Low-Frequency Infrared Bands and Chain Conformations of Polypeptides

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Synopsis

Infrared spectra of polypeptides were measured in the region of 1800–400 cm^{-1}. For the α-helical form, disordered form, and antiparallel-chain β-form, amide V bands arising from N-H out-of-plane bending modes were observed at 610–620, around 650, and 700–705 cm^{-1}, respectively, and amide V' bands arising from N-D out-of-plane bending modes were observed at 455–465, around 510, and 515–530 cm^{-1}, respectively. These correlations are useful for conformation diagnoses, particularly for copolyamino-acids or proteins which are not oriented. The nature of low-frequency amide bands are discussed with reference to potential energy distributions calculated for the α-helical form and β form.

INTRODUCTION

Polypeptides and proteins exhibit characteristic infrared bands at about 3300 (amide A), 3100 (amide B), 1650 (amide I), and 1550 cm^{-1} (amide II). Conformational changes of polypeptide chains may be studied by observing amide bands. It was first remarked by Ambrose and Elliott[1,2] that the frequencies and dichroism of these amide bands vary with chain conformations and then amide bands were extensively used for structure diagnoses.[3,4] Later, the nature of the amide I and II vibrations was elucidated by normal vibration treatments.[5] Also, correlations between amide bands and conformations of polypeptides were analyzed theoretically with the coupled-oscillator model and some additional correlations were found.[6-8] Accordingly, the α-helical form, parallel-chain β form, antiparallel-chain β form and disordered form may be distinguished by amide I and II bands observed for oriented samples of polypeptides or fibrous proteins.[9-12]

However, if various conformations coexist in unoriented samples, the fraction of each conformation may not necessarily be estimated by intensity measurements of the amide I and II bands only, because these amide bands of the α-helical form are hardly separated from those of disordered form. In view of these situations, characteristic amide bands in the low-frequency region were studied.

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Low-frequency amide bands were previously studied for model molecules[5,13] and the amide IV (627 cm^{-1}), V (725 cm^{-1}), and VI bands (600 cm^{-1}) of N-methylacetamide were found to arise from the C=O inplane bending mode, N-H out-of-plane bending mode, and C=O out-of-plane mode, respectively. These assignments were, in fact, confirmed by theoretical treatments of the in-plane[5] and out-of-plane normal vibrations[14] of N-methylacetamide. The atomic displacements involved in these amide vibrations are not highly localized within the CONH group, and infrared bands due to these vibrational modes are expected to vary sensitively with chain conformational changes. In fact, infrared absorption spectra of polypeptides were measured in the low-frequency region of 800–400 cm^{-1}, and amide V bands were found useful for conformation diagnoses of polypeptide chains as reported in a previous communication.[15] In the present paper, infrared spectra in the low-frequency region will be reported in detail and the nature of amide bands will be discussed with reference to vibrational potential energy distributions calculated for the α and β forms.[16] Also new correlations will be given for chain conformations and amide V' bands arising from N-D out-of-plane bending modes.

EXPERIMENTAL

Poly (γ-methyl L-glutamate) (PMLG) and poly-L-alanine (PLA) were synthesized by the N-carboxyamino acid anhydride method, as described previously for poly (γ-benzyl L-glutamate) (PBLG).[17-19] Samples of sodium poly-α,L-glutamate (SPLG), poly(γ-isoamyl L-glutamate) (PALG) and poly (γ-ethyl L-glutamate) (PELG) were generously given by Prof. Blout, Prof. Noguchi, and Ajinomoto, Ltd., respectively. N-Deuterated samples were prepared by dissolving undeuterated samples into O-deuterated trifluoroacetic acid[20] and was precipitated with ether, filtered, and finally dried. Infrared absorption measurements were made by use of a Perkin-Elmer Model 221 spectrophotometer and a Japan Spectroscopic Model IR-G spectrophotometer. Dichroic measurements of oriented solid films were made with a pair of silver chloride polarizers.

α-Helical Form

The polarized infrared spectra of high molecular weight PMLG (anhydride/initiator = 200) are shown in Figure 1 for the regions of 1800–1350 and 800–400 cm^{-1}. The amide I bands are observed at 1654 cm^{-1} (parallel) and 1659 cm^{-1} (perpendicular) and the amide II bands are observed at 1550 cm^{-1} (perpendicular) and 1519 cm^{-1} (parallel), characteristic of the α-helical conformation.[1,7] The polarized infrared spectra of the N-deuterated derivatives (Fig. 2) exhibit the parallel amide I' band at 1645 cm^{-1} and the perpendicular amide II' band at 1445 cm^{-1}.

In the low-frequency region, a strong perpendicular band of PMLG is observed at 615 cm^{-1}, which is shifted, on N-deuteration, to 462 cm^{-1}

got PMLG-d (Figs. 1 and 2). The frequency ratio of these amide bands (615/462 = 1.33) indicates that these bands arise largely from the N-H and N-D out-of-plane bending modes (amide V and V'), respectively. The perpendicular dichroism of these bands is also in accord with these assignments, since the amide CONH (or COND) planes of the α-helical conformation are nearly parallel to the helix axis and the transition moment
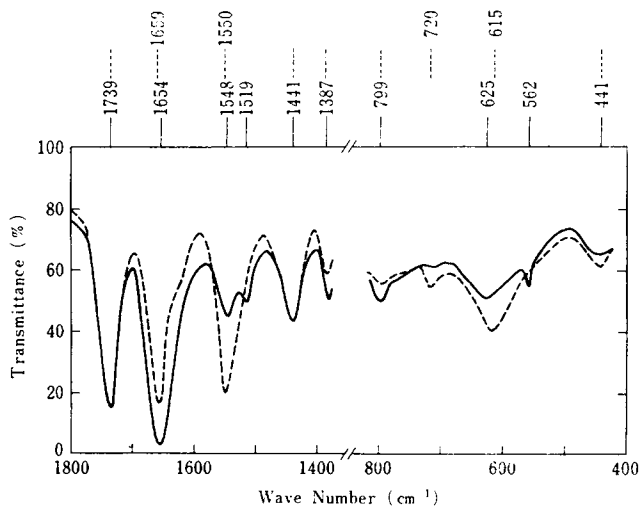
Fig. 1. Polarized infrared spectra of PMLG (A/I = 200) oriented film cast from chloroform solution: (——) electric vector parallel and (– –) perpendicular to the direction of orientation.
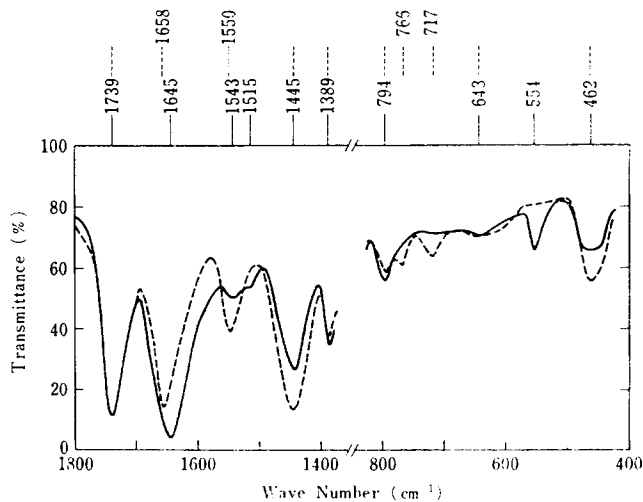
Fig. 2. Polarized infrared spectra of PMLG d (A/I = 200) oriented film cast from chloroform solution: (——) electric vector parallel and (– –) perpendicular to the direction of orientation.
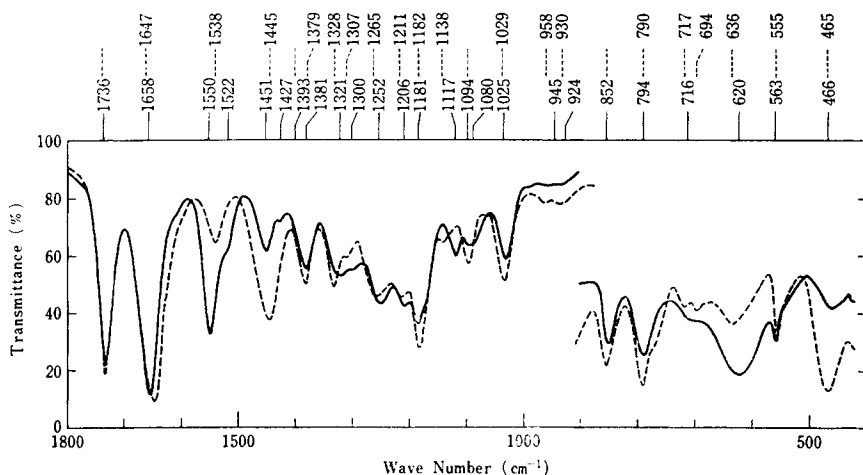
Fig. 3. Infrared spectra of (——) PELG and (– –) PELG-*d*. Films were cast from chloroform solution.

of the N-H (or N-D) out-of-plane bending mode is expected to be nearly perpendicular to the helix axis. The infrared spectra of high molecular weight PELG are shown in Figure 3. Again, the amide V and V′ bands are observed at 620 cm^{-1} and 465 cm^{-1}, respectively, similar to the cases of PBLG[19] and PALG (Table I).

TABLE I

Characteristic Amide Frequencies

	Frequency, cm^{-1}[a]			
	I(‖)	II(⊥)	V(⊥)	V′(⊥)
α-Helical Form				
Poly(γ-methyl L-glutamate)	1658	1550	620	462
Poly(γ-ethyl L-glutamate)	1658	1550	620	465
Poly(γ-isoamyl L-glutamate)	1656	1550	615	461
Poly(γ-benzyl L-glutamate)	1653	1550	615	458
Poly-L-alanine	1658	1548	610	456
Antiparallel-chain *β* Form				
Poly(γ-methyl L-glutamate)	1629	1531	700	531
Poly(γ-benzyl L-glutamate)	1629	1524	[b]	518
Poly-L-alanine	1632	1548	703	520
Disordered Form				
Sodium poly-L-glutamate	1655	[c]	650	510

[a] Frequencies are listed for the main component of amide I, II, and V bands of undeuterated species and amide V′ bands of *N*-deuterated species.

[b] Overlapped by the band due to benzyl groups.

[c] Overlapped by the band due to COO$^-$ groups.

The amide V band of α-helical poly-L-alanine (PLA) is observed at 610 cm^{-1}, which corresponds to the E vibration (phase difference of 100°) calculated at 612 cm^{-1} for the right-handed α-helical form.[16] In fact, the potential energy of this E vibration is associated primarily with the N-H out-of-plane bending mode. The amide V' band of N-deuterated derivative (PLA-d) is observed at 456 cm^{-1}. This band corresponds to the E vibration calculated at 442 cm^{-1}, which is largely associated with the N-D out-of-plane bending mode.[16]

Amide I, II, and V frequencies of PMLG, PELG, PALG, PBLG, and PLA are listed for comparison in Table I, together with amide V' frequencies of the N-deuterated derivatives. The frequencies of perpendicular amide V bands of the α-helical form are thus observed in a narrow frequency region of 610–620 cm^{-1} and perpendicular amide V' bands in the region of 455–465 cm^{-1}. Recently, amide V bands were also observed at 615 cm^{-1} for the α-helical form of poly(L-α-amino-n-butyric acid), poly-L-norleucine, and poly-L-leucine.[21]

The perpendicular band of PMLG at 720 cm^{-1} is shifted, on N-deuteration, to the perpendicular band of PMLG-d at 717 cm^{-1}. These bands appear to correspond to the bands of PLA at 656 cm^{-1} of PLA-d at 648 cm^{-1} (reference 16, Fig. 1). The nature of these bands may be discussed on the basis of the potential energy distributions calculated for the corresponding vibrations; the E vibrations calculated for PLA at 655 cm^{-1} is due to the N-H out-of-plane bending mode (amide V) strongly coupled with the C=O out-of-plane bending mode (amide VI) and E vibration calculated for PLA-d at 636 cm^{-1} is associated with the C=O out-of-plane bending mode (amide VI) and the N—C=O bending mode (amide IV).

For PLA and PLA-d, well-defined bands were observed at 528 and 524 cm^{-1}, respectively (reference 16, Fig. 1). As for potential energy distributions, the A vibration (phase difference of 0) of PLA calculated at 524 cm^{-1} and of PLA-d at 515 cm^{-1} are primarily due to the Cα—C=O bending modes. Corresponding parallel bands were observed at 562 cm^{-1} for PMLG, PELG, and PBLG and at 554 cm^{-1} for N-deuterated derivatives. For copoly(γ-methyl D,L-glutamate)[22] and copoly(γ-benzyl D,L-glutamate).[23] the band at 562 cm^{-1} was found to weaken markedly as the D/L ratio was raised from 0 to 1, and this band was used for estimating the fraction of "regular" helical form.[23]

Disordered Form

The amide I and II bands of sodium poly-α,L-glutamate in disordered form were observed at 1655 and 1535 cm^{-1}, respectively, for a solid film cast from aqueous solution in a dry atmosphere without unidirectional orientation.[7,24] Infrared absorption of a like film was measured in the low frequency region of 850–450 cm^{-1} and is shown in Figure 4. Since the infrared absorption of water abosrbed in solid films predominates in the region around 600 cm^{-1}, the solid film of sodium polyglutamate was dried in vacuum. The amide V band of the disordered form was now observed
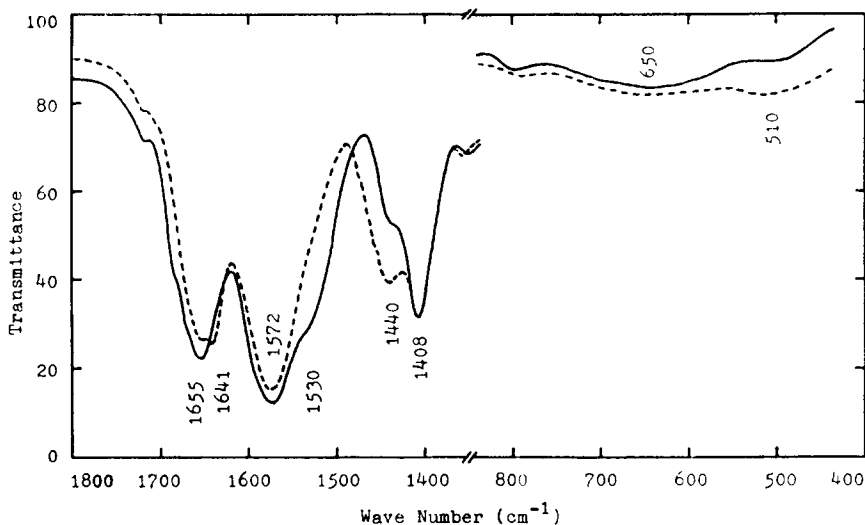
Fig. 4. Infrared spectra of solid films of (——) SPLG and (– –) SPLG-*d* in disordered form. Films were cast from aqueous solution and were dried *in vacuo*.

with the band center around 650 cm⁻¹, which is shifted on *N*-deuteration, to 510 cm⁻¹. For poly-DL-alanine (solid film),[22] the amide V band of the disordered region was observed at 665 cm⁻¹. The amide V band of the disordered form is quite diffuse and may readily be distinguished from the sharp amide V band of the α-helical form.

β-Form

For low molecular weight PMLG (anhydride/initiator = 4), the amide I bands (Fig. 5) were observed at 1695 and 1629 cm⁻¹ and the amide II band was observed at 1531 cm⁻¹, indicating that this polypeptide is in the antiparallel-chain β form.[1,7] In the low-frequency region, the strong band of PMLG at 700 cm⁻¹ is shifted, on *N*-deuteration, to the medium-intensity band of PMLG-*d* at 531 cm⁻¹. From the frequency ratio of 700/531 = 1.31, these bands are identified as the amide V and V′ bands of the β form.

For poly-L-alanine in the β form (Fig. 6), the amide V band is observed at 703 cm⁻¹, which is assigned to the *B* vibration (phase difference of 180°) calculated at 688 cm⁻¹, the N–H out-of-plane bending vibration coupled with the C=O out-of-plane bending mode.[16] The amide V′ band of PLA-*d* (*N*-deuterated with deuterium oxide) at 520 cm⁻¹ is assigned to the *B* vibration calculated at 542 cm⁻¹, which is primarily due to the N–D out-of-plane bending mode.[16] Similarly the amide V band of polyglycine I[14] in the β form is observed at 700 cm⁻¹.

The amide I, II, and V frequencies of PMLG, PBLG, and PLA and the amide V′ frequencies of *N*-deuterated derivatives are listed for comparison in Table I. The amide V bands of the antiparallel-chain β form
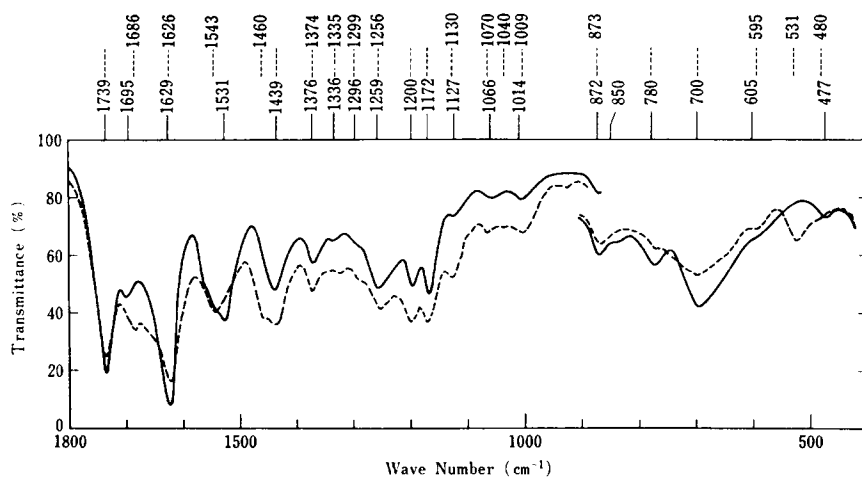
Fig. 5. Infrared spectra of solid films of (———) PMLG (A/I = 4) and (– –) PMLG *d*.
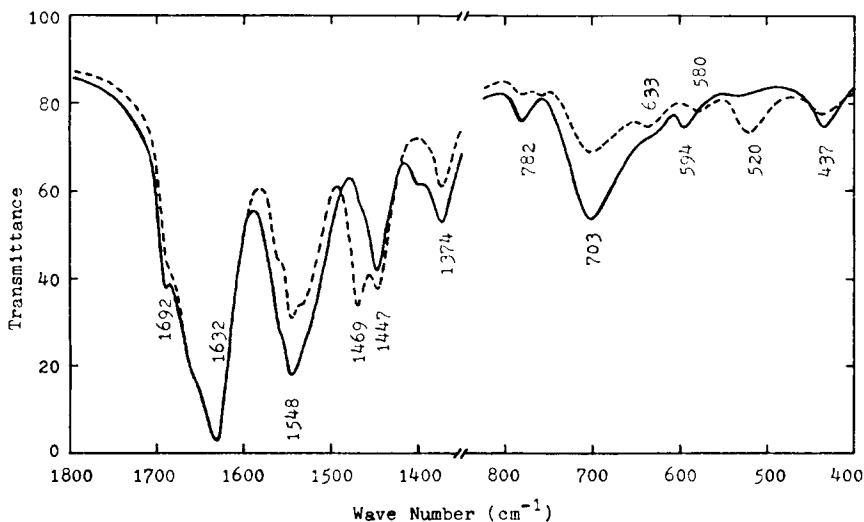Films were cast from chloroform solution.

Fig. 6. Infrared spectra of KBr disks of (———) PLA (A/I = 4) and (– –) PLA-*d*.

are thus observed in a narrow frequency region of 700–705 cm^{-1} and the
amide V′ bands are observed in the region of 515–530 cm^{-1}. For PBLG-*d*,
the amide V′ band of the β-form was observed at 518 cm^{-1}, whereas the
amide V band of PBLG in the β form was overlapped by the strong band
of benzyl groups at 696 cm^{-1}.[14]

CONCLUSION

Amide V bands arising from N-H out-of-plane bending modes of poly-
peptides were thus found to vary with chain conformations: α-helical form,

610-620 cm^{-1}; disordered form, around 650 cm^{-1}; antiparallel-chain β form, 700–705 cm^{-1}. These amide V bands of various forms are widely separated from each other and these correlations anre useful for conformation studies of polypeptides and proteins. Conformation analyses with amide V bands do not necessarily require dichroic measurements and therefore are also applicable to copolypeptides or proteins which are not oriented. In fact, the amide V bands due to the α-helical form, disordered form, and antiparallel-chain β form were observed for a solid film of lysozyme.[16]

Amide V bands of the α-helical form are well-defined and make sharp contrast with diffuse amide V bands of disordered form. Accordintly, the intensity of amide V bands at 610–620 cm^{-1} may be used for estimating the fraction of the α-helical form, although amide V bands of disordered form partially overlap. In fact, the right-handed and left-handed α-helical forms and disordered forms of copoly(γ-benzyl D,L-glutamate)[23] and copoly(γ-methyl D,L-glutamate)[22,25] were studied, from measurements of the intensity of amide V bands and the optical rotatory dispersion.

Amide V′ bands arising from N-D out-of-plane bending modes of N-deuterated polypeptides were also found to vary with chain conformations: α-helical form, 455–465 cm^{-1}; disordered form, around 510 cm^{-1}; antiparallel-chain β form, 515–530 cm^{-1}. These correlations for amide V′ bands are useful for conformation studies on polypeptides whose amide V bands are overlapped by side-chain bands or for confirming the conformations derived from analyses of amide V bands.

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