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Vibrational Spectra of *N*-Acyl-L-Glutamic Acid Oligomers and the Long *N*-Acyl Chain Effect

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Trimers and tetramers of *N*-acyl-L-glutamic acid oligomers (Glu-oligomers) with various acyl chains have been prepared. For these Glu-oligomer molecules the vibrational spectra were measured and compared with those of two β-forms (β_1 and β_2) and the α-helical form of poly(L-glutamic acid). For these samples the molecules take up a β_2-type structure in the solid state. The long acyl chain in the oligomers induces stronger hydrogen bonds between the side-chain COOH and the amide linkages; this effect is due to the close packing of the long acyl chains accompanying the reduction of the intersheet spacing. Moreover, the long acyl-chain effect strongly depends upon the residue number of the oligomer. For cast films of Glu-oligomers made onto an NaCl plate, these oligomer molecules assume an α-helical type structure. For the α-helical type oligomers, it is also found that the long acyl chain induces stronger hydrogen bonds in the side-chain COOH groups, and that the effect depends on the residue number.

Membrane proteins have been crystallized as protein–detergent complexes in the presence of detergent molecules.[1—3] Furthermore, it has been elucidated that the ordered packing of the protein–surfactant complexes is promoted by surfactant molecules.[4,5] Recently, the structure of the surfactant assembly in the crystals of a bacterial photosynthetic reaction center complex has been demonstrated.[6] The results provide a model for the interaction between the lipid molecules and membrane proteins. The possibility of a conformational change in the protein molecules induced by the lipid molecules is present in such a lipid–protein complex. However, very little is known about how the conformational change of the peptide can occur in such a hydrophobic environment.

In order to aid in our understanding of the role of the hydrocarbon chain in the lipid–protein complex, *N*-acylglycine oligomers with various acyl chains have been synthesized and the conformation of the glycine oligomer parts has been investigated in terms of the vibrational spectra and the X-ray powder diffraction patterns.[7] The results have demonstrated that the long acyl chains induce an additional polyglycine II-type helical structure in the NH···O=C and peptide skeletons. However, such a long acyl-chain effect may depend on the nature of the residues in the peptides. A study of the residue dependence of a long acyl-chain effect is thus highly desirable.

In our previous paper,[8] we reported on the conformations of *N*-octanoyl-L-glutamic acid oligopeptides (residue number, N=3—6, 8, 10, 12, 14, 16, 18, 20, and 22) in the solid state. These oligomers assume a β_1- or β_2-type structure similar to that of the two β-forms of poly(L-glutamic acid) (poly(Glu)). The preferential stabilization of the β_1- or β_2-forms is strongly dependent on the residue number. Films of the oligomers, made by casting these samples from a dimethylformamide solution onto NaCl or KBr plates, assume an α-helical structure.

Conformational studies of the two β-forms for poly(Glu) have also been made by X-ray diffraction.[9—11] The results show that the intersheet distance is 9.03 Å for β_1 and 7.83 Å for β_2. Itoh et al.[11] have assumed that in the β_1 structure there are larger cavities between the neighboring sheets, and that water molecules within the cavities are hydrogen-bonded to either the peptide groups or to the carboxyl groups, thereby stabilizing the β_1 form. The pleated sheets of the β_2 form are more tightly packed, compared with those of the β_1 form. For β_2-poly(Glu), X-ray powder patterns have provided a structural model in which the carboxyl groups lie in planes normal to the chain axis. This arrangement enables the side chains to interpenetrate deeply within each other.

In the present work, *N*-acyl-L-glutamic acid trimer and tetramer having various acyl chains were synthesized; the long acyl-chain effect on the conformations of these molecules is discussed in terms of the use of the vibrational spectra.

Experimental

Materials. *N*-Acyl-L-glutamic acid oligopeptides (trimer and tetramer) having acetyl, butanoyl, hexanoyl, octanoyl, and decanoyl groups were synthesized by a stepwise procedure described in our previous study.[8] The films of samples were made by casting from a dimethylformamide solution onto NaCl plates. Samples were identified by elemental analysis; the agreement between the calculated and observed values for these oligomers was within 0.5—0.6%. The abbreviations for the oligomers used are listed in Table 1.

Methods. Raman spectra below 4000 cm^{-1} were measured with a Perkin–Elmer 1700 FT Raman spectrometer attached to an indium gallium arsenide (InGaAs) detector, using near-infrared laser (1064 nm, 500 mW) excitation at room temperature. The infrared (IR) absorption spectra were recorded on a Perkin–Elmer 1600 Fourier-transform infrared (FTIR) spectrometer (4000—600 cm^{-1}) at 20 °C and 60% relative humidity. X-Ray powder diffraction patterns were obtained by using an RAD-RC diffractometer with a counter monochromator (Cu $K\alpha$, 60 kV, 200 mA).

Results and Discussion

For the *N*-acyl-L-glutamic acid oligomers (Glu-trimer and -tetramer) having various acyl chains, X-ray powder diffraction patterns were measured and compared with those of β_2-poly(Glu). For HeGl4H, the lattice spacings characteristic of the β_2-type structure for poly(Glu)[9—11] were observed, as listed in Table 2. The lattice spacing at 7.70 Å for HeGl4H closely corresponds to that at 7.83 Å for the β_2-type poly(Glu), which have been assigned to the intersheet spacing.[9—11] This observation shows that the *N*-hexanoyl-Glu-tetramer assumes a sheet-type structure similar to that of β_2-poly(Glu). For the other *N*-acyl-Glu-tetramers and -Glu-trimers, diffraction lines characteristic of β_2-poly(Glu) were also observed.

For these *N*-acyl-Glu-oligomers, the Raman and IR spectra were measured and compared with those of β_2-poly(Glu)[11] in the solid state. The characteristic bands of β_2-poly(Glu)[11] were observed in common for these oligomers, as listed in Table 3. Figure 1 shows the representative Raman and IR spectra of HeGl4H; the vibrational bands characteristic of β_2-poly(Glu) are indicated by asterisks. The characteristic bands of β_2-poly(Glu) are also briefly summarized in Table 3, together with those of the *N*-acyl-Glu-oligomers.

Table 1. Abbreviations[a)] for *N*-Acyl-L-glutamic Acid Oligomers[b)]

		Trimer (N=3)	Tetramer (N=4)
Acetyl	(m=0)	AcGl3H	AcGl4H
Butanoyl	(m=2)	BuGl3H	BuGl4H
Hexanoyl	(m=4)	—	HeGl4H
Octanoyl	(m=6)	OcGl3H	OcGl4H
Decanoyl	(m=8)	DeGl3H	DeGl4H

a) H denotes acid type and the numbers (3, 4) in abbreviations indicate the residue number. b) $\mathrm{CH_3(CH_2)_{\mathit{m}}CO(NH\text{-}CH(CH_2CH_2COOH)\text{-}CO)_{\mathit{N}}\text{-}OH}$.

Table 2. Observed Lattice Spacing (Å) of HeGl4H Characteristic of β_2-Poly(Glu) [a,b)]

HeGl4H	β_2-Poly(Glu)
7.70 vw	7.83 w
4.73 s	4.74 m
3.97 vs	3.90 vs
3.67 m	3.61 s
3.58 m	3.43 w
3.14 w	3.17 vs-d
2.59 w	2.60 s
2.28 vw	2.31 w
1.96 vw	1.98 w

a) From Refs. 9—11. b) vw, w, m, s, vs, have the usual meanings: very weak through moderate to very strong; d, indicates the band was diffuse.

For HeGl4H the Raman band at 1734 cm^{-1} and the corresponding IR band at 1736 cm^{-1} are assigned to the C=O stretching mode of the side-chain COOH group. These C=O stretching bands are characteristic of the β_2-type structure.[11]

The Raman bands at 1605 and 1643 cm^{-1} and the IR band at 1610 cm^{-1} for HeGl4H are assigned to the amide I modes. The two Raman bands closely correspond to those at 1603 and 1647 cm^{-1} for β_2-poly(Glu).[11] The 1610 cm^{-1} IR band also corresponds well to that at 1601 cm^{-1} for β_2-poly(Glu).[11] However, the IR band at 1643 cm^{-1} may be regarded as being the amide I band characteristic of this oligomer (Fig. 1[B]), since the IR band at 1643 cm^{-1} is not observed for β_2-poly(Glu).[11]

The strong IR band at 1552 cm^{-1} and the weak Raman bands at 1540 and 1560 cm^{-1} for HeGl4H are assigned to the amide II modes. The former IR band corresponds well to the IR band at 1554 cm^{-1} for β_2-poly(Glu),[11] and the latter two bands to the Raman bands at 1540 and 1560 cm^{-1} for β_2-poly(Glu),[11] respectively.

The amide III modes for HeGl4H appear at 1278 and 1294 cm^{-1} (Raman) and at 1275 and 1295 cm^{-1} (IR), corresponding well to the Raman and IR bands at 1280 and 1295 cm^{-1} for β_2-poly(Glu),[11] respectively.

The 642 cm^{-1} band (IR) for HeGl4H is very close to the amide IV band at 638 cm^{-1} characteristic of β_2-poly(Glu), and is also characteristic of the β_2-type structure.

For HeGl4H the Raman (IR) bands at 1444(1443), 1418(1415), (1410), 1230(1238), 1198(1192), 1167(1168) and 784(793) cm^{-1} arise from the side-chain $\mathrm{CH_2}$ groups, and are also characteristic of the β_2-type structure.[11] The Raman (IR) bands at 1061(1064) and 1018 cm^{-1}, arising from the skeletal stretching modes, are evidently characteristic of the β_2-type structure.

For the other *N*-acyl-Glu-trimers and -tetramers, the vibrational bands characteristic of the β_2-type structure were also observed (Table 3).

Furthermore, it was found that the spectral features in the NH and C=O stretching, amide I and low-frequency regions are markedly dependent on the acyl chain length. In particularly the present study, the long acyl-chain effect on the IR spectra of a series of *N*-acyl-Glu-oligomers is emphasized. The structural change is discussed in detail.

NH Stretching Region. Figure 2 shows the *N*-acyl chain-length dependence of the IR bands for *N*-acyl-Glu-trimers and -tetramers in the NH stretching region. The spectral features for these oligomers in the NH stretching mode (amide A) are found to depend on

Table 3. Observed Vibrational Band Frequencies (cm^{-1}) Characteristic of β_2-Type N-Acyl-Glu-oligomers in the Solid State and Tentative Assignment[a)]

β_2-Type						
N-Acyl-Glu-trimers		N-Acyl-Glu-tetramers		Poly(Glu)[b)]		
Raman	IR	Raman	IR	Raman	IR	Assignment[b,c)]
1731—1734 m	1734—1736 vs	1732—1734 m	1735—1736 vw	1732 m	1732 vs	ν[C=O(COOH)]
1639—1641 m		1642—1644 s		1647 s		Amide I
1603—1605 vw	1597—1600 m,sh	1603—1609 vw	1608—1610 vs	1603 vw	1601 vs	
1556—1562 vw		1558—1563 w		1560 w		Amide II
1539—1543 sh	1545—1551 s	1540—1549 sh	1552 s	1540 sh	1554 s	
1442—1445 s	1444—1446 w	1443—1444 m	1443—1444 w	1443 m	1441 w	
1418—1419 s	1413—1416 m	1417—1419 s	1411—1415 m	1412 s	1417 m	s(CH_2)
	1404—1410 sh		1404—1408 sh		1408 m	
1318—1321 m,sh		1319—1322 m		1318 m		t(CH_2)+w(CH_2)
1293—1300 m	1292—1296 w	1294—1298 m	1294—1296 w	1295 m	1295 w	Amide III
1275—1278 m,sh	1272—1274 m	1274—1279 m	1274—1275 m	1280 m	1280 m	
1231—1239 m	1234—1238 w	1229—1235 m	1237—1238 w	1230 s	1236 w	
1189—1197 w	1190—1193 w,sh	1196—1200 w	1192—1193 w	1200 w	1190 m	w(CH_2)
1172—1175 vw	1168—1173 m	1166—1171 w	1168—1170 s	1172 w	1168 s	
1115—1121 w		1118—1119 w		1118 w		
1061—1067 s	1062—1065 vw	1061—1064 vs	1064 vw	1059 vs	1065 vw	ν(C–N,C–O)
1015—1021 m		1017—1019 m		1021 m		
955— 959 m		960— 961 m		965 m		ν(C–C)
941— 947 sh	947— 950 vw	931— 937 vw	948— 949 w	941 vw	952 vw	
898— 905 w		898— 900 m		900 w		
884— 892 sh	894— 901 vw	876— 880 vw	877— 882 vw	889 vw	885 vw	
821— 832 vw		820— 829 w		826 w		r(CH_2)
775— 782 vw	792— 794 m	782— 784 vw	791— 793 m	772 vw	789 m	
664— 675 vw		674— 677 w		686 vw		Amide V
625— 643 vw	629— 631 vw	640— 644 vw	634— 643 m	645 vw	638 m	Amide IV
559— 567 vw		554— 564 w		562 vw		Amide VI

a) s, strong; m, medium; w, weak; v, very; sh, shoulder. Only the main vibrational bands characteristic of β_2-type are listed. b) From Ref. 11. c) ν, stretching; s, scissoring; t, twisting; w, wagging; r, rocking; amide I, mainly C=O stretching vibration; amide II, N–H in-plane bending vibration coupled with amide C–N stretching; amide III, mainly amide C–N stretching; amide IV, mainly skeletal deformation of peptide skeleton; amide V, mainly N–H out-of-plane bending; amide VI, mainly out-of-plane deformation of peptide skeleton.

both the residue number and the acyl chain length.

The spectral patterns of the NH stretching mode for BuGl3H, OcGl3H, and DeGl3H are different from that of AcGl3H. For BuGl3H, in addition to the bands at 3321 and 3348 cm^{-1}, a very broad band appears at 3236 cm^{-1}, indicating that each NH group participates in a different hydrogen-bonding environment. However, in the case of OcGl3H, three IR bands appear at 3287, 3326, and 3339 cm^{-1}; the former two bands tend to increase in intensity. For DeGl3H with a longer hydrocarbon chain, the 3286 and 3340 cm^{-1} bands are observed. The former band becomes stronger in intensity than the latter one. These observations reveal that strong hydrogen bonds are formed upon increasing the length of the acyl chain.

For the N-acyl-Glu-tetramers, the spectral features in the NH stretching region are completely different from those for the Glu-trimers. Very broad bands at ca. 3240—3273 cm^{-1} and the sharp bands at 3340—3344 cm^{-1} are observed in common for AcGl4H, BuGl4H, OcGl4H, and DeGl4H. The former bands are assigned to the strongly hydrogen-bonded NH groups and the latter ones to the weakly hydrogen-bonded NH groups. However, for the Glu-tetramers it is found that such a long acyl-chain effect is not so marked.

Amide I and II Regions. The N-acyl chain length affects the IR spectral patterns in the amide I region (Fig. 3). For AcGl3H a very broad band at 1644 cm^{-1} appears and is assigned to the amide I mode. Since the 1644 cm^{-1} band is not observed in the IR spectrum of the β_2-poly(Glu), it may be regarded as being an amide I band characteristic of a very short oligomer. However, the shoulder bands at ca. 1600 cm^{-1} closely correspond to the amide I band at 1601 cm^{-1} for β_2-poly(Glu).[11)]

The IR spectral features in the amide I region for BuGl3H, OcGl3H, and DeGl3H are quite different from that for AcGl3H. For BuGl3H, three IR bands at 1597, 1618, and 1639 cm^{-1} appear in the amide I region,

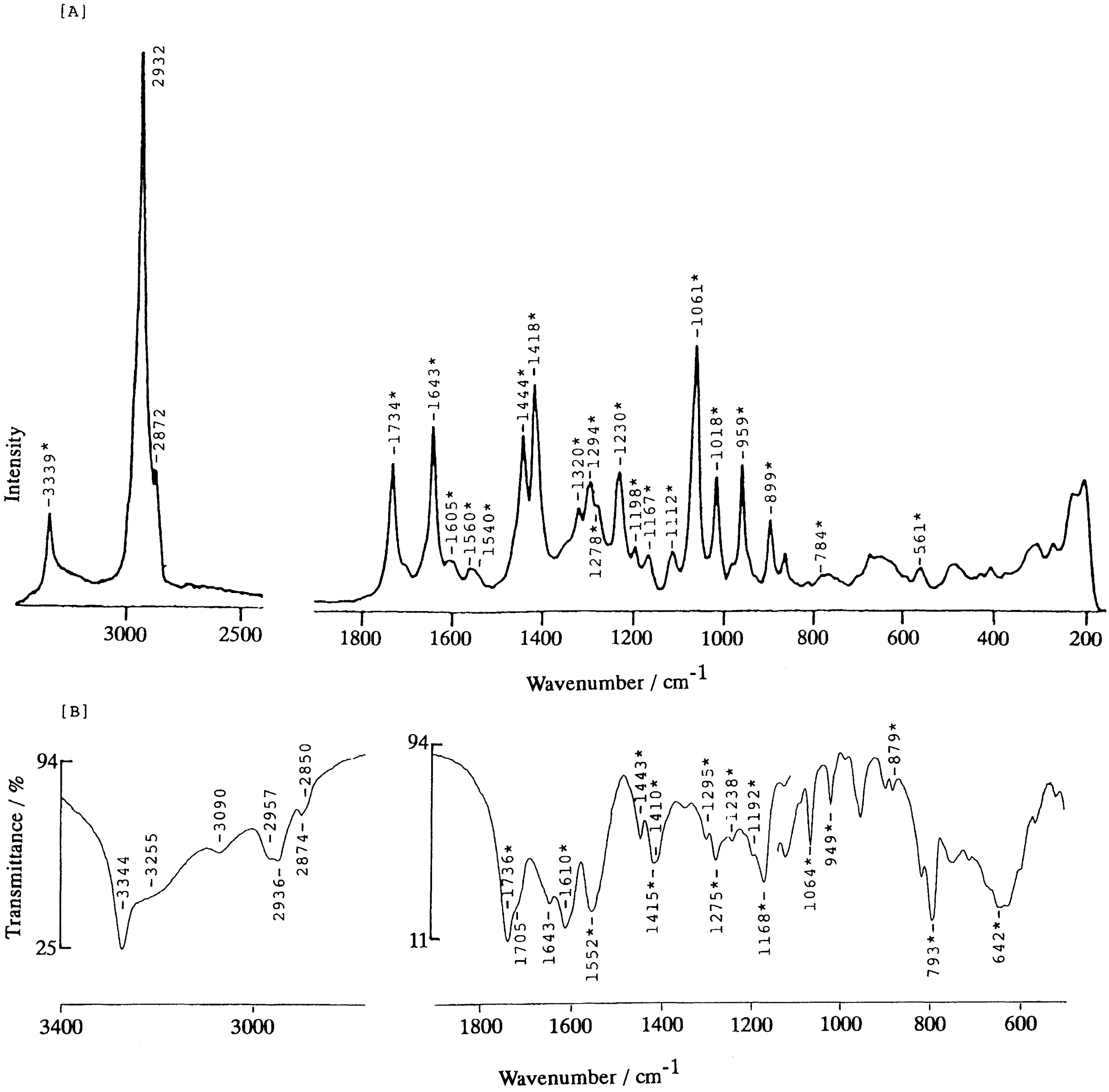

Fig. 1. Raman [A] and IR [B] spectra of HeGl4H in the solid state at room temperature (the bands characteristic of the β_2-type structure are marked with asterisks).

and the 1618 cm^{-1} band increases in intensity. It is found that a long acyl chain brings about a further pronounced variation in the amide I region. For OcGl3H, in addition to the shoulder bands at 1600 and 1640 cm^{-1}, the 1628 cm^{-1} band appears in this region and increase in intensity. In the case of DeGl3H, the amide I bands are observed at 1598, 1625, and 1638 cm^{-1}, which correspond well to those at 1600, 1628, and 1640 cm^{-1} for OcGl3H; the 1638 cm^{-1} band, however, is predominant in intensity. Such a band splitting and intensity change in the amide I region should be ascribed to the long acyl-chain effect.

For AcGl4H, BuGl4H, OcGl4H, and DeGl4H the IR bands at 1608—1609 and 1642—1643 cm^{-1} are observed in common. The former bands are characteristic of the β_2-type structure, and the latter bands can be regarded as being amide I bands characteristic of the oligomers. Thus, for the *N*-acyl-Glu-tetramers the spectral feature in the amide I region does not depend upon the acyl chain length in frequency and intensity.

In the IR spectra of the *N*-acyl-Glu-trimers, as the acyl chain becomes longer the frequency of the amide II band slightly shifts to lower frequency; the long acyl chain effect, however, is not so marked. In the case of the Glu-tetramers, a variation of the acyl chain length does not bring about any shifting of the amide II band frequency. Thus, the long acyl chain affects the spectral features in the amide I and II regions for the IR spectra of the Glu-trimers, while for the Glu-tetramers this effect seems to be extremely small.

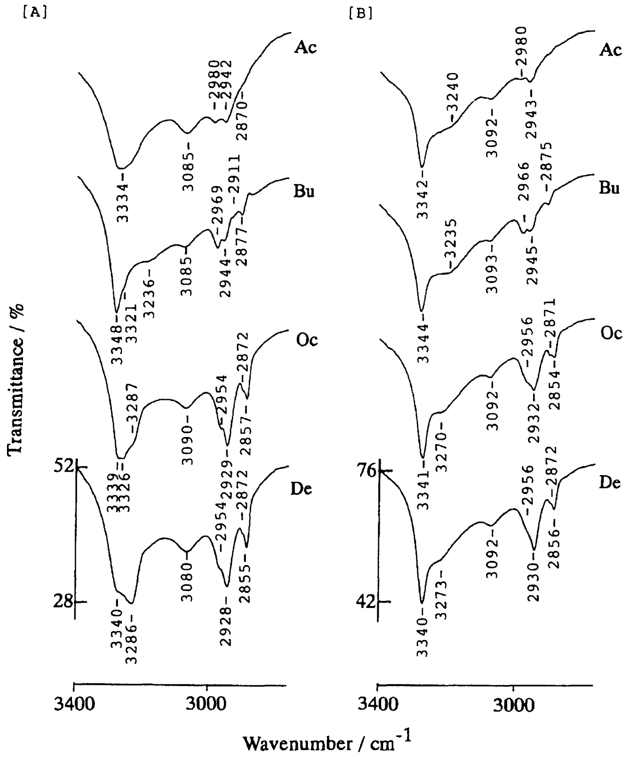

Fig. 2. *N*-Acyl chain length dependence of the IR spectra for *N*-acyl-Glu-trimers [A] and -tetramers [B] in the NH stretching region: Ac, acetyl; Bu, butanoyl; Oc, octanoyl; De, decanoyl.

The mechanism of an amide I splitting induced markedly by long acyl chains should be due to a transition dipole interaction.[12,13] The splitting patterns reflect the steric configuration of the peptide groups in these oligopeptides.[14—16] For the Glu-oligomers having long acyl chains the reduction of the intersheet spacing occurs due to the close packing of the long acyl chains in the solid state, as discussed later. This must change the peptide configuration, resulting in the amide I splitting.

C=O Stretching Region. In the β_2-type structure model of poly(Glu),[11] the side-chain COOH groups lie in a plane normal to the polymer chain axis; this arrangement enables the side chains to interpenetrate deeply within each other. Therefore, one would expect an interaction between the hydrogen-bonded peptide linkages in one β-sheet and the carboxyl groups of the side chains in an adjacent β-sheet. The C=O stretching band at 1732 cm^{-1}, observed in the vibrational spectra of the β_2-poly(Glu), reflects such an environment of the side-chain COOH groups in the β_2-type structure.

Figure 3 also shows the acyl chain length dependence of the IR spectral feature in the C=O stretching region. The 1734—1737 cm^{-1} bands are observed in common for AcGl3H, BuGl3H, OcGl3H, and DeGl3H. These bands correspond well to the 1732 cm^{-1} band for β_2-poly(Glu).[11] However, these bands for the Glu-trimers are at slightly higher frequencies compared with that of β_2-poly(Glu). Furthermore, it should be noted that the 1700—1708 cm^{-1} bands appear for BuGl3H, OcGl3H, and DeGl3H and increase in intensity with an increase in the acyl chain length. This observation shows that in the side-chain COOH hydrogen bond system stronger hydrogen bonds are induced by the long acyl chain.

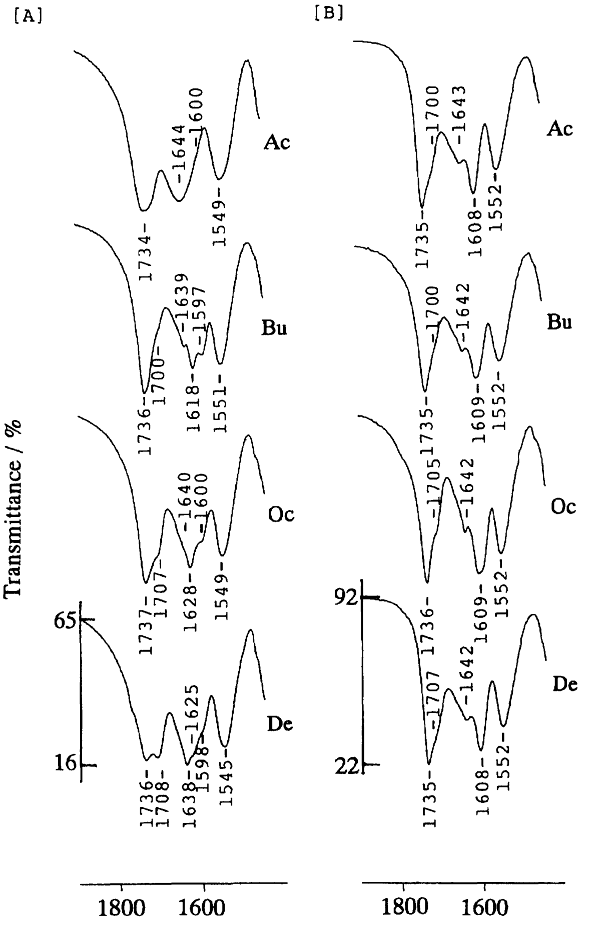

Fig. 3. *N*-Acyl chain length dependence of the IR spectra for *N*-acyl-Glu-trimers [A] and -tetramers [B] in the 1500—1800 cm^{-1} region: Ac, acetyl; Bu, butanoyl; Oc, octanoyl; De, decanoyl.

For the *N*-acyl-Glu-tetramers, shoulder bands at ca. 1700—1707 cm^{-1} in addition to the 1735—1736 cm^{-1} bands are also observed in common. These shoulder bands have a tendency to increase in intensity with an increase in the acyl chain length. However, the long acyl-chain effect is very small for the Glu-tetramer. Therefore, the long acyl chain strongly affects the C=O stretching mode for the Glu-trimers, while this effect is very small for the Glu-tetramer.

Low-Frequency Region. Figure 4 shows the IR spectra of the *N*-acyl-Glu-trimers and -tetramers in the low-frequency region, in which the bands characteristic of β_2-type structure are indicated by asterisks. For the Glu-tetramers, the IR bands at 239—240, 316—320, 444—449, 483—486, and 494—499 cm^{-1} closely correspond to those at 240, 318, 450, 484, and 500 cm^{-1}

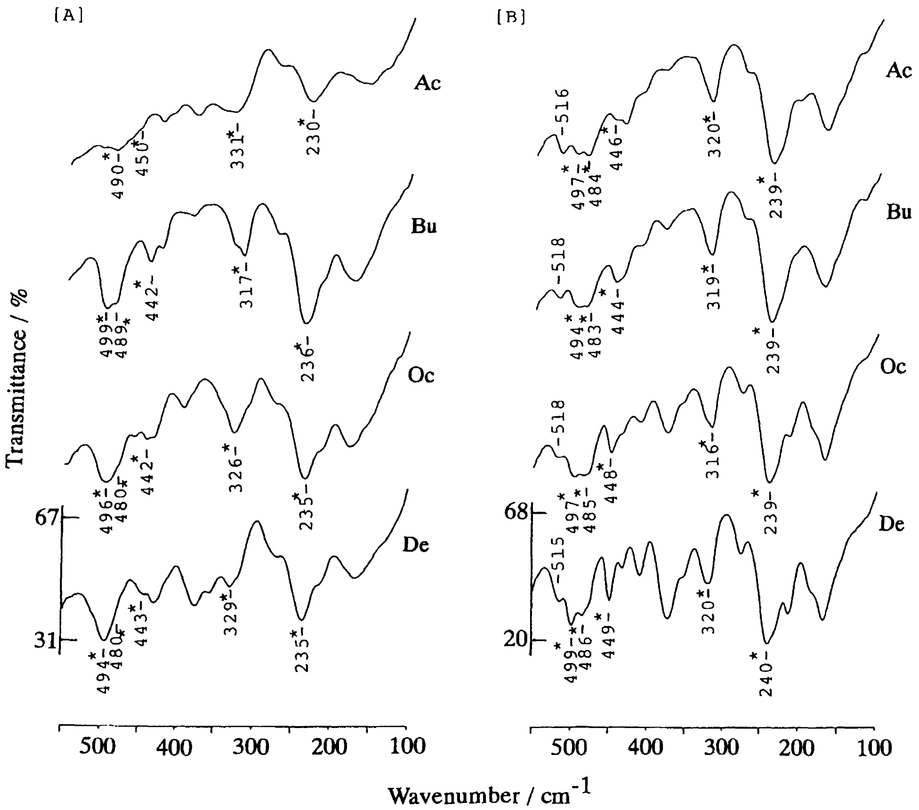

Fig. 4. *N*-Acyl chain length dependence of the IR spectra for *N*-acyl-Glu-trimers [A] and -tetramers [B] in the low frequency region: Ac, acetyl; Bu, butanoyl; Oc, octanoyl; De, decanoyl. The IR bands characteristic of the α-helical structure are marked with asterisks.

Table 4. Acyl Chain Length Dependence of Intersheet Spacing (Å)[a,b]

N-Acyl-Glu-oligomers									
Trimer (*N*=3)				Tetramer (*N*=4)					β_2-
Ac	Bu	Oc	De	Ac	Bu	He	Oc	De	Poly(Glu)[c]
7.72 w	7.74 w	7.19 vw	6.94 vw	7.78 w	7.82 w	7.70 vw	7.60 w	7.66 w	7.83 w

a) Ac, acetyl; Bu, butanoyl; He, hexanoyl; Oc, octanoyl and De, decanoyl. b) See footnote to Table 2.
c) From Refs. 9, 10, and 11.

for the β_2-poly(Glu),[11] respectively. This observation provides ample evidence that these Glu-tetramers take up a sheet structure similar to that of β_2-poly(Glu), since the vibrational bands in the low-frequency region directly reflect the structures in the hydrogen bonding and peptide skeleton.[17—19]

For the Glu-trimers, the IR bands at 230—236, 317—331, 442—450, 480—490, and 494—499 cm^{-1} are also characteristic of the β_2-type structure. In particular, the IR spectrum of AcG13H in this region is different from those of the other Glu-trimers regarding their features. The bands at 230 and 331 cm^{-1} are definitely different from the 239 and 320 cm^{-1} bands of AcG14H in frequency; moreover, the 450 and 490 cm^{-1} bands for AcG13H are very weak in intensity compared with those at 446, 484, and 497 cm^{-1} for AcG14H. This may indicate that the number of residues in the AcG13H molecule is too small to provide the complete β_2-type spectral feature in this region.

For the IR spectra of the Glu-trimers, an increase in the acyl chain length brings about a marked variation in the low-frequency region. For BuG13H, OcG13H, and DeG13H the characteristic bands at 235—236, 317—329, 442—443, 480—489, and 494—499 cm^{-1} increase in intensity as the acyl chain length becomes longer, showing that the β_2-type skeletal structures of the Glu-trimers are promoted by the long acyl chain.

For the Glu-tetramers, the bands characteristic of the β_2-type structure in the low-frequency region are not affected by the long acyl chain in frequency. However, the bands at 444—449, 483—486, and 494—499 cm^{-1} strongly depend upon the acyl chain length in intensity. That is, these bands increase in intensity with an increase in the acyl chain length. For the IR spec-

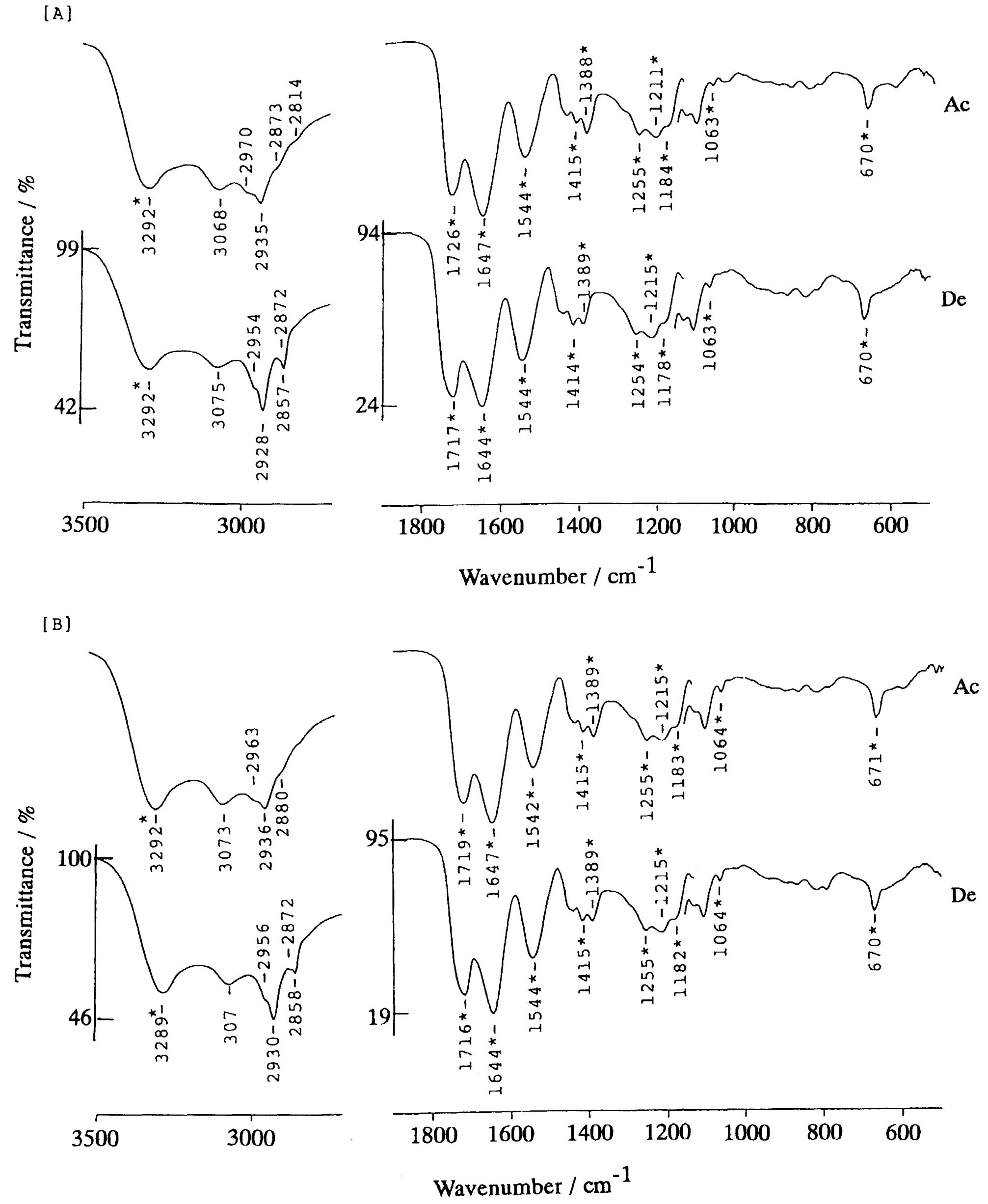

Fig. 5. *N*-Acyl chain length dependence of the IR spectra of α-helical type cast films (AcGl3H and DeGl3H [A] and AcGl4H and DeGl4H [B]) in the 600—4000 cm^{-1} region: Ac, acetyl; De, decanoyl. The IR bands characteristic of the α-helical structure are marked with asterisks.

tra of the Glu-tetramers in this region, the bands at 239—240 and 316—320 cm^{-1} are not affected by the long acyl chain in frequency and intensity. These bands may be assigned to the skeletal backbone deformation modes.[11] Therefore, regarding the Glu-tetramer it may be assumed that the long acyl chain does not affect the skeletal backbone structure.

The bands at 480—489 and 494—499 cm^{-1} for the Glu-trimer and the corresponding bands (483—486 and 494—499 cm^{-1}) for the Glu-tetramer may be assigned to the C=O in-plane and out-of-plane deformation modes arising from the side chain, respectively, judging from the assignments of the 483 and 500 cm^{-1} bands for the β_2-poly(Glu).[11] Accordingly, the marked variation in intensity for these IR bands may reflect a structural change in the hydrogen bonding system in which the side-chain COOH groups participate. Thus, we may assume that the longer acyl chain groups in these oligomers strongly induce the β_2-type structure in the side-chain COOH hydrogen bonding system.

Close Packing of Long Acyl Chains and Its X-Ray Powder Diffraction Pattern Evidence. As mentioned above, the diffraction line at 7.83 Å, observed in the X-ray powder diffraction pattern for the β_2-poly(Glu),[11] reflects the intersheet spacing for the β_2-type structure. The intersheet spacings for the *N*-acyl-Glu-trimers and -tetramers having various acyl chain lengths are listed in Table 4. For the Glu-tetramers, as the acyl chain length becomes longer the intersheet spacing has a tendency to decrease, though the magnitude of the decrease is very small. However, for the Glu-trimers, the intersheet spacing evidently strongly depends upon the chain length, and is diminished by the long acyl chain. We may thus conclude that the reduction in the intersheet spacing brings about a close packing of the long acyl chains in the *N*-acyl-Glu-oligomers.

It was also found that the intersheet spacings observed for these oligomers gradually change with time. A detailed study of the X-ray powder patterns for these oligomers will be reported separately.

Vibrational Spectra of α-Helical Type *N*-Acyl-Glu-oligomers. In our previous study,[8] the FTIR spectra of the films of the *N*-octanoyl-Glu-oligomers (residue number, $N=1-14$), made by casting them from a dimethylformamide solution, were measured and compared with those of the two β-forms[11] and the α-helical structure of poly(Glu).[20,21] The IR spectra of the Glu-oligomers could be satisfactorily explained in terms of the α-helical type structure.

In the present study, the IR spectra for the cast films of a series of the *N*-acyl-Glu-trimers and -tetramers were measured in order to investigate the long acyl-chain effect on the α-helical type IR spectra for these oligomers. Figure 5 shows the representative IR spectra of cast films for AcGl3H, DeGl3H, AcGl4H, and DeGl4H, which can be interpreted by allocating the spectral features to the α-helical structure. The IR bands characteristic of the α-helix are indicated by asterisks in Fig. 5.

For the IR spectra of the Glu-trimers in the 500—4000 cm^{-1} region, as the acyl chain becomes longer the C=O stretching band frequency (1726 cm^{-1} for AcGl3H) shifts to lower frequency (1717 cm^{-1} for DeGl3H), but the long acyl chain does not markedly affect the NH stretching and amide I modes. Therefore, for the α-helical type cast films of the Glu-trimers, we may assume that the long acyl chain mainly induces the formation of stronger dimeric COOH hydrogen bonds in the side chains, and does not affect the NH hydrogen bonding system. For the case of the Glu-tetramers, such a long acyl-chain effect on the C=O and NH stretching and amide I modes is not marked, compared with the case of the Glu-trimers.

In our previous paper,[22] we have reported that, in the α-helical type cast film for the *N*-octanoyl-Glu-oligomers, the octanoyl chains might be in a loosely packed state rather than in a densely packed state. This may be responsible for the difference in the long acyl-chain effect between the α-helical and the β_2-type structures.

Conclusion

Trimers and tetramers of *N*-acyl-L-Glu-oligopeptides having acetyl, butanoyl, hexanoyl, octanoyl, and decanoyl groups have been prepared. The vibrational spectra and X-ray powder diffraction patterns of these molecules have been measured and compared with those of two β-forms (β_1 and β_2) and α-helical form of poly(Glu). The vibrational spectra and X-ray powder diffraction patterns provide ample evidence that the *N*-acyl-Glu-oligomers having various acyl chains take up a β_2-type conformation in the solid state and that the oligomer films made by the casting method onto an NaCl plate take up an α-helical structure.

The long acyl chain induces a splitting of the amide I band for the Glu-trimers. This observation is due to the steric configurational change of the peptide moieties induced by a reduction of the intersheet spacing accompanying the close packing of a long hydrocarbon chain, which was confirmed by the X-ray powder diffraction patterns. The IR spectra in the NH and C=O stretching regions also reveal that for the β_2-type oligomers the long acyl chains induce strong hydrogen bonds between the peptide linkages and the side-chain COOH groups. Furthermore, such a structural change in the hydrogen bonding system upon the close packing of the long acyl chain is strongly reflected in the low-frequency region. This effect depends upon the residue number in the oligomers. For the α-helical type films of the Glu-trimers, only the C=O stretching mode is affected by the long acyl chain. For the NH stretching and amide I modes, however, the long acyl-chain effect is not so marked. In the case of the Glu-tetramers of the α-helical structure, the long acyl-chain effect may be very small, due to the loosely packed state of the acyl chain.

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