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Vibrational Spectra of *N*-Octanoyl-L-glutamic Acid Oligomer Barium Salts and Their Conformations

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Barium salts of *N*-octanoyl-L-glutamic acid oligomers (oct-oligomer salts, residue number, N=2—6, 8, 10, 12, 14, 16, 18, 20, and 22) have been prepared. The vibrational spectra of these molecules were measured and compared with those of β-Ca-poly(L-glutamic acid). For the vibrational spectra of these oligomer salts, the very broad amide I bands at 1644—1655 cm^{-1} (IR), amide III bands at 1240—1256 cm^{-1} (Raman) and at 1240—1250 cm^{-1} (IR) are due to a disordered structure. The Raman bands at 840—855 cm^{-1} and their counter part at 835—848 cm^{-1} in the IR spectra may also come from disordered structures. In the C$^{\alpha}$C stretching-mode region, which strongly reflects the skeletal backbone structure, the Raman bands at 941—944 cm^{-1} were observed in common for those oligomer salts having residue numbers of N=4—22, and correspond well to the 943 cm^{-1} band characteristic of the extended helical conformation of charged poly(L-glutamic acid). We may conclude that the oligomer salts (N=2—22) have disordered structures. However, the oligomer chains having at least residue numbers above N=4 are locally relatively regular and may take up an extended helical structure.

Infrared (IR) absorption and Raman scattering spectroscopy have provided a powerful approach for the conformational analysis of polypeptides and proteins. In particular, poly(L-glutamic acid)(poly(Glu)) has offered a very interesting case for the vibrational analysis of secondary structures. Many experimental studies have been carried out concerning poly(Glu) using various techniques, including X-ray and electron diffraction, IR, Raman, and circular dichroism spectroscopic methods.[1—11)] The results have revealed that the secondary structure of poly(Glu) strongly depends on such conditions as the temperature, pH, and salt concentration. In particular, the local main chain conformation of polypeptides with charged side chains has been the subject of considerable discussion. Tiffany and Krimm[2)] have proposed that the local conformations of polypeptide chains having charged side chains are not random, but consist of relatively ordered regions in which the polypeptide chain takes up conformations similar to that of a left-handed 3_1-helix. Krimm and Mark[3)] indicated based on simplified conformational energy calculations that such a charged helix should have about 2.5 residues/turn; this helical structure has been referred to as the so-called extended helix (EH) conformations. Furthermore, based on CD spectroscopic studies[12—23)] of a variety of systems including synthetic polypeptides, fibrous proteins, globular proteins and other proteins, it has been elucidated that locally random conformations are quite different from those of the EH conformations. Raman and normal-mode studies of the EH structure for a charged poly(Glu), which has been reported by Sengupta and Krimm,[23)] have provided very strong support for such an EH structure. The EH conformation has been found by X-ray diffraction analysis even in a crystalline globular protein.[24)] However, very little is known about the critical size of a relatively regular region adopting such an EH conformation.

To the critical size for the appearance of a secondary structure of L-glutamic acid oligopeptides in an aqueous solution and in the solid state, much attention has been denoted.[25—27)] However, further study along this line seems to be desirable. In our previous work,[28)] the chain-length dependence of the conformations of *N*-octanoyl-L-glutamic acid oligopeptides (acid types) synthesized by a stepwise procedure was investigated in detail using the vibrational spectra. The results showed that these oligomers take up a β_1- or β_2-like structure, similar to that of the two β-forms of poly(Glu) and, that preferential stabilization of the β_1- or β_2-forms is strongly dependent on the residue number.

In the present work, the conformations of the barium salts of *N*-octanoyl-L-glutamic acid oligomers were examined by IR and Raman spectra as a basis for further understanding the EH conformation. The conformations of these oligomer salts are discussed on the basis of a comparison with the vibrational studies of the calcium salt of poly(Glu).[11)]

Experimental

Materials. *N*-Octanoyl-L-glutamic acid oligopeptides (acid types) were prepared by the stepwise procedure described in our previous paper.[28)] The barium salts of these oligomer acid types were then prepared according to a previous study.[10)] The residue numbers (N) of the barium salts of the *N*-octanoyl-L-glutamic acid oligomers (oct-oligomers) synthesized for the present study were 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20, and 22.

Methods. Raman spectra below 4000 cm^{-1} were measured with a Perkin–Elmer 1700 FT Raman spectrometer having 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.

Results and Discussion

For a series of the oct-oligomer barium salts, the FT Raman and FTIR spectra were measured and a band assignment was made on the basis of a comparison with the vibrational spectra of α-poly(Glu)[29] and calcium poly(Glu)[11] and their normal-mode analyses.[11,29] Figures 1 and 2 show the FT Raman and FTIR spectra of these oct-oligomer salts in the solid state. The observed frequencies ($\tilde{\nu}$/cm^{-1}) are listed in Table 1. For calcium, strontium and barium salts of poly(Glu), the crystal structures, based on the antiparallel-chain pleated sheet structure proposed by Pauling & Corey, has been investigated by Keith et al.,[4,5] using an analysis of the X-ray powder diffraction patterns and the selected-area electron diffraction patterns. They have concluded from the observed intersheet spacings and electrostatic considerations that the β-sheet structures of all three salts for poly(Glu) are essentially the same, and that the side chains are fully extended with the planes of the COO$^-$ groups almost parallel to the chain axis. Therefore, a comparison of the vibrational spectra of these oct-oligomer barium salts with those of calcium salt of poly(Glu) provides structural information concerning the oct-oligomer barium salts.

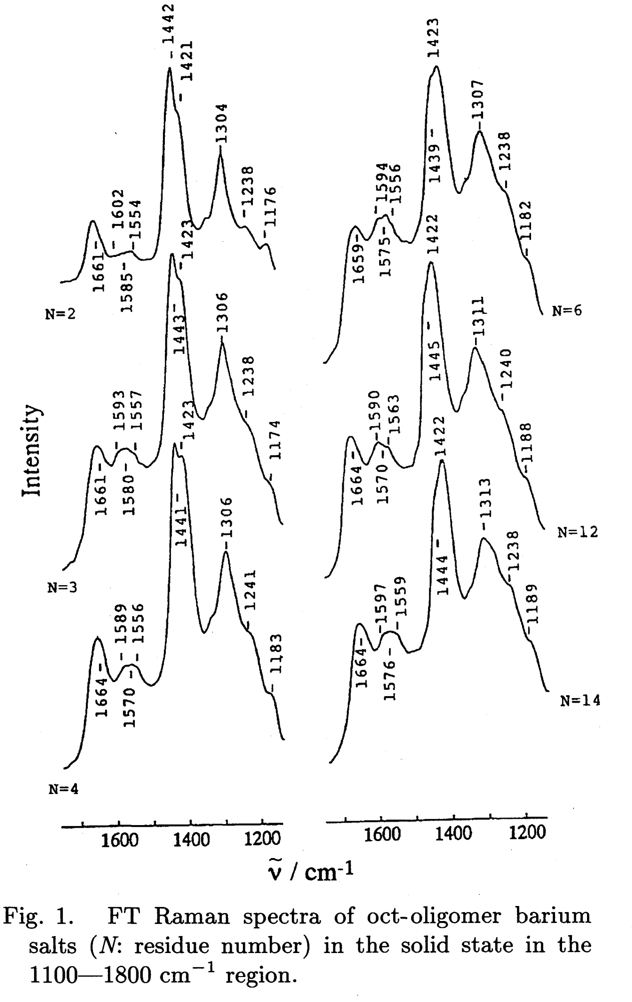

Fig. 1. FT Raman spectra of oct-oligomer barium salts (N: residue number) in the solid state in the 1100—1800 cm^{-1} region.

Apparently, it can be seen that the vibrational bands for these oct-oligomer salts closely correspond to those of the β-type calcium poly(L-glutamate) (Table 1). However, their Raman and IR spectra are extremely broadened and less distinct, as shown in Figs. 1 and 2. This may be due to the presence of a disordered structure in the oligomer salts, which provides the very broad bands in the spectra, resulting from a wide distribution of the dihedral angles ϕ about NC$^\alpha$ and ψ about C$^\alpha$C in the peptide linkage, and from the strong Ba^{2+}-binding to the COO$^-$ groups of the oct-oligomers. Therefore, in the oct-oligomer salts the possibility for the existence of disordered conformations is present. The Raman and IR bands and their assignment are discussed in detail concerning the conformations.

Amide I Modes. For the oct-oligomer barium salts, very broad Raman bands at 1659—1668 cm^{-1} and very broad IR bands at 1644—1655 cm^{-1} are observed in common (Fig. 1), and correspond well to the amide I bands (mainly the C=O stretching) at 1665 (Raman) and 1650—1660 (IR) cm^{-1} for β-Ca-poly-(Glu),[11] respectively. However, in the IR spectra of the oct-oligomer salts (Fig. 2), the bands corresponding to the 1624 cm^{-1} band characteristic of an antiparallel-chain pleated β-sheet structure of Ca-poly(Glu)[5,11] are not found. Since the broad IR band at 1650—1660 cm^{-1} of Ca-poly(Glu) is due to the presence of some disordered Ca-poly(Glu),[11] the IR bands at 1644—1655 cm^{-1} for the oligomer barium salts may indicate the presence of disordered structures in oligomer samples.

Amide II Mode and COO$^-$ Characteristic Band. For the IR spectra of the oct-oligomer barium salts, the assignment of the amide II mode (NH inplane bending plus CN stretching) is difficult, since the IR spectra, in which the amide II bands are strongest, are overlapped in this region due to the strong COO$^-$ antisymmetric stretching mode at 1549—1556 cm^{-1} (Fig. 2).

For the FT Raman spectra of simple oct-oligomer salts (N=2—6) (Fig. 1), three bands at 1554—1561, 1570—1585, and 1589—1602 cm^{-1} are observed, and closely correspond to the Raman bands at 1556, 1569, and 1595 cm^{-1} for β-Ca-poly(Glu).[11] This may imply the presence of β-sheet structures in these oct-oligomer barium salts.

The Raman bands at 1554—1561 cm^{-1} might be due to the COO$^-$ antisymmetric stretching modes (ν_{as}(COO$^-$)), and the Raman bands at 1570—1585 and 1589—1602 cm^{-1} are assignable to the amide II modes on the basis of a normal-mode analysis for β-type Ca-poly(Glu).[11] As was elucidated in our previous paper,[30] the antisymmetric stretching bands aris-

Table 1. Observed Vibrational Band Frequencies[a] ($\tilde{\nu}$/cm^{-1}) Characteristic of Oct-Oligomer Barium Salts (Residue Number, N=2—22)

Oct-oligomer barium salts (N)												
2		3		4		5		6		8		
Raman	IR	Raman	IR	Raman	IR	Raman	IR	Raman	IR	Raman	IR	Assignment[b,c]
3406sh		3421sh		3418w		3423w		3433s		3419w		Amide A
3300vw	3300m	3253w	3293m	3262m	3284m	3262m	3286m	3267s	3293m	3256m	3288m	
3059vw	3076w	3072sh	3078w	3084sh	3070w	3064sh	3076w	3072s	3070w	3065m	3079w	Amide B
	2956w		2956w		2956w		2958w		2953w		2964w	ν(CH)
2932vs	2929w	2930vs	2930w	2932vs	2931w	2932vs	2934w	2930vs	2936w	2930vs	2946w	
2874sh	2872sh	2872sh	2872sh	2880sh	2871sh	2876sh	2872w	2879sh	2873w	2872sh		
2859sh	2858w		2857w		2860w		2861w		2861sh			
1661m	1644s	1661m	1648s	1664m	1652s	1666m	1655s	1659m	1654s	1660sh	1652s	Amide I
										1640m		
										1620sh		
1602vw		1593sh		1589sh		1593sh		1594m		1594m		Amide II
1585sh		1580m		1570m		1581m		1575m		1576sh		
1554vw	1552vs	1557sh	1553vs	1556sh	1551vs	1561sh	1549vs	1556sh	1555vs	1558sh	1553vs	$\nu_{as}(CO_2^-)$
1442s	1446sh	1443s	1445sh	1441s	1442sh	1440s	1443sh	1439sh	1443sh	1446sh	1444sh	s(CH_2)
1421sh		1423sh		1423s		1424s		1423s		1422s		
	1408s		1408s		1406s		1406s		1406s		1407s	$\nu_s(CO_2^-)$
1347w	1344w	1349sh	1349w	1349sh	1350w	1348sh	1351w	1353sh	1351w	1351m	1350w	w(CH_2)
1304m	1310w	1306m	1310w	1306m	1306w	1306m	1310w	1307m	1311w	1304m	1314w	t(CH_2)
1238w	1237sh	1238sh	1240sh	1241sh	1240sh	1245sh	1248sh	1238sh	1247sh	1240sh	1249sh	Amide III
1176vw	1178vw	1174sh	1181vw	1183sh	1182vw	1187w	1184vw	1182sh	1183vw	1189sh	1184vw	w(CH_2)
	1128vw	1118w	1122vw	1116w	1118vw	1119sh	1126vw	1117w	1126vw	1128w	1126vw	
1077w	1087sh	1081w	1087sh	1077w	1088sh	1075w	1089vw	1075w	1088vw	1080w	1076sh	Skeletal and side-chain stretching
1065sh		1059sh		1068sh		1046sh		1050sh		1051sh		
1031w	1026vw	1034sh	1029vw	1021sh	1035vw	1025sh	1036vw	1029sh	1037vw	1019sh	1037vw	
945w	940vw	938w	934vw	943w	934vw	944w	934vw	941w	934vw	944w	937vw	
894w	888vw	894w	888vw	894w	888vw	894w	887vw	894w	887vw	898w	888vw	
842vw	837vw	844vw	839vw	847vw	838vw	850vw	835vw	850vw	841vw	851vw	840vw	r(CH_2)
792sh		794sh		787vw		803vw		801vw		794sh		
783vw	776vw	781vw	777vw	776sh	776vw	784vw	779vw	782vw	775vw	783vw	782vw	

Oct-oligomer barium salts (N)												
10		12		14		18		20		22		
Raman	IR	Raman	IR	Raman	IR	Raman	IR	Raman	IR	Raman	IR	Assignment[b,c]
3421w		3423w		3416sh		3416w		3421w		3416sh		Amide A
3258m	3282m	3260m	3281m	3258w	3296m	3258m	3282m	3258m	3304m	3256w	3282m	
3067m	3078w	3071m	3076w	3049sh	3079w	3070m	3082w	3058m	3074vw	3077sh	3081w	Amide B
	2962sh		2974vw		2978w		2978vw		2975vw		2980sh	ν(CH)
2933vs	2948vw	2933vs	2939vw	2935vs	2944w	2930vs	2943vw	2930vs	2938vw	2933vs	2940vw	
2874sh		2872sh		2872sh		2881sh		2874sh		2872sh		
1662m		1664m		1664m				1668m		1666sh		Amide I
	1652s		1652s		1654s	1647sh	1652s	1655m	1654s	1650m	1654s	
								1638m		1633sh		
1589m		1590m		1597sh		1594m		1601sh		1606m		Amide II
1576m		1570sh		1576m		1580m		1581m		1582m		
1555sh	1553vs	1563m	1556vs	1559sh	1551vs	1548m	1556vs	1559sh	1556vs	1559m	1554vs	$\nu_{as}(CO_2^-)$
1444sh	1442sh	1445sh	1444sh	1444sh	1438sh	1441sh	1443sh	1444sh	1444sh	1449sh	1440sh	s(CH_2)
1421s		1422s		1422s		1424s		1421s		1424s		
	1407s		1406s		1406s		1408m		1407m		1408m	$\nu_s(CO_2^-)$
1351sh	1352vw	1350sh	1352vw	1354sh	1351w		1351vw	1353m	1352vw	1362m	1352vw	w(CH_2)
1311m	1312w	1311m	1314w	1313m	1310w	1312m	1311vw	1309m	1311w	1311m	1313w	t(CH_2)
1240sh	1249sh	1240sh	1250sh	1238sh	1249sh	1250sh	1250sh	1256m	1250sh	1246m	1250sh	Amide III
1185sh	1184vw	1188sh	1184vw	1189sh	1183vw	1187sh	1185vw	1194sh	1184vw	1181sh	1186vw	w(CH_2)
1133w	1126vw	1122w	1122vw	1136w	1122vw		1126vw			1132w	1125vw	
1109w		1087sh	1091sh		1096sh	1089w	1097sh	1087sh		1081w		Skeletal and side-chain stretching
1076w	1076sh	1070w		1076m		1067sh		1074w		1068w		
	1037vw	1044sh	1040vw	1048sh	1039vw	1044sh	1030vw	1038sh	1031vw	1037sh	1034vw	
944w	937vw	943w	935vw	944w	936vw	944vw	936vw	942w	936vw	943vw	938vw	
895vw	888vw	894vw	888vw	895vw	886vw	888vw	887vw	891vw	888vw	893vw	888vw	
855vw	840vw	855vw	841vw	853vw	840vw	850vw	840vw	856vw	848vw	849vw	842vw	r(CH_2)
799vw		805sh		800sh		802vw		800vw		805sh		
781vw	782vw	775vw	777vw	769vw	784vw	786vw	777vw	787vw	782vw	789vw	780vw	

a) s, strong; m, medium; w, weak; v, very; sh, shoulder; Only the main vibrational bands are listed. b) From Ref. 11. c) ν, stretching; s, scissoring; t, twisting; w, wagging; r, rocking, amide I, mainly C=O stretching; amide II, N–H in-plane bending coupled with amide C–N stretching; amide III, mainly amide C–N stretching; amide A, N–H stretching and amide B, the overtone mode of amide II.

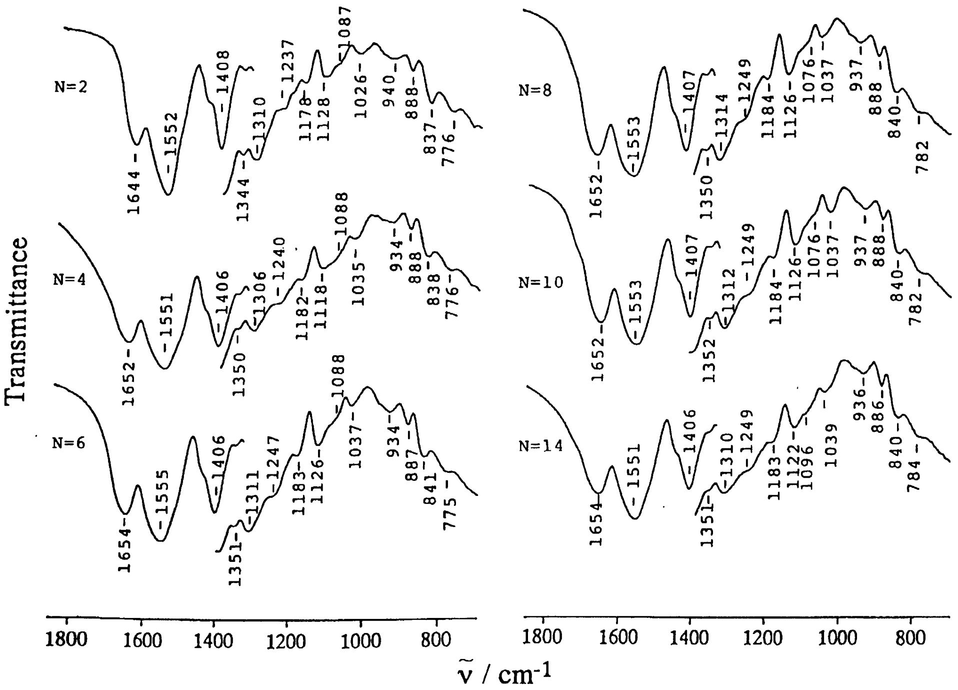

Fig. 2. FT IR spectra of oct-oligomer barium salts (*N*: residue number) in the solid state in the 700—1800 cm^{-1} region.

ing from the C-terminal α-COO$^-$ group are quite different from those of the side-chain COO$^-$ groups in frequency. For an α-COO$^-$ group, the ν_{as}(COO$^-$) band appears at 1590—1600 cm^{-1}, while that for a γ-COO$^-$ group appears at 1560—1570 cm^{-1}. For the vibrational spectra of the oct-oligomer salts the contribution from the ν_{as}(α-COO$^-$) mode becomes greater as the residue number decreases.

For these oligomer salts, the Raman bands at 1421—1424 cm^{-1}, which increase in intensity with an increase in the residue number, and the IR bands at 1406—1408 cm^{-1} closely correspond to the bands at 1424 (Raman) and 1414 (IR) cm^{-1} arising from the COO$^-$ symmetric stretching modes for β-Ca-poly(Glu).[11]

The Raman band at 1439—1449 cm^{-1} are assigned to the CH$_2$ scissoring modes of the *N*-octanoyl chain.

Amide III Modes. For the Raman and IR spectra of β-Ca-poly(Glu), the vibrational bands observed at 1260 cm^{-1} have been assigned to the amide III modes (CN-stretching plus NH in-plane bending). For the oct-oligomer salts, no vibrational bands corresponding to the 1260 cm^{-1} band of β-Ca-poly(Glu)[11] are observed, and the Raman bands at 1238—1256 cm^{-1} and the IR bands at 1237—1250 cm^{-1} are observed in common for these salts. These bands may be due to disordered structures, since for the case of β-Ca-poly(Glu)[11] the weak Raman band at 1238 cm^{-1} and weak IR band at ca. 1248 cm^{-1} result from disordered structures.

Skeletal and Side-Chain Stretching Modes and Raman Evidence for an Extended Helix Conformation. The modes at 900—1200 cm^{-1} have major contributions from the skeletal backbone and side-chain stretching for the oligomer salts. Figure 3 shows the FT Raman spectra of the oct-oligomer barium salts in the skeletal backbone and side-chain stretching region.

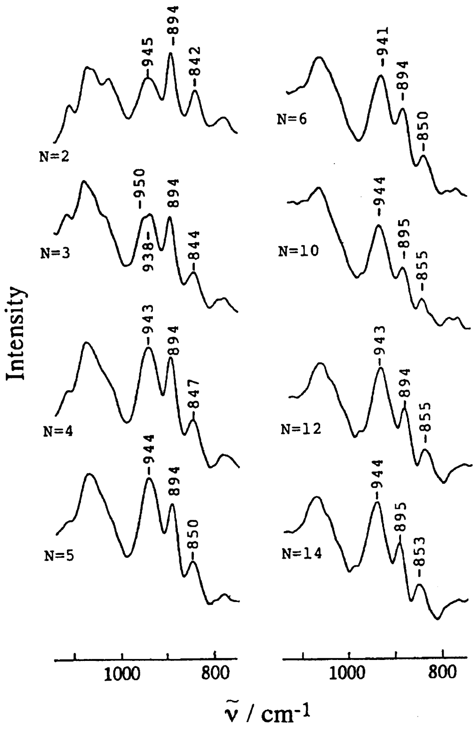

Fig. 3. FT Raman spectra of oct-oligomer barium salts (*N*: residue number) in the solid state in the 700—1100 cm^{-1} region.

Sengupta and Krimm[23] analyzed the normal modes for charged poly(Glu), indicating that the frequency of the skeletal $C^{\alpha}C$ stretching band in the 900—1000 cm^{-1} region of the Raman, spectra is linearly correlated with the value of the dihedral angle (ϕ), and is highly sensitive to the polypeptide backbone conformation. For α-poly(Glu)[29] and the calcium salt of poly(Glu),[11] the Raman bands in the 900—1000 cm^{-1} region are associated with a skeletal $C^{\alpha}C$ stretching mode coupled with CN and CO stretching modes. This mode is observed at 956 cm^{-1} for β-Ca-poly(Glu)[11] and at 924 cm^{-1} for α-helical poly(Glu).[29] Moreover, their normal-mode calculations have shown that the band at 943 cm^{-1} in the Raman spectrum of crystalline Ca-poly(Glu) can be assigned to an EH conformation.

For barium salts of the oct-oligomers, a comparison of the Raman-band frequencies in the 900—1000 cm^{-1} region with the observed and calculated band frequencies for charged poly(Glu)[11] and α-helical poly(Glu)[29] in the same region may provide information concerning the local conformation of the oligomer salts. This attempt is very important, since very little is known about the critical size for taking up an EH conformation.

Figure 3 shows the Raman spectra of barium salts of the oct-oligomers in the 900—1000 cm^{-1} region, reflecting the skeletal $C^{\alpha}C$ stretching mode combined with the CN and CO stretching modes. The Raman bands at 938—945 cm^{-1} for all barium salts correspond well to the 943 cm^{-1} band assigned to the EH form of Ca-poly(Glu), implying that these oct-oligomer barium salts may take up locally an EH conformation. However, the 938—945 cm^{-1} bands of these oligomer salts ($N=4$—22) have a half width (full width at half maximum, $\Delta\tilde{\nu}_{1/2}$) of ca. 42 cm^{-1}, which is very close to the $\Delta\tilde{\nu}_{1/2}$ value (ca. 40 cm^{-1}) of the 949 cm^{-1} band observed for the charged helix in solution,[31] indicating a broader distribution in the dihedral angle (ϕ). Therefore, for these oligomer salts we may expect that there would be a larger distribution in ϕ than would be the case in the ordered environment of a crystal.

For the Raman spectra of the dimer and trimer salts, the bands at ca. 950 cm^{-1} may arise from the N-terminal octanoyl chain, since these Raman bands increases in intensity with a decrease in the residue numbers.

Below 900 cm^{-1}, the Raman bands at 888—898 cm^{-1} may be tentatively assigned to the $C^{\alpha}C$ and CN stretching modes.[11,29] However, these bands increase in intensity as the number of glutamyl residues decreases, implying that for very simple oct-oligomers these bands may result from the terminal octanoyl group. The weak Raman bands at 842—856 cm^{-1} for the oct-oligomer salts may result from disordered structures, since the 850 cm^{-1} Raman band for β-Ca-poly(Glu)[11] was assigned to disordered structures coexisting with the β-sheet-type structure.

CH_2 Stretching Region. Five vibrational modes are predicted in the CH_2 stretching region. For very simple oct-oligomer salts ($N=2$—6), the IR bands at 2857—2861, 2871—2873, 2929—2936, 2953—2958 cm^{-1} are mainly observed in this region (Fig. 4), and can easily be assigned to four of these modes. Only two of these vibrational modes are seen in the Raman spectra. In the IR spectrum of β-calcium poly(Glu),[11] only three bands at 2875, 2936, and 2965 cm^{-1} are observed. Therefore, the IR bands at 2871—2873, 2929—2936, and 2953—2958 cm^{-1} mainly come from the CH_2 groups of the glutamyl residues. However, in the IR spectra of very simple oct-oligomer salts ($N=2$—4), the CH_2 stretching modes have a greater contribution from the octanoyl CH_2 groups.[32]

For the IR spectra of these oligomer salts ($N=2$—22), on the whole, those bands arising from the CH_2 stretching modes are broadened. This may result from some disorder in the side chains. However, since some bands are still evident, we may assume that most of the side chains are in an extended conformation.

Conclusion

The amide I band at 1624 cm^{-1}, a characteristic of the antiparallel-chain pleated-sheet β-structure for Ca-poly(Glu), does not appear in the IR spectra of these oct-oligomer barium salts. Moreover, for these oligomers the very broad amide I bands at 1644—1655 cm^{-1} are observed in common and correspond to the 1650—1660 cm^{-1} (IR) bands resulting from the disor-

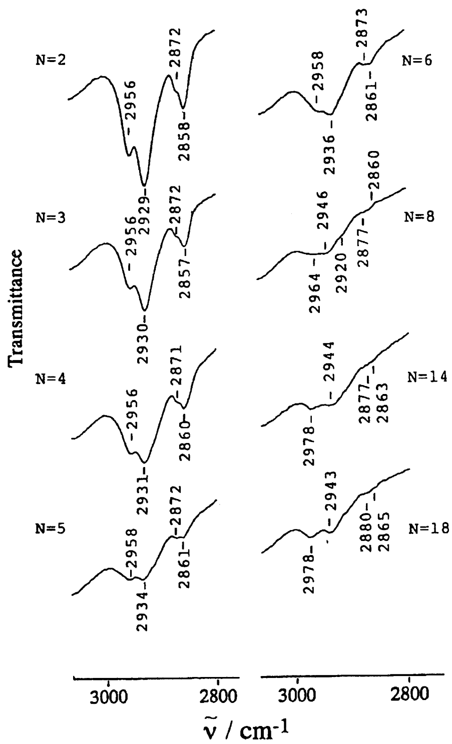

Fig. 4. FT IR spectra of oct-oligomer barium salts (N: residue number) in the solid state in the 2800—3000 cm^{-1} region.

dered structure of Ca-poly(Glu).

No amide III bands (Raman and IR bands at 1260 cm^{-1}) characteristic of β-Ca-poly(Glu) have been observed, and the vibrational bands at 1238—1256 cm^{-1} (Raman) and at 1237—1250 cm^{-1} (IR) observed in the amide III region have been assigned to disordered structures.

However, in the $C^{\alpha}C$ stretching mode, which exhibits a sensitivity to a skeletal backbone conformation, the Raman bands at 938—945 cm^{-1} are observed in common for the oct-oligomer salts, and closely correspond to the 943 cm^{-1} band characteristic of the extended helix conformation of Ca-poly(Glu).

These results show that for a series of the oct-oligomer salts the peptide chains are disordered, but are locally relatively regular and may take up an extended helical conformation, even though there is a larger distribution in ϕ.

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