Spectrosc.* **1998**, *29*, 81.

(39) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B. G.; Chen, W.; Wong, M. W.; Andres, J. L.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*, revision A.7; Gaussian, Inc.: Pittsburgh, PA, 1998.

(40) Pople, J. A.; Scott, A. P.; Wong, M. W.; Radom, L. *Isr. J. Chem.* **1994**, *33*, 345.

(41) Choi, J.-H.; Ham, S.; Cho, M. *J. Chem. Phys* **2002**, *117*, 6821.

(42) Cha, S.; Ham, S.; Cho, M. *J. Chem. Phys* **2002**, *117*, 740.

(43) Kubelka, J.; Keiderling, T. A. *J. Am. Chem. Soc.* **2001**, *123*, 6142.

(44) Huelsekopt, M.; Ludwig, R. *Magn. Reson. Chem.* **2001**, *39*, S127.

(45) Brauner, J. W.; Dugan, C.; Mendelsohn, R. *J. Am. Chem. Soc.* **2000**, *122*, 677.

(46) Hamm, P.; Lim, M.; Hochstrasser, R. M. *J. Phys. Chem. B* **1998**, *102*, 6123.

(47) Scheurer, C.; Piryatinski, A.; Mukamel, S. *J. Am. Chem. Soc.* **2001**, *123*, 3114.

(48) Asplund, M. C.; Zanni, M. T.; Hochstrasser, R. M. *Proc. Natl. Acad. Sci.* **2000**, *97*, 8219.

(49) Ge, N.-H.; Zanni, M. T.; Hochstrasser, R. M. *J. Phys. Chem. A* **2002**, *106*, 962.