

Raman and normal mode studies of the polypeptide chain conformations in crystalline magnesium and calcium poly(L-glutamate)s*

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Abstract—An analysis of the Raman spectra of dry crystalline magnesium and calcium poly(L-glutamate)s, based on a normal mode calculation of α -helical poly(L-glutamate), shows that the polypeptide chains in both salts have an α -helix conformation. In the wet crystalline state, another chain conformation is also present for the calcium salt.

INTRODUCTION

KEITH *et al.* [1] have shown that, under appropriate conditions, alkaline earth salts of poly(L-glutamic acid) ((GluH)_n) can form lamellar, chain-folded single crystals. The mode of crystal packing, and the conformation adopted by the polypeptide chain, are determined primarily by the nature of the alkaline earth cation and the temperature at which crystallization occurs. We have recently reported a detailed vibrational spectroscopic analysis on the β -sheet forms of the Ca²⁺ salt (β -(GluCa)_n) [2]. In addition to confirming the antiparallel-chain pleated sheet structure which had been proposed [1], this work led to considerable insight into the influence of the side chain on main chain modes, and has stimulated our interest in examining other conformations present in different crystalline forms of (GluH)_n salts.

It is known [1, 3] that, at or below room temperature, both the Mg²⁺ and Ca²⁺ salts of (GluH)_n crystallize in the hexagonal form, which suggested a helical conformation of the polypeptide chain [3]. Indirect arguments, derived from measurements of the *a* dimensions in X-ray powder diffraction patterns of wet as well as dry crystals, led to proposals for an α -helix conformation for the Mg²⁺ salt [1], and a possible "extended-helix" structure for the Ca²⁺ salt [3]. The latter was based on earlier suggestions [4, 5] that in polypeptides with charged side chains there should be local regions in which the main chain adopts an ordered conformation similar to the left-handed 3₁-helix of poly(L-proline)II. However, since the *c*-axis (fiber axis) repeat distance of these crystals could not be measured, there was no direct crystallographic evidence for either of these proposals.

In the present study, we have used Raman spectroscopy to examine the chain conformations existing in

hexagonal crystals of the Mg²⁺ and Ca²⁺ salts of (GluH)_n. Using the force field recently refined for α -helical poly(L-alanine) (α -(Ala)_n) [6], we have calculated normal modes for a standard α -helical structure of (GluH)_n [6], assuming a fully extended side chain as in β -(GluCa)_n [1]. These theoretical results, together with our recent work on β -(GluCa)_n, have provided a reliable basis for the interpretation of the observed Raman spectra.

EXPERIMENTAL

Lamellar, hexagonal crystals of (GluMg)_n and (GluCa)_n were obtained by following published procedures [1, 3], which involved dropwise addition of ethanol to a mixture of aqueous solutions containing the Na⁺ salt of (GluH)_n and Mg²⁺- or Ca²⁺-acetate. To prepare the *N*-deuterated derivative, H₂O and ethanol were replaced by D₂O and ethanol-D, respectively. The crystals were dried from slurries in ethanol (or ethanol-D), and Raman spectra were recorded using a spectrometer described previously [2]. The sample of (GluNa)_n (MW ~ 60,000, DP 380, Lot No. 34F-5011) was obtained from Sigma Chemicals, and Mg²⁺- and Ca²⁺-acetate, reagent grade, were supplied by J. T. Baker and Allied Chemical, respectively.

The details of the normal mode calculations for α -helical (GluH)_n will be presented elsewhere (P. K. SENGUPTA and S. KRIMM, to be published.)

RESULTS AND DISCUSSION

Raman spectra of crystalline (GluMg)_n and its *N*-deuterated derivative are shown in Fig. 1A and B, respectively. The corresponding spectrum for (GluCa)_n is given in Fig. 1C. Table 1 lists the observed frequencies for the principal conformationally sensitive bands of (GluMg)_n and (GluCa)_n, together with our corresponding calculated frequencies for an α -helical structure.

The amide A mode (NH stretch perturbed by Fermi resonance [7]) for (GluMg)_n is observed as a weak, broad band with a maximum at ~ 3311 cm⁻¹. This value is indicative of a hydrogen-bonded NH group, and is comparable to that of other α -helical polypeptides [7]. In β -(GluCa)_n, amide A occurs at 3274 cm⁻¹, the lower frequency being consistent with

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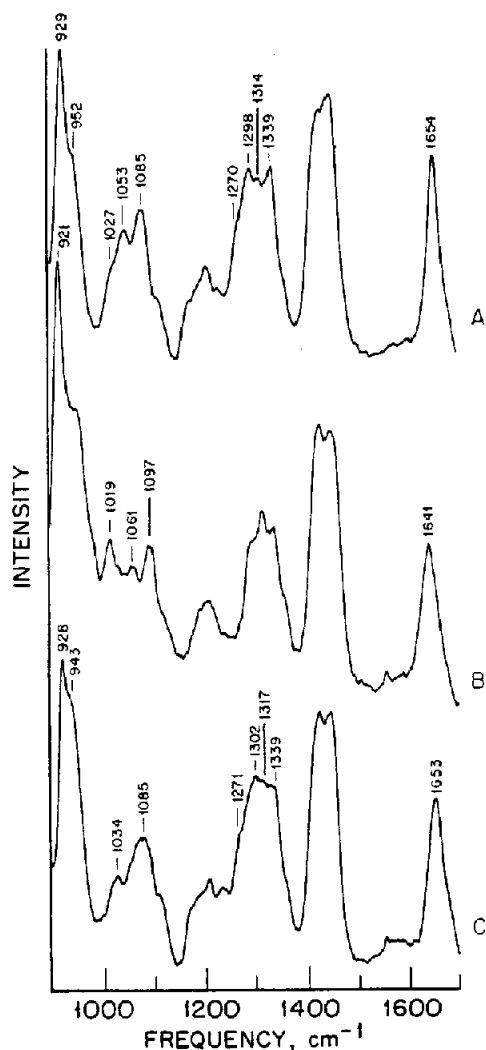


Fig. 1. Raman spectra of dry (A) crystalline $(\text{GluMg})_n$, (B) crystalline N -deuterated $(\text{GluMg})_n$, (C) crystalline $(\text{GluCa})_n$ ($\lambda_{\text{exc}} = 5145 \text{ \AA}$, spectral slit width $\sim 5.5 \text{ cm}^{-1}$, laser power 80 mW for (A) and (B), 60 mW for (C).)

Table 1. Observed and calculated frequencies (in cm^{-1}) for conformationally sensitive Raman bands of $(\text{GluMg})_n$ and $(\text{GluCa})_n$

Mode	Observed		Calculated*
	$(\text{GluMg})_n$	$(\text{GluCa})_n$	
Amide A	$\sim 3311 \text{ W}$	$\sim 3309 \text{ W}$	$3279 (A, E_1, E_2)^\dagger$
Amide I	1654 S	1653 S	$1657 (A)$
Amide III	1339 M	1339 M	$1343\text{--}1326^\ddagger$
	1298 M	1302 M	$\{1299 (E_2)$ $\{1287 (E_1)$
	$\sim 1270 \text{ sh}$	$\sim 1271 \text{ sh}$	$1263 (A)$
Skeletal stretch	929 VS	928 VS	$922 (A)$
		$\sim 943 \text{ sh}$	\S

*For standard α -helix with fully extended side chain (P. K. SENGUPTA and S. KRIMM, to be published).

† NH stretch frequency unperturbed by Fermi resonance.

‡ CH_2 twist + NH in-plane bend modes (see text).

\S Non- α -helix band (see text).

the much stronger hydrogen bond in a β -sheet structure [2]. The amide I (mainly C=O stretch) frequency for $(\text{GluMg})_n$ appears at 1654 cm^{-1} in the Raman spectrum, which is identical to the i.r. value [1], and is in the typical range ($1650\text{--}1657 \text{ cm}^{-1}$) for α -helical polypeptides and proteins [8]. In the N -deuterated derivative, this mode is found at 1641 cm^{-1} . (Our calculations predict a downshift of 10 cm^{-1} .) Amide III (CN stretch plus NH in-plane bend) generally occurs in the $1200\text{--}1300 \text{ cm}^{-1}$ region, and, on the basis of N -deuteration studies, we identify this mode with the band at 1298 cm^{-1} . This is very close to the amide III bands of α - $(\text{GluH})_n$ [9] and α -poly(γ -benzyl-L-glutamate) [9], which appear in both near 1295 cm^{-1} . In the present case, the observed 1298 cm^{-1} band can be assigned, by analogy with α - $(\text{Ala})_n$ [6], to calculated amide III modes at $1287(E_1)$ and $1299(E_2)$, both of which are Raman active. The weak shoulder at $\sim 1270 \text{ cm}^{-1}$ is probably assignable to the calculated A species amide III mode at 1263 cm^{-1} . We note that, in addition to the 1298 cm^{-1} band, the 1339 cm^{-1} band also decreases in intensity on N -deuteration. Our calculations predict two kinds of modes in this region: CH_2 twist modes at $1343(A)$, $1341(E_1)$ and $1339(E_2) \text{ cm}^{-1}$, and modes at $1326(E_1, E_2) \text{ cm}^{-1}$ that include significant contributions from NH in-plane bend. It is likely that the 1339 cm^{-1} band represents an overlapping of these two kinds of modes. This interpretation was also suggested by KOENIG and SUTTON [10] with regard to the 1336 cm^{-1} Raman band in α -poly(γ -benzyl-L-glutamate).

In addition to the amide I and III modes, a band which occurs in the $900\text{--}1000 \text{ cm}^{-1}$ region, and is associated primarily with skeletal stretching vibrations, also seems to be sensitive to the polypeptide backbone conformation [11, 12]. A strong band in this region is characteristic of the α -helix structure [8], and is observed at $908(A) \text{ cm}^{-1}$ in α - $(\text{Ala})_n$ [6]. In the spectrum of $(\text{GluMg})_n$, we observe a very strong band at 929 cm^{-1} , which is in reasonably close agreement with a calculated mode at $922(A) \text{ cm}^{-1}$. In α - $(\text{GluH})_n$ [9] this band appears at 924 cm^{-1} , whereas in β - $(\text{GluCa})_n$ the corresponding frequency is 956 cm^{-1} [2]. The origin of the shoulder at $\sim 952 \text{ cm}^{-1}$ is not immediately obvious. It may be due to the presence of a small amount of β -sheet structure, or to some other conformation that occurs on the crystal surface, where the polypeptide chain undergoes folding.

The above results provide strong support for the assignment of an α -helix conformation to the polypeptide chain in the hexagonal form of $(\text{GluMg})_n$, consistent with the conclusions from X-ray studies [1, 3].

For crystalline $(\text{GluCa})_n$, the amide A, I, III and characteristic skeletal modes are observed at ~ 3309 , 1653 , 1302 and 928 cm^{-1} , respectively. These are almost identical to the corresponding frequencies for $(\text{GluMg})_n$, the slight differences being well within experimental error. This close agreement between the

principal Raman bands of crystalline (GluMg)_n and (GluCa)_n, indicates that, at least in the dry state, the polypeptide backbone conformations of these two salts are similar, i.e. α -helical. However, we do find differences in widths and relative intensities of some bands. In the 1000–1100 cm⁻¹ region of (GluMg)_n, we find two distinct peaks at 1053 and 1085 cm⁻¹ (which are in close agreement with calculated values of 1047(A), 1048(E₁, E₂) and 1085(A) cm⁻¹), and a shoulder at \sim 1027 cm⁻¹. For (GluCa)_n, these are replaced by a broad band at \sim 1085 cm⁻¹ plus a peak at 1034 cm⁻¹. This region is dominated by side chain CC stretch vibrations, and it is possible that some differences exist in the side chain conformations of the two salts. Since the calculations for the extended side chain are in quite good agreement with the observed bands for (GluMg)_n, we infer the presence of such extended side chains in this salt; this is in agreement with deductions from X-ray diffraction studies [1, 3]. The dissimilar spectra of (GluCa)_n in this region therefore imply that in this salt the side chains are not fully extended. It is interesting that this is one possibility suggested by the smaller *a*-axis dimension of hexagonal (GluCa)_n [3].

It is also of interest that the 928 cm⁻¹ band of (GluCa)_n exhibits a broad shoulder on the high frequency side. In view of the likely sensitivity of this region to backbone conformation [11, 12], and the spectroscopic evidence [11–14] in favor of an "extended-helix" conformation [4, 5], it is important to consider the possibility that the above shoulder may be associated with a chain conformation other than the α -helix. Since solvent can have a strong influence in stabilizing a chain conformation, we examined a slurry of wet crystals of (GluCa)_n in a 10% ethanol-water mixture. (Crystals of (GluMg)_n were not sufficiently stable in the wet state to obtain spectra.) The Raman spectrum exhibits, in addition to a strong band at 928 cm⁻¹, a medium intensity band at 943 cm⁻¹ (plus a weak shoulder at \sim 953 cm⁻¹, as in (GluMg)_n). This band seems to be similar to the \sim 949 cm⁻¹ band of (GluH)_n in the charged form [11, 15]. In a subsequent

publication we will consider the relation of this band to the "extended-helix" structure.

In summary, Raman studies of hexagonal (GluMg)_n and (GluCa)_n, combined with normal mode calculations, establish conclusively that in both salts in the dry state the main chain conformation is α -helical, with the side chains being extended in the former case and not in the latter. For (GluCa)_n there is evidence for the simultaneous presence of a different backbone conformation, particularly in the wet state.

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