Cooperativity in Amide Hydrogen Bonding Chains. A Comparison between Vibrational Coupling through Hydrogen Bonds and Covalent Bonds. Implications for Peptide Vibrational Spectra

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Vibrational frequencies of the coupled N−H, C═O stretches (amide I), and C−N stretch/CNH bend (amide II) have been calculated by density functional theory (DFT) at the B3LYP/D95** level and compared for polyglycines in β-strands and chains of H-bonding formamides. The amide groups of the polyglycines are connected by covalent bonds but not by extended H-bonding interactions. Conversely, the H-bonding chains are connected by an extended H-bonding interaction, but not by covalent bonds. All three types of vibrations couple more strongly and are more red (amide I and N−H) or blue shifted (amide II) in the formamide chains than in polyglycine. In contrast to polycglycine chains, the C═O's nearest the center of the formamide are elongated compared to those near the ends. As a result, the C═O's near the center of the chain of 10 formamides no longer couple effectively with the C═O's near the ends. Isotopic substitutions of ^{13}C, ^{14}C, ^{18}O, and ^{2}H (deuterium) at individual sites allowed us to probe the natural frequencies of individual C═O's and N−H's. The central ^{13}C═O's and ^{14}C═O's of the formamide chains (but not polyglycine) are significantly more shifted than those at the ends from those of a model containing only one C═O. The greatest intensity for the H-bonded C═O's comes from the lowest frequency fully delocalized stretch. ^{14}C isotopic substitution confines the vibrations to either side of the position of substitution, effectively restricting the extent of delocalization and lowering the intensity of the vibration. In general, the vibrations studied couple more effectively through the H-bonds than through the covalent bonds.

Vibrational spectra have been extensively used to characterize the secondary structure of proteins. In particular, the coupling of the frequencies and intensities of the amide I infrared absorption has been used as an indication of the nature of the secondary structure.[1−4] Both the frequency shift and the intensity of the absorption are sensitive to the coupling of the carbonyl stretches in the polypeptide chain. These couplings result from the interaction between the amide carbonyls either through covalent bonds, through space (via classical dipole−dipole interactions), through hydrogen bonds, or some combination of these. Spectroscopists often use a model that assumes the through-bond or through-space dipole−dipole coupling to be dominant,[5,6] although some recent reports have found some fault with this model.[7,8] However, we have demonstrated the H-bond cooperativity in amide H-bonding chains to be unusually extensive.[9,10] The central hydrogen bond in a chain of 15 H-bonding formamides is approximately 2.9 times as strong as that of a dimer. Coupling of the amide vibrations of H-bonding amides in solution has been reported by Fillaux over 30 years ago.[11]

In this paper, we examine the separate effects of several vibrational couplings through the covalent bonds and through the H-bonds. To do this, we shall compare the carbonyl stretching frequencies in single β-strands of oligoglycines of varying sizes and in chains of hydrogen-bonding formamides containing equivalent numbers of interacting carbonyl groups (Figure 1) using density functional theory (DFT) molecular orbital (MO) calculations. The extended single β-strands can only form weak, cyclic H-bonds that are insulated from cooperativity through the π-system by a CH_2 group. Thus, all coupling between the carbonyl groups must come through the covalent bonds or through space. On the other hand, the H-bonding chains have no covalent linkages between the formamide molecules. Thus, all coupling between these carbonyl groups must come through the H-bonds or through space. We present results for the coupled C═O (amide I), H-bonding N−H stretching, and C−N stretching/C−H bending (amide II) vibrations. Ab initio[12−18] and DFT[19] studies on the coupling of these vibrations have previously been restricted to relatively small oligopeptides.

TABLE 1: Vibrational Frequencies for Formamide Monomer (1FA), Dimer (2FA), and Decamer (10FA)

	N−H stretch		C═O stretch		C−N stretch/N−H bend	
	wave number, cm^{-1}	intensity, km/mol	wave number, cm^{-1}	intensity, km/mol	wave number, cm^{-1}	intensity, km/mol
1FA	3608	41	1813	406	1608	64
2FA	3549	418	1798	824	1614	79
	3604	52	1812	202	1628	33
10FA	3337	7150	1757	5747	1615	68
	3356	24	1765	0	1634	64
	3369	1207	1773	799	1635	73
	3381	16	1779	4	1639	29
	3384	457	1784	307	1640	142
	3410	652	1787	41	1642	31
	3416	695	1790	219	1643	4
	3488	591	1791	92	1644	4
	3495	556	1802	210	1645	13
	3603	59	1803	251	1645	10

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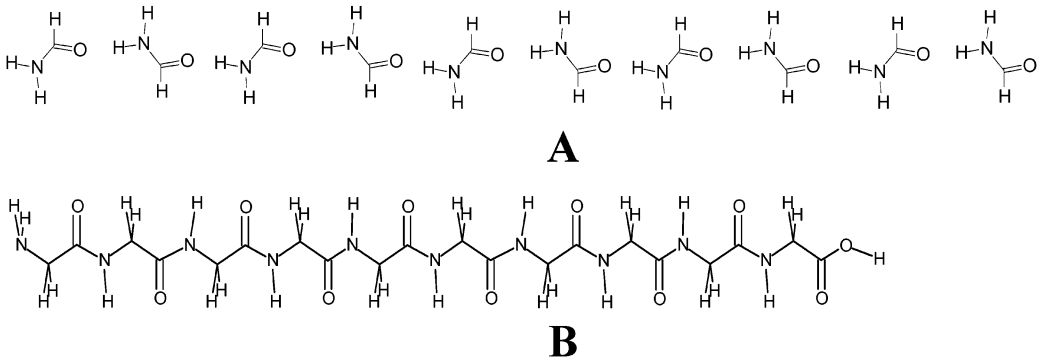


A

B

Figure 1. Chains of formamides (A) and extended single β-strand (B). The numbering convention used in the text starts from the left.

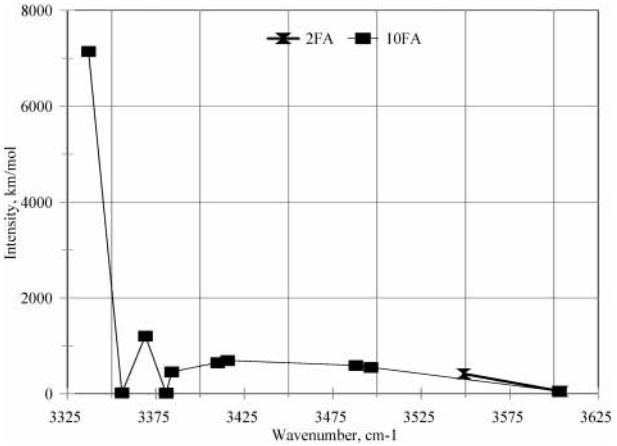


Figure 2. Variation of frequencies and intensities for the H-bonding N—H stretches in formamide chains.

Isotopic substitutions by individual heavy nuclei of H, C, N, and O provide added insight into the interpretation of the vibrational spectra by partially or (almost) completely attenuating the vibrational coupling.

Methods

Molecular orbital calculations were performed using a hybrid DFT method at the B3LYP/D95(d,p) level using the GAUSSIAN 98 suite of programs.[20] This method combines Becke's three-parameter functional,[21] with the nonlocal correlation provided by the correlation functional of Lee, Yang, and Parr.[22] The geometries were completely optimized with the sole constraint that all the structures were of C_s symmetry (all atoms are coplanar except for the hydrogens of the terminal NH_2 and of CH_2 groups in the polyglycines, which are symmetrically placed above and below the plane). We used our cluster of Intel and AMD powered computers that are parallelized using LINDA[23] for these calculations. The number of nodes used for each calculation varied with the sizes of the systems studied. The vibrational frequencies were calculated using the normal

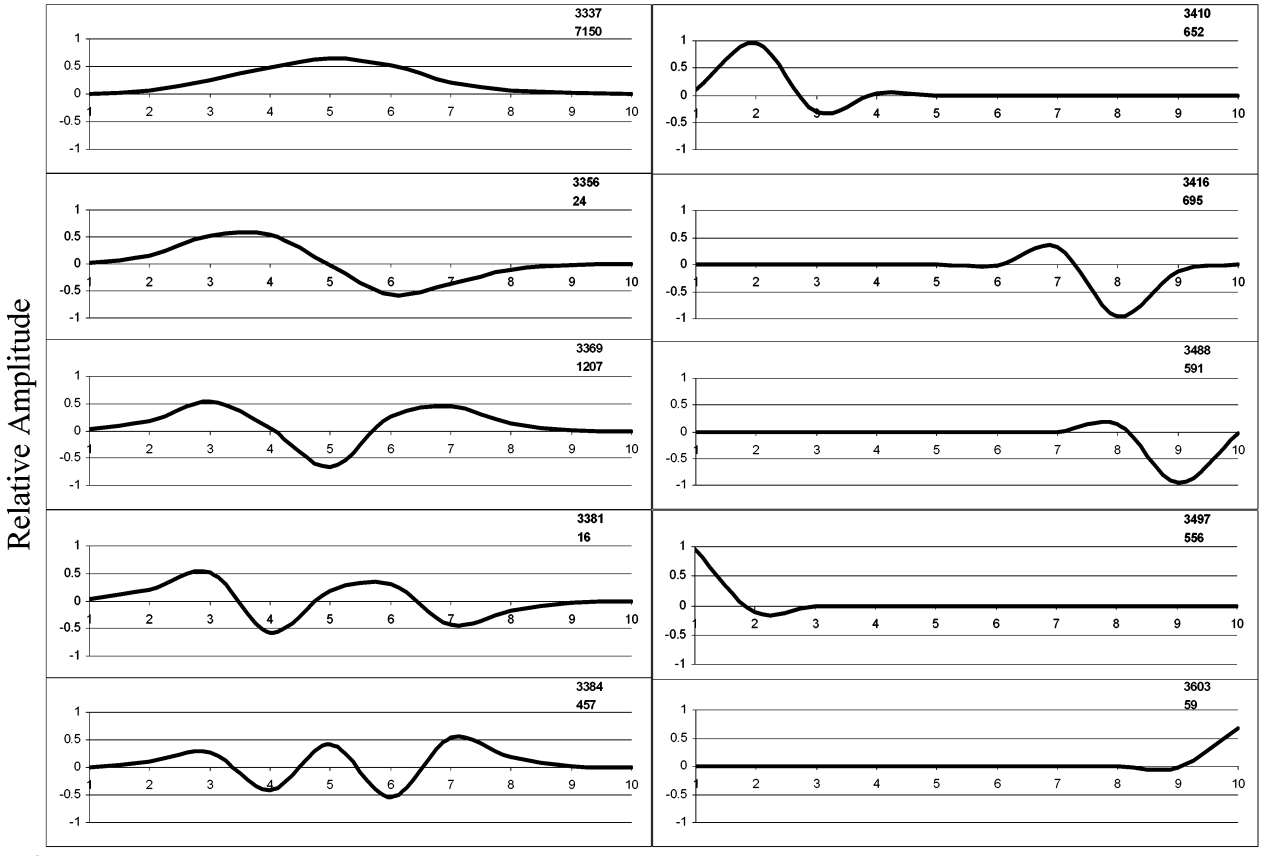


Figure 3. Amplitudes for each formamide in the coupled N—H stretches. See text for explanation; see Figure 1 for numbering.

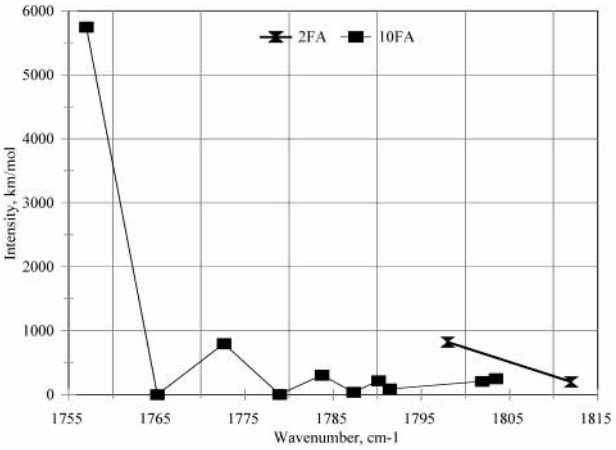


Figure 4. Variation of frequencies and intensities for the C═O (amide I) stretches in formamide chains.

harmonic approximations employed in the GAUSSIAN 98 program. All frequencies were real except for some very low imaginary frequencies that involved out-of-plane twists between pairs of formamides in some of the longer formamide chains and inversion of the terminal groups in polyglycines. All these imaginary frequencies were less than 21 and 64 cm^{-1}, respectively. The frequencies reported here have not been modified by any scaling factor. B3LYP frequency calculations are usually scaled by a factor of 0.96. When scaled by this factor, the calculated frequencies for the formamide monomer are in good agreement with the experimental IR spectra in the gas phase.[24]

TABLE 2: Variation of Bond Lengths (Å) for Formamide Monomer (1FA), Dimer (2 FA), and Decamer (10FA)[a]

bond	1FA	2FA	10FA,center
C═O	1.252	1.225	1.238
N—H	1.020	1.016	1.028
C—N	1.368	1.360	1.344
O- -H		1.920	1.786

[a] The bond lengths for the dimer to those most involved in the H-bond, for the decamer, for those most involved in the central H-bond.

TABLE 3: Comparison of Vibrational Frequencies and Absorption Intensities for Formamide with Different Isotopic Substitutions

	N—H stretch		C═O stretch		C—N stretch/ N—H bend	
	wave number, cm^{-1}	intensity, km/mol	wave number, cm^{-1}	intensity, km/mol	wave number, cm^{-1}	intensity, km/mol
normal	3608	41	1813	406	1608	64
^{13}C	3608	40	1770	388	1607	56
^{14}C	3608	40	1732	375	1605	48
^{2}H	2695	37	1811	399	1469	30
^{15}N	3603	39	1812	400	1600	61
^{18}O	3608	41	1785	401	1606	55

Results and Discussion

We shall first present and discuss the results for the H-bonding chains and the β-strands separately and then compare them.

H-Bonding Chains. The results for the H-bonding chains are gathered in Tables 1−4 and Figures 2−13.

The most significant vibrations for the H-bonding chains are the carbonyl stretch and the N—H stretch associated with the

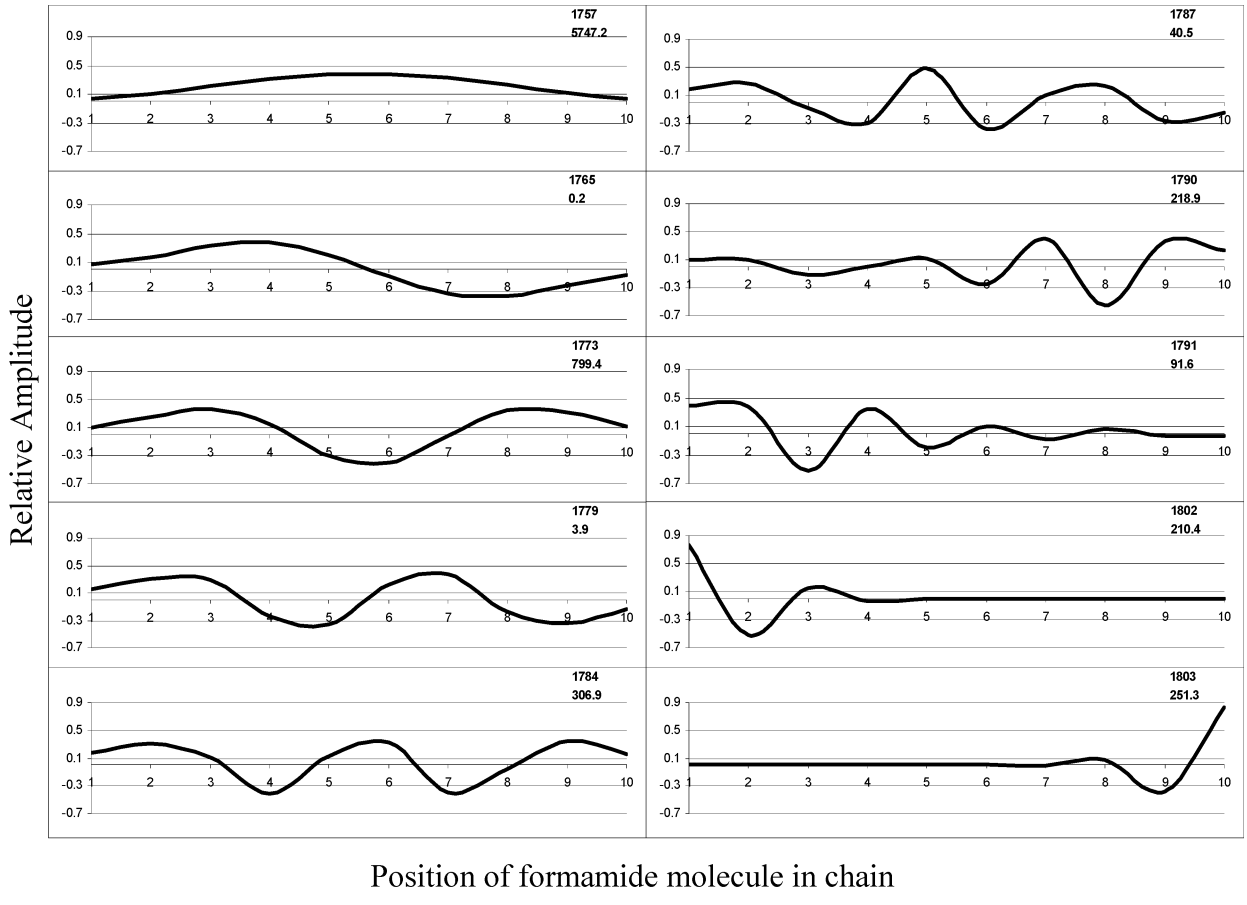


Figure 5. Amplitudes for each formamide in the coupled C═O stretches (amide I). See text for explanation; see Figure 1 for numbering.

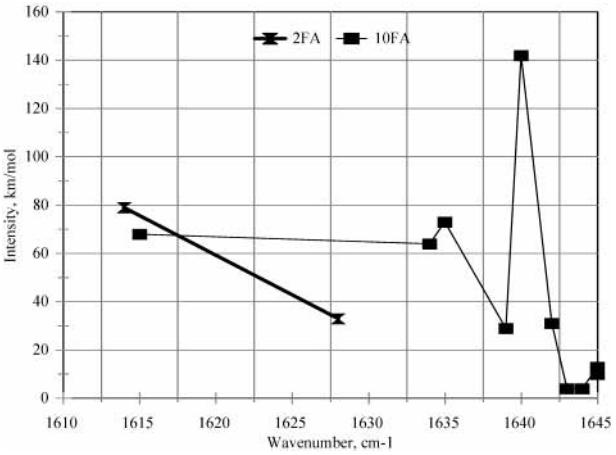


Figure 6. Variation of frequencies and intensities for the C—N stretch/CNH bend (amide II) vibrations in formamide chains.

H-bond (see Table 1). We have previously noted that the H-bonding interactions near the center of the formamide chain increase dramatically in strength as the H-bonding chains increase in length.[9,10] This behavior is reflected in the N—H stretching frequencies associated with the H-bonds. There is one fewer H-bond than formamide units in the H-bonding chain. The NH_2 group at one end of the chain will not H-bond. The N—H stretching frequency for the H-bond in the dimer is calculated to be 3549 cm^{-1} (see Table 1). As the chain becomes longer the lowest frequency vibration decreases markedly to 3337 cm^{-1} in the chain of 10 formamides, a red shift of 212 cm^{-1} from that of the dimer and 269 cm^{-1} from that of the monomer. This lowest vibration is composed of a linear combination of N—H stretches that are all in phase. As might be expected from a positive combination of all, this vibration has the highest intensity (see Figure 2 and Table 1). It grows in intensity as the chain lengthens from 421 km/mol for the dimer to 7150 km/mol for the decamer. The second lowest frequency in this group has half of the N—H stretches of one end of the chain in-phase with each other but out-of-phase with those at the other side. Thus, its intensity should be quite small, but not zero as there is no symmetry element that relates one end of the chain to the other. These two vibrations clearly can be thought of as the lowest two wave functions of the coupled N—H stretches. The amplitudes of the N—H stretches for each formamide in a chain of 10 were approximated by the changes of the individual N—H bonds, as determined from the relative displacements of the N and H atoms. These are shown in Figure 3 for each of the coupled vibrations. The lowest has no nodes, whereas the second has one node. One might expect this pattern to continue for all the linear combinations. However, this is not the case. The central H-bonds become increasingly stronger than the terminal ones as the chains become longer. As a result, the coupling between the central and terminal N—H stretches decreases with increasing chain length. Thus, the higher frequency N—H stretches become localized at each end of the longer chains (see Figure 3).

The C═O stretching frequencies behave in a manner qualitatively similar to those of the N—H's. However, because the H-bond strength affects the C═O less than the N—H, the effects are somewhat attenuated compared to those of the N—H stretches. For example, the lowest frequency C═O stretch decreases by only 41 cm^{-1} from 1798 to 1757 cm^{-1} on going

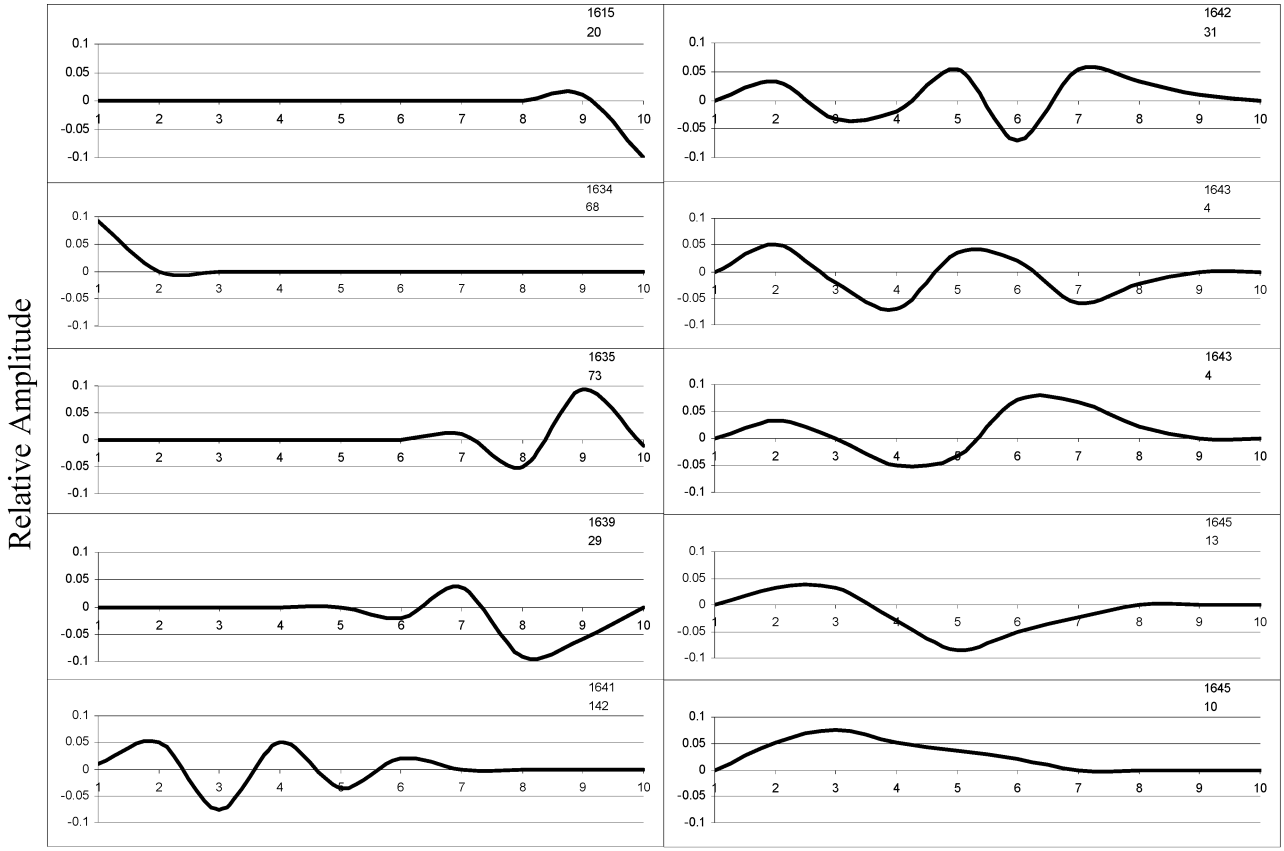


Figure 7. Amplitudes for each formamide in the coupled C—N stretch/CNH bend (amide II). See text for explanation; see Figure 1 for numbering.

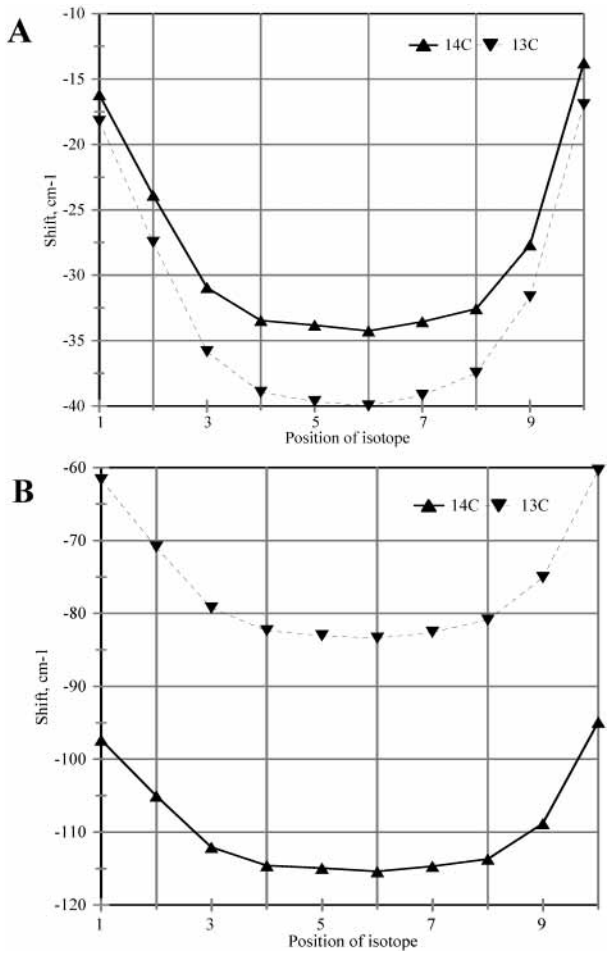


Figure 8. Frequency shift for ^{13}C and ^{14}C from isotopically substituted (A) and unsubstituted (B) formamide for monosubstituted decamers with same isotopic substitution at indicated formamide. See Figure 1 for numbering.

from the dimer (and 55 cm^{-1} from the monomer) to the decamer chain whereas the corresponding N—H stretch decreases by 212 cm^{-1} (see Table 1, Figure 4). The intensity pattern for the C═O's is similar to that for the N—H's. Once again, the linear combinations of amplitudes of the individual C═O stretches for the lowest frequencies resemble the lowest wave function for the coupled C═O's. Like the N—H stretches, in long chains, the higher frequency C═O's tend to be localized at the ends (see Figure 5).

The intensities of the lowest frequency C═O stretches for the short chains are larger than those for the N—H stretches. However, the intensities of the N—H stretches increase more with increasing chain length than do the intensities of the C═O's (see Table 1). Chains with six or more formamides have more intense low frequency, delocalized, N—H than C═O stretches.

The formamide chains also have deformations that resemble the amide II deformations of peptides. They involve the C—N bond stretches and N—H bond bends. These vibrations also couple, but to a lesser extent than the N—H and C═O stretches. In the decamer, the deformations involved in the nine H-bonds vary from 1634 to 1645 cm^{-1} compared with 1628 cm^{-1} for the dimer. The intensities of these vibrations, which are much less than those of the N—H and C═O stretches, do not change substantially with increasing chain length (see Figure 6). In contrast to the C═O and N—H stretches, the frequencies with amplitudes of the individual amides concentrated near the center of the decamer are more blue shifted than those near the ends

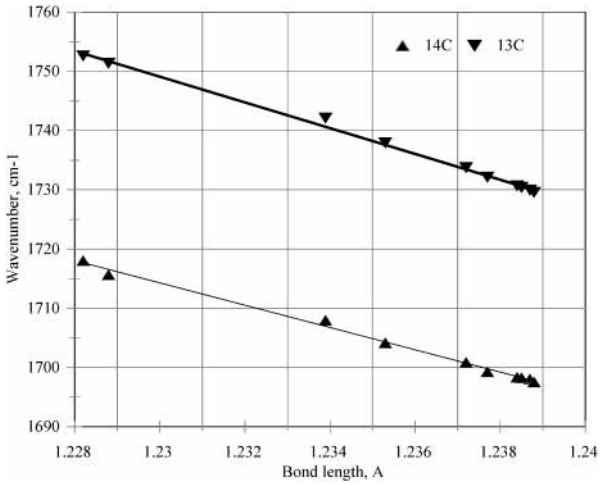


Figure 9. Correlation between C═O bond lengths and stretching frequencies for formamide decamers individually ^{13}C and ^{14}C subsituted at various positions.

(the individual amplitudes are taken from the C—N displacements; see Figure 7). Likewise, the lowest frequencies of this coupled set are more localized near the ends of the formamide chains. Unlike the N—H and C═O coupled stretches, the terminal deformation is largely unperturbed on going from the dimer to the decamer as it is blue shifted by only 1 cm^{-1}.

We note that the N—H and C═O stretches undergo substantial red shifts (in the case of N—H very substantial). In fact, all the coupled transitions for both of these modes are red-shifted from the corresponding modes in the monomer and dimer. This reflects the cooperative stabilization of the H-bonds, which causes both the N—H and C═O bonds to lengthen with increasing chain length (see Table 2). On the other hand, the modes that resemble the amide II vibration undergo blue shifts as the chain length increases. This again reflects the cooperative stabilization as the C—N bond lengths decrease with increasing chain length. These effects are larger near the center than near the ends of the chains. This observation reflects the polarization of the π-system of the formamides that accompanies the cooperative interactions of the H-bonds. As the C═O bonds lengthen, they lose some of their π-character, whereas the C—N bonds contract as they acquire more π-character.

Isotopic Substitutions. We explored the effects of isotopic substitutions of individual nuclei in the various chains of H-bonding formamides. Several studies have recently appeared describing the effect of ^{13}C substitution at the C═O upon the amide I absorptions of peptides and proteins.[16,18,19,25−28] For the purpose of illustration, we shall use the data for the decamer. We considered substitution of ^{14}C, ^{15}N, ^{18}O, and ^{2}H (deuterium) individually at each possible position in the decamer (only the H-bonding N—H's were substituted by deuterium). ^{14}C substitution reduces the C═O frequency of monomer formamide by 81 cm^{-1} from 1813 to 1732 cm^{-1} (see Table 3). The H-bonding within the decamer chain effectively changes the environments of the individual C═O's. However, these changes upon their respective native C═O stretching frequencies are masked by the extensive coupling between them. ^{14}C substitutions effectively decouple the ^{14}C═O stretches from the corresponding ^{12}C═O values due to the isotopic shifts. Comparison of the ^{14}C═O stretching vibrations as a function of the position of isotopic substitution in the decamer allows us to assess the effects of H-bonding and H-bonding cooperativity upon the C═O stretches of individual carbonyls. Figure 8 displays the shift in ^{14}C═O and ^{13}C═O frequencies from the monomer as a function of the

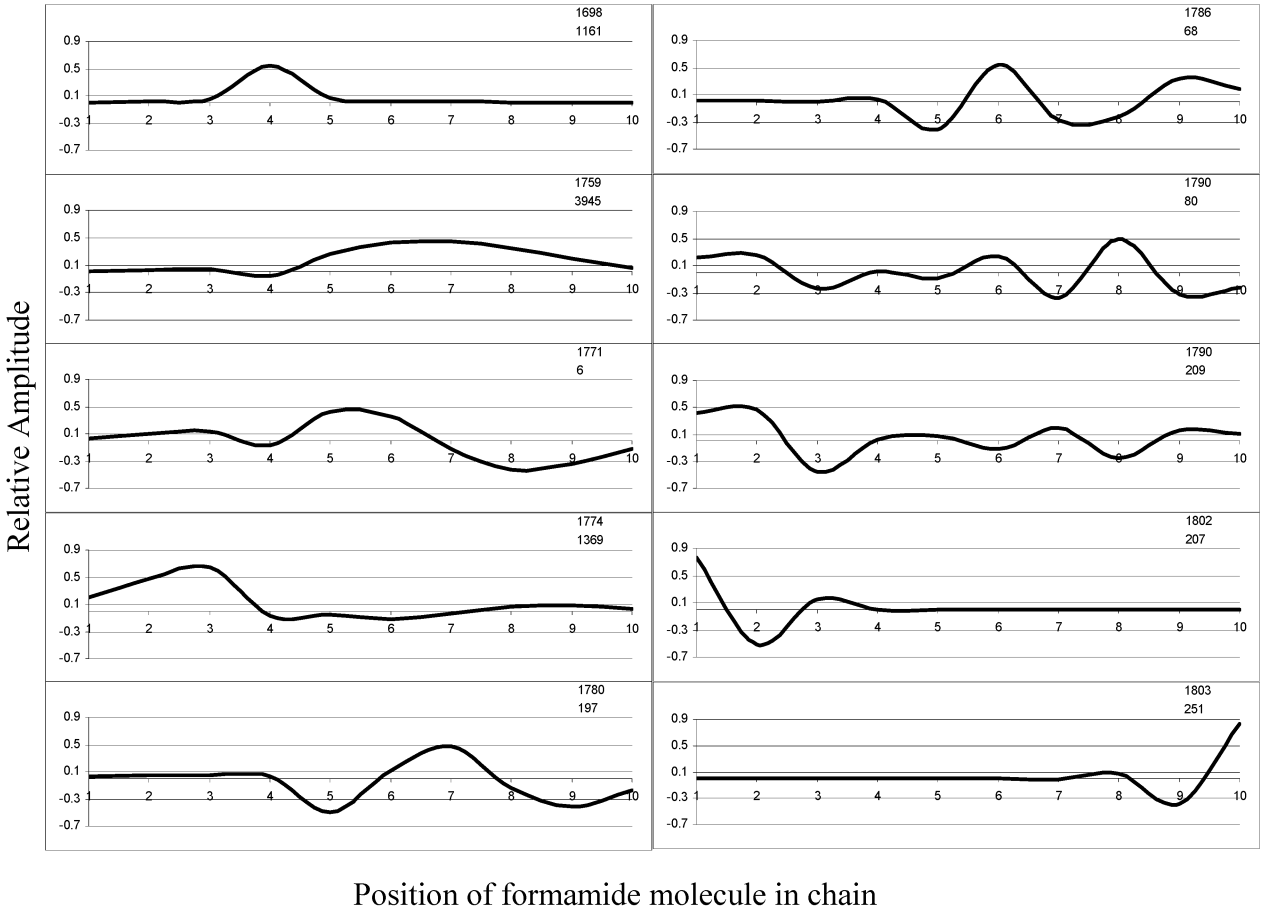


Figure 10. Amplitudes for each formamide in the coupled C═O stretches (amide I) with ^{14}C in the fourth formamide. See text for explanation; see Figure 1 for numbering.

^{14}C and ^{13}C positions in the decamer. The frequency shifts are largest when the isotopic substitution is near the middle of the H-bonded chain. We have already noted that the C═O bonds lengthen more for those carbonyls near the center of the H-bonding chain. The ^{14}C═O and ^{13}C═O stretching frequencies at different positions in the decamer correlate linearly with the corresponding calculated C═O bond lengths (see Figure 9).

The ^{14}C═O also acts as a barrier to coupling of the other C═O's. Their couplings are effectively constrained to the C═O's on either side of the ^{14}C═O, as illustrated for the decameric chain with ^{14}C═O in the fourth formamide (see Figure 10). Coupling through the ^{14}C═O is minimal. Compare Figure 10 with the respective frequencies of the normal decamer (Figure 5). Furthermore, intensities decrease upon ^{14}C substitution. These decreases are greatest when the substitution is near the center of the H-bonding chain, as exemplified by the data for ^{14}C substitution at position 5 (Figure 11). Because the coupled vibrations are essentially constrained to one side or the other of the ^{14}C-substituted amide (as noted above; see Figure 10) the most delocalized of these vibrations involve fewer C═O's when the substitution is near the center. Table 4 indicates the variation of the highest intensity transitions with the position of substitution for both isotopes of carbon.

Isotopic substitution by ^{18}O causes a smaller shift (-28 cm^{-1}) in monomeric formamide than does ^{14}C (see Table 3). Because of this, substituting individual C═^{18}O's in the decamer does not decouple the C═O stretches to the same extent as ^{14}C═O's (Figure 12). In fact, when C═^{18}O is substituted in formamide number 2, the shift caused by the isotope moves its "natural" frequency closer to those C═O's near the middle of the

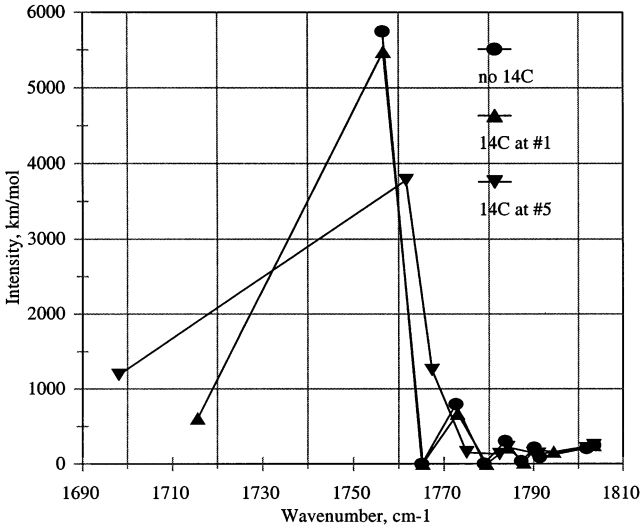


Figure 11. Variation of frequencies and intensities for the C═O (amide I) stretches in formamide chains upon ^{14}C substitution.

H-bonding chain. Consequently, the coupling increases between this C═O and the more central C═O's (Figure 13).

Substitution of the H-bonding N—H by D (^{2}H) causes the lowest N—H stretch (now a localized N—D stretch) to be substantially red shifted by 760−860 cm^{-1} depending upon the position of substitution. Once again, we observe the greatest shifts when the D is substituted at the middle of the chain. Unlike the ^{14}C substitutions, The D substitutions have little effect upon the coupling of the N—H and C═O vibrations on either

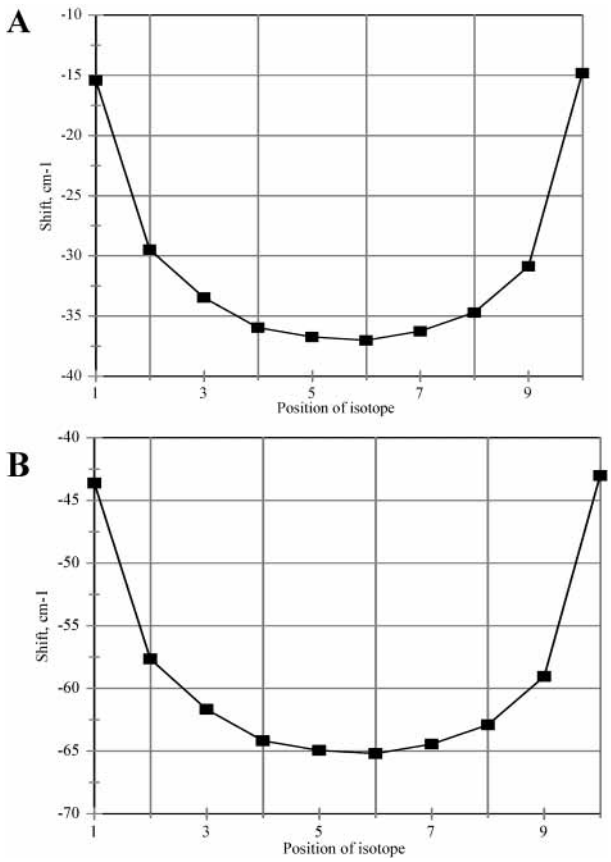


Figure 12. Frequency shift for C═^{18}O from isotopically substituted (A) and unsubstituted (B) formamide for monosubstituted decamers with same isotopic substitution at indicated formamide. See Figure 1 for numbering.

TABLE 4: Frequencies of the Highest Intensity Absorptions for the Coupled C═O Vibrations (cm^{-1}) in Formamide Chains

position of isotope	highest intensity	
	^{14}C	^{13}C
none	5747	5747
1	5480	4368
2	4982	4270
3	4440	3906
4	3945	3360
5	3782	2865
6	4199	2919
7	3781	3122
8	4224	3694
9	4772	4186
10	5263	3421[a]

[a] This vibration is coupled with another.

side of the substitution.

The frequencies of the other N—H's (non-H-binding) and the C═O's are not appreciably affected by D substitution. For example, the lowest C═O stretch varies less than 1 cm^{-1} whereas the second lowest N—H stretch (lowest after the N—D) varies by only 14 cm^{-1} upon substitution of D for any H-bonding H.

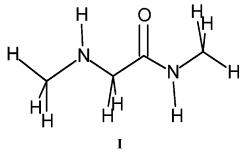
Extended β-Strands. The results for the β-strands are collected in Table 5 and Figures 14 and 15.

In contrast to the formamide chains, the carboxyl C═O (amide I) stretches remain little changed in oligoglycines containing from 2 to 10 glycine residues. The amide C═O stretching modes do not extensively couple with the higher frequency C═O stretch of the carboxyl group.

TABLE 5: Vibrational Frequencies and Absorption Intensities for the Model Compound, I, Glycine (1Gly), the Dipeptide (Gly−Gly), and the Polyglycine Decapeptide

	N—H stretch		C═O stretch (amide I)		C—N stretch/ N—H bend (amide II)	
	frequency, cm^{-1}	intensity, km/mol	frequency, cm^{-1}	intensity, km/mol	frequency, cm^{-1}	intensity, km/mol
I			1769	243		
1Gly			1833	296		
2Gly	3622	77	1764	305	1536	232
			1829	244		
10Gly	3563	511	1741	1185	1515	3970
	3566	2	1743	176	1525	880
	3567	128	1746	185	1536	31
	3570	17	1750	18	1546	73
	3570	234	1755	85	1552	9
	3575	154	1760	1	1557	13
	3576	146	1764	85	1559	4
	3587	120	1767	60	1561	13
	3610	107	1769	686	1562	13
			1831	255		

The coupling of the amide C═O stretches differs somewhat from those of the H-bonding chains. Unlike the H-bonding chains (where all the C═O's are aligned in approximately the same direction as the long axis of the chain), the C═O's of the β-strand are roughly perpendicular to the long axis of the strand and oriented in opposite direction to that of its nearest neighbors (see Figure 1). Because there are no cooperative H-bonds in the β-strand, all coupling must come via a through-bond (or through-space) mechanism involving the amide linkages. To model a single C═O in β-strand, we used α-(methylamino)-methylacetamide, (CH_3—NH—CH_2—**C═O**—NH—CH_3), **I**, which



I

avoids the coupling between the amide C═O and the COOH. Using this model, the lowest amide I frequency shifts from 1769 cm^{-1} for a single C═O bond to 1741 cm^{-1} in the decapeptide. At the same time the highest frequency stays unchanged at 1769 cm^{-1} compared to that of **I** (Table 5). The lowest, most intense, frequency increases further in intensity as the strand increases in size (Figure 14A). However, the intensities (from 305 km/mol in the dipeptide to 1185 km/mol in the decapeptide) are much lower than those of the H-bonding chains of comparable sizes.

The variation of the C═O bond lengths is very small compared to that of the H-bonding chains. All, except the two terminal bonds, vary only from 1.2348 to 1.2352 Å, the terminal bonds are 1.2324 and 1.2322 Å.

The amide II frequencies involve C—N stretching (as well as N—H deformation) that is more aligned with the length of the β-strand than the C═O stretches. The frequency moves from the dipeptide value of 1536 cm^{-1} to a range of 1515−1562 cm^{-1} in the decapeptide. The most intense frequency is always the lowest, which involves the in-phase C—N stretches approximately aligned along the length of the strand (in contrast to the amide chains; see below). This transition increases with intensity as the strand becomes larger, becoming more intense than the lowest amide I transition for tripeptides and larger strands. Although the range of amide II vibrations extends both above (26 cm^{-1}) and below (21 cm^{-1}) the frequency of the dipeptide,

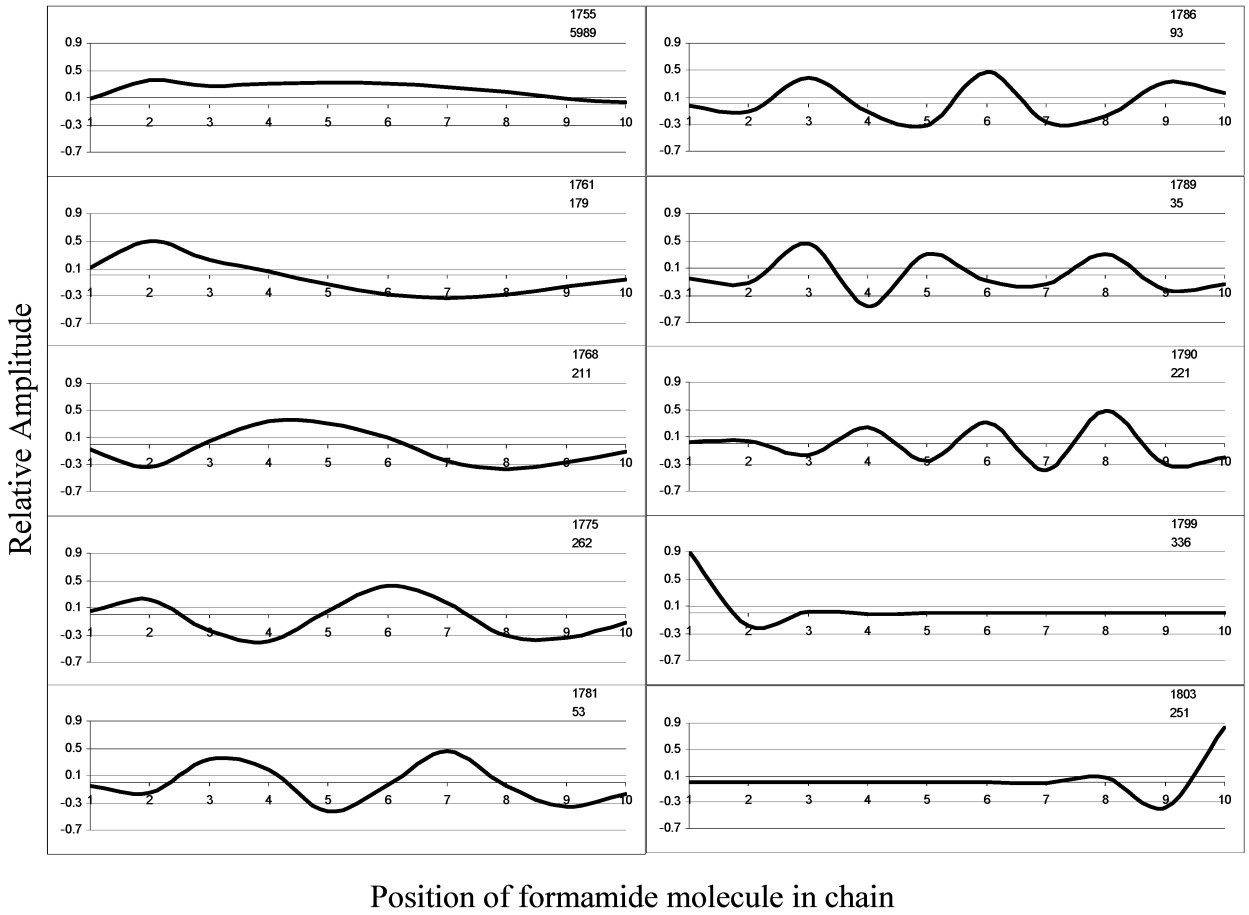


Figure 13. Amplitudes for each formamide in the coupled C═O stretches (amide I) with ^{18}O substitution in second formamide. See text for explanation; see Figure 1 for numbering.

the most intense transition has the largest red shift (21 cm^{-1}). Thus, although the centroid of the amide II frequencies exhibits a slight blue shift, the most intense band exhibits a somewhat larger red shift (Figure 14B).

Upon isotopic substitution with ^{14}C at individual positions within the chain containing 10 glycine residues, the carbonyl frequency at the substituted position decouples with the others. The terminal C═O's are different due to the effects of the chain ends. The "natural" (uncoupled) C═O could be obtained from the frequencies of the ^{14}C-substituted decaglycines in a manner analogous to that used for the H-bonding chains. However, unlike the case of the formamide chains, the ^{14}C═O stretching vibrations varied only within a range of 5 cm^{-1} upon ^{14}C substitution at positions 2−9 in the polyglycine chain. Thus, the natural frequencies of each of these carbonyls are not significantly perturbed by the change in the environment from one position to another within the polyglycine chain. The wave functions for decaglycine are displayed in Figure 15, along with the same wave functions for the decaglycine chain substituted with ^{14}C in positions 4 and 6. Similar to the case for the formamide chains, the lowest frequency is delocalized among the all the carbonyls. Unlike the case of the chain of formamides, where the ^{14}C-labeled carbonyl acted as a blocker to coupling of the other carbonyls (see Figure 10), the substituted carbonyl in the chain of dacaglycine does not block coupling of the carbonyls on either side of it. This observation is consistent with a model in which the C═O's couple through the C−C and C−N (but not C═O) bonds.

We have not made any direct comparisons with the through-space dipole−dipole coupling model. However, this model using equivalent dipoles for all C═O's in either the formamide chain or polyglycine should not lead to any shift of the centroid of the amide I frequencies (for example). Clearly, the formamide chain vibrations (which exhibit a large red shift) cannot be adequately described by such a model. We should emphasize that the dipole coupling theory is simply a model. All the appropriate interactions are implicitly included in the DFT calculations. Furthermore, the observations that the isotopic shifts of the vibrations are quite dependent on the position of the isotope in the formamide chain, and the observations that the C═O bond distances are also dependent on the position in the chain suggest that the C═O dipoles should not be taken as equivalent.

Comparison of Amide I and II Vibrations in H-Bonding Chains and β-Strands. *Amide I.* The coupling of the C═O's in both the H-bonding chains and the β-strands leads to red shifts and increased intensities with increasing size. However, the effects are predicted to be significantly greater for the H-bonding chains than for the β-strands. All frequencies are red shifted in the H-bonding chains. For the β-strands, all but the highest are red shifted. The intensity is concentrated in the lowest (in-phase) frequency for the H-bonding chains. No other frequency reaches 15% of this for the decamer. For the β-strands, the lowest frequency is most intense but generally less than 25% of the corresponding intensities of the H-bonding chains. The highest (slightly blue shifted from the dimer, but unshifted from **I**) frequency is roughly half as intense as the lowest frequency in the decapeptide.

Amide II. The amide II (like) frequencies of the H-bonded chains are all blue shifted whereas those of the β-strands extend

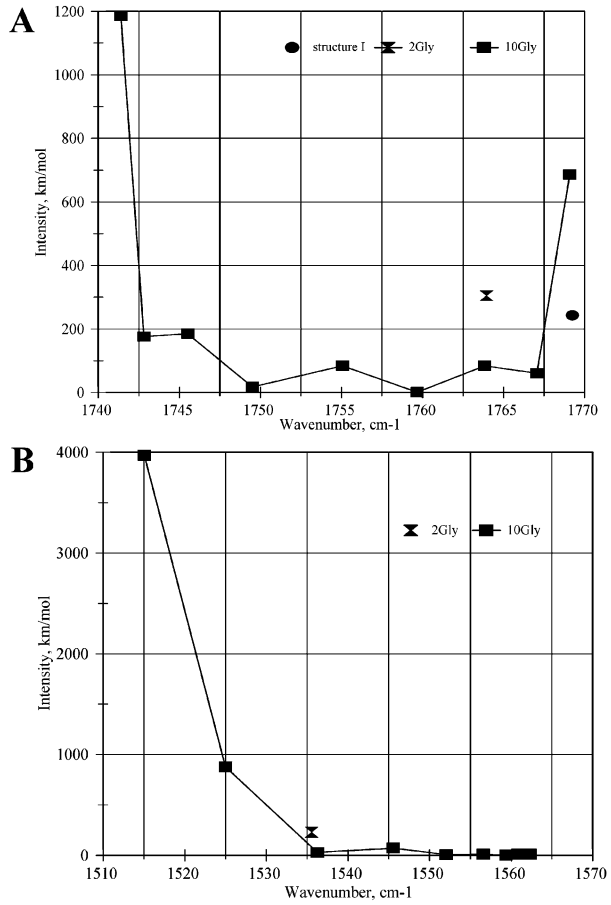


Figure 14. Variation of frequencies and intensities for the amide I (C═O) stretch (A) and amide II bend (B) in diglycine and decaglycine.

above and below that of the dipeptide with the collective center of the frequencies only slightly blue shifted (see Figure 6 and Figure 14B). The intensities of these vibrations in the H-bonded chain are all relatively low. They do not change appreciably with chain length. In contrast to the C═O stretches, the lowest frequencies are localized at the ends of the chains, whereas the highest frequencies are those more delocalized and central.

On the other hand, the intensities of the lowest vibrations of the β-strands are by far the largest in this series. They increase with the size of the β-strand. The second most intense band is the second lowest frequency, which had about 20% of the intensity of the lowest one. Because the most intense vibration is red shifted, the observed spectrum ought to appear red shifted as the β-strands grow.

Conclusions

Through H-bond coupling of the C═O stretches is clearly stronger than the through (covalent) bond coupling for the models studied. The lowest frequency vibrations are the most red-shifted and most intense. To the extent that these model studies approximate the amide I frequencies in proteins, the implications are clear:

(1) Longer H-bonding chains lead to lower frequency, more intense amide I vibrations.

(2) When long H-bonding chains are present, the intensities of these low-frequency amide I vibrations might dominate the spectra.

(3) Isotopic substitution at the C═O carbon will lower the red shifts and the intensities of the amide I frequencies of the H-bonded chains. Consequently, the amide I vibrations of such isotopically labeled proteins might be dominated by the parts of the protein containing chains that are not labeled. The

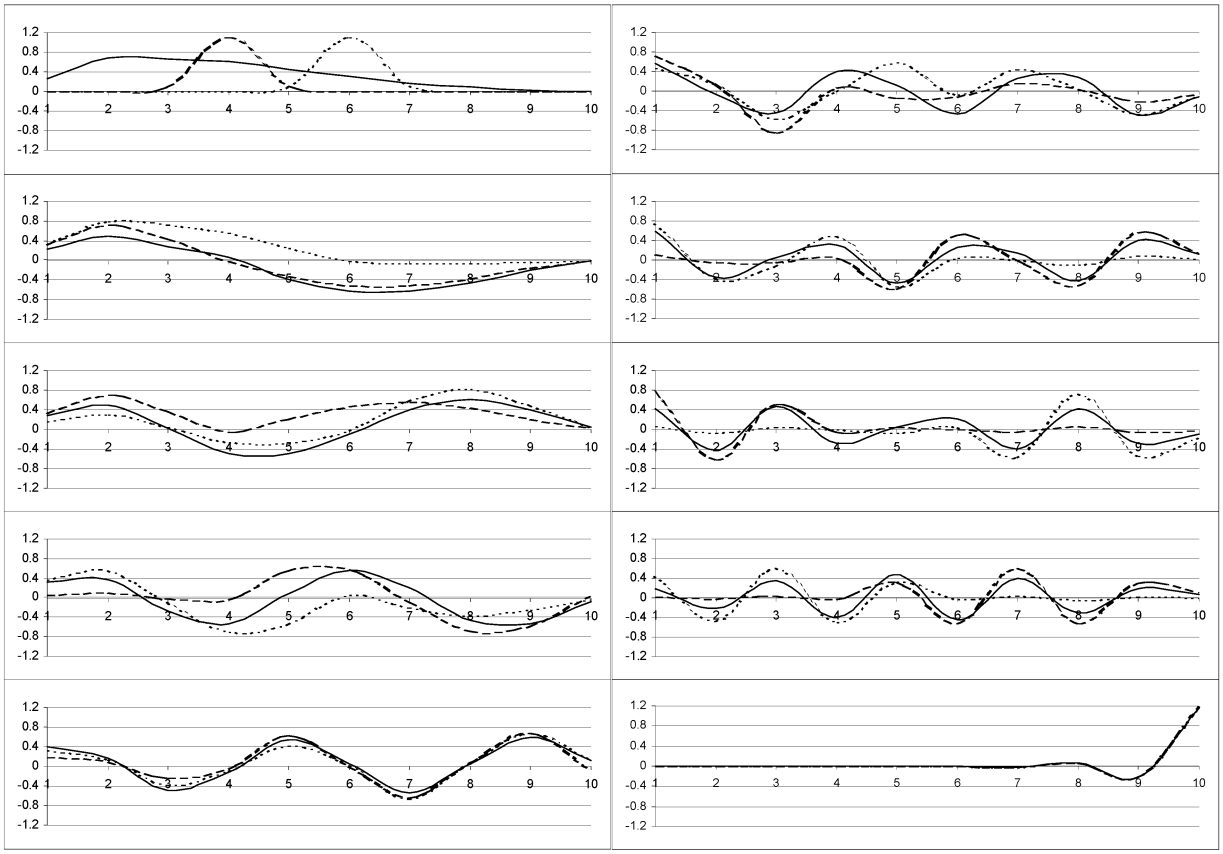


Figure 15. Amplitudes for each C═O in the coupled stretches (amide I) with C═O position in decaglycine with no isotopic substitution (solid line), ^{14}C at the fourth C═O (dashed line) and ^{14}C at the sixth C═O (dotted line).

isotopically labeled regions that have the label near the end of a H-bonding chain will be more prominent than those with the label near the middle of the H-bonding chain. The natural abundance ^{13}C in proteins will have similar effects upon the amide I frequencies.

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