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The β-Type Structures of Very Simple N-Octanoyl-L-glutamic Acid Oligomers (Residue Number, N=2—6) and Their $\beta_1\rightarrow\beta_2$-Type Transition

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N-Octanoyl-L-glutamic acid oligomers (residue number, $N=2$—6) have been synthesized in order to study their molecular conformations in the solid state. The X-ray powder diffraction patterns and vibrational spectra of these oligomers have been investigated and compared with those of the α-helical and β-sheet conformations of poly-L-glutamic acid. For fresh samples of these oligomers, the tetramer, pentamer, and hexamer take up a conformation similar to the β_1-form of poly-L-glutamic acid. However, the β_1-type conformation of these oligomers is rapidly transformed to a β_2-type conformation with time at room temperature, indicating that a transition similar to the $\beta_1\rightarrow\beta_2$ transition of poly-L-glutamic acid occurs, even in the tetramer, pentamer, and hexamer. For fresh samples of the dimer and trimer, these molecules take up the β_2-type structure. This means that the critical size of the oligomer for the formation of the $\beta_1\rightarrow\beta_2$-type transition is the tetramer.

A great number of conformational studies of poly-L-glutamic acid (poly(Glu)) have been made using various techniques, and the conformations of the α-helix, random-coil, and some intermediate states have been elucidated to depend on such experimental conditions as the pH, ionic strength, solvent, and temperature.[1) For example, a random-coil structure exists at the neutral and high pH, where the side-chain COOH groups of the glutamyl residues are negatively charged. However, the random-coil structure can be easily transformed to an α-helical structure under lower pH conditions at room temperature. Poly(Glu) molecules in the α-helical structure have a strong tendency to form an associated state. For poly(Glu) in the associated state, Itoh, Foxman, and Fasman[2) have found a temperature-induced transition from α-helix to β-sheet, in which two β-forms (β_1 and β_2) are obtained. The β_1-poly(Glu) is produced by heating α-helical poly(Glu) from 40 to 85 °C, and the β_2-poly(Glu) by heating above 85 °C. The conformations of these two β-forms for poly(Glu) have been studied by X-ray diffraction as well as IR and CD spectroscopy.[2)

Many studies of small oligopeptides have been made, and the critical size for the appearance of such secondary structures has been discussed. Rinaudo and Domard[3,4) have synthesized the L-glutamic acid oligomers, [CH_3CO–$(Glu)_n$–$NHCH_2CH_3$], having a degree of polymerization (DP) of 1—50; they have also investigated their CD spectra in aqueous solutions. The CD spectra of their aqueous solutions have been interpreted in terms of α-helix, β-sheet, random, and extended structures. Furthermore, they have demonstrated that the critical range of DP values is 8—10 for β-structure formation. Mutter[5) concluded that for the poly(oxyethylene) (POE)-bound L-α-glutamic acid oligomers (residue number, $N\leqq 20$) the formation of an α-helix starts at $N=7$. It has been shown by Uehara et al.[6) that the long acyl chain effect causes the critical size of the secondary structure appearance to change when the long acyl groups, rather than POE, and bound to L-α-glutamic acid oligomers.

Futhermore, it has also been elucidated by Uehara et al.[6) that for a series of N-octanoyl-L-glutamic acid oligomers (residue number, $N=3$—22) the shorter oligomers ($N=3$—6) take up a β_2-type conformation, and that the longer ones take up a β_1-type conformation and the $\beta_1\rightarrow\beta_2$-type transition is possible for the longer oligomers.

From the present study we report that even the very simple N-octanoyl-L-glutamic acid oligomers ($N=4$—6) take up the β_1-type conformation for fresh samples, and that the $\beta_1\rightarrow\beta_2$-type transition rapidly occurs until the β_2-type conformation is preferentially stabilized. In particular, the vibrational bands and X-ray powder diffraction patterns characteristic of the secondary structures of polypeptides[7—10) are used to analyze the conformations of these simple oligomers.

Experimental

N-Octanoyl-L-glutamic acid oligomers (abbreviated to oct-oligomers hereafter) were prepared by the following stepwise procedure. N-Butoxycarbonyl(BOC)-L-glutamic acid α,γ-dibenzyl ester oligomers were prepared from N-BOC-L-glutamic acid γ-benzyl ester and L-glutamic acid α,γ-dibenzyl ester p-toluenesulfonate in dichloromethane (DCM) in the presence of triethylamine. The usual 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) method[11) was used for the condensation reaction. The BOC groups were removed by the action of hydrogen chloride in ethyl acetate. The BOC-free oligomer benzyl esters were coupled with an octanoic acid in DCM by the EDC method.[11) The coupling reaction was confirmed by thin-layer chromatography. The obtained oct-oligomer α,γ-benzyl esters were recrystallized in DCM–ethanol. These oligomer benzyl esters ($N=2$—6) were identified by elemental analysis.

For the oligomer dibenzyl esters, debenzylation was performed in *N*,*N*-dimethylformamide (DMF) in the presence of Pd black, and was confirmed by observing the disappearance of the vibrational bands arising from the benzyl groups. The residue was then dissolved in DMF and dried diethyl ether was added to the solution until a precipitate appeared. The precipitate of oct-oligomer acid-types was then collected by filtration and dried.

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

X-Ray Powder Diffraction Patterns of Very Simple Oct-Oligomers. For the samples of very simple oct-oligomers ($N=2$—6), which were kept at −20 °C for about one month, X-ray powder diffraction patterns were measured and were compared with those of the two β-forms (β_1 and β_2) of poly-L-glutamic acid,[2] as well as those of two β-forms for longer oct-oligomers ($N=8$—14).[12] The lattice spacings observed for these simple oct-oligomers are listed in Table 1. For ease of discrimination between the β_1- and β_2-structures the lattice spacings characteristic of the β_1- and β_2-poly(Glu)[2] are also listed in Table 1. The observed lattice spacings for these oligomers closely correspond to those of the β_2-poly(Glu),[2] indicating that these very simple oct-oligomers take up a sheet structure similar to the β_2-conformation of poly(Glu).[2]

In the X-ray diffraction pattern of β_2-poly(Glu),[2] the reflections observed at 7.83 and 2.60 Å have been assigned to the intersheet spacing (001) and a third-order reflection from the intersheet spacing (003), respectively; the crystal structure of β_2-poly(Glu) has been proposed, based on the antiparallel-chain pleated sheet structure proposed by Corey and Pauling.[13] In this model (Fig. 1[B]), the carboxyl groups lie in planes normal to the chain axis; this arrangement enables the side chains to interpenetrate deeply within each other. Therefore, in the β_2-structure we may expect an interaction between the hydrogen-bonded peptide linkage in one sheet and the COOH groups of the side chains in an adjacent sheet. The reflections at 7.72—7.85 and 2.61—2.65 Å observed for the simple oct-oligomers ($N=2$—6) can be regarded as being those characteristic of a β_2-type sheet structure. It should be noted that even the oct-dimer takes up a β_2-type sheet structure.

In a previous paper[18] we showed that the long acyl chains affect the conformations of *N*-acylglycine oligopeptides (residue number, $N=3$—5), due to the close packing of the long acyl chain in the solid state.

For the oct-oligomers the *N*-octanoyl chain may promote the formation of a β_2-type sheet structure. In particular, as the number of residues decreases, such a long *N*-acyl chain effect may be strengthened.

For the oct-pentamer and hexamer, the X-ray powder diffraction patterns were measured almost immediately after these samples were prepared at room temperature. For fresh samples, intense diffraction lines characteristic of the β_1-type structure[2,14,15] were observed at 9.01—9.05 Å(Table 1). The diffraction lines at 9.01—9.05 Å closely correspond to the diffraction at 9.03 Å for the β_1-poly(Glu), which has been assigned to the intersheet spacing.[2,14,15] This observation shows that fresh samples of the pentamer and hexamer take up a sheet-structure similar to that in β_1-poly(Glu) (Fig. 1[A]).[2]

In the β_1-structure of poly(Glu),[2,14,15] the planes of the COOH groups of the side chains lie almost parallel to the chain axis and hydrogen bonds are formed between pairs of carboxyl groups belonging to different neighboring sheets. Furthermore, it is assumed from the X-ray analysis that in the β_1 structure there are large cavities between neighboring sheets.[2]

For fresh sample of the oct-tetramer the X-ray powder diffraction pattern was also measured. However, only the powder pattern characteristic of the β_2-type structure was obtained, as listed in Table 1, indicating that for a fresh sample of the tetramer the rapid $\beta_1 \rightarrow \beta_2$-type transition occurs while measuring the powder pattern.

IR Spectra of Very Simple Oct-Oligomers and a β_2-Type Structure. The IR spectra of these simple oct-oligomer acid-types in the solid state, in which the lattice spacings characteristic of the β_2-type structure were confirmed, are shown in Fig. 2; the observed IR band frequencies are listed in Table 2. A tentative assignment of the IR bands for the two β-forms of poly(Glu)[2] was used for the assignment of the IR bands of these oct-oligomers. In the IR spectra of the simple oct-oligomers ($N=2$—6), the bands characteristic of the β_2-poly(Glu) can be observed (Fig. 2 and Table 2). The bands at 1593—1605, 1546—1555, and 635—642 cm^{-1} are assigned to the amide I, amide II, and amide IV modes, respectively, and correspond well to those at 1601, 1554, and 638 cm^{-1} for β_2-poly(Glu),[2] respectively. The bands at 1294—1302 and 1272—1279 cm^{-1}, arising from the amide III modes, are very close to those at 1295 and 1280 cm^{-1} for β_2-poly(Glu),[2] respectively. In particular, it should be emphasized that the bands at 635—642 cm^{-1} due to the amide IV mode provide ample evidence of the β_2-type skeleton structure of the oct-oligomers.[16]

For the IR bands arising from the side-chain $\mathrm{CH_2}$ groups, the bands at 1168, 1191—1192, and 1237—1245 cm^{-1}, which are assigned to the wagging modes of the $\mathrm{CH_2}$ group, correspond well to those at 1168, 1190, and 1236 cm^{-1} for β_2-poly(Glu),[2] respectively. Furthermore, the 790—792 cm^{-1} bands observed in common for these very simple oct-oligomers ($N=2$—6), result from the rocking modes of the side-chain $\mathrm{CH_2}$ groups, and are characteristic of β_2-poly(Glu).[2]

Table 1. Observed Lattice Spacings (Å) of *N*-Octanoyl-L-glutamic Acid Oligomers (Acid Type) Characteristic of β_2-Type Structures[a)]

β_1-Type oligomers $(N)^{b)}$		β_1-Poly(Glu)$^{c)}$	β_2-Type oligomers $(N)^{b)}$					β_2-Poly(Glu)$^{c)}$
$N=5^{d)}$	$N=6^{d)}$		$N=2^{e)}$	$N=3^{e)}$	$N=4^{d)}$	$N=5^{e)}$	$N=6^{e)}$	
9.01w	9.05w	9.03s						
			7.72m	7.81w	7.85m	7.77m	7.84m	7.83w
4.66vs	4.66vs	4.73vs	4.76m	4.70vs	4.69s	4.71s	4.70s	4.74m
3.95s	3.96s	3.89s	3.91s	3.96vs	3.93vs	3.93vs	3.93vs	3.90vs
			3.64w	3.57m	3.62m	3.61m	3.60m	3.61s
		3.47w	3.38w		3.41w	3.44w	3.45m	3.43w
		3.03m						
			3.22w	3.18vw	3.22w	3.19vw	3.18w	3.17vs
2.76vw	2.74vw	2.80m						
			2.65vw	2.61w	2.62w	2.61w	2.61w	2.60s
			2.47vw	2.31vw	2.35vw	2.30vw	2.30vw	2.31w
		2.22w						
					1.99vw	1.98vw	2.00vw	1.98w

a) s, strong; m, medium; w, weak; v, very. b) N=residue number. c) Refs. 11 and 12. d) Measured almost immediately after these samples were prepared. e) These samples were kept at −20 °C for one month.

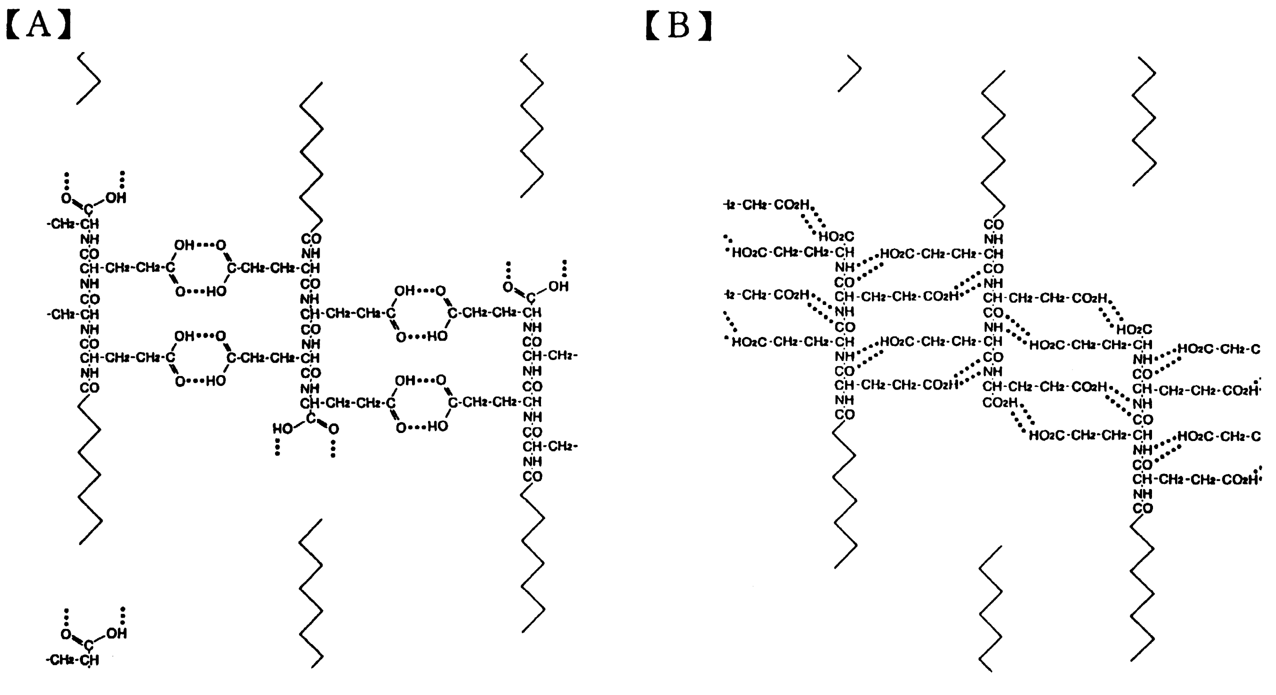

Fig. 1. β_1-Type ([A]) and β_2-type ([B]) structural models for the oct-tetramer.

In the region of 850—1100 cm^{-1}, reflecting the skeletal stretching modes, the bands at 890—893, 947—951, and 1063—1065 cm^{-1}, corresponding to those at 889, 952, and 1065 cm^{-1} for β_2-poly(Glu),[2)] respectively, are characteristic of the β_2-structure.

The C=O stretching bands at 1734—1739 cm^{-1} for the side-chain COOH groups are very close to the 1732 cm^{-1} band of β_2-poly(Glu).[2)] However, these band frequencies are at the higher frequency side, compared with those participating in the strongly hydrogen-bonded COOH cyclic dimer,[11)] indicating that the 1734—1739 cm^{-1} bands arise from the weakly hydrogen-bonded COOH groups.

The IR bands at 3324—3344 cm^{-1} for these oct-oligomers are assigned to the NH stretching mode of a peptide linkage, and are at the higher frequency side, compared with those of the α-helical poly(Glu).[17)] This observation reveals that the NH groups of these β_2-type oct-oligomers take part in a weakly hydrogen-bonded system. Although the OH stretching mode of the COOH group is expected in the same region as the NH stretching, no sharp band has generally been ob-

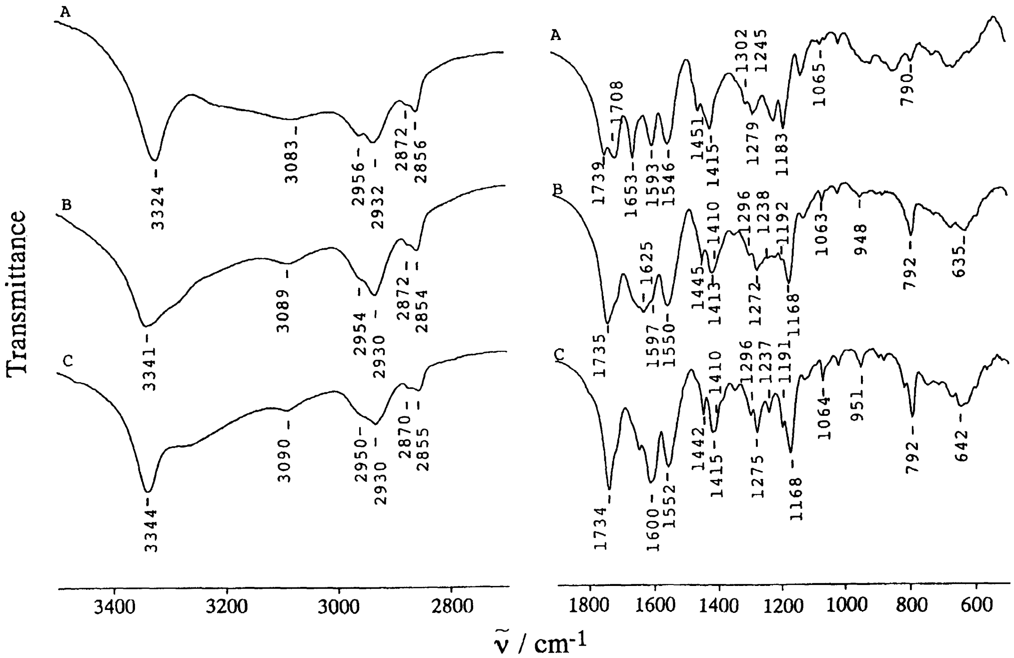

Fig. 2. Infrared absorption spectra of the β_2-type oct-oligomers (A, oct-dimer; B, oct-trimer; C, oct-tetramer) in the solid state at 20 °C and 60% r.h.

served. Thus, the IR observations indicate that very simple oct-oligomers (N=2—6) are very similar to β_2-poly(Glu) in skeletal and side-chain structures.

In the β_2-type structure of simple oct-oligomers, as mentioned above, an interaction between the peptide linkage in one sheet and the side chain COOH groups in an adjacent sheet may be expected, since the side chains interpenetrate deeply within each other. This interaction may result from weak hydrogen bondings between the peptide linkage in one sheet and the γ-COOH groups in an adjacent sheet, as schematically shown in Fig. 1[B], reflecting the band frequencies arising from the C=O (COOH) and NH stretching modes.

Evidently, the β_2-type structures of these simple oct-oligomers (N=2—6) strongly reflect in the IR spectra. However, in the IR spectrum of the oct-dimer (Fig. 2 and Table 2), from which its β_2-type sheet structure was assumed based on the X-ray powder pattern, the bands corresponding to those at 638, 885, 952, 1168, and 1190 cm^{-1} characteristic of β_2-poly(Glu)[2] disappear. Probably, the number of glutamyl residues in the oct-dimer is too small for appearance of these β_2-characteristic bands.

Amide I bands are also observed at 1653 cm^{-1} for the oct-dimer, at 1654, 1640, and 1625 cm^{-1} for the trimer and at 1640—1644 cm^{-1} for the longer oligomers (N=4—6), in addition to the amide I bands at 1593—1605 cm^{-1} characteristic of the β_2-type structure. These bands should be due to the amide I bands characteristic of the oligopeptides.[16]

β_1-Type Structure, $\beta_1\rightarrow\beta_2$-Type Transition of Simple Oct-Oligomers and Their IR Evidence.

For simple oct-oligomers (N=2—6), the IR spectra were also measured almost immediately after samples were prepared. It was found that the IR spectra of the oct-dimer and trimer are in good agreement with those of two samples which were kept at −20 °C for one month. However, for fresh samples of shorter oct-oligomers (N=4—6), the IR spectra were found to be quite different from those of samples kept for a long time.

Figure 3 shows the IR spectra for fresh samples of the tetramer, pentamer, and hexamer. The observed band frequencies are listed in Table 3. These IR spectra are definitely different from those of the β_2-type oct-oligomers. The amide I bands at 1632—1638 cm^{-1}, amide II bands at 1537—1543 cm^{-1} and amide III bands at 1256—1260 cm^{-1} for the shorter oct-oligomers (N=4—6) closely correspond to the bands at 1631—1638, 1535—1541, and 1257—1260 cm^{-1} for the longer β_1-type oct-oligomers[6] (N=8—14), respectively, and also correspond to the 1625, 1523, and 1254 cm^{-1} bands for β_1-poly(Glu),[2] respectively.

The C=O stretching (ν(C=O)) bands at 1715—1716 cm^{-1} for the shorter oct-oligomers are very close to the 1714—1716 cm^{-1} bands of the longer β_1-type oct-oligomers[6] and the 1711 cm^{-1} bands of the β_1-poly(Glu) in frequency.[2] The ν(C=O) bands at 1715—1716 cm^{-1} for the shorter oct-oligomers are at the lower frequency side, compared with those at 1734—1735 cm^{-1} for the shorter β_2-type oligomers,[6] indicating that the side-chain COOH groups form a strongly hydrogen-bonded COOH cyclic dimer. As can be seen from Table 3, it was found that the IR bands for the shorter oct-oligomers arising from the side-chain CH_2

Table 2. Observed IR Band frequencies (cm^{-1})$^{a)}$ Characteristic of β_2-Type Simple Oct-Oligomers (Residue Number, N=2—6) in the Solid State and Tentative Assignments

β_2-Type oct-oligomers (N)$^{b)}$			β_2-Poly(Glu)$^{d)}$	Assignment$^{c,d)}$
2	3	4—6^{c}		
3324$^{f)}$vs	3341$^{f)}$vs	3339—3344$^{f)}$vs		Amide A
(3315)$^{e)}$	(3330)$^{e)}$	(3324—3327)$^{e)}$		
3083$^{f)}$m	3089$^{f)}$m	3090$^{f)}$m		Amide B
2956m	2954m	2944—2950m		ν(CH)
2932s	2930s	2930—2931s		
2872w	2872w	2870—2872w		
2856w	2854w	2852—2855m		
1739vs	1735vs	1734—1735vs	1732vs	ν[C=O(COOH)]
1653vs	1654sh			Amide I
	1640sh	1640—1644m		
1625w,sh	1625s			
(1593 s)$^{g)}$	(1597 s,sh)$^{g)}$	(1600—1605vs)$^{g)}$	1601vs	
1546s	1550s	1551—1555s	1554s	Amide II
1451m	1445m	1442m	1441w	s(CH_2)
1415$^{g)}$s	1413$^{g)}$m	1414—1416m	1417m	
	1410m	1410$^{g)}$m	1408m	
1302m	1296w	1294—1295w	1295w	Amide III
1279m	1272m	1274—1276m	1280m	
1245sh	1238vw	1237w	1236w	w(CH_2)
1214m	1215vw			
	1192m	1191m	1190m	
1183s				
	1168s	1168s	1168s	
1065vw	1063vw	1063—1064vw	1065vw	ν(C–N, C–O)
	948vw	947—951vw	952vw	ν(C–C)
	893vw	890—893vw	885vw	
790$^{g)}$vw	792$^{g)}$m	790—792$^{g)}$m	789m	r(CH_2)
	635vw	642m	638m	Amide IV

a) s, strong; m, medium; w, weak; v, very; sh, shoulder; ν, stretching; s, scissoring; w, wagging; r, rocking; t, twisting. Only the main IR bands are listed. b) N is the residue number of oct-oligomers. c) From Ref. 3. d) From Ref. 2. e) Corrected for Fermi resonance. f) Observed frequency. g) From Ref. 17 the bands at 1593—1605, 1410—1416, and 790—792 cm^{-1} are overlapped with the asymmetric stretching, symmetric stretching, and scissoring modes of an α-COO$^-$ group, respectively.

groups, closely correspond to those of the longer β_1-type oligomers (N=8—14)[6] as well as those for the β_1-poly(Glu).[2] Thus, the IR spectra of these fresh oct-oligomer samples are characteristic of the β_1-type structure.[2,6]

In order to confirm the $\beta_1 \rightarrow \beta_2$-type transition for the shorter oct-oligomers (N=4—6), the time-dependence of the IR spectra for these samples was measured immediately after the samples were prepared. Figure 4 shows the time-dipendence of the IR spectra for the β_1-type tetramer and pentamer at 20 °C and 60% relative humidity (r.h.). Evidently, the IR bands characteristic of the β_2-type structure (indicated by an asterisk in Fig. 4) increase in intensity with time, until the β_2-type IR spectral feature remains unchanged. A similar observation was made for the oct-hexamer (spectra not shown).

The rate of formation of a $\beta_1 \rightarrow \beta_2$ type transition for the β_1-type oct-oligomers can be easily assumed based on the time-dependence of the value of ln $[(1-f\beta_2^0)/(1-f\beta_2)]$ (Fig. 5), where $f\beta_2$ denotes the fraction of the β_2-type structure and $f\beta_2^0$ is the initial value of $f\beta_2$. It was found that the data can be approximated by a straight line at the beginning of the transition at 20 °C. This shows that the $\beta_1 \rightarrow \beta_2$-type transition in its ealrier stage obeys pseudo-first-order kinetics. The values of the pseudo-first-order rate constant (k_{init}, min^{-1}) are 3.11×10^{-3} for the tetramer and 7.37×10^{-3} for the pentamer at 20 °C and 60% r.h.

For the $\beta_1 \rightarrow \beta_2$ transition of poly(Glu), Itoh et al.[2] have followed the time course of the $\beta_1 \rightarrow \beta_2$ transition by measuring the IR spectra of a poly(Glu) sample. They have indicated that the transition curve (time-dependence of the fraction ($f\beta_1$) of the β_1 structure) is sigmoidal and that the rate process is quite different from that of the $\alpha \rightarrow \beta_1$ transition for poly(Glu). Furthermore, the kinetic theory of phase transitions developed by Avrami[19—21] could be successfully applied to explain the $\beta_1 \rightarrow \beta_2$ transition for poly(Glu).

For the oct-hexamer, it was found that the transition curve has a tendency to take a sigmoidal shape, and that

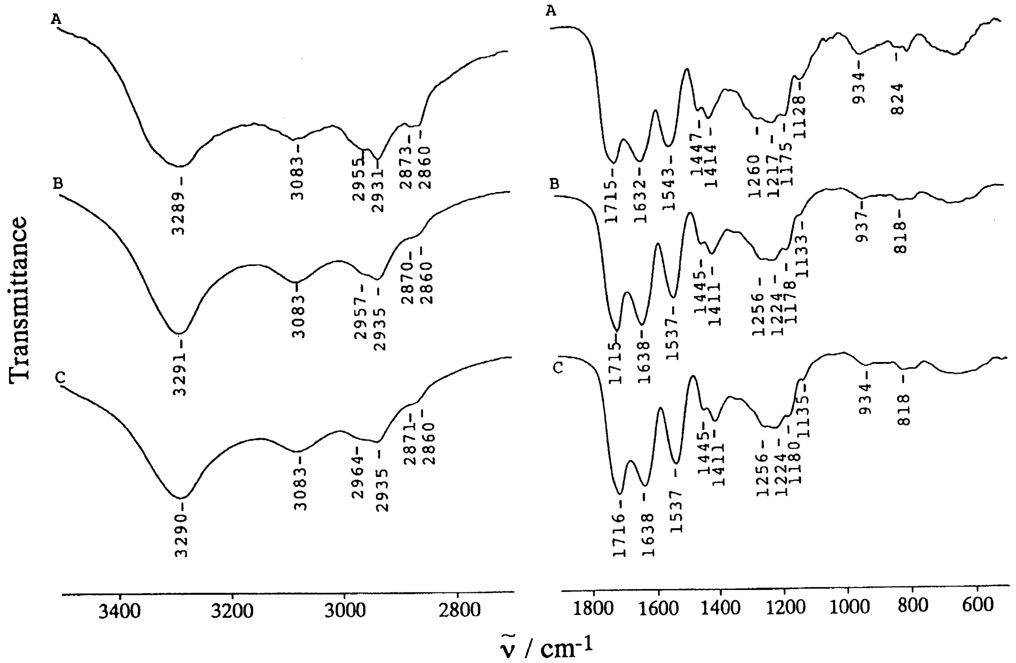

Fig. 3. Infrared absorption spectra of the β_1-type oct-oligomer acid-types: A, oct-tetramer; B, oct-pentamer; C, oct-hexamer.

Table 3. Observed IR Band Frequencies (cm^{-1})[a)] Characteristic of β_1-Type Simple Oct-Oligomers (Residue Number, N=4—6) in the Solid State and Tentative Assignments

β_1-Type oct-oligomers (N)[b)]				β_1-Poly(Glu)[d)]	Assignment[c),d)]
4	5	6	(8—14)[c)]		
3289[f)]vs	3291[f)]vs	3290[f)]vs	3292[f)]		Amide A
(3286)[e)]	(3300)[e)]	(3297)[e)]	(3297)[e)]		
3083[f)]m	3083[f)]m	3083[f)]m	3081—3084[f)]m		Amide B
2955sh	2957sh	2964sh	2978—2980m		ν(CH)
2931s	2935m	2935m	2940—2944m		
2873w	2870sh	2871sh	2871—2874sh		
2860w	2860w	2860w	2862—2864sh		
1715vs	1715vs	1716vs	1714—1716vs	1711vs	ν[C=O(COOH)]
1632vs	1638vs	1638vs	1631—1638vs	1625vs	Amide I
1543s	1537s	1537s	1535—1541s	1523s	Amide II
1447m	1445m	1445m	1444—1448m	1444m	s(CH_2)
1414m	1411m	1411m	1409—1414m	1414m	
1260m	1256m	1256m	1257—1260m	1254m	Amide III
1217m	1224m	1224m	1218—1232m	1230w	w(CH_2)
1175m	1178m	1180m	1172—1181m	1175w	t(CH_2)
1128w	1133w	1135w	1133—1136w	1137w	
934vw	937vw	934vw	932—939vw	932w	ν(C–O)
824vw	818vw	818vw	819—825vw	822w	r(CH_2)

a) See footnote to Table 2. b) N is the residue number of oct-oligomers. c) From Ref. 3. d) From Ref. 2. e) Corrected for Fermi resonance. f) Observed frequency.

the rate process is different from that of the $\beta_1 \rightarrow \beta_2$-type transition for the oct-tetramer and pentamer.

For the $\beta_1 \rightarrow \beta_2$-type transition in the oct-oligomers, we found that the rate process is quite different from that of the $\beta_1 \rightarrow \beta_2$ transition for poly(Glu), and is very similar to that of the $\alpha \rightarrow \beta_1$ transition for poly(Glu).[2)]

For the $\alpha \rightarrow \beta_1$ transition of poly(Glu), which has been investigated by Itoh et al.[2)] using the IR spectra, the values of ln $[(1-f\beta_1^0)/(1-f\beta_1)]$ could be approximated by a straight line at the beginning of the transition, indicating that the $\alpha \rightarrow \beta_1$ transition of poly(Glu) in its earlier stage obeys pseudo-first-order kinetics. For the $\alpha \rightarrow \beta_1$ transition of poly(Glu),[2)] the values of the pseudo-first-order rate constant (k_{init}), 3.0×10^{-3} min^{-1}

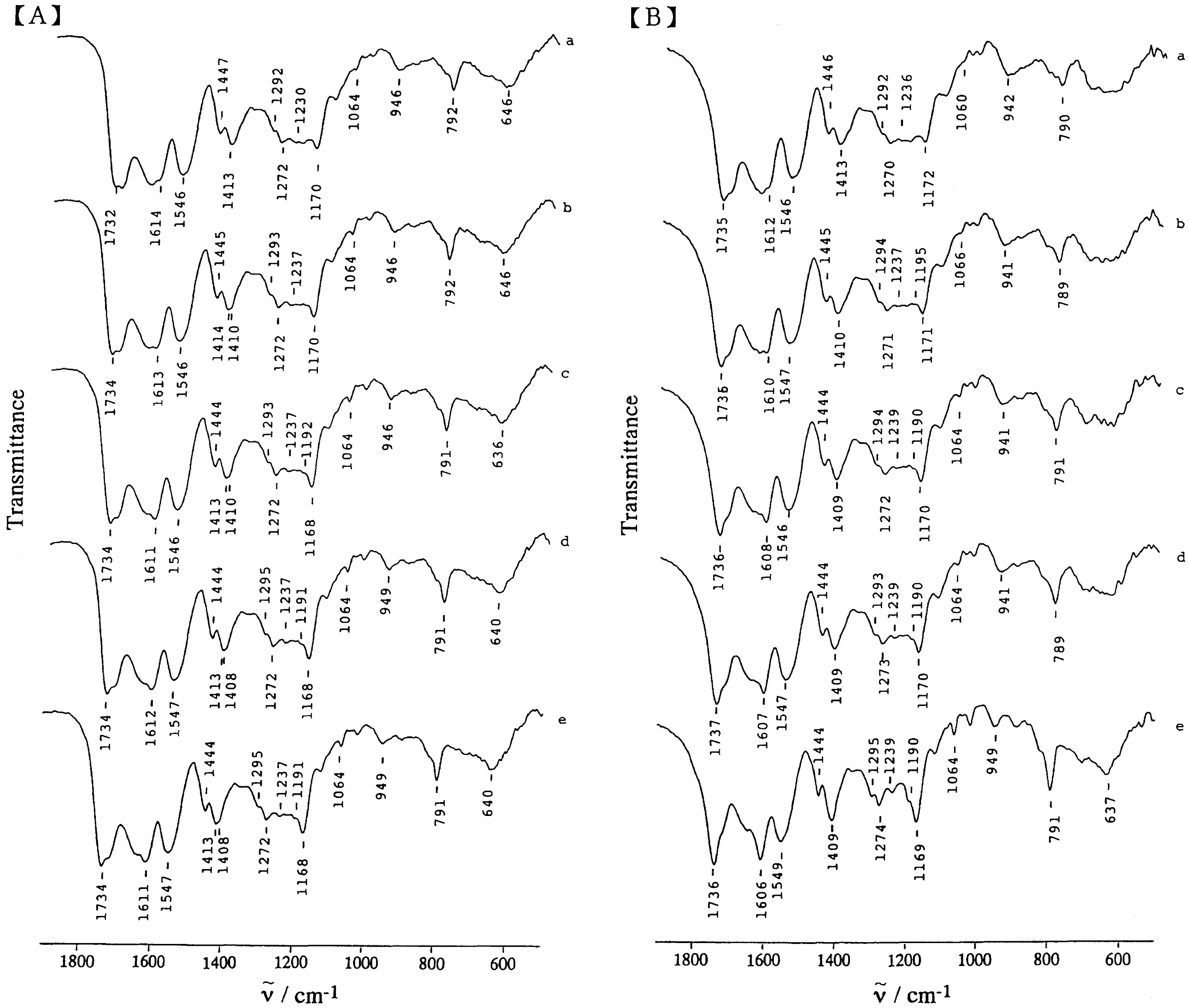

Fig. 4. Time-dependence of the IR spectra for the β_1-type oct-tetramer ([A] a, 0.17 h; b, 1 h; c, 6 h; d, 12 h; and e, 48 h) and oct-pentamer ([B] a, 0.17 h; b, 1 h; c, 6 h; d, 12 h; and e, 48 h) at 20 °C and 60% r.h. The numbers in these IR spectra indicate the observed frequencies of the β_2-characteristic bands.

at 47.0 °C, are very close to those of the oct-oligomers obtained at the present study. Therefore, we may assume that the rate process of the $\beta_1 \rightarrow \beta_2$-type transition for the oct-oligomers is strongly dependent on the residue number. The kinetics of the $\beta_1 \rightarrow \beta_2$-type transition for these oct-oligomers is not discussed herein, but will be reported separately in detail.

Conclusion

For the samples of very simple oct-oligomers (N=2—6), which were kept at −20 °C for one month, the X-ray powder diffraction patterns were measured and the diffraction patterns characteristic of the β_2-poly(Glu) were obtained. This observation shows that these simple oct-oligomers take up a β-sheet structure similar to that of β_2-poly(Glu). For these oligomer samples, the X-ray powder diffraction patterns were also measured almost immediately after the samples were prepared, and were compared with those of the two β-forms for poly(Glu). The results indicate that fresh samples of the pentamer and hexamer provide diffraction patterns which are characteristic of the β_1-poly(Glu), while the fresh samples of the dimer, trimer, and tetramer provide the β_2-type diffraction patterns. It may thus be assumed on the basis of the X-ray powder diffraction patterns that fresh samples of the pentamer and hexamer take up a sheet-type structure similar to that of β_1-poly(Glu), but that the β_1-type sheet structure for these oligomers is transfered to the β_2 sheet-type structure with time until the β_2-type structure is preferentially stabilized.

From the IR specroscopic evidence we may conclude that even the oct-tetramer as well as the pentamer and hexamer take up a β_1-type structure for fresh samples in the solid state. However, for the β_1-type samples of these oct-oligomers, the IR spectra provide evi-

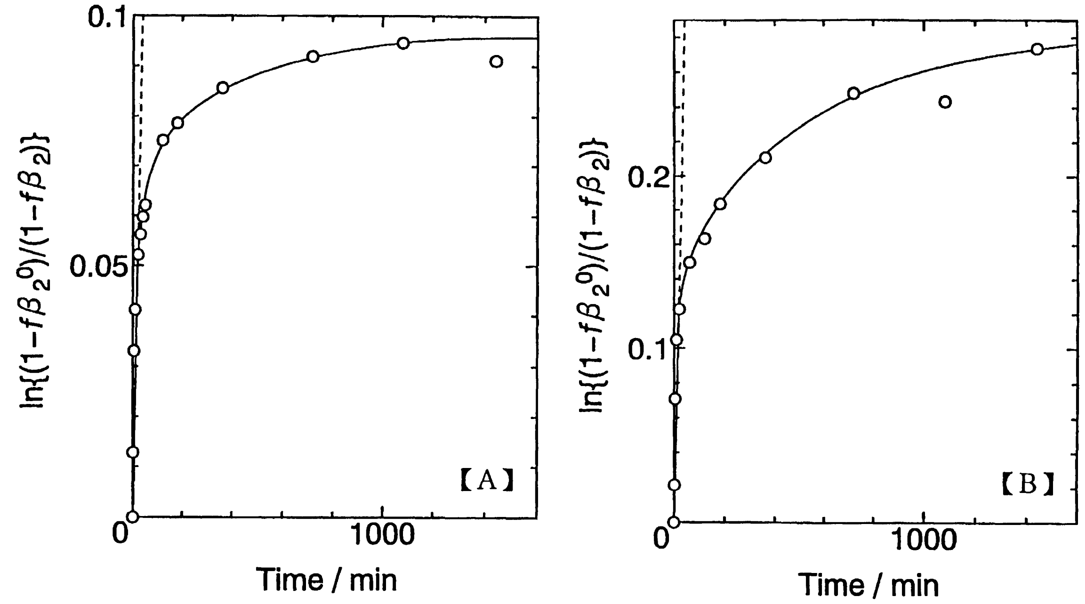

Fig. 5. Value of ln $[(1-f\beta_2^0)/(1-f\beta_2)]$ for the oct-tetramer [A] and oct-pentamer [B], as a function of time (t) in minutes at 20 °C and 60% r.h. The fraction ($f\beta_2$) of the β_2-type form was calculated using the equation ($f\beta_2=[\beta_2]/([\beta_1]+[\beta_2])$), where $[\beta_1]$ and $[\beta_2]$ are the concentrations of the β_1- and β_2-type structure, respectively. The absorbance of the β_1- and β_2-type amide I bands was used for calculations of the values of $f\beta_2$: the bands at 1631—1632 and 1611—1614 cm^{-1} for the β_1- and β_2-type oct-tetramer, respectively, and those at 1627—1630 and 1605—1612 cm^{-1} for the β_1- and β_2-type oct-pentamer, respectively.

dence that a transition similar to the $\beta_1 \rightarrow \beta_2$ transition for poly(Glu) occurs with time. In particular, for the tetramer a $\beta_1 \rightarrow \beta_2$-type transition rapidly occurs compared with the case of the pentamer and hexamer.

The time-dependence of the vibrational spectra for the β_1-type oct-oligomers was measured in detail, and the rate of formation of a $\beta_1 \rightarrow \beta_2$-type transition could be assumed to be as follows. The $\beta_1 \rightarrow \beta_2$-type transition for these oligomers in its earlier stage obeys pseudo-first-order kinetics and is very similar to that of the $\alpha \rightarrow \beta_1$ transition for poly(Glu) in the kinetics. The rate process is quite different from that of the $\beta_1 \rightarrow \beta_2$ transition for poly(Glu). Therefore, a further investigation of the molecular weight-dependence of the kinetics for the $\beta_1 \rightarrow \beta_2$-type transition of the oct-oligomers is highly desirable.

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