# Conformational Structure of an L-Asparagine/L-Aspartate Random Copolypeptide as Revealed by Vibrational and CD Spectroscopy

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**The infrared and Raman spectra of an L-asparagine/L-aspartate random copolypeptide with an L-asparagine residue content of 69% mol have been measured in aqueous solution and in the solid state and analyzed for the first time. The infrared amide I, I′, and II bands have been related to the conformational structure of the polypeptide backbone with the aid of CD spectroscopy, whereby it has been concluded that this biopolymer adopts a random coiled secondary structure. The backbone conformation remains unordered even when side-chain carboxyl groups are in the undissociated form (-COOH). The fact that this polypeptide takes this secondary structure in preference to α-helices and β-sheets can be explained by weak hydrophobic bonding between side chains and/or by hydrogen bonding between peptide CONH groups and side-chain polar groups. Another interesting site is the amide III Raman region, which in spite of the above results shows a band in the range usually attributed to α-helical structure. This finding is due to contributions from methine bonds and $CH_2$ groups and indicates that the side chains can influence the position of the bands in the amide III region. Consequently, this result reveals that associating polypeptide backbone conformation with characteristic frequency ranges in the amide III region may be incorrect.**

Index Headings: **Infrared; Raman spectroscopy; L-asparagine/L-aspartate random copolypeptide.**

## INTRODUCTION

The structural information available from the vibrational spectra of proteins is potentially substantial but depends on frequency assignments and interpretation. The analysis of the vibrational spectra of proteins is difficult because of the myriad of amino acid residues and protein chain conformations. A strategy that has been used has been examination of the spectra of homopolypeptides or simple copolymers as spectral models for protein molecules.[1-3] In this way, a comparison of the protein spectra with the aid of model compounds can assist in determining the conformationally sensitive spectral features and the relation between the amino acid sequence of a protein segment and the conformation which it may have induced.

In this work we measure and analyze for the first time the Raman and infrared spectra of an L-asparagine/L-aspartate random copolypeptide with an L-asparagine residue content of 69% mol. The amide bands of the spectra measured in solution and in the solid state are related to the conformation of the polypeptide backbone. As an aid to interpretation, the CD spectra of this biopolymer in aqueous solution were also obtained. At first sight, an apparent difficulty might arise because the carbonyl stretching vibrational mode of the polypeptide side-chain -$CONH_2$ group lies in the same frequency region as the amide I band of the polypeptide backbone. However, the use of appropriate resolution and the comparison of the spectra of this biopolymer with those of model compounds containing the -$CONH_2$ group have allowed us to assign these two vibrational modes. As there has been virtually no work to date that has studied the conformational structure of this polypeptide, the results obtained in this investigation may contribute new data relative to the role of asparagine and aspartate residues in the secondary structure of proteins.

## EXPERIMENTAL

Poly-L-(Asn, Asp) (mol wt 5000–15,000) was obtained from Sigma. This compound was dried under vacuum and kept in a refrigerator prior to the preparation of its aqueous solutions. After a fitting program for band resolution had been used, infrared $\nu COO^-$ bands were evaluated, whereby the percentage of Asp residues was found to be 31%.

Stock water solutions for vibrational spectroscopy were used in cacodylate buffer (pH 6.5). As the molecule weights of -$CONH_2$ and -$COO^-$ groups are equivalent, the concentrations of poly-L-(Asn, Asp) were expressed as 0.1 M in peptide monomer. The heavy water solutions were prepared by dissolving the copolymer in $D_2O$, followed by drying under vacuum and redissolving in fresh $D_2O$.

Film specimens of the polypeptide in the solid state for infrared spectroscopy were cast from aqueous solution. After being dried under vacuum for 2–3 hours, the specimens were equilibrated over saturated salt solutions at the desired humidities. A 25-mm cylindrical cell was prepared to include a small reservoir, which contained the saturated salt solution. The polymer films were cast on one of the removable $F_2Ca$ windows and equilibrated for about 12 h. After the spectra were recorded, the sample window was replaced by a $F_2Ca$ blank, and the spectra of the moist air were also recorded. These spectra were then subtracted from those of the specimens.

Samples were scanned in a Perkin-Elmer FT-IR 1725X spectrometer, with the use of $F_2Ca$ cells and an Epson AX2 computer for data acquisition for analysis. Sixty-four scans at 2 $cm^{-1}$ resolution were averaged. Buffer spectra were recorded under the same scanning conditions as the corresponding spectra of the polypeptide in

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solution, in order to obtain by subtraction the net spectra of the biopolymer. Subtractions of $H_2O$ absorption from the spectra of solutions of poly-L-(Asn, Asp) were based on the criterion of obtaining a straight baseline from 2200 to 1725 $cm^{-1}$. Second-derivative spectra were generated by using an Obey program available from Perkin-Elmer, allowing overlapping bands to be resolved.

For Raman spectroscopy, aqueous solutions of the polypeptide were transferred to a sample cell (1.0-mm glass capillary tube). The Raman spectrometer consisted of a Jobin-Yvon monochromator, Model U-1000, and a Spectra-Physics argon-ion laser operating at 514.5 nm. Slit widths were chosen to give a spectral resolution of 3 $cm^{-1}$, and usually three scans were accumulated.

CD spectra were acquired on a Jobin-Yvon Mark III spectrophotometer. A 0.05-cm-pathlength cuvette was used, and polypeptide samples were examined in aqueous solution containing 0.6 mg/mL.

## RESULTS AND DISCUSSION

Examination of the spectrum of the copolymer in aqueous solution (Fig. 1) shows that the absorption in the amide I region appears to be composed of two major parts, a relatively sharp band at 1652 $cm^{-1}$ (Table I) and a high-wavenumber broad shoulder which causes a second-derivative peak at 1677 $cm^{-1}$. On the basis of the infrared spectrum of acetamide in aqueous solution we have assigned the high-wavenumber component to the stretching motion of the C=O bonds in side-chain -$CONH_2$ groups. The 1652-$cm^{-1}$ strong band is the polypeptide amide I mode, and this frequency is consistent with either a random coil or $\alpha$-helix, as deduced from the well-known spectrum-structure correlations.[4–6] No amide I band attributable to $\beta$-sheet structures is visible in this spectrum. Aspartic acid residues, which in this case are present as anions, cause the asymmetric ($COO^-$) stretching mode at 1590 $cm^{-1}$. This band is in the region where the $\delta NH_2$ vibration of primary amides used to appear. In fact, we have observed that acetamide and butiramide in aqueous solution exhibit the $\delta NH_2$ vibrational mode with medium-weak intensity near 1600 $cm^{-1}$. The relative intensity of this mode and the fact that only 69% of amino acid residues are asparagine result in the masking of the asparagine $\delta NH_2$ band by the $\nu_{as}COO^-$ one. A broad shoulder is observed at the slope of this band toward smaller wavenumbers that runs from about 1560 $cm^{-1}$ to about 1510 $cm^{-1}$. This broad shoulder results from overlapping of the polypeptide amide II band components which, as shown by the second-derivative spectrum (Fig. 1), appear at 1557, 1537, and 1521 $cm^{-1}$.

Some authors have studied spectrum-structure correlations experimentally and used the result to determine conformations of polypeptides and proteins.[4–6] Concerning the amide II region, the following spectral assignments were proposed: $\alpha$-helix, 1550–1545 $cm^{-1}$; antiparallel $\beta$-structure, 1530–1520 $cm^{-1}$; random coil, 1545–1520 $cm^{-1}$; turns, 1562–1536 $cm^{-1}$. In addition, in two reports[7,8] based on spectra and calculations of fibrous proteins and polypeptides, the position of unordered structure in the amide II region was assigned to 1520 $cm^{-1}$. These assignments are consistent with our data. In fact, the spectral profile in the amide I region (Fig. 1) precludes $\beta$-structure polypeptide segments, and on the other hand no band is present in the 1550–1545 $cm^{-1}$ range as an indicator of $\alpha$-helix. Moreover, the CD spectrum, as described below, does not show any signal of helical structure. Therefore, the 1557-$cm^{-1}$ band suggests that turns are present to some extent, and the 1537- and 1521-$cm^{-1}$ bands can be unambiguously assigned to unordered structure.

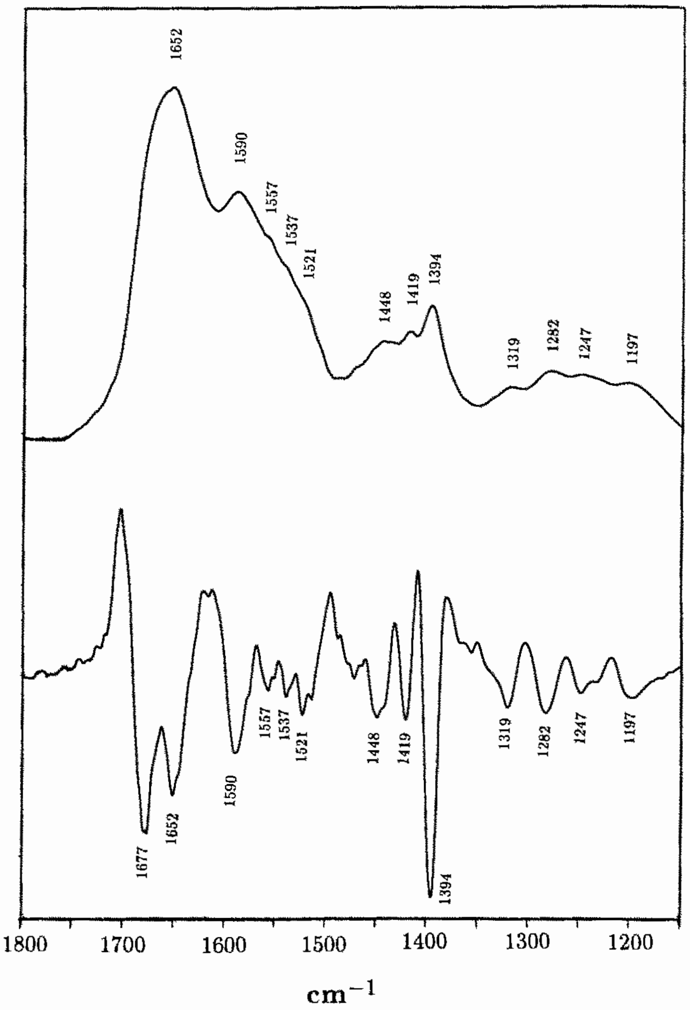

FIG. 1. Infrared spectra of poly-L-(Asn, Asp) in $H_2O$ solution. Upper absorption spectrum; lower: second-derivative spectrum.

The CD data are consistent with infrared results. In fact, poly-L-(Asn, Asp) has a CD spectrum (Fig. 2) typical of an unordered polypeptide backbone. The same conclusion relative to the secondary structure can be drawn from the infrared spectra in $D_2O$ solution (Fig. 3). The course of deuteration exchange in poly-L-(Asn, Asp) at room temperature has been followed in the amide I and amide II regions. After a 5-min incubation no additional spectral changes occur, because the exchange has gone to completion (Fig. 3).

A result has emerged from studies of hydrogen exchange in aqueous solutions of proteins, polypeptides, and model compounds,[4,9,10] in the sense that amide hydrogens in polypeptide backbones that exchange very slowly are thought to be either in hydrogen-bonded structures such as helices and $\beta$-strands or buried in internal domains inaccessible to solvent. By contrast,

**TABLE I. Infrared and Raman bands of poly-L-(Asn, Asp).**[a]

| IR | | Raman | | Assignment |
|---|---|---|---|---|
| $H_2O$ solution | $D_2O$ solution | $H_2O$ solution | $D_2O$ solution | |
| 1677 sh | | 1670 sh | | $\nu$C=O (-$CONH_2$) |
| (1676) s | | (1670) s | | |
| 1652 vs | | 1655 vs | | Amide I, $\delta H_2O$ |
| | 1652 sh | | | |
| | 1646 vs | | 1653 vs | Amide I′, random coil |
| | 1639 sh | | 1639 sh | $\nu$C=O (-$COND_2$) |
| | (1637) vs | | (1638) s | |
| 1590 s | 1596 s | 1599 sh | 1603 w | $\nu_{as}COO^-$, $\delta NH_2$ |
| (1608) m | | (1603) m | | |
| 1557 sh | | | | Amide II, turns |
| 1537 sh | | | | Amide II, random coil |
| 1521 sh | | | | |
| 1448 w | | 1447 sh | | $\delta CH_2$ |
| | 1450 m | | 1450 sh | $\nu C\text{-}ND_2$, $\delta CH_2$ |
| | 1433 sh | | 1432 s | |
| | 1421 sh | | | |
| 1419 w | | 1415 sh | | $\nu C\text{-}NH_2$ |
| 1394 m | 1395 m | 1395 s | 1403 sh | $\nu_{as}COO^-$ |
| | 1347 w | | 1342 sh | tw$CH_2$ + $\omega CH_2$ + $\delta C_\alpha H$ |
| | | | 1328 w | |
| 1319 w | | 1315 vw | | $\delta NH$ + $\delta C_\alpha H$ + tw$CH_2$ + $\omega CH_2$ |
| 1282 w | | 1274 w | | |
| 1247 w | | 1248 w | | Amide III |
| | | | 1239 sh | $\delta ND_2$ |
| | 1208 s | | 1204 m | $\delta D_2O$ |
| 1197 w | | 1186 w | | $\nu C_\alpha N$ + $\nu CC$ |
| | | 1107 m | | |
| | | (1130) s | | r$NH_2$ |
| | | 1069 m | | |
| | | 1041 vw | 1059 vw | $\nu CC$ |
| | | 992 m | | $\nu C_\alpha N$ + $\nu CC$ |
| | | | 975 vs | $\nu C_\alpha N$ + $\nu CC$ + Amide III′ |
| | | 938 vw | | $\nu CC$ |
| | | | 932 m | r$ND_2$ |
| | | | (931) m | |
| | | 879 m | 874 m | $\nu CC$ |
| | | 830 m | 836 m | $\nu CC$ |

[a] Note: Values between parentheses correspond to acetamide in aqueous solution. Abbreviations: s, strong; vs, very strong; m, medium; w, weak; vw, very weak; sh, shoulder; $\nu$, stretching; $\delta$, in plane deformation; $\omega$, wagging; r, rocking; tw, twisting.

amide hydrogens in turns or randomly coiled polypeptides exchange rapidly, as is the case in poly-L-(Asn, Asp). The band at 1652 $cm^{-1}$ in $H_2O$ solution assigned to helical or unordered polypeptide segments shifts 6 $cm^{-1}$ towards lower wavenumbers upon deuteration (Fig. 3, Table I). Polypeptide fragments in a nonordered conformation are usually associated with an infrared amide I band which in spectra measured in $D_2O$ is located in the 1648–1640 $cm^{-1}$ range.[4,11]

We assign the 1639-$cm^{-1}$ shoulder to the amide groups in the polypeptide side chains, as this frequency is very close to the $\nu$C=O frequency (1637 $cm^{-1}$) of acetamide in heavy water. Another amide I′ band component appears as a shoulder at 1652 $cm^{-1}$. The amide I modes that remain practically at the same frequency upon deuteration are generally assigned to ordered structures, such as $\alpha$-helices and $\beta$-sheets.[4,11] One could, then, assign the 1652-$cm^{-1}$ shoulder to $\alpha$-helical segments, according to well-established spectrum-structure correlations.[4–6,11,12] However, the application of a fitting program[13] for resolution of the amide I′ spectral profile reveals that the percentage of this structure could be about 18%, and therefore the attribution of this structural percentage to $\alpha$-helices is not consistent with the CD spectra of the biopolymer in aqueous solution. The presence of the amide I′ band component at 1652 $cm^{-1}$ may, then, be caused by the asparagine side-chain carbonyl groups that are buried to some extent in internal domains of the polypeptide and therefore are less solvated than the ones causing the 1639-$cm^{-1}$ shoulder.

The Raman spectra (Fig. 4) show the following features. The frequency of the amide I vibration of peptide groups falls in the 1700–1600 $cm^{-1}$ range, but is obscured by the water band at 1650 $cm^{-1}$. The shoulder at 1670 $cm^{-1}$ may be caused by the $\nu$C=O vibrations of side-chain -$CONH_2$ groups, as deduced from comparison with the Raman spectrum of acetamide in aqueous solution. As the $\delta NH_2$ vibration is mixed with the $\nu$C=O vibration in -$CONH_2$ groups, the 1670-$cm^{-1}$ shoulder shifts to 1639 $cm^{-1}$ (Table I) when the polypeptide is dissolved in heavy water. The same phenomenon occurs for acetamide.

On the other hand, we assign the most prominent band component (1655 $cm^{-1}$) to the polypeptide amide I vibrational mode. An interesting aspect of the Raman spectra of this biopolymer concerns the amide III region. In fact, the $H_2O$ solution spectrum gives two bands in the 1300–1200 $cm^{-1}$ range located at 1248 and 1274 $cm^{-1}$. The former is in the spectral range assigned to polypep-

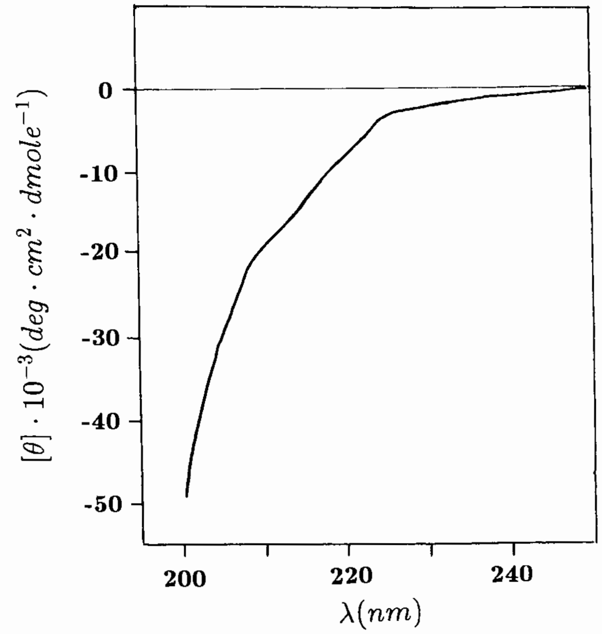

FIG. 2. CD spectrum of poly-L-(Asn, Asp) in $H_2O$ solution.

tide disordered conformation,[1] and the position of the latter could be considered as an indicator of the presence of $\alpha$-helical secondary structure. However, the 1274-$cm^{-1}$ band is the strongest in the 1300–1200 $cm^{-1}$ region, and its attribution to $\alpha$-helices is not consistent with our CD result. We, then, suspect that the appearance of these two overlapping bands in the amide III region is due to splittings or mixing of the modes that involve the in-plane NH bend, the bends of methine protons, and the twisting and wagging vibrations of side-chain $CH_2$ groups.

In fact, Diem and co-workers[14–16] in their Raman spectroscopic studies on alanyl dipeptides and various N- and/or $C_\alpha$-deuterated isotopomers found experimental evidence for the mixing of $\delta$NH and $\delta C_\alpha H$ vibrations. Moreover, the calculations carried out by Krimm and Bandekar[5] for the calcium salt of poly-L-(glutamic acid) showed that a nonweak amide III band located at 1260 $cm^{-1}$ can be assigned to a mode where the $\delta$NH, $\delta C_\alpha H$, and twisting and wagging vibrations of side-chain $CH_2$ groups are involved. In addition, the finding that the 1274-$cm^{-1}$ Raman band of poly-L-(Asn, Asp) shifts to 1328 $cm^{-1}$ upon N-deuteration (Fig. 4, Table I) means that the in-plane bend of polypeptide backbone NH groups may be mixed with the $\delta C_\alpha H$, twisting $CH_2$, and wagging $CH_2$ motions, as the 1328-$cm^{-1}$ band falls in the ranges of the last three vibrations. Consequently, our spectroscopic results constitute an experimental proof that not only the polypeptide secondary structure can influence the position of the bands in the amide III region, but also the side chains of the polypeptide.

In this connection, a recent Raman spectroscopic study[17] of peptides containing two amino acids—glycine-*X*, alanine-*X*, and serine-*X*, where *X* is an uncharged amino acid—shows that the amide III band shifts systematically as the side chain of the *X* amino acid changes in size. It is, then, not possible to expect a simple general relationship between amide III frequencies and polypeptide backbone conformations. Besides the amide III

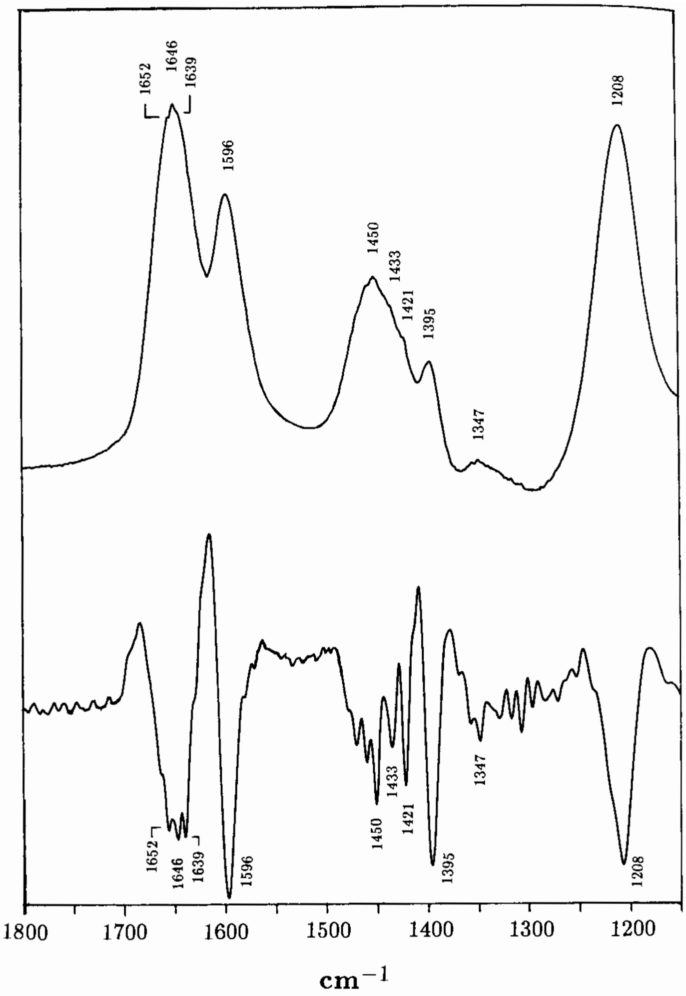

FIG. 3. Infrared spectra of poly-L-(Asn, Asp) in $D_2O$ solution. Upper: absorption spectrum; lower: second-derivative spectrum.

region, the 890–945 $cm^{-1}$ region is also sensitive to structural changes.[1] A strong band assigned to the C-C stretching vibration appears in this range of the Raman spectra of all $\alpha$-helical polypeptides studied up to now.[1,18] However, a large decrease in the intensity of this band during the helix to random coil transition has been observed for some homopolymers.[19] In addition, the Raman spectrum of poly-L-(Asn, Asp) in aqueous solution displays C-C stretching broad bands (Fig. 4) with weak–medium intensity, these broad bands implying that the rotational $\phi$ angles of the peptide linkages have a wide distribution.

The unordered secondary structure remains, even in the solid state. Broad amide I′ bands characteristic of unordered polypeptide backbone appear in the infrared spectra of the polymer at 0, 45, and 82% relative humidity (Fig. 5). No significant spectral changes indicative of the $\alpha$-helix of $\beta$-sheet formation occur as humidity increases. These results contrast with the reversible conformational transitions found in sodium salts of poly-L-glutamic acid, where $\alpha$-helical segments increase upon increasing relative humidity.[20] On the other hand, we have also observed that the polypeptide backbone in aqueous solution remains unordered, even when aspartate carboxyl groups are present in the undissociated form (-COOH). This result also contrasts with the ten-

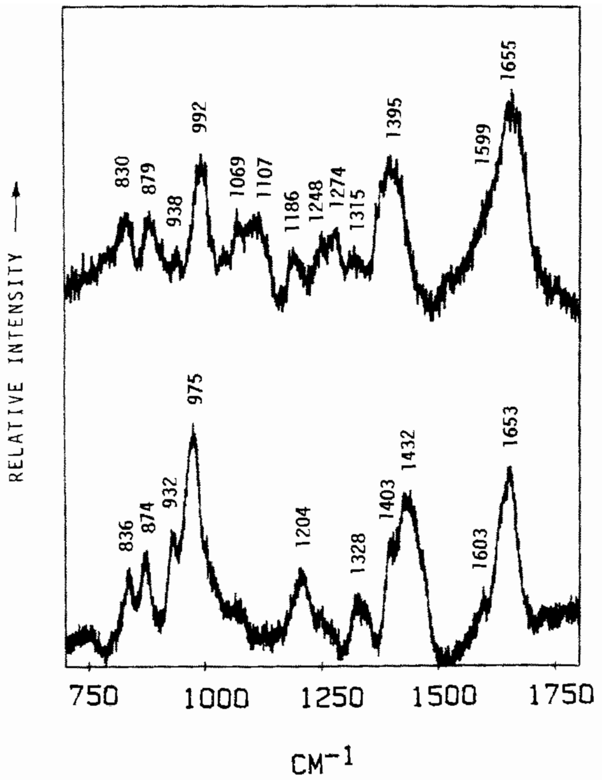

FIG. 4. Raman spectra of poly-L-(Asn, Asp) in $H_2O$ (upper) and $D_2O$ (lower) solutions.

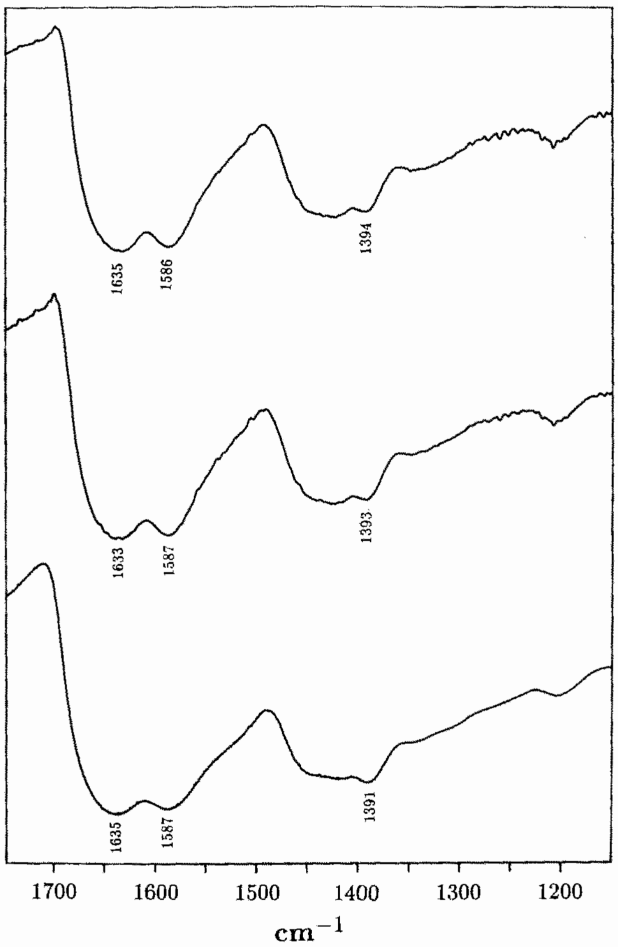

FIG. 5. Infrared spectra of N-deuterated poly-L-(Asn, Asp) films equilibrated with $D_2O$ at 82% (upper) and 45% (middle) relative humidities. The lower spectrum corresponds to the N-deuterated polypeptide equilibrated with $P_2O_5$.

dency of some salts of poly-L-(glutamic acid) to form $\alpha$-helices at acid pH.[21]

These conformational differences are in accord with expectations arising from consideration of hydrophobic bonding.[22] In fact, hydrophobic bonding between side chains of polypeptides in the helical form makes a positive contribution to the total change in entropy, $\Delta S^0$, of the transition from coil to helix: water molecules structured about the side chains of the polypeptide in the coil form would be released on formation of $\alpha$-helices, because in these there appears to be greater possible contact between the side chains as the number of $CH_2$ groups increases.[23] Although we lack thermodynamic data, we suspect that the presence of only one side-chain methylene group in poly-L-(Asn, Asp) causes an unfavorable $\Delta S^0$, which consequently renders this polypeptide incapable of forming the helical configuration.

On the other hand, with asparagine and aspartate residues, the hydrogen bonding interactions of the polar side chains with the peptide dipole can prevent $\alpha$-helix and $\beta$-sheet formation. This observation is consistent with our spectroscopic results. In fact, CPK molecular models constructed with the asparagine side-chain -$NH_2$ group hydrogen bonded to the peptide C=O group in the same amino acid residue result in a cycle which makes the amide III, wagging $CH_2$, and twisting $CH_2$ coordinates in the cycle nearly parallel and therefore strongly coupled, as described above. This type of hydrogen bond can be a reason why this polypeptide has the tendency to form random coils. This preference of the asparagine for the random coil conformation also has been found by examining some protein crystal structures[24] with the goal of determining conformational preferences for the various amino acids. Thus, certain residues are more likely than others to be found in $\alpha$-helical sections.

This tendency is expressed through the conformational parameter, $P_\alpha$, which in the case of asparagine and aspartate residues has the values of 0.73 and 0.98, respectively. $P_\alpha$ values less than 1 mean a less than average frequency of occurrence of the $\alpha$-helical conformation for these residues, whereby these amino acids are considered to be helix breakers. Another analogous conformational parameter for amino acid residues with a $\beta$-sheet-making tendency, $P_\beta$, has been defined.[24] Its values for asparagine and aspartate residues are 0.65 and 0.80 ($P_\beta < 1$), whereby these residues are also classified as $\beta$-sheet breakers in proteins. Therefore, the weak hydrophobic character of the side chains and/or hydrogen bonding of -$CONH_2$ and $COO^-$ groups with the polypeptide backbone amide groups can be the factors that determine the tendency of poly-L-(Asn, Asp) to form random coils.

## ACKNOWLEDGMENTS

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