

Study of Poly(L-aspartic acid). I. Laser Raman Spectrometry

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ABSTRACT: The conformational properties of poly(α -L-aspartic acid) (L-Asp)_n have been investigated in water, in deuterium oxide and also in the solid state, by Raman scattering. The conformational transition induced by changing the pH of the solutions has been given special attention. The spectra exhibit a mixture of different conformations. Random-coil and β -antiparallel pleated-sheet conformations exist in both acidic and ionized forms of the polyacid. In addition a local β_1 helical conformation has been detected in the ionized form. The α helical conformation appeared as the acidic form only.

KEY WORDS Poly(α -L-aspartic acid) / Raman Scattering / Conformational Transition /

Only a few studies have been carried out up till now on (L-Asp)_n.^{1–3} Patrone and Conio⁴ showed that only 20% of the α helical structure exists in H₂O–methanol solutions.

A complete study of the structure of (L-Asp)_n has been carried out using infrared, X-rays, circular dichroism, NMR and Raman techniques.⁵ The aim of this paper is to present the results of the laser Raman investigations and discuss them, making reference especially to the results of Koenig and Frushour on (L-Glu)_n⁶ and Yu, *et al.*, on (L-Lys)_n.⁷

EXPERIMENTAL

(β -Benzyl L-aspartate)_n was synthesised by the *N*-carboxyanhydride method and then debenzylated with dry HBr in benzyl alcohol. The molecular weight of this sample was about 7,000 and determined by tonometric measurement (solvent, DMF; rebenzyl M_w , 210). The total debenzylation was controlled by UV spectroscopy (λ , 260 nm; ϵ , 200 l mol⁻¹ cm⁻¹) and residual benzyl groups were less than 1%. Debnylation was also controlled by infrared spectroscopy and no characteristic band due to the presence of an α - β copolypeptide was recorded. Kowacs, *et al.*,¹⁶ have recorded the IR

spectrum of an α - β copolypeptide of (L-Asp)_n. No such bands were present in our spectra or if present, they were too small to be detected.

Generally, an α - β shift occurs when the debenzylation takes place in a solvent such as DMSO or DMF and gives rise to the formation of succinimide rings and the appearance of two negative dichroic bands at 258 and 242 nm. Debnylation of (L-Asp)_n was performed in CHCl₃ by HBr and no such bands appeared in our spectrum. Another (L-Asp)_n sample was obtained from Sigma (molecular weight M_w = 14,000). A small difference between these two materials was detected in their circular dichroism spectra. Raman spectra were recorded using a CODERG T 800 triple monochromator. The 514.5 nm line of a Spectraphysics Argon ion laser was used as the exciting source, with 400–600-mW power at the sample. The spectra were obtained at a constant temperature of 25°C.

In order to avoid fluorescence phenomena, the solutions and solids investigated were maintained one week on charcoal then the solutions were dialysed and lyophilized in H₂O (or D₂O). The solids were maintained in the laser beam several hours before recording. The solutions were prepared 24 h before use. They were comparatively less fluorescent than the solids.

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Table I. Frequencies (cm⁻¹) and assignments of Raman bands for (L-Asp)_n^a

(Asp Na) _n H ₂ O (10%) pH 10	(Asp) _n H ₂ O (10%) pH 4.7	(Asp) _n Solid	(Asp Na) _n Solid	(Asp Na) _n D ₂ O (20%) pD 11.4	(Asp Na) _n D ₂ O (20%) pD 7.8	(Asp) _n D ₂ O pD 4.7	Assignments
					2979 S 2959 W 1938 M, sh		ν(CH) _n
	1722 W 1701 VS, P 1698 sh	1722 S	1755 M 1730 M 1710 M 1708 W				ν(C=O)
1680 M	1677 S	1670 S, s	1682 M	1672 M	1672 M	1670 S	
1666 M	1670 S	1654 br	1672 W				
1660 M	1655 M		1668	1653 M	1653 M	1658 S	Amide I + δ(H ₂ O)
1644 M(H ₂ O)	1635 S, P	1638 W, br	1652 M		1638 M	1644 M	
1621 W	1613		1640 S	1640 S	1621 W		
1592 W		1583 W	1584 S 1562 W 1545 W	1569 W	1569 W	1589 W	ν _a (COO ⁻)
1524 W	1553 W 1529 W	1513 M 1489 W		1479 W	1471 VW	1471 VS 1454 S	Amide II (NH+(ND) Amide II')
1449 W			1461 M	1447 br		1421 M	δ(CH ₂)
1419 S, sh	1427 S, P	1412 S	1417 S	1421 S	1421 M	1421 M	δ(CH)
1404 VS			1405 W	1403 VS	1403 VS	1407 M	ν _s (COO ⁻)
1338 W	1363 S	1342 1336	1346 W	1346 W		1358 W 1346	γ _w (CH ₂)
1321 M		1326 W	1315 W 1306 W 1300 W	1321 M	1321 M	1324 M	γ _t (CH ₂)
1276 W	1276 W	1279 W	1285 M 1257	1259 W		1254 M	Amide III
1250 M	1250 M						Amide III
1242 M, br	1242 S, P	1229 S	1235 M, br 1205 M	1209 M	1202 W	1209 S, br	
1205 M			1192 W 1168 br	1201 M			
	1189 W	1169 W 1150 W	1138 W	1136 VW	1133 W 1124 W		
1085	1102 W		1092 M	1086 VW		1088 W	ν(C-N) terminal
1076 Sh	1076 S, s, P		1060 M, br	1067 W	1067 W		
1068 S, s		1044 S					ν(C-C)
1021 M	1033		1020 M		1021 M 1010 W		Amide III'
981 S, br	1002 W 981 W	1005 W 969 S, s	1005 W	1003 W 970 M, br	1001 VW 983 W	1002 W 965 VS	ν(C-C) aromatic ν(C-C) ν(C-C-N)
935 S	941 M, P			937 VS	938 S	938 S, sh	ν(C-C)
898 M	879 S, s			889 W	897 W	889 W	γ _t (CH ₂)
836 W	822 sh			819 W			

^a VS, very strong; S, strong; M, medium; W, weak; VW, very weak; s, sharp; br, broad; sh, shoulder; L, large; δ, deformation mode; γ_t, twisting mode; γ_w, wagging mode; γ_r, rocking mode; ν_s, symmetrical stretching mode; ν_a, antisymmetrical mode; P, polarized band.

RESULTS AND DISCUSSION

Solid Acid Form (L-Asp)_n

In Table I, the frequencies of the Raman bands observed for poly(aspartic acid) in the solid state are reported along with the Raman bands of solutions in H₂O and D₂O.

The amide I band appears in three lines at 1670, 1654, and 1638 cm⁻¹ (Figure 1). The first is assignable to a β antiparallel structure as reported for (L-Lys)_n.⁷ The second corresponds to the random coil conformation observed by Koenig and Frushour at

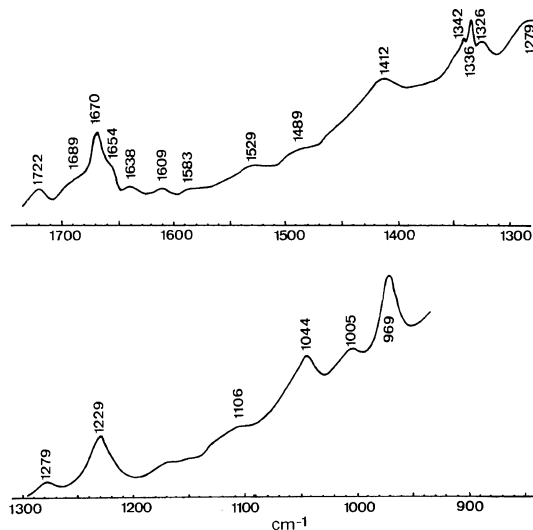


Figure 1. Raman spectra of neutral (L-Asp)_n in the solid state.

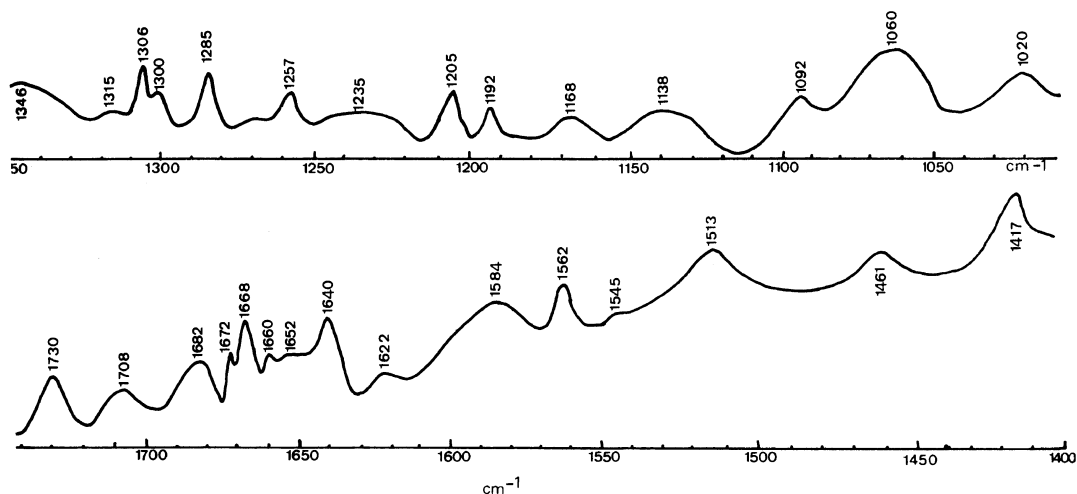


Figure 2. Raman spectra of (L-Asp Na)_n in the solid state.

1656 cm⁻¹ for (L-Glu)_n.⁶ The last one is due to the helical conformation (1650 cm⁻¹ in IR).

The band at 1689 cm⁻¹ indicates the $\nu_{C=O}$ stretching of non hydrated C=O groups. The one at 1722 cm⁻¹ is due to the $\nu_{C=O}$ stretching of neutral carboxylic groups. However a small number of ionized carboxylic groups is shown to exist, by the presence of a band at 1583 cm⁻¹. Moreover, the small bands at 1609, and 1005–1002 cm⁻¹ may be assigned to the residual benzyl rings¹⁰ (less than 1% as determined by UV spectrometry) (Figure 1).

The sharp and intense amide III band is found at 1229 cm⁻¹ (1270 cm⁻¹ for (L-Asp Na)_n and 1230 cm⁻¹ for (L-Asp)_n in IR in the solid state), and we have assigned it to the amide III vibrations of antiparallel sheets. A weak band was observed at 1279 cm⁻¹, is discussed in the following paragraph.

Solid Ionized Form (L-Asp Na)_n

The spectra of (L-Asp Na)_n were difficult to obtain due to intense fluorescence (Figure 2). The analysis of the bands could be made for (L-Asp)_n. The amide I bands at 1672–1668 cm⁻¹ were assigned to a β antiparallel conformation (1690–1672 cm⁻¹ in IR). The band at 1652 cm⁻¹ corresponds to the random coil conformation as observed at 1649 cm⁻¹ for (L-Glu Na)_n by Koenig and Frushour.⁶ The band at 1638 cm⁻¹ of the α helix conformation was not present here but a strong band at 1640 cm⁻¹ was observed. It is surprising that such a band occurs at this frequency and we have not been able to identify it.

A band at 1682 cm⁻¹ corresponds to the stretching

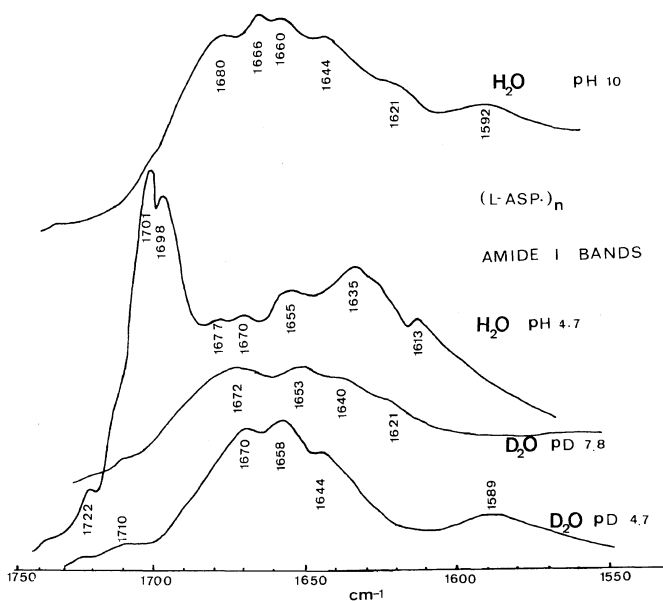


Figure 3. Raman spectra of (L-Asp)_n amide I bands in both H₂O and D₂O.

of non-hydrated C=O groups. A point of discussion is the presence in the spectra of (L-Asp Na)_n of many bands between 1755 and 1708 cm⁻¹ corresponding to the C=O stretching. These bands do not originate in the presence of oligomers, the samples being dialyzed and lyophilized several times before recording the spectra. The strong band at 1584 cm⁻¹ was assigned to the ionized carboxylic groups.

The amide III band was intense like for (L-Asp)_n but shifted somewhat at 1235 cm⁻¹ for the β anti-parallel pleated sheets. Another sharp band appears at 1285 cm⁻¹. (This band is weak at 1279 cm⁻¹ in the acidic (L-Asp)_n form.) This band can be related to the one at 1283 cm⁻¹ for the (Gly)_n II 3₁ helix structure found by Small, Fanconi, Peticolas in the solid state.¹³ It is unusual to find a band which seems to be in the ionized form (1279 cm⁻¹) in the spectrum of the acidic form (L-Asp)_n. However, it should be pointed out that all the carboxylic functions were not in the neutral carboxylic form in these experiments. If the pH is further lowered, the precipitation takes place before completion of the neutralization.

Aqueous Solutions

Only basic solutions can be prepared directly. Their concentration must be near 10% in weight for the purpose of Raman spectrometry. Acidic solutions can be prepared by neutralization of basic ones. The spectra recorded from aqueous solutions

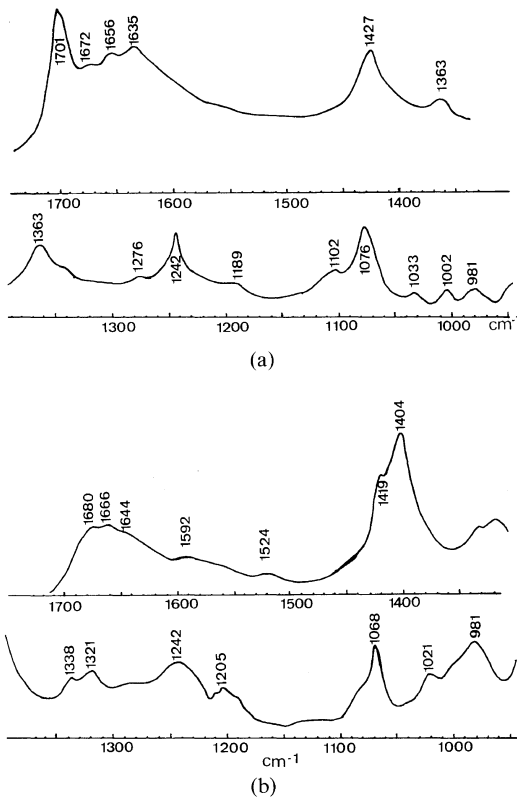


Figure 4. Raman spectra of (L-Asp): a, in H₂O at pH 4.7 (10% in weight); b, in H₂O at pH 10 (10% in weight).

were not very different from those obtained in the solid state (Figures 1 and 4).

Whatever the pH of the solution is, the amide I bands are obscured by water (1644 cm^{-1}). The two bands corresponding to the β antiparallel pleated sheets appeared at 1680 and 1666 cm^{-1} for basic solutions (Figure 4 b), and shifted to 1677 and 1670 cm^{-1} when the pH was lowered (Figure 4 a). A comparison can be made with the β structures of $(\text{Gly})_n$ I¹³ and $(\text{L-Val})_n$ in the β -form¹⁵ which exhibit bands at 1674 – 1665 cm^{-1} and 1666 cm^{-1} , respectively. The random coil conformation, observed at 1660 cm^{-1} in basic solutions does not disappear in acidic conditions but is shifted at 1655 cm^{-1} with a lower intensity. This random coil is obtained at 1656 cm^{-1} for $(\text{L-Glu Na})_n$ ⁶ and at 1654 cm^{-1} for feather keratin.¹¹

By lowering the pH, a strong highly polarized band at 1635 cm^{-1} appeared and was assigned to the α helical conformation

The amide III band does not shift on lowering the pH of the solutions, but at basic pH, this band is very broad at 1242 cm^{-1} and was assigned to the β antiparallel conformation⁹ (in good agreement with the observation of Painter and Koenig¹² (1239 cm^{-1} for $(\text{L-Lys})_n$).⁷

The constant intensity of this band conforms that the structure does not disappear when the polyacid is dissolved in its ionized form and then neutralized.

Finally the strong band observed at 1285 cm^{-1} for solid $(\text{L-Asp Na})_n$ (Figure 3) appears in solution at 1276 cm^{-1} (very weak) and does not shift on lowering the pH.

In basic solutions, a strong band at 981 cm^{-1} corresponds to the stretching of C–C–N bonds of the backbone. This band decreases in intensity in acidic solutions. In the same way, a strong band at 935 cm^{-1} in basic solutions decreases and a band at 941 cm^{-1} (highly polarized) increases when lowering the pH.

It is difficult to give an assignment to these bands. However for $(\text{L-Lys})_n$, Yu⁷ found a band at 945 cm^{-1} for the α helix and another at about 1002 cm^{-1} for the β structure (C–C–N)_s skeletal motion. We assigned the band at 941 cm^{-1} to the α helix (in acidic pH) and the band at 981 cm^{-1} for the random coil (for both basic and acidic pH).

D₂O Solutions

In D_2O , the apparent pH values of the solutions

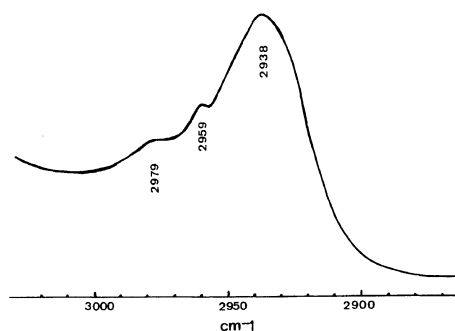


Figure 5. Raman spectra of $(\text{L-Asp Na})_n$ in D_2O at pD 7.8 in the region of CH_2 stretching.

have been corrected by adding a value of 0.4 so $\text{pD} = \text{pH} + 0.4$.

In D_2O , under the ionized form at pD 7.8, three bands were recorded at 2979, 2938 (shoulder) and 2959 cm^{-1} (weak) (Figure 5). The first two can be assigned to a 3_1 helical conformation at 2980 and 2940 cm^{-1} according to Small, *et al.*¹³ The third one is compatible with a β conformation as for $(\text{Gly})_n$ I.¹³ For basic solutions at pD 11.4 (Figure 6) and pD 7.8 (Figures 1 and 7 b) three amide I' bands appear at 1672, 1653, and 1638 – 1640 cm^{-1} . The strong band at 1640 cm^{-1} may be related to the 3_1 helical *N*-deuterated type $(\text{Gly})_n$ II conformation.¹³ The band at 1653 cm^{-1} seems to correspond to the random coil conformation; however, the corresponding random coil conformation of $(\text{L-Lys, HCl})_n$ is found at 1660 cm^{-1} .⁷ If the band at 1670 cm^{-1} is to be comparable with the amide I of the β antiparallel conformation, it will have to shift towards a lower frequency upon *N*-deuteration and this will be discussed below.

For "Acidic Solutions" (Figure 7 a) at pD 4.7, only a very weak band is seen at 1702 cm^{-1} (COOD), being just at the transition point. Below this pD, the polypeptide precipitates. The band at 1644 cm^{-1} might be assigned able to the α helical conformation (Amide I'). The band at 1653 cm^{-1} at basic pD shifted to 1658 – 1660 cm^{-1} by lowering the pD and it may be concluded that some random coil conformation persists in acidic solutions to some extent. At this frequency, it was surprising to find a band at 1672 cm^{-1} . It corresponds to the β antiparallel conformation and should shift at 1658 cm^{-1} through *N*-deuteration. This observation can be correlated with the fact that a very compact β_2 antiparallel conformation is possible. Such a conformation has

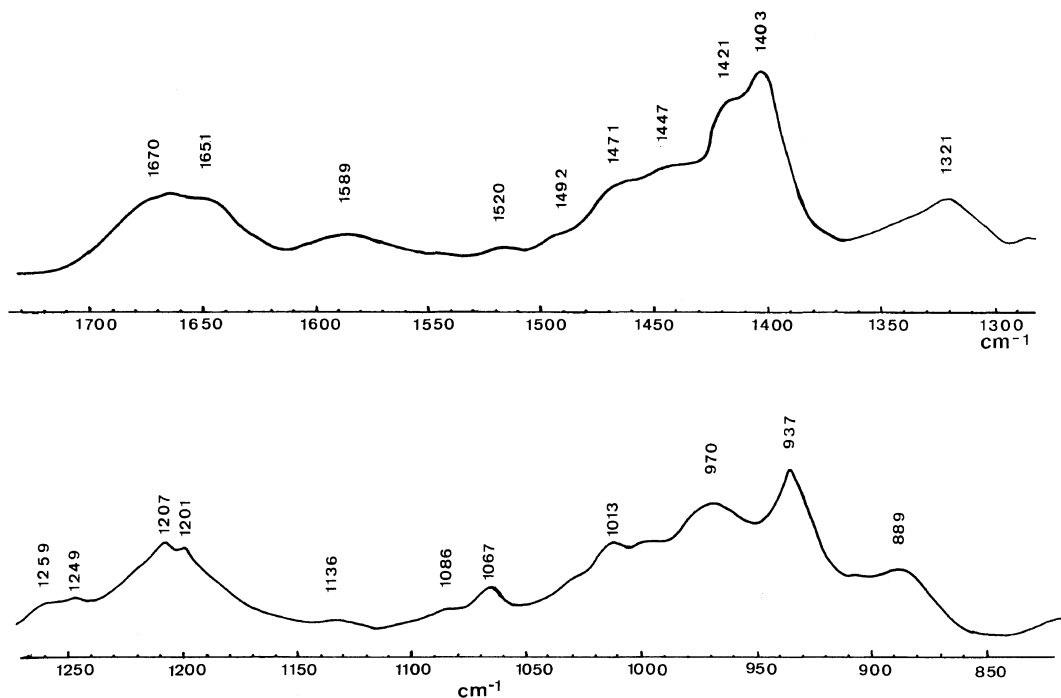


Figure 6. Raman spectra of $(L\text{-Asp Na})_n$ in D_2O in highly basic medium pD 11.4 (20% in weight).

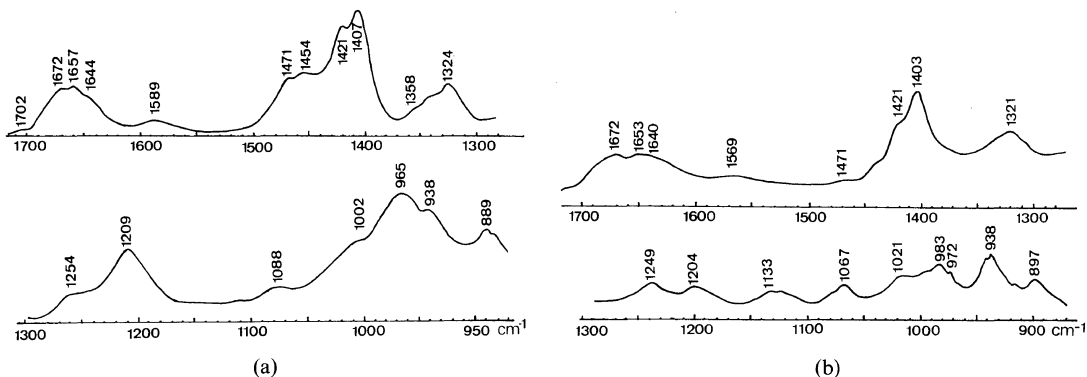


Figure 7. Raman spectra of $(L\text{-Asp})_n$: a, in D_2O at pD 4.7 (20% in weight); b, in D_2O at pD 7.8 (20% in weight).

been found for $(L\text{-Glu})_n$, in the acidic form, in D_2O solutions.⁸ In the case of $(L\text{-Asp})_n$, the measurement of near the IR spectrum in basic solutions show that no molecules of the solvent are bound to $(L\text{-Asp})_n$ in the β structure⁵ which appears similar to the β_2 structure of $(L\text{-Glu})_n$.⁵⁻⁸

Our X-ray studies⁵ showed that the parameters given for Itoh, *et al.*,⁸ for the β_2 form of the antiparallel β pleated-sheet structure of $(L\text{-Glu})_n$ in acidic form could explain a part of the rays obtained

for $(L\text{-AspNa})_n$. (Monoclinic cell, $a=9.75 \text{ \AA}$, axis of the hydrogen bonds, $b=6.84 \text{ \AA}$ axis of the β chain, and $c=8.16 \pm 0.1 \text{ \AA}$ for $(L\text{-AspNa})_n$ and $c=8.06 \text{ \AA}$ for $(L\text{-Glu})_n$ is the intersheets distance and $\beta=104^\circ$ angle between the a and c axis.) The d_{001} rays were the most intense for $(L\text{-AspNa})_n$, and changes in the value of c or β did not agree with the obtained results, despite the fact that an additive $-\text{CH}_2$ occurs in the side-chain of $(L\text{-Glu})_n$.

The amide III' bands are very weak. A band at

1013 cm^{-1} (medium) is recorded for very basic solutions and a weak band at 1010 cm^{-1} appears at pD 4.7.

In the region of 1470–1400 cm^{-1} , bands appear at 1471–1454–1421 and 1407 cm^{-1} at acidic pD and 1471–1421 and 1403 cm^{-1} at pD 7.8.

The bands at 1403 and 1407 cm^{-1} might be due to the symmetric stretching of $(\text{COO}^-)_s$ (residual at pD 4.7). The band at 1421 cm^{-1} keeps its intensity constant for both acidic and basic pD and can be assigned to the CH_2 bending vibration of the methylene of the side chain.

Band around 1471 cm^{-1} may be assigned to the amide II' bands. This is in close agreement with the recent investigations of Harada¹⁴ who studied the preresonance Raman effect of $(\text{L-Lys})_n$ and $(\text{L-Glu})_n$ and found that the amide II' band was strongly enhanced in the UV excited spectrum. The band corresponds to one of the shoulders on the higher frequency side of the $(\nu \text{CO}_2^-)_s$ stretching band around 1410 cm^{-1} in the ordinary Raman spectra (ref 14). This band is very weak in basic solution but very intense when the pD is lowered to 4.7.

Some bands at 938 and 983 cm^{-1} in basic solution correspond to the C–C–N skeletal stretching vibrations of β antiparallel sheets and random coils respectively. In acidic pD, a strong band appears at 965 cm^{-1} and was assigned to the (C–C–N) skeletal motion of the α helix conformation.

CONCLUSION

Good Raman spectroscopy arguments allow for the following conclusions.

(1) In the same ionization state, relative amounts of different conformations of polyaspartic acid do not change when going from the solid state to solutions.

(2) In the ionized state, the random coil, compact β antiparallel and 3_1 helical $(\text{Gly})_n$ II type conformations are found.

(3) In the neutral state, the random coil, β anti-

parallel, and α helical conformations are found.

These results explain that only a small amount of the helical contents of $(\text{L-Asp})_n$ has ever been found by previous authors.

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