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A Quantitative Anharmonic Analysis of the Amide A Band in α -Helical Poly(L-Alanine)

Abstract: Polarized ir spectra of oriented films of α -helical poly(L-alanine) (α -PLA) have been obtained as a function of residual solvent dichloroacetic acid (DCA). The amide A, B, II, and V regions exhibit multiple bands whose structure depends on the residual DCA content, and those associated with the α_1 -PLA structure have been identified. A calculation of the relevant cubic anharmonic force constants indicates that, contrary to previous assignments, the overtone of amide II(A) is in Fermi resonance with the NH stretch fundamental, whose unperturbed frequency we now find to be at 3314 cm^{-1} , significantly higher than the previously suggested 3279 cm^{-1} . The presence of a structure in addition to the standard α_1 -PLA is indicated by our analysis. © 1999 John Wiley & Sons, Inc. Biopoly 49: 195–207, 1999

Keywords: amide A; α -helical poly(L-alanine); anharmonic analysis; fermi resonance

INTRODUCTION

Although the amide A band in polypeptides and proteins represents a highly localized mode, being nearly a pure NH stretch (s) vibration, it has thus far not been fully utilized in spectroscopic structure analyses of these molecules because of the difficulties in the interpretation of the complex band structure in this region. In fact, its vibrational nature is highly anharmonic compared to other amide modes, and also many overtones or combinations of lower frequency modes fall in this region. For an accurate interpretation of this region, it is indispensable that, in addition to good spectra, relevant anharmonic force constants be computed so that quantitative conclusions about such perturbations can be deduced. In an effort to produce a more reliable spectroscopic force field for polypeptides and proteins, we recently obtained good

polarized vibrational (ir¹ and Raman²) spectra on uniaxially oriented samples of α -helical poly(L-alanine) (PLA) and its N-deuterated analogue, and also developed an anharmonic formalism for a regular helical molecule.³ As a result of this work, we are able to present here a quantitative anharmonic analysis of the observed amide A region of α_1 -PLA.

Early polarized ir studies on synthetic polypeptides and fibrous proteins⁴ showed that in the (folded) α -form both the amide A and the amide I (mainly CO s⁵) bands are characterized by strong parallel (\parallel) dichroism, while in the (extended) β -form both bands are characterized by perpendicular (\perp) dichroism. Due to the difficulty in making an oriented sample, only a few polarized ir studies have so far been done for the PLA molecule,^{6–9} while polarized spectra for the amide A region can be found only in the work by

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Elliott.⁶ He observed two main bands in the NH s frequency region: at 3293S(\parallel) (broad) and 3067W(\parallel) cm^{-1} for α -PLA and at 3283S(\perp) (broad) and 3076W(\perp) cm^{-1} for β -PLA. These bands are related to the stretching of the NH bond since they disappear on N-deuteration. The stronger one is called the amide A and the weaker one the amide B band, and two similar bands are present in the ir spectra of peptides, polypeptides, and proteins. As a reasonable explanation of the observed weak band (which is considered too strong to be only an overtone or a combination of lower frequency bands), it is widely accepted that it derives from an overtone of amide II (NH in-plane angle bend, *ib*), or a combination of amide I and amide II, that interacts with the strong NH s fundamental through a cubic anharmonic potential, acquiring enough intensity to be observable through a Fermi resonance (FR) and resulting in two bands (amide A and amide B) whose separation is proportional to the interaction strength.^{10–12} The previous FR analyses in this region for α -PLA^{12,13} were based on unpolarized ir spectra on an unoriented sample.¹³ Rabolt et al.¹³ observed the two bands at 3307S (somewhat broad) and 3058W cm^{-1} , and considered the weak band to be an overtone of the amide II band at 1545(E_1) cm^{-1} . (In general for α -helical structures, optically active vibrations are classified into three symmetry species—A, E_1 , and E_2 —where A (\parallel dichroism) and E_1 (\perp dichroism) modes are both ir and Raman active, while E_2 modes are only Raman active.³) On N-deuteration, they observed two new bands related to the stretching of the ND bond that appeared at 2464S and 2407M cm^{-1} . Krimm and Dwivedi¹² considered the band at 2407 cm^{-1} to be a combination of the bands at 1439(E_1) and 974(E_1) cm^{-1} .

Our new polarized study on α -helical PLA,¹ which differs from previous results, shows two bands with strong \parallel ir dichroism corresponding to the NH s vibration when the residual solvent dichloroacetic acid (DCA) is almost completely washed out from the sample films. We find that this doublet feature in the amide A band depends on the amount of residual DCA in the sample: for samples with more DCA this doublet nature disappears and the same spectral features are found as in the previous study.⁶ Corresponding to this change in the amide A band, spectral changes depending on the DCA amount are also observed in other amide bands, viz., amides B, II, and V (NH out-of-plane bend plus CN torsion). Judging from its strong \parallel dichroism, this amide A doublet seems to arise from two different structures, the stronger of the two components being associated with the standard α_1 -helical structure in the chain.

In this paper we present detailed features of the newly observed polarized ir spectra in the amide A region, including band-resolved spectra. In order to do a more accurate anharmonic analysis in this region for the α_1 -PLA structure than was previously possible,¹⁴ cubic anharmonic force constants related to the NH s mode have been computed. Based on these results, a rigorous analysis has been done for this region, and a reasonable unperturbed NH s frequency for α_1 -PLA has been obtained through an FR analysis.^{10–12}

EXPERIMENTAL METHODS

The PLA samples used in this study were purchased from Sigma Chemical Co. and used as received (molecular weight based on viscosity determination \approx 23,000). The experimental details, including the method of making oriented sample films from concentrated solutions of PLA in DCA and of preparing N-deuterated PLA, α -PLA-ND, are given elsewhere.¹ The sample films used for ir measurements were prepared on silver chloride (AgCl) plates. The residual DCA in a sample film can be removed by extraction with diethyl ether or water followed by drying in a vacuum oven. The observed ir spectra are labeled in three different ways depending on the residual DCA amount in the sample: a spectrum with “no DCA” is taken from a sample in which the residual DCA is nearly completely washed out, the one “with DCA” is taken from a sample dried only by vacuum extraction without any treatment with diethyl ether or water, and one with “intermediate DCA” is taken from a sample containing an amount of DCA intermediate between the above two. The intensity of the 1752 cm^{-1} (C=O s) DCA band is consistent with these designations.

COMPUTATIONAL METHODS

Curve Fitting

The observed ir spectrum in the amide A and amide B regions for an oriented PLA sample was resolved into component bands by using the Voigt function,¹⁵ which is a convolution of a Gaussian (G) and a Lorentzian (L):

$$I(\nu) = \int_{-\infty}^{\infty} G(\nu - t)L(t) dt \quad (1)$$

The Lorentzian represents the true intensity distribution of a spectral line, while the Gaussian takes account of the slit distortion. Specifically, the observed intensity was fitted to the function¹⁵

Table I Peptide Unit Cubic Force Constants in Internal Coordinates^a (≥ 0.05 mdyne/Å³) for *trans*-N-Methylacetamide^b

1	1	1	-25.80	2	1	1	-0.82	2	2	1	-2.08	2	2	2	-98.12	3	1	1	-0.73
3	2	1	-0.27	3	2	2	-2.85	3	3	1	-0.81	3	3	2	-2.47	3	3	3	-49.23
4	1	1	0.07	4	2	2	-0.30	4	3	1	0.12	4	3	2	0.16	4	3	3	-0.27
4	4	1	0.05	4	4	2	0.07	4	4	3	0.20	4	4	4	-53.32	5	2	2	-0.15
5	3	2	0.30	5	3	3	-0.47	5	4	3	-0.14	5	4	4	0.25	5	5	1	-0.06
5	5	2	0.13	5	5	3	-0.37	5	5	4	-0.19	5	5	5	-32.88	12	1	1	-0.49
12	2	1	0.21	12	2	2	1.24	12	3	1	-1.05	12	3	2	0.55	12	4	1	0.14
12	4	2	0.07	12	4	3	0.33	12	5	2	-0.07	12	5	3	-0.06	12	5	5	-0.10
12	12	1	-1.50	12	12	2	-0.59	12	12	3	-1.46	12	12	4	0.32	12	12	5	-0.09
12	12	12	-1.78	13	1	1	-0.58	13	2	1	-0.75	13	2	2	0.27	13	3	2	1.22
13	3	3	0.96	13	4	2	0.13	13	4	3	0.08	13	4	4	-0.08	13	5	2	-0.07
13	5	5	-0.13	13	12	1	0.36	13	12	2	-0.20	13	12	3	-0.59	13	12	4	-0.05
13	12	12	0.19	13	13	1	-0.59	13	13	2	-1.93	13	13	3	-1.02	13	13	4	-0.11
13	13	12	1.09	13	13	13	0.46	14	2	2	0.17	14	3	2	0.08	14	3	3	-0.49
14	4	4	-0.23	14	5	2	0.18	14	5	3	-0.65	14	5	4	0.09	14	5	5	-0.24
14	12	2	-0.05	14	12	3	0.51	14	12	4	-0.15	14	13	2	-0.12	14	13	3	0.49
14	13	5	0.08	14	13	12	-0.20	14	13	13	-0.34	14	14	1	-0.05	14	14	2	0.08
14	14	3	-1.24	14	14	5	-1.11	14	14	12	0.21	14	14	13	0.56	14	14	14	-1.59
15	2	1	-0.05	15	2	2	-0.22	15	3	2	0.12	15	3	3	-0.45	15	4	1	0.06
15	4	3	-0.14	15	5	2	0.05	15	5	3	-0.06	15	5	4	0.13	15	5	5	0.59
15	12	1	-0.13	15	12	2	0.05	15	12	3	-0.20	15	12	4	0.17	15	12	12	-0.28
15	13	2	0.11	15	13	3	-0.05	15	14	3	0.21	15	14	5	-0.24	15	15	1	-0.05
15	15	3	-0.33	15	15	4	-0.09	15	15	5	-0.35	15	15	12	-0.06	15	15	13	0.06
15	15	14	0.41	15	15	15	-0.07	28	28	1	-0.48	28	28	2	-0.69	28	28	3	-0.62
28	28	12	-0.61	28	28	13	0.07	29	28	5	-0.06	29	29	2	0.15	29	29	3	-0.60
29	29	4	-0.10	29	29	5	-0.28	29	29	14	-0.17	31	28	2	0.08	31	28	3	0.11
31	28	12	-0.13	31	28	13	-0.14	31	28	14	0.06	31	29	1	0.06	31	29	5	0.07
31	29	12	0.10	31	29	14	0.10	31	31	2	0.22	31	31	3	-0.79	31	31	5	-0.12
31	31	13	0.14	31	31	14	-0.11												

^a Internal coordinate indices are the same as those defined in Ref. 1.^b Calculated at HF/6-31+G* level.

$$I(\nu) = b_0 + b_1\nu + b_2\nu^2 + \sum_i h_i K \sigma_i U[2K(\nu - t_i), K\sigma_i] \quad (2)$$

where b_j are the linear background parameters,

$$U(x, y) = \frac{y}{\sqrt{\pi}} \int_{-\infty}^{\infty} \frac{e^{-s^2}}{y^2 + (x - s)^2} ds \quad (\text{for } y \neq 0) \quad (3)$$

$K = \sqrt{\ln 2/S}$ with S = full width at half maximum of the Gaussian slit function, and h_i , σ_i , and t_i are parameters related to the i th peak height, width, and position, respectively. The intensity I_i of the i th peak (within a proportionality factor) was computed by the area of the deconvoluted Lorentzian component:

$$I_i = h_i \int_{-\infty}^{\infty} \frac{d\nu}{1 + [2(\nu - t_i)/\sigma_i]^2} = \frac{\pi}{2} h_i \sigma_i \quad (4)$$

Calculation of Cubic Force Constants for α -PLA

In order to accurately determine the unperturbed NH s frequency associated with the observed amide A band, which is in FR with overtones or combinations of lower frequency bands, it is first necessary to compute the relevant anharmonic force constants, since the frequency shift due to an FR between two accidentally degenerate states is roughly proportional to the anharmonic interaction strength,¹⁶ $|\phi_{rst}|$, where ϕ_{rst} is the corresponding cubic interaction force constant. If $|\phi_{rst}|$ is small, then no significant FR can occur between the two related states, even though the two frequency values are very close and the modes have proper symmetries for an FR. Specifically, we need to compute such anharmonic force constants in dimensionless normal coordinates as $\phi_{rst}(0, 0, 0)$, the interaction strength within A-species modes, and $\phi_{rst}(-\theta, \theta, 0)$, the interaction strength between an A-species mode and an overtone or a combination from other (E_1 or E_2) species, where the phase differ-

Table II Cubic Force Constants in Dimensionless Normal Coordinates ($\geq 1 \text{ cm}^{-1}$) Related to the Amide A, I, and II Modes for α_1 -PLA

NH-(A, A, A): $\phi_{rst}(0, 0, 0)^a$															
1	1	1	3008	6	1	1	16	6	6	1	4	6	6	6	-501
7	1	1	8	7	6	1	-51	7	6	6	193	7	7	1	194
7	7	6	-457	7	7	7	254								
NH-(E ₁ , E ₁ , A): $ \phi_{rst}(-\delta, \delta, 0) $															
1	1	1	5	6	6	6	591	7	6	6	72	7	7	6	88
7	7	7	22												
NH-(E ₂ , E ₂ , A): $ \phi_{rst}(-2\delta, 2\delta, 0) $															
1	1	1	5	6	6	6	577	7	6	6	102	7	7	6	76
7	7	7	22												
ND-(A, A, A): $\phi_{rst}(0, 0, 0)$															
5	5	5	1856	6	5	5	69	6	6	5	-13	6	6	6	-452
9	5	5	-43	9	6	5	25	9	6	6	-143	9	9	5	99
9	9	6	-276	9	9	9	-451	16	5	5	53	16	6	5	-9
16	6	6	6	16	9	5	45	16	9	6	-45	16	9	9	-51
16	16	5	77	16	16	6	9	16	16	9	-53	16	16	16	533
ND-(E ₁ , E ₁ , A): $ \phi_{rst}(-\delta, \delta, 0) $															
5	5	5	1873	6	5	5	64	6	6	5	13	6	6	6	613
9	5	5	34	9	6	5	12	9	6	6	71	9	9	5	26
9	9	6	61	9	9	9	72	16	5	5	46	16	6	6	114
16	9	5	8	16	9	6	22	16	9	9	69	16	16	5	4
16	16	6	30	16	16	9	13	16	16	16	48				
ND-(E ₂ , E ₂ , A): $ \phi_{rst}(-2\delta, 2\delta, 0) $															
5	5	5	1873	6	5	5	63	6	6	5	14	6	6	6	606
9	5	5	30	9	6	5	10	9	6	6	51	9	9	5	26
9	9	6	62	9	9	9	67	16	5	5	41	16	6	6	97
16	9	5	9	16	9	6	23	16	9	9	57	16	16	5	6
16	16	6	33	16	16	9	8	16	16	16	46				

^a Shown are the normal coordinate indices of α_1 -PLA. For α -PLA-NH, 1 = NH s, 6 = amide I (CO s), and 7 = amide II (NH ib plus CN s), while for α -PLA-ND, 5 = ND s, 6 = amide I' (CO s), 9 = amide II' (CN s plus CH₃ ab2), and 16 = amide III' (ND ib), where the internal coordinate names in the parenthesis represent the main potential energy distribution components of that mode. Since $\phi_{rst}(-2\delta, 2\delta, 0)$ and $\phi_{rst}(-\delta, \delta, 0)$ ($\delta = 99.57^\circ$, rotation per residue) are complex numbers, only their absolute values are shown.

ence between adjacent groups is $\theta = \delta$ (E₁ species) or 2δ (E₂ species) with $\delta = 99.57^\circ$ (rotation per residue) for α_1 -PLA. Following the general vibrational formalism for an infinite regular helix,³ these quantities are found to be

$$\begin{aligned} & \phi_{rst}(0, 0, 0) \\ &= \frac{1}{hc} \sum_{\mu, \nu, \lambda=1}^p \sum_{i, j, k=1}^3 \left\{ \sum_{\alpha, \beta, \gamma=1}^{N_R} \mathbf{F}_{\alpha\beta\gamma}(0, 0) \mathbf{B}_{\mu'}^{\alpha}(0) \mathbf{B}_{\nu'}^{\beta}(0) \mathbf{B}_{\lambda'}^{\gamma}(0) \right. \\ &+ \sum_{\beta, \gamma=1}^{N_R} \mathbf{F}_{\beta\gamma}(0) \{ \mathbf{B}_{\mu'\nu'}^{\beta}(0, 0) \mathbf{B}_{\lambda'}^{\gamma}(0) + \mathbf{B}_{\mu'\lambda'}^{\gamma}(0, 0) \mathbf{B}_{\nu'}^{\beta}(0) \} \\ &+ \left. \sum_{\alpha, \gamma=1}^{N_R} \mathbf{F}_{\alpha\gamma}(0) \mathbf{B}_{\nu'\lambda'}^{\gamma}(0, 0) \mathbf{B}_{\mu'}^{\alpha}(0) \right\} \mathbf{I}_r^{\mu'}(0) \mathbf{I}_s^{\nu'}(0) \mathbf{I}_t^{\lambda'}(0) \quad (5) \end{aligned}$$

$$\phi_{rst}(-\theta, \theta, 0) = \frac{1}{hc} \sum_{\mu, \nu, \lambda=1}^p \sum_{i, j, k=1}^3$$

$$\left\{ \sum_{\alpha, \beta, \gamma=1}^{N_R} \mathbf{F}_{\alpha\beta\gamma}(\theta, 0) \mathbf{B}_{\mu'}^{\alpha}(-\theta) \mathbf{B}_{\nu'}^{\beta}(\theta) \mathbf{B}_{\lambda'}^{\gamma}(0) \right\}$$

$$\mathbf{I}_r^{\mu'}(-\theta) \mathbf{I}_s^{\nu'}(\theta) \mathbf{I}_t^{\lambda'}(0) \quad (6)$$

where

$$\mathbf{F}_{\beta\gamma}(\theta) \equiv \sum_{n=-\infty}^{\infty} \mathbf{F}_{\beta\gamma}^{0,n} e^{in\theta} \quad \text{and}$$

$$\mathbf{F}_{\alpha\beta\gamma}(\theta', \theta) \equiv \sum_{m, n=-\infty}^{\infty} \mathbf{F}_{\alpha\beta\gamma}^{0,m,n} e^{i(m\theta' + n\theta)} \quad (7)$$

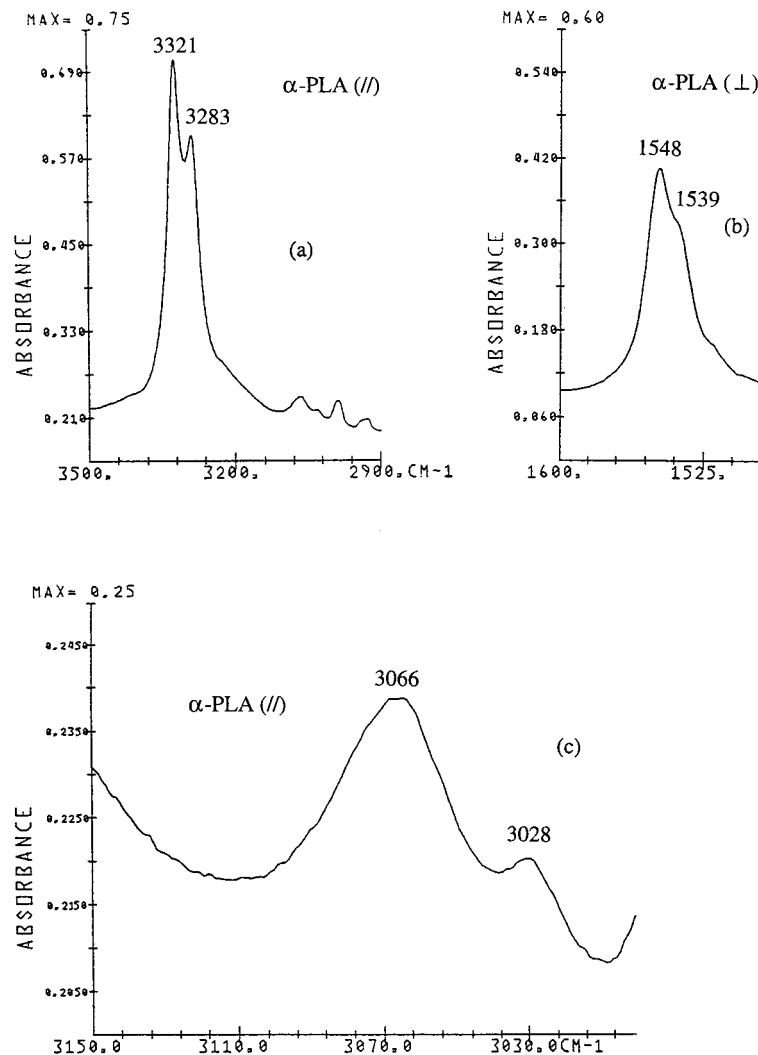


FIGURE 1 Polarized ir spectra of oriented α -poly(L-alanine) films with varying amounts of residual DCA. (a)–(c) With no DCA (see text): (a) amide A(A), (b) amide II(E_1), and (c) amide B(A) regions. (d)–(f) With intermediate amount of DCA (see text): (d) amide A(A), (e) amide II(E_1), and (f) amide B(A) regions. (g)–(i) With DCA (see text): (g) amide A(A), (h) amide II(E_1), and (i) amide B(A) regions.

are force constants in internal symmetry coordinates, $\mathbf{B}_{\lambda^k}^{\alpha}(\theta)$ and $\mathbf{B}_{\nu^j, \lambda^k}^{\alpha}(\theta', \theta)$ are the first- and second-order \mathbf{B} -matrix elements, respectively, and $I_X(\theta)$ is the Cartesian eigenvector matrix in dimensionless normal coordinates, $\mathbf{q}^r(\theta)$. Here, we are interested especially in the case when t designates the coordinate index of the NH s mode, and r and s represent those of amide I or amide II modes.

In applying Eqs. (5) and (6) to the α_1 -PLA structure, the values of quadratic force constants $\mathbf{F}_{\beta\gamma}^{0,n}$ were taken from the newly refined force field for α_1 -PLA.¹ Since the values of cubic force constants for intergroup interactions, viz., $\mathbf{F}_{\alpha\beta\gamma}^{0,m,n}$ for $m \neq 0$ or $n \neq 0$, are not available at present, as an approximation we

used only those for intragroup interactions, viz., $\mathbf{F}_{\alpha\beta\gamma}^{0,0,0}$, which can be transferred from those of an ab initio calculation for a *trans*-N-methylacetamide (*trans*-NMA) molecule [i.e., $\mathbf{F}_{\alpha\beta\gamma}(\theta, 0)$ in Eq. (6) is approximated by $\mathbf{F}_{\alpha\beta\gamma}^{0,0,0}$]. We believe this approximation will still preserve the main anharmonic features related to the NH s , amide I, and amide II modes, since their vibrations are fairly localized. The initial unscaled HF/6-31+G* ab initio cubic force field for *trans*-NMA in Cartesian coordinates was transformed to a force field in internal coordinates (Table I) by using an inverse \mathbf{B} -matrix transformation, since force constants in internal coordinates are essentially transferable between similar local structures. Second-order

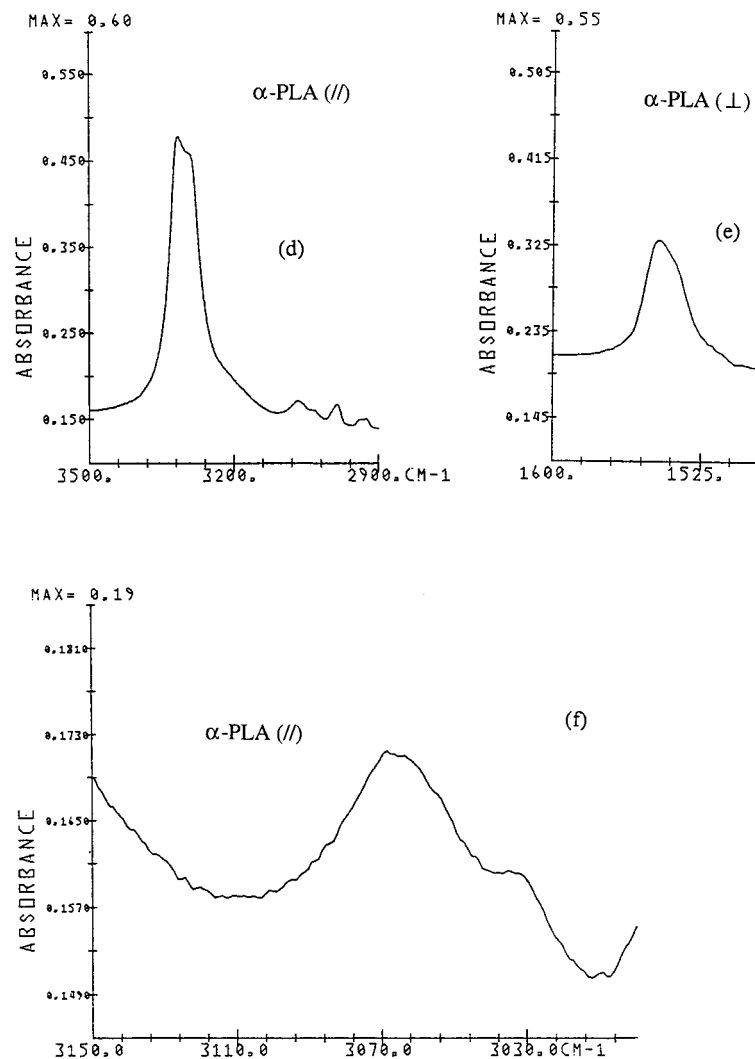


FIGURE 1 (Continued from the previous page)

B-matrix elements were computed following a new procedure.¹⁷ The resulting cubic force constants (related to the amide A, I, and II modes) in dimensionless normal coordinates for α_1 -PLA that are greater than 1 cm^{-1} are listed in Table II. The most important result of this calculation is that, different from previous α -PLA studies^{13,14} in which the NH s mode was considered to be in FR with the first overtone of amide II(E_1), only the first overtone of amide II(A) is dominantly in FR with the fundamental: $\phi_{771}(0, 0, 0) = 194 \text{ cm}^{-1}$ while $|\phi_{771}(-\delta, \delta, 0)| < 1 \text{ cm}^{-1}$ ($7 = \text{amide II}, 1 = \text{NH s}$).

RESULTS AND DISCUSSION

Observed Spectral Features in the Amide Band Regions

The most important new finding in the NH s frequency region of α -PLA is the fact that, different

from previous observations, our polarized ir spectra of an oriented sample with no DCA [Figure 1(a)] show two doublets of nearly the same separation with \parallel dichroism¹: amide A at 3321VS and 3283S cm^{-1} and amide B near 3066MW (broad) and 3028W cm^{-1} . These features are also found in our ordinary spectrum of an unoriented sample with no DCA (Figure 2). A similar amide A doublet is also observed in a polarized Raman spectrum,² with components at 3311MS(A) and 3288M(A) cm^{-1} . Curve resolution of this region [Figure 3(a)] permits the identification of ten component bands, whose position, shape, and intensity are given in Table III. It is interesting to note that the average frequency of the amide A ir doublet is close to the observed value of 3307 cm^{-1} for an unoriented sample found by Rabolt et al.¹³ With increasing amounts of DCA, the 3321 cm^{-1} band weakens and the overall peak position shifts to 3295 cm^{-1} [Figure 1(g)], reflecting the increased contribution of

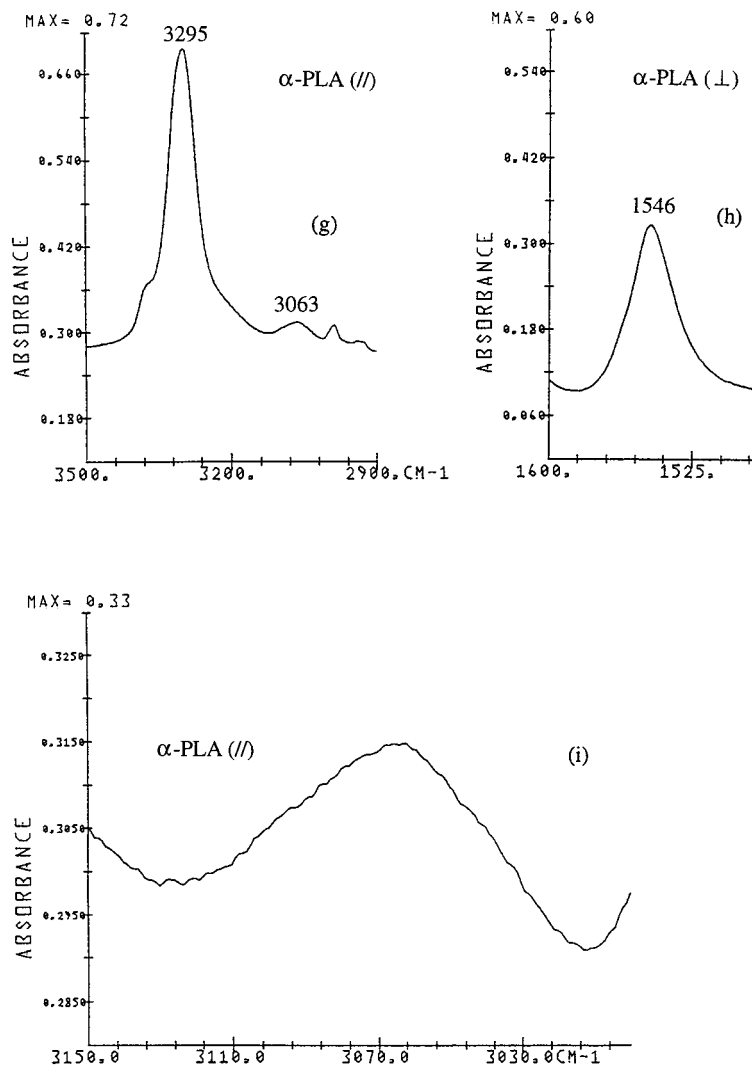


FIGURE 1 (Continued from the previous page)

the 3283 cm^{-1} component, while the 3066 cm^{-1} band shifts to 3063 cm^{-1} [Figure 1(i)].

Although the 3283 cm^{-1} component is coincident with the characteristic β -PLA band,⁶ its strong \parallel dichroism is opposite to the strong \perp dichroism expected of an oriented β -PLA structure. Also, no comparably strong characteristic β bands [e.g., 1632(\perp), 1524(\parallel), and 1222(\parallel) cm^{-1}] appear in the lower frequency spectrum.¹ Considering the observed very weak intensity at 1632(\perp) and 1221(\parallel) cm^{-1} in our sample,¹ the relative intensity at 3283(A) cm^{-1} to that at 3321(A) cm^{-1} is too high ($I_4/I_2 = 0.656$; see Table III) to attribute it to β -PLA. [Furthermore, the weak 1632(\perp) cm^{-1} band is reasonably assignable to a combination.¹] We therefore exclude a significant β -PLA origin for the 3283(A) cm^{-1} band. Considering its strong \parallel dichroism, this additional band seems to be due to a (probably helical) structure

different from the standard α_1 -helix. We assume that the higher intensity component (3321VS cm^{-1}) is due to the α_1 -helix structure. The weaker band (at 3283 cm^{-1}) we associate with another structure, whose origin we consider elsewhere.¹⁸ From the resolved intensity ratio, the additional structure constitutes about 40% of this sample with no DCA.

The observed 1658VS(A) cm^{-1} amide I band¹ does not show an obvious doublet structure, but its broad appearance indicates that it is definitely composite, which would suggest that the amide I bands of the α_1 -helix and of the additional structure have frequencies close together. However, this is not the case for amide II. A noticeable difference from the previous observations is found in the amide II(E_1) region: in a polarized spectrum of an oriented sample with no DCA [Figure 1(b)], a new band with \perp dichroism centered near 1539MS(E_1) cm^{-1} is present as a shoul-

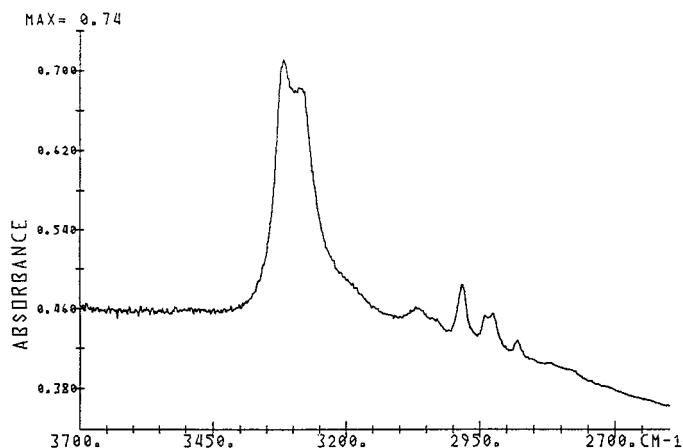


FIGURE 2 Unpolarized ir spectrum of unoriented α -poly(L-alanine) film with no DCA (see text) in 2600–3700 cm^{-1} region.

der on the very strong amide II band at 1548VS(E_1) cm^{-1} , with about the same intensity ratio as the weaker component in the amide A doublet. With increasing DCA content, the doublet character diminishes and the overall peak position shifts to 1546 cm^{-1} [Figure 1(h)]. These amide A, B, and II features with DCA were the ones observed in the previous study.⁶ In the amide II region of the \perp spectrum of an α -PLA-ND sample,¹ we find that the residual α -PLA-NH exhibits a doublet in which the 1539 cm^{-1} band is stronger compared to that at 1548 cm^{-1} . Since the 3283 cm^{-1} band is also comparably stronger with respect to that at 3321 cm^{-1} , this is compelling evidence that the 1539 cm^{-1} band should be assigned to the additional structure. Both amide V modes, at 612MS(E_1) and 639M(A) cm^{-1} in α_1 -PLA,¹ have shifted to 621M(E_1) and 677MS(A) cm^{-1} with increasing amounts of DCA (Figure 4).

Thus, the bands at 3321(A), 1548(E_1), 612(E_1), and 639(A) cm^{-1} , which are prominent in oriented samples with no DCA, are assumed to be due to the α_1 -helix structure, while the bands at 3283(A), 1539(E_1), 621M(E_1), and 677MS(A) cm^{-1} are assumed to be associated with the additional structure.

Fermi Resonance Analysis in the Amide A and B Regions

Before we can do an FR analysis,^{10–12} we have to properly assign the first overtones of the three observed amide II modes at 1518W(A), 1539MS(E_1), and 1548VS(E_1) cm^{-1} to the observed amide B bands. If we assign the stronger band near 3066MW(A) cm^{-1} to an overtone of 1548VS(E_1) cm^{-1} , the weaker band at 3028W(A) cm^{-1} is at too low a frequency to be assigned to the first overtone of

1539MS(E_1) cm^{-1} : the observed separation of 38 cm^{-1} is too large compared to the expected (maximum) separation of 18 ($=2 \times [1548-1539]$) cm^{-1} . With residual DCA, the 3028W(A) cm^{-1} band, which also disappears on N-deuteration, appears to move up to near 3035 cm^{-1} , with decreased intensity so as to be hardly discernible because of overlap with the shifted 3066MW(A) cm^{-1} band. Recalling the intensity decrease with DCA of the band at 3321VS, the 3028W(A) cm^{-1} band is thus reasonably considered to be in FR with the amide A band of the α_1 -helix. It is better assigned to the first overtone of the amide II band at 1518W(A) cm^{-1} , acquiring its intensity mostly from an FR with the strong NH s mode. This is also in excellent agreement with the prediction of our cubic interaction force constant calculation, i.e., $\phi_{771}(0, 0, 0) = 194 \text{ cm}^{-1}$ and $|\phi_{771}(-\delta, \delta, 0)| < 1 \text{ cm}^{-1}$.

Although the above assignment may seem strange in view of the larger intensity of the 3066MW(A) cm^{-1} band, a more careful look at this band reveals that it is actually composed of more than one band. Although the fitting may be somewhat uncertain, this band is well resolved into at least two bands at 3070MW and 3062W cm^{-1} [Figure 3(a)]. While the 3062W cm^{-1} band does not shift much with changes in the amount of residual DCA, the band at 3070MW cm^{-1} seems to decrease in intensity in proportion to the residual DCA. Thus, we assign the 3070MW cm^{-1} band to an overtone of the 1548VS(E_1) cm^{-1} band of the α_1 -helix and the 3062W cm^{-1} band to an overtone of the 1539MS(E_1) cm^{-1} band of the additional structure. The observed overtone intensity at 3070MW(A) cm^{-1} seems to be due mainly to the pure anharmonic interaction term related to $|\phi_{777}(-\delta, \delta, 0)| = 22 \text{ cm}^{-1}$ together with the very strong

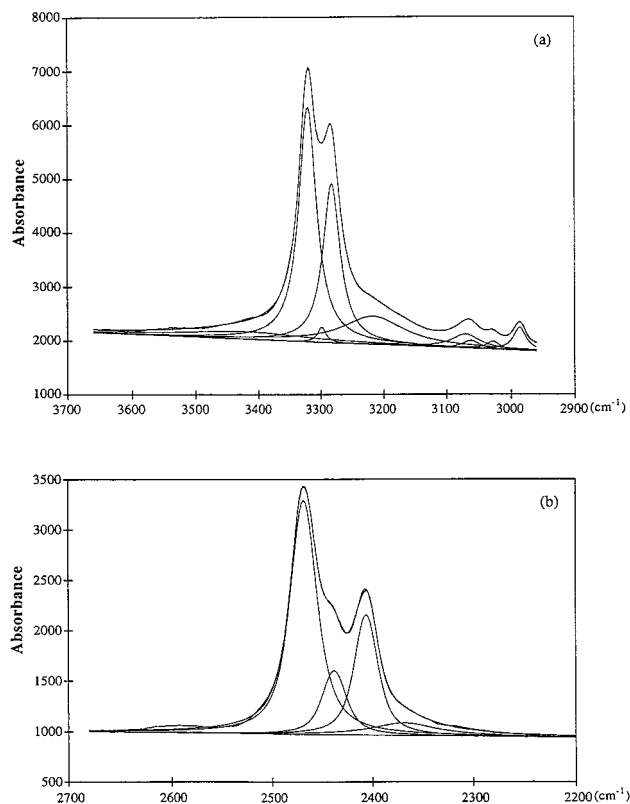


FIGURE 3 Curve-resolved infrared spectra of \parallel -polarized infrared spectra in amide A and amide B region of (a) α -poly(L-alanine) and (b) α -poly(L-alanine-ND). Note that the sum of the resolved components is almost exactly equal to the experimentally observed curve.

$1548\text{VS}(\text{E}_1) \text{ cm}^{-1}$ amide II fundamental intensity,¹⁹ rather than from an FR with the NH s fundamental (since $|\phi_{771}(-\delta, \delta, 0)| < 1 \text{ cm}^{-1}$).

The two unidentified very broad bands at 3419W and $3217\text{M} \text{ cm}^{-1}$ in Figure 3(a) seem to originate from the background water residing in the AgCl on which the oriented sample films are made. During the curve fitting process, it turned out that a new band at $3299\text{W}(\text{A}) \text{ cm}^{-1}$, which was completely hidden between the two strong amide A bands, was essential for a satisfactory fit in this region. Although this band, presumably an overtone of the amide I band at $1658\text{VS}(\text{A}) \text{ cm}^{-1}$, is much closer than the others to the amide A band at $3321(\text{A}) \text{ cm}^{-1}$, it does not seem to be in FR with NH s since the corresponding cubic anharmonic interaction force constant is found to be small, viz., $\phi_{661}(0, 0, 0) = 4 \text{ cm}^{-1}$ ($6 = \text{amide I}$), and its intensity may arise from the interaction term related to $\phi_{666}(0, 0, 0) = -501 \text{ cm}^{-1}$ together with the very strong amide I(A) fundamental intensity.¹⁹ In fact, there are some other bands, such as a combination of amide I(A) and amide II(A) and a combination of amide I(E₁) and amide II(E₁), which are also within

close range of an FR with NH s, although our approximate calculation predicts small interaction strengths. Thus, the real interaction in the amide A region may be complex enough that a multilevel FR mechanism²⁰ should be used in order to calculate the most accurate unperturbed frequency of the NH s mode.

As a simple approximation, however, we consider an FR only between the amide A band at $3321\text{VS}(\text{A}) \text{ cm}^{-1}$ and the amide B band at $3028\text{W}(\text{A}) \text{ cm}^{-1}$, an overtone of amide II(A) at $1518\text{W} \text{ cm}^{-1}$, since the corresponding interaction is found to be the most effective one among the possibilities related to NH s. On the basis of such a two-level FR,^{10,11} the unperturbed frequencies, ν_{A}^0 and ν_{B}^0 , of the two related bands are given in terms of the observed (perturbed) frequencies, ν_{A} and ν_{B} , by

$$\nu_{\text{A}}^0 = \nu_{\text{A}} - \frac{1}{2}(d - d^0) \quad (8)$$

$$\nu_{\text{B}}^0 = \nu_{\text{B}} + \frac{1}{2}(d - d^0) \quad (9)$$

where $d \equiv \nu_{\text{A}} - \nu_{\text{B}}$ and $d^0 \equiv \nu_{\text{A}}^0 - \nu_{\text{B}}^0$, which is also determined by^{10,11}

Table III IR (A Species) Band Components in the Amide A Region and Fermi Resonance Analysis

		ν^{obs}	h^{a}	σ^{a}	I^{a}	Assignment	
NH	1	3419	138.2	234.5	50890	From background H ₂ O in AgCl	
	2	3321	4368.3	34.0	233253	Amide A of α_1 -helix	
	3	3299	288.5	15.3	6920	$2 \times [1658(\text{A})] = 3316(\text{A})$	
	4	3283	2960.8	32.9	152989	Amide A of additional structure	
	5	3217	520.9	117.8	96369	From background H ₂ O in AgCl	
	6	3164	56.3	50.7	4487	$1658(\text{A}) + 1518(\text{A}) = 3176(\text{A})$	
	7	3070	260.0	52.7	21513	$2 \times [1548(\text{E}_1)] = 3096(\text{A})$	
	8	3062	147.1	30.1	6962	$2 \times [1539\text{sh}(\text{E}_1)] = 3078(\text{A})$	
	9	3028	149.7	23.8	5591	$2 \times [1518(\text{A})] = 3036(\text{A})$	
	10	2986	431.9	23.9	16222	CH ₃ antisymmetric stretch	
ND	1	2468	2778.6	24.6	107200	Amide A' of α_1 -helix	
	2	2438	806.7	20.2	25603	Amide A' of additional structure	
	3	2406	1533.3	19.3	46432	$1429(\text{A}) + 1000(\text{A}) = 2429(\text{A})$	
	4	2368	127.9	74.7	15004	$1377(\text{A}) + 1000(\text{A}) = 2377(\text{A})$	
FR Analysis							
	$R = I_{\text{B}}/I_{\text{A}}$	$\Delta\nu^{\text{b}}$	ν_{A}	$\nu_{\text{A}}^{\text{0}}$	ν_{B}	$\nu_{\text{B}}^{\text{0}}$	
NH	$I_9/I_2 = 0.024$ 0.127^{c}	6.85 28	3321 3307	3314 3279	3028 3058	3035 3086	$2 \times [1518(\text{A})] = 3036(\text{A})$ $2 \times [1545(\text{E}_1)] = 3090(\text{A})$
ND	$I_3/I_1 = 0.433$ 0.35^{d}	18.70 15	2468 2464	2449 2449	2406 2407	2425 2422	$1429(\text{A}) + 1000(\text{A}) = 2429(\text{A})$ $1439(\text{E}_1) + 974(\text{E}_1) = 2413(\text{A})$

^a h = Peak height; σ = peak width; I = intensity proportionality factor.

^b $\Delta\nu \equiv (d - d^{\text{0}})/2 = (\nu_{\text{A}} - \nu_{\text{B}} - \nu_{\text{A}}^{\text{0}} + \nu_{\text{B}}^{\text{0}})/2$ (see text).

^c From Ref. 13.

^d From Ref. 12.

$$d^{\text{0}} = d \frac{1 - R}{1 + R} \quad (10)$$

where $R \equiv I_{\text{B}}/I_{\text{A}}$, the intensity ratio of the two observed bands. From our measured intensities of these bands (Table III), the predicted unperturbed amide A frequency $\nu_{\text{A}}^{\text{0}}$ is found to be 3314(A) cm^{-1} , which is significantly higher than the value of 3279(A) cm^{-1} predicted in the previous α -PLA study¹³ (Table III). (This results in an unperturbed amide B frequency $\nu_{\text{B}}^{\text{0}}$ of 3035 cm^{-1} .) In this connection, it is very interesting to consider the assignment of observed combinations with the NH s fundamental in the polarized near-ir spectra of an oriented α -PLA⁷: the 4860S(E₁) cm^{-1} band is assignable to the combination of amide II at 1548VS(E₁) cm^{-1} and NH s(A) (1548 + 3314 = 4862), and the 4965M(A) cm^{-1} band may arise from the combination of amide I at 1658VS(A) cm^{-1} and NH s(A) (1658 + 3314 = 4972). Since no significant FR is present in these two combination bands, and a pure anharmonic interaction mostly results in the corresponding observed (perturbed) frequency being lower than the unper-

turbed one, they reasonably provide the lower bound of the unperturbed NH s frequency independent of the more complicated FR analysis of the amide A fundamental frequency region. These two combination bands predict the value of the unperturbed amide A frequency for α_1 -PLA to be at least greater than 3307 and 3312 cm^{-1} , respectively, consistent with the result of our new FR analysis. Coincidentally, the predicted unperturbed amide A frequency of 3314(A) cm^{-1} from the FR analysis is very close to the observed Raman frequency² of 3311MS(A) cm^{-1} . The difference may be due to a frequency shift related to the larger amount of residual DCA in the thick Raman sample compared to the fairly thin ir sample, which contains nearly no residual DCA after extraction with diethyl ether or water.

In the case of the additional structure, the unperturbed NH s frequency cannot be determined unambiguously from the present data. This is because, although this structure should presumably have such bands, we cannot clearly separate its amide II(A) and first overtone from the α_1 -PLA bands; furthermore, the resolved 3062W cm^{-1} band is best assigned to an

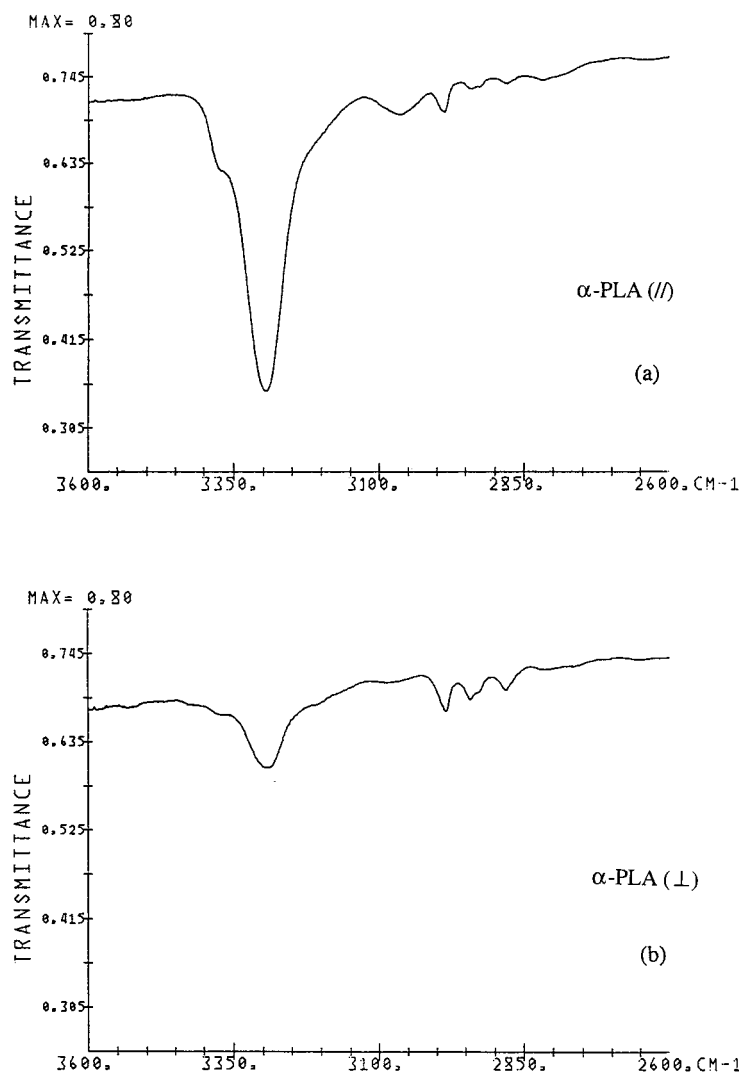


FIGURE 4 Polarized ir spectra of an oriented α -poly(L-alanine) film with DCA (see text). (a) and (c) Electric vector parallel to orientation direction. (b) and (d) Electric vector perpendicular to orientation direction.

amide II(E_1) overtone and thus cannot be in effective FR with its NH s(A) mode. If the situation is similar to that in the α_1 -helix, the unperturbed frequency of the additional structure might be lower than the observed value of 3283 cm^{-1} by the same order ($\sim 7\text{ cm}^{-1}$) as for the α_1 -helix.

On N-deuteration, we observed three bands related to the ND s mode, at 2468S(A) , 2438sh(A) , and $2406\text{MS(A)}\text{ cm}^{-1}$ [Figure 3(b)]. The medium shoulder at 2438 cm^{-1} seems to originate from the additional structure, and we assign the $2468\text{S(A)}\text{ cm}^{-1}$ band to amide A' and the $2406\text{MS(A)}\text{ cm}^{-1}$ band to amide B'. Even though the latter, as in the previous analysis,¹² can be considered as a combination of the bands at $1445(E_1)$ and $974(E_1)\text{ cm}^{-1}$ of α_1 -PLA-ND, the corresponding anharmonic interaction strength of

$|\phi_{16,9,5}(-\delta, \delta, 0)| = 8\text{ cm}^{-1}$ (16 = ND ib, 9 = amide II', 5 = ND s; see Table II) is small enough to suggest looking for other possibilities. Considering that $\phi_{16,9,5}(0, 0, 0) = 45\text{ cm}^{-1}$, the combination of amide II'(A) at 1429 cm^{-1} (CN s plus ND ib)¹ and the band at $1000\text{M(A)}\text{ cm}^{-1}$ (CH_3 rock plus ND ib)¹ is a more plausible assignment for the observed amide B' band at $2406\text{MS(A)}\text{ cm}^{-1}$, which is in FR with ND s. Even though the interaction strength in α_1 -PLA-ND, i.e., $|\phi_{16,9,5}(-\delta, \delta, 0)| = 45\text{ cm}^{-1}$, is smaller than $\phi_{7,7,1}(0, 0, 0) = 194\text{ cm}^{-1}$ of α_1 -PLA-NH, the overall FR effect is stronger in α_1 -PLA-ND than in α_1 -PLA-NH (i.e., amide B' is relatively stronger than amide B) because the unperturbed frequencies are much closer together in the former case than in the later. (It should be noted that the calculated cubic interaction force

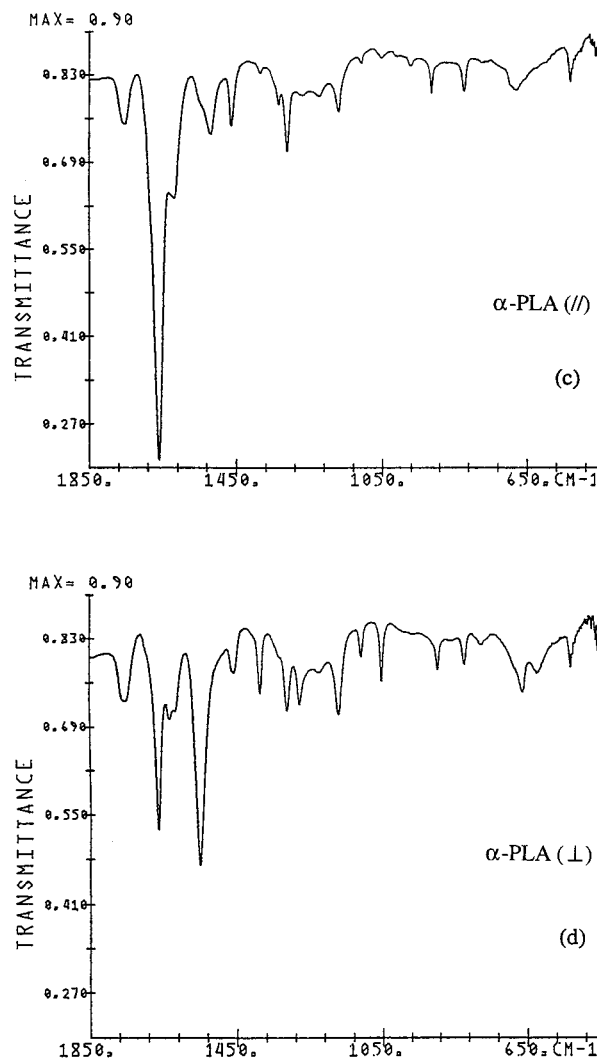


FIGURE 4 (Continued from previous page)

constant values for α_1 -PLA-ND are incomplete in that they were computed without full consideration of cubic force constants related to the side-chain methyl group. In α_1 -PLA-ND, ND is well mixed with CH_3 rock.¹) A similar FR analysis¹⁰⁻¹⁴ places the unperturbed frequency of NDs for the α_1 -helix at 2449 cm^{-1} . This is higher by 17 cm^{-1} than the value (2432 cm^{-1}) calculated by using the NH stretch force constant, 5.989 mdyn/\AA , that was refined to the predicted unperturbed NHs frequency of $3314(\text{A})\text{ cm}^{-1}$ for α_1 -PLA-NH. As was suggested in the previous vibrational analysis,¹³ this discrepancy may be due mainly to the difference in anharmonicity between NHs and NDs, since $\phi_{111}(0, 0, 0)(\text{NH}) = 3008\text{ cm}^{-1}$ and $\phi_{555}(0, 0, 0)(\text{ND}) = 1856\text{ cm}^{-1}$.

The unperturbed NDs of the additional structure is again difficult to determine because the corresponding amide B' band cannot be identified unambiguously

from the present data. Although the resolved broad band at 2368 cm^{-1} may be considered as the amide B' of the additional structure, it could arise from the combination of $1377\text{MW}(\text{A})$ and $1000\text{M}(\text{A})\text{ cm}^{-1}$ rather than from an FR with NDs [$|\phi_{16105}(0, 0, 0)| < 1\text{ cm}^{-1}$, 10 = normal coordinate index for the $1377(\text{A})\text{ cm}^{-1}$ band].

CONCLUSIONS

The calculation of cubic anharmonic force constant values for α_1 -PLA has enabled us to reasonably interpret the observed complex band structure in the amide A region and to predict that the FR with the NHs fundamental is most plausible with the first overtone of amide II(A), different from previous α -PLA studies^{13,14} in which it was considered to be in FR with the

first overtone of amide II(E_1). The predicted unperturbed NH s frequency of 3314 cm^{-1} (A), which is significantly higher than the previously proposed value of 3279 cm^{-1} , is within the predicted range from polarized near-ir spectra.⁷

According to the analysis of our polarized ir spectra, the spectrum of helical PLA depends on the amount of residual solvent DCA. For our samples containing almost no DCA, about 40% of the sample is a structure other than the standard α_1 -helix, with an NH s frequency that is lower than that of α_1 -PLA. The nature of this structure is the subject of a further study.¹⁸

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