Metal Isotope Effects on the Vibrational Spectra of [Bis(glycylglycinato)calcium Chloride]_n¹⁾

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The infrared and Raman spectra of [bis(glycylglycinato)calcium chloride]_n and its isotopic complexes containing metal and hydrogen isotopes have been measured. A large isotope shift of more than 4 cm⁻¹ on ⁴⁰Ca and ⁴⁴Ca substitution has been observed in the infrared-active bands at 345, 267 and 242 cm⁻¹. These bands have been assigned to Ca–O antisymmetric stretching vibrations. The Raman-active bands at 305, 158 and 111 cm⁻¹ have been assigned to Ca–O symmetric stretching vibrations with no displacement of the calcium atom. The result of a normal coordinate analysis is consistent with both the empirical assignments and the experimental isotope shifts. These results indicate that Ca–ligand bond forces are almost similar to those of copper- and zinc-amino acid complexes.

Keywords infrared spectra; Raman spectra; metal isotope effect; [bis(glycylglycinato)calcium chloride]_n; normal coordinate analysis; ⁴⁰Ca–⁴⁴Ca substitution; amino acid; deuteration; glycylglycine, calcium complex

Calcium-binding proteins, such as calmodulin, troponin and parvalbumin, are of much interest because of their biological properties,^{3–5)} and a relationship between their biological activities *in vivo* and a coordination structure of the calcium atom has been investigated by various methods.^{6–8)} Calcium–amino acid complexes are suitable substances, as models of calcium-binding proteins, for investigating the coordination structure around the calcium atom. Although vibrational spectroscopy provides information about coordination of metal complexes, infrared (IR) and Raman spectra of calcium complexes have been investigated in less detail than X-ray analyses and nuclear magnetic resonance (NMR) spectra.^{9–12)} It seems, however, rather difficult to analyze the vibrational spectra of calcium complexes, since they frequently have a complicated polymeric structure. Previously, it has been shown for complicated polymeric copper- and zinc-complexes that the metal isotope technique is very useful for assigning the vibrations involving displacement of the metal atom.^{13–15)}

In the present work, the infrared and Raman spectra of a complicated polymeric [bis(glycylglycinato)calcium chloride]_n (Ca-GG) were investigated by the metal isotope technique (⁴⁰Ca and ⁴⁴Ca substitution) in order to study the metal-ligand stretching vibrations. The metal isotope shifts observed indicate that the frequencies of the Ca–ligand stretching frequencies are relatively higher than those of the six-coordinated Ca–amino acid complexes. These frequencies are in almost the same region as those for the copper- and zinc-amino acid complexes, although the

Ca–ligand bond-lengths of Ca-GG are longer than those of the copper- and zinc-amino acid complexes.

Experimental

Materials Commercially available glycylglycine (GG) was purified several times by recrystallization from a mixture of water and ethanol. *N,N'*-Deuterated glycylglycine (GG-*d*₄) was obtained from the purified GG by the usual exchange reaction with heavy water (Merck, A.G., 99.8%). [Bis(glycylglycinato)calcium chloride]_n was prepared from the purified GG and CaCl₂·2H₂O according to the method of Meulemans *et al.*¹⁶⁾ For the preparation of the *N,N'*-deuterated complex (Ca-GG-*d*₈), CaCl₂·2D₂O was reacted with GG-*d*₄ in heavy water. The complex was precipitated by the addition of ethanol-*d*₁, filtered, washed with a mixture of heavy water and ethanol-*d*₁ and dried on phosphorous pentoxide under reduced pressure. For the preparation of the complexes containing ⁴⁰Ca and ⁴⁴Ca, ⁴⁰CaCO₃ and ⁴⁴CaCO₃ (Oak Ridge National Lab., U.S.A.) were converted into the corresponding calcium chloride dihydrate by

Fig. 1. IR Spectra of Ca-GG in the Region between 4000 and 400 cm⁻¹ Ca-GG (—) and Ca-GG-*d*₈ (---).

Fig. 2. Raman Spectra of Ca-GG in the Region between 4000 and 400 cm⁻¹ Ca-GG (—) and Ca-GG-*d*₈ (---).

reaction with dilute hydrochloric acid on a milligram scale. ^{40}Ca -GG and ^{44}Ca -GG were prepared from the corresponding calcium chloride dihydrate in a similar way. The infrared and Raman spectra of each complex containing a metal isotope coincided with that of the complex containing the metal of natural abundance except for the metal isotope shifts. The isotopic purities were more than 98% for $^{40}\text{CaCO}_3$ and $^{44}\text{CaCO}_3$.

Measurements The IR spectra were recorded on a JASCO DS-403G IR spectrophotometer ($4000\text{--}200\text{ cm}^{-1}$). Measurements of the solid samples were made in Nujol and hexachlorobutadiene (Merck, AG., Uvasol) mulls. The frequencies were calibrated by use of the standard absorptions of polystyrene, indene and water vapor. For measuring small shifts of band centers on ^{40}Ca and ^{44}Ca (^{40}Ca - ^{44}Ca) substitution, the frequency scale was expanded ten times over the desired frequency region. The measurements were repeated at least three times to check the reproducibility of the frequencies. The IR spectra of Ca-GG and its N,N' -deuterated analog in the region above 400 cm^{-1} are shown in Fig. 1, and parts of the expanded spectra in the region between 420 and 200 cm^{-1} are shown in Fig. 3.

The Raman spectra were recorded for the solid samples in a glass capillary tube by using a JASCO NR-1000 Raman spectrophotometer and an NEC 3300 Ar^+ ion laser (514.5 nm). The frequencies were calibrated by use of the standard bands of indene. The Raman spectra of Ca-GG and its N,N' -deuterated analog in the region above 400 cm^{-1} and parts of the expanded spectra in the region between 450 and 50 cm^{-1} are shown in Figs. 2 and 4, respectively.

Normal Coordinate Analysis

According to X-ray analysis,¹⁶⁾ Ca-GG takes a complicated polymeric structure chained by the bonds between the calcium atom and carbonylic and carboxylic oxygens of the neighboring glycylglycinates. Since the complex belongs to the C_i point group, the normal vibrations of the complex are classified into Raman-active A_g and IR-active A_u species. The optical active normal frequencies of Ca-GG were calculated by using the same computer program as reported in the previous paper.¹⁷⁾ By assuming the bond-lengths, $r_{\text{CH}} = 1.08$ and $r_{\text{NH}} = 1.02\text{ \AA}$, structure parameters were constructed on the basis of the X-ray data reported by Meulemans *et al.*¹⁶⁾ The calculation was carried out for a full chain molecule, neglecting the chloride atoms. A modified Urey-Bradley force field was used with a few valence-type force constants for the torsional and out-of-plane bending coordinates. The force constants for glycylglycinates were initially transferred from DL-serine,¹⁸⁾ and those concerning the calcium atom were transferred from $\text{Cu}(\text{DL-serinate})_2$ reported by Inomata *et al.*¹⁹⁾ In this calculation, only the intramolecular forces were taken into account. The force constants used in the final calculation are listed in Table I.

The calculated frequencies in the final calculation are

TABLE I. Modified Urey-Bradley Type and Valence Type Force Constants

Modified Urey-Bradley type force constants (mdyn/ $\text{\AA}$)							
$K(\text{C-O})$	7.55	$H(\text{HCH})$	0.422	$H(\text{OCaO})$	0.0	$F(\text{CCN})$	0.60
$K(\text{C-C})$	1.95	$H(\text{NCH})$	0.25	$H(\text{OCaO})$	0.0	$F(\text{CNC})$	0.50
$K(\text{C-N})$	2.45	$H(\text{CCN})$	0.55	$\kappa(\text{N})^a$	-0.05	$F(\text{CNH})$	0.50
$K(\text{C-H})$	4.00	$H(\text{CNC})$	0.40	$\kappa(\text{C})^a$	0.01	$F(\text{NCO})$	0.50
$K(\text{N-H})$	5.45	$H(\text{CNH})$	0.30	$F(\text{OCO})$	2.52	$F(\text{CNH})$	0.50
$K(\text{Ca-O})$	0.50	$H(\text{NCO})$	0.40	$F(\text{OCC})$	0.70	$F(\text{HNH})$	0.02
$H(\text{OCO})$	0.10	$H(\text{CNH})$	0.30	$F(\text{CCH})$	0.40	$F(\text{CaOC})$	0.10
$H(\text{OCC})$	0.24	$H(\text{HNH})$	0.55	$F(\text{HCH})$	0.076	$F(\text{OCaO})$	0.0
$H(\text{CCH})$	0.16	$H(\text{CaOC})$	0.05	$F(\text{NCH})$	0.54	$F(\text{OCaO})$	0.0
Valence type force constants (mdyn $\cdot \text{\AA} / \text{rad}^2$)							
$f(\omega\text{CO}_2, \omega\text{CO}_2)$	1.20	$f(\delta\text{NH}, \delta\text{NH})$	0.50	$f(\delta\text{C=O}, \delta\text{C=O})$			0.50
$f(\tau\text{CC}, \tau\text{CC})$	0.15	$f(\tau\text{CN}, \tau\text{CN})$	0.45	$f(\tau\text{C=O}, \tau\text{C=O})$			0.50

a) mdyn $\cdot \text{\AA}$.

shown in Table II, together with the observed frequencies and the approximate intensities.

Vibrational Assignments and Discussion

The Region above 400 cm^{-1} All the bands of Ca-GG and Ca-GG- d_8 in this region showed no appreciable shift on ^{40}Ca - ^{44}Ca substitution, and are accordingly assigned to the vibrations involving no displacement of the calcium atom. By referring to the frequency shifts on N,N' -deuteration and the assignments for GG by previous authors,^{20,21)} the bands in this region were assigned to the vibrations of glycylglycinates. As shown in Table II, the agreement of the calculated and the observed frequencies was satisfactory for the A_g and A_u species.

The Region below 400 cm^{-1} According to X-ray analysis,¹⁶⁾ the coordination structure around the calcium atom of Ca-GG is square-bipyramidal, including six oxygen atoms of the carbonyl and carboxyl groups, and the central calcium atom is on a center of symmetry. Consequently, three Ca-ligand antisymmetric stretching vibrations are IR-active (A_u species) and three Ca-ligand symmetric stretching vibrations are Raman-active (A_g species). The metal isotope effect on ^{40}Ca - ^{44}Ca substitution is expected for only three Ca-ligand antisymmetric stretching vibrations (A_u species) involving displacement of the calcium atom.

For the IR spectra in the region between 400 and 200 cm^{-1} , five bands are observed at 391 , 345 , 309 , 267 and 242 cm^{-1} as seen in Fig. 3. On ^{40}Ca - ^{44}Ca substitution, the centers of the 345 , 267 and 242 cm^{-1} bands shift to lower frequencies by 6.5 , 5.4 and 4.3 cm^{-1} , respectively, and these

Fig. 3. IR Spectra of Ca-GG in the Region between 420 and 200 cm^{-1} . A, ^{40}Ca -GG (—) and ^{44}Ca -GG (---). B, Ca-GG (—) and Ca-GG- d_8 (---).

TABLE II. Observed and Calculated Frequencies and Assignments for Ca-GG and Its N,N' -Deuterated Analogues in the Region between 4000 and 50 cm^{-1}

A _u species (IR)				A _g species (Raman)				Assignments ^{b)}
Ca-GG		Ca-GG- <i>d</i> ₈		Ca-GG		Ca-GG- <i>d</i> ₈		
Obs. ^{a)}	Calc.	Obs. ^{a)}	Calc.	Obs. ^{a)}	Calc.	Obs. ^{a)}	Calc.	
3215 s	{ 3297 3242 3242	2370 s	2408	3225 w	{ 3297 3242 3242	2375 w	{ 2408 2395 2393	νNH(ND) ν _d NH ₃ (ND ₃) ν _d NH ₃ (ND ₃)
3080 s	3151	2170 s	2258	3105 w	{ 3151 3030	2250 vw	2258	ν _s NH ₃ (ND ₃)
3000 s	3030	2995 w	3030	2995 s	2871	3105 vw	3030	ν _a CH ₂
2920 sh	{ 2871 2845 2845	2940 w	2872	2940 sh	2845	2998 s	2872	ν _a CH ₂
		2930 sh	2845	2927 s	2845	2940 sh	2845	ν _s CH ₂
		2860 sh	2845			2928 s	2845	ν _s CH ₂
1647 s	1654	1638 m	1653	1662 m	1654	1652 m	1652	ν _a CO ₂
1607 sh	1631	1593 m	1597	1630 w	1631	1572 m	1596	νC=O
1595 sh	1626			1608 w	1626	1170 vw	1167	δ _d NH ₃ (ND ₃)
1568 s	1623	1181 m	{ 1167 1163	1575 m	1623	1150 vw	1163	δ _a NH ₃ (ND ₃)
1493 s	{ 1506 1493	1138 m	1124	1470 m	1506	1126 w	1123	δNH(ND)in-plane
1447 s	1452	1160 m	1158	1446 w	1493	1105 w	1158	δ _s NH ₃ (ND ₃)
1424 w	1444	1502 s	1457	1430 m	1452	1503 m	1457	βCH ₂
1402 s	1364	1447 s	1455	1405 s	1444	1446 m	1455	βCH ₂
1325 sh	1310	1402 s	1364	1395 sh	1364	1405 s	1362	ν _s CO ₂
1317 m	1294	1367 s	1309	1330 sh	1310	1330 m	1308	ωCH ₂
1269 m	{ 1239 1249	1319 s	{ 1294 1287 1237	1320 m	1293	1322 sh	1294	ωCH ₂
1241 m	1231			1276 m	{ 1238 1249	1280 sh	1286	νC=O in-plane
1123 m	1122	1242 m	{ 1229 1229			1271 m	1236	νCH ₂
1105 w	1108	755 m	756	1248 m	1231	1249 m	1229	νCH ₂
1081 m	1077	708 m	708	1126 m	1122	715 w	756	ρNH ₃ (ND ₃)
1042 m	1008	699 m	708	1102 m	1108	708 w	703	ρNH ₃ (ND ₃)
999 m	962	1027 m	{ 1054 997	1077 w	1076	1044 m	1054	νskel
953 m	947	965 sh	945	1044 m	1008	1021 vw	997	νskel
		991 m	955	998 m	962	972 w	943	νskel
895 m	{ 861 860	927	915	952 s	947	997 m	955	ρCH ₂
		933 m	927	897 m	{ 858 860	956 s	926	νskel
		892 w	915			899 m	915	ρCH ₂
721 m	{ 767 694	839 m	810	815 w	765	840 w	807	νskel
		480 w	444	710 m	694	520 vw	437	δNH(ND) out-of-plane
653 w	661	622 w	659	655 w	656	600 w	651	βCO ₂
596 m	582	558 m	568	600 m	578	560 vw	561	ρCO ₂
579 w	536	598 m	611	574 m	535	574 m	610	ωCO ₂
551 m	524	520 m	513	557 sh	529	530 vw	501	δC=O out-of-plane
500 w	494	480 w	408	520 w	485		408	δskel
391 w	367	383 w	363	386 w	362	384 w	354	δskel
346 w	327	344 w	325	— ^{d)}	— ^{d)}	— ^{d)}	— ^{d)}	ν _{as} Ca-O
— ^{d)}	— ^{d)}	— ^{d)}	— ^{d)}	305 w	301	302 w	296	ν _s Ca-O
— ^{c)}	353	287 sh	329	320 sh	329	320 w	307	δskel
309 w	250	299 w	245	212 m	237	210 w	231	δskel
267 vw	238	268 vw	235	— ^{d)}	— ^{d)}	— ^{d)}	— ^{d)}	ν _{as} Ca-O
— ^{d)}	— ^{d)}	— ^{d)}	— ^{d)}	158 m	169	156 m	167	ν _s Ca-O
242 w	203	242 w	200	— ^{d)}	— ^{d)}	— ^{d)}	— ^{d)}	ν _{as} Ca-O
— ^{d)}	— ^{d)}	— ^{d)}	— ^{d)}	111 m	135	110 m	129	ν _s Ca-O
— ^{e)}	190	— ^{e)}	184	135 vw	154	122 vw	148	δskel
— ^{e)}	138	— ^{e)}	132	115 sh	114	— ^{c)}	108	δskel
— ^{e)}	124	— ^{e)}	117	89 m	79	88 m	78	δskel
— ^{e)}	84	— ^{e)}	81	75 m	76	75 w	73	δskel
— ^{e)}	46	— ^{e)}	44	53 m	41	53 m	40	δskel

a) s, Strong; m, medium; w, weak; vw, very weak; sh, shoulder. b) ν , Stretching; β , bending; δ , deformation; t , twisting; ω , wagging; ρ , rocking. c) Hidden by a neighboring band. d) Not expected. e) Not observed.

bands are undoubtedly assigned to the vibrations involving displacement of the calcium atom. Since these bands remain almost unshifted on N,N' -deuteration, these bands are clearly assigned to the Ca-ligand antisymmetric stretching vibrations. The remaining bands were assigned to the skeletal deformation vibrations of glycyglycinates because of small isotope shifts on ^{40}Ca - ^{44}Ca substitution and large shifts on N,N' -deuteration.

In the Raman spectra, eleven bands are observed in the

region between 400 and 50 cm^{-1} . Since three Ca-ligand symmetric stretching vibrations belong to the A_g species, these vibrations involve no displacement of the calcium atom, and are expected to show no metal isotope effect on ^{40}Ca - ^{44}Ca substitution. Actually, all eleven bands showed no metal isotope shift on ^{40}Ca - ^{44}Ca substitution, as shown in Fig. 4. By referring to the shifts on N,N' -deuteration and the result of the normal coordinate analysis, the relatively strong bands at 305, 158 and 111 cm^{-1} were assigned to

Fig. 4. Raman Spectra of Ca-GG in the Region between 450 and 50 cm^{-1}

A, Ca-GG (—) and Ca-GG- d_8 (---). B, ^{40}Ca -GG (—) and ^{44}Ca -GG (---).

the Ca-ligand symmetric stretching vibrations. The remaining bands in this region were assigned to the skeletal deformation vibrations of glycylglycinates. The bands corresponding to the Ca-ligand antisymmetric stretching vibrations were not observed in the Raman spectra, as expected from the mutual exclusion rule.

The observed and calculated isotope shifts for the vibrations of IR-active A_u species are summarized in Table III, together with their assignments. Table III indicates that the final calculation reproduced well the observed isotope shifts on ^{40}Ca - ^{44}Ca substitution and N,N' -deuteration, although the calculated frequencies were generally lower than those observed. Small isotope shifts of the skeletal deformation vibrations on ^{40}Ca - ^{44}Ca substitution may be caused by a small contribution of the Ca-ligand stretching vibrations. The calculated L matrices actually reveal that the Ca-ligand stretching vibrations couple with most of the skeletal deformation vibrations. The calculated frequencies for the A_g species also correspond well not only to the observed Ca-ligand stretching vibrations but also to the observed skeletal deformation vibrations, as shown in Table II. No frequency shifts on ^{40}Ca - ^{44}Ca substitution were calculated for all the vibrations of the Raman-active A_g species, supporting the present assignments.

Previously, the metal-ligand stretching vibrations of copper- and zinc-amino acid complexes were comprehensively investigated by the metal isotope technique (^{63}Cu - ^{65}Cu and ^{64}Zn - ^{68}Zn substitution, respectively) and were observed in the region between 450 and 200 cm^{-1} .^{13-15,17)} Since the Ca-ligand bond-lengths of Ca-GG are longer than those of the copper- and zinc-amino acid complexes, the Ca-ligand stretching vibrations of Ca-GG are expected to be observed in the region below 200 cm^{-1} . However, the metal-isotope sensitive Ca-ligand stretching vibrations of Ca-GG were observed in almost the same region as those of copper- and zinc-amino acid complexes. The force

TABLE III. Observed and Calculated Infrared-Active Frequencies, Isotope Shifts and Assignments of Ca-GG in the Region between 400 and 200 cm^{-1} (A_u species)

Ca-GG		$\Delta\nu_m^a)$		$\Delta\nu_h^b)$		Assignments ^{c)}
Obs.	Calc.	Obs.	Calc.	Obs.	Calc.	
391	367	1.6	1.1	7.2	4.4	δskel
345	327	6.5	3.0	0.9	2.3	$\nu_{as}\text{Ca-O}$
— ^{d)}	353	— ^{d)}	1.8	ca. 20	23.3	δskel
309	250	1.5	1.9	9.7	5.8	δskel
267	238	5.4	5.0	0.2	2.4	$\nu_{as}\text{Ca-O}$
242	203	4.3	3.2	ca. 0	2.9	$\nu_{as}\text{Ca-O}$

a) Shift on ^{40}Ca and ^{44}Ca substitution for Ca-GG. b) Shift on N,N' -deuteration. c) δ , deformation; ν_{as} , antisymmetric stretching. d) Hidden by 309 cm^{-1} band.

constants for the metal-ligand stretching mode of Ca-GG used in this calculation were somewhat larger than those for $\text{Zn}(\text{L-serinate})_2$.¹⁷⁾

In conclusion, the Ca-ligand stretching vibrations are observed around 300 cm^{-1} , which is higher than those expected from their bond-lengths. This result is useful for investigations of calcium-amino acids and -drugs as fundamental data.

Acknowledgments The authors are very grateful to Prof. Katsunosuke Machida, Faculty of Pharmaceutical Sciences, Kyoto University, for his kind offering of the computer program. The calculations were carried out using an ACOS 1000 computer of the Okayama University Computer Center. This study was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

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- 1) This work was presented at the 25th Meeting of the Chugoku-Shikoku Branch, Pharmaceutical Society of Japan, Tottori, Japan, 1986.
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