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Raman Scattering Study on Coordination Structures of Cu(II)–L-Histidine(1 : 2) in Aqueous Solutions

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The pH and pD dependences of the Raman spectra of Cu(II)–L-histidine (1:2) and Cu(II)–L-histidine-d_{3-5} (1:2) were analyzed based on the pH titration data. Several ring vibrations of the imidazolyl group with cationic, neutral and anionic forms, the out-of-plane bending vibration of the bound carboxylate, and the Cu(II)–N stretching vibrations of the bound amino groups are used to study the chelation structures of the species taken by the solutions. From the results it was concluded that MHA^{2+}, the major species below pH 3, assumes a glycine-like chelation structure with an unbound imidazolium cation. The results also indicate that the species MHA_2^+, which is prominent in the pH region 4.0—4.5, takes a mixed-type structure, in which one histidine molecule coordinates to Cu(II) as a terdentate ligand and another binds as a substituted glycine. MA_2, the major species around pH 7, is determined to have the chelation structure in which two nitrogen atoms of the amino groups and two nitrogen atoms of the imidazole rings are bound to the Cu(II) ion in the square-planar trans position and at least one carboxyl group in the axial position.

The imidazolyl group, as a histidine moiety, functions as a ligand toward transition metal ions in a variety of metalloproteins including carbonic anhydrase,[1] carboxypeptidase,[2] and thermolysine.[3] Yoshida *et al.*[4] and Salama and Spiro[5] measured the Raman spectra of cobalt(II)–imidazole and cobalt(II)–L-histidine complexes as model compounds of metalloproteins and studied the relationship between the spectra and the coordination geometries of the complexes. Recently we also analyzed the Raman spectra of hexakis(4-methylimidazole)cobalt(II) complex, $Co(4MeImH)_6^{2+}$, and bis(L-histidinato) metal complexes, M(L-His)$_2$ (M=Ni(II), Zn(II)), in solid state.[6] From this study we concluded that some vibrational modes of the bound imidazolyl group clearly reflect the coordination site of this group to central metal ions. We also mentioned the possibility of elucidating the coordination geometries of the above-mentioned metalloproteins by means of Raman spectroscopy.

In this paper we measured Raman spectra and studied the coordination structures of Cu(II)–L-histidine(1:2) in aqueous solutions. Physiological studies suggest that a fraction of Cu(II) ions in human plasma is bound to human serum albumin and at the same time bound to amino acids consisting mostly of L-histidine.[7,8] Therefore, many physicochemical methods have been applied to Cu(II)–L-histidine(1:2) in aqueous solutions. Several pH-titration studies indicate that the solution contains MHA^{2+}, MA^+, MHA_2^+ and MA_2 as major species near and below the physiological pH[9,10] (Cu(II) abbreviated to M^{2+} and anionic histidine to A^-). The precise structures of these species, however, are still uncertain because the physical parameters obtained for the system (*e.g.*, formation constants, absorption maxima, and circular dichroism extrema) give only indirect informations about the coordination geometries. The use of ^{1}H-NMR spectroscopy is restricted only to the Cu(II)–L-histidine system with the ligand to metal ion ratio greater than 10^3 because the long electron spin relaxation time of Cu(II) ion causes too large line broadening of ligand resonances to permit their observation at stoichiometric concentration ratios.[11,12] The analysis of the Raman spectra provide us with some new and direct informations about the coordination structures of several species. The results clearly show that Raman spectroscopy is one of the powerful method for the structural study of coordination compounds containing L-histidine molecules and L-histidine residues as ligands.

Experimental

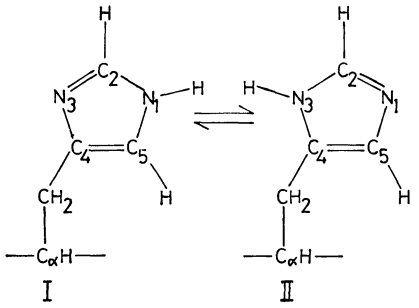
Materials. L-Histidine monohydrochloride monohydrate and $CuCl_2 \cdot H_2O$ were of reagent grade and obtained from Kanto Chemical Co., Inc. D_2O (99.75%), and concentrated D_2O solutions of NaOD (40%), and DCl (37%) were from E. Merck AG, Darmstadt. The measurement of the Raman spectra was made on the aqueous solutions of L-histidine and those containing L-histidine (0.4 M†) and $CuCl_2$ (0.2 M). The measurement was also performed on the D_2O solutions of L-histidine-d_{3-5} and those containing L-histidine-d_{3-5} (0.4 M) and $CuCl_2$ (0.2 M). L-Histidine-d_{3-5} and $CuCl_2 \cdot D_2O$ were prepared by Iyophilizing two times the D_2O solutions of L-histidine and $CuCl_2 \cdot H_2O$. The pH and pD values of the sample solutions were adjusted with 2—5 M NaOH and HCl and 2—5 M NaOD and DCl, respectively.

Measurement of Raman Spectra. All the spectra were taken by using a JEOL 400D Raman spectrophotometer equipped with a Spectra Physics model 164 argon ion laser. The 488.0 nm emission line was used as an excitation source. A spinning cell or a flow technique was used for the measurement.

Results and Discussion

Species Distribution of Cu(II)–L-Histidine (1:2) in Aqueous Solutions. A histidine molecule is considered to have three potential coordination sites in aqueous solutions. As pH rises, the carboxyl oxygen (pK_a=1.9), the imidazole nitrogen (pK_a=6.1) and the amino nitrogen (pK_a=9.1) become available for coordination to a metal ion.[10,13] The neutral imidazolyl group exists in the tautomeric equilibrium between the N(1) protonated form and the N(3) protonated one (Scheme 1).[14] Either one of these nitrogens

† 1 M=1 mol dm^{-3}.

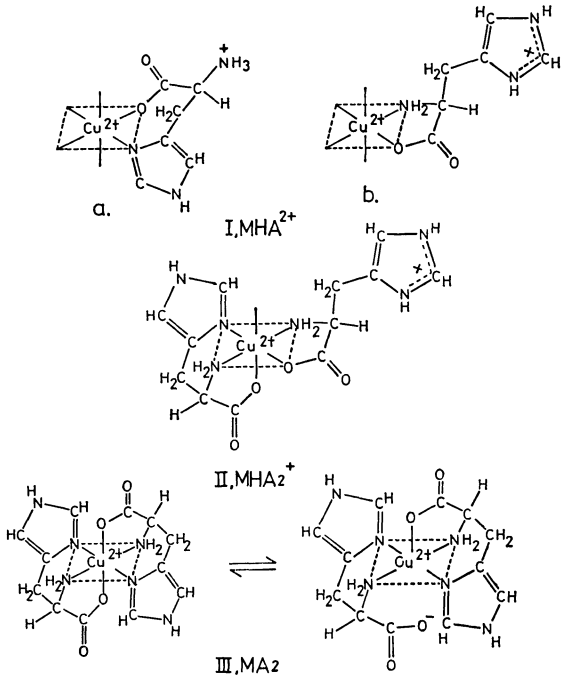


Scheme 1.

forms the chelation site.

In order to analyze the Raman spectra of Cu(II)–L-histidine complexes in aqueous solutions, it is first necessary to know the type of species which appears in various pH values of the solutions. As mentioned in the previous section, the solutions possess the predominant species, MHA^{2+}, MA^{+}, MHA_2^{+}, and MA_2. Below pH 3 the only major species is MHA^{2+} with a frequency approaching 50%. Just above pH 3, the solution contains the species MA^{+} in its maximum amount where about 25% of the Cu(II) ion is so bound. The species MHA_2^{+} appears to the extent of 90% from pH 4 to 4.5, and its deprotonation leads to MA_2 with $pK_a=5.5$. From pH 7 to 8 the species MA_2 is the most stable and its existence ratio approaches to 100%. In a pH region higher than 9, the formation of species $MH_{-1}A_2^{-}$, $MH_{-1}A$, and $M_2H_{-2}A_2$ are possible due either to hydroxylation or pyrrole hydrogen ionization ($pK_a=11.7$).[15,16]

It is known that a Cu(II) ion prefers a square-planar or a grossly distorted octahedral geometry to bind a maximum of two amino acid ligands. For a steric reason the three donor atoms of a terdentate histidine anion (A^{-}) cannot all occupy a planar site simultaneously, and these atoms have the equal possibility of being bound to the Cu(II) ion in the axial position. It has been strongly favored that the species MA^{+} involves the amino and imidazolyl groups in the square plane (histamine-like) with the carboxyl group in an apical position.[17] In contrasts, the site of protonation in MHA^{2+} is more controversial. The most popular view has been that the Cu(II) ion is bonded through an imidazole nitrogen and a carboxyl oxygen, while the amino nitrogen is protonated (Scheme 2 Ia).[9,18–20] The imidazole-carboxylate chelation, however, forms an unstable 7-membered ring and the binding at the glycine locus with an unbound protonated imidazolium cation has also been suggested to MHA^{2+} (Scheme 2 Ib).[17] Scheme 2 II shows one of the structures proposed to the species MHA_2^{+}, in which one histidine molecule coordinates to the Cu(II) ion as a terdentate ligand while another binds as a substituted glycine with an unbound imidazolium cation (mixed type).[15] The structure of the species MA_2 has so far received the most attention as a model for the transport form of Cu(II) ions in human blood. Great controversy, however, has been centered around its structure and several different proposals have been made to it.[17,21–23] The structure shown in Scheme 2 III is one of the proposals.[15]



Scheme 2.

Raman Spectra of L-*Histidine and Cu(II)*–L-*Histidine (1:2) in* H_2O. Figure 1 shows the Raman spectra of L-histidine in H_2O at the pH values of 3.90(A), 8.15(B), and 11.98(C). Ashikawa and Itoh analyzed the pH dependence of Raman spectra of L-histidine.[24] On the basis of the normal coordinate treatment they assigned the bands observed at 1627, 1264, and 994 cm^{-1} in Fig. 1(A) to the C=C stretching, the ring breathing, and the C–H in-plane bending vibrations of the protonated imidazolium cation, respectively. They also stated that the doublet bands around 1580, 1270, and 990 cm^{-1} observed in Fig. 1(C) reflect the tautomerism of the neutral imidazolyl group of L-histidine, the 1570-, 1285-, and 988-cm^{-1} components corresponding to the tautomer I and the 1585-, 1262-, and 1004-cm^{-1} to the tautomer II (Scheme 1). At pH 8.15, where the tautomer I is predominant, the spectrum shown in Fig. 1(B) gives rise to the strong and sharp bands at 1570, 1282, and 988 cm^{-1}. These results are useful for monitoring the coordination mode of each species assumed by the Cu(II)–L-histidine (1:2) system.

Figure 2 shows the Raman spectra of the Cu(II)–L-histidine(1:2) aqueous solutions with various pH values. The spectrum observed at pH 2.48 (Fig. 2(F)), where the species MHA^{2+} is predominant, shows the prominent bands at 1628, 1483, 1268, 1189, 990, and 629 cm^{-1}. The spectrum is quite similar to that of the ligand containing a protonated imidazolium cation (Fig. 1(A)) with the exception that a well-defined band at 561 cm^{-1} is observed in Fig. 2(F). The 561-cm^{-1} band is observed throughout the pH range 2.48—7.23 (Figs. 2(C)—(F)), where the carboxyl group of the ligand is bound to the Cu(II) ion. This band can be assigned to the out-of-plane bending mode of the bound carboxylate by comparing with the same vibrational mode observed at 570 cm^{-1} for bis-

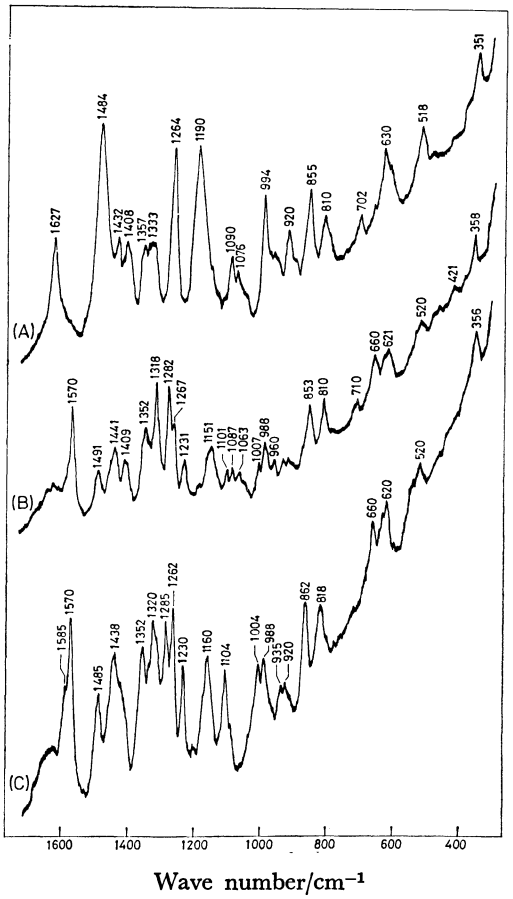


Fig. 1. Raman spectra of L-histidine in aqueous solutions (*ca.* 10 wt%) at a room-temperature: (A) pH= 3.90, (B) 8.15, (C) 11.98.

(glycino) complex of Cu(II).[25] From these results it is clear that in the species MHA^{2+} the Cu(II) ion is bound through an amino nitrogen and a carboxyl oxygen (glycine-like chelation), while the protonated imidazolium cation is not involved in the coordination. Therefore, the structure of this species is concluded to be the one shown in Scheme 2 Ib.

On raising the pH values (Figs. 2(D) and (E)) the band at 1628 cm^{-1} decreases its intensity and another band begins to appear at about 1585 cm^{-1} (the C=C stretching vibration of the neutral imidazole ring). The latter band is attributable to the bound imidazolyl group because the group with the pK_a value of 6.1 cannot exist as a free and neutral form at pH 4.55 and 3.72. One of the pH titration studies[10] reports that at pH 3.72 there exist appreciable amounts of three species, MA^+, MHA^{2+}, and MHA_2^+ in the Cu(II)-L-histidine(1:2) system. Therefore, it is difficult to assign each Raman scattering band in Fig. 2(E) to either one of the species. At pH 4.55, MHA_2^+ is the only major species. As Fig. 2(D) shows, the intensities of the two C=C stretching bands at 1628 and 1583 cm^{-1} are almost identical with each other, which is consistent with the mixed-type chelation structure shown in Scheme 2 II.

The Raman spectrum measured at pH 7.23 (Fig. 2(C)), where the species MA_2 exists almost exclusively, exhibits the vibrational modes of the neutral imidazole

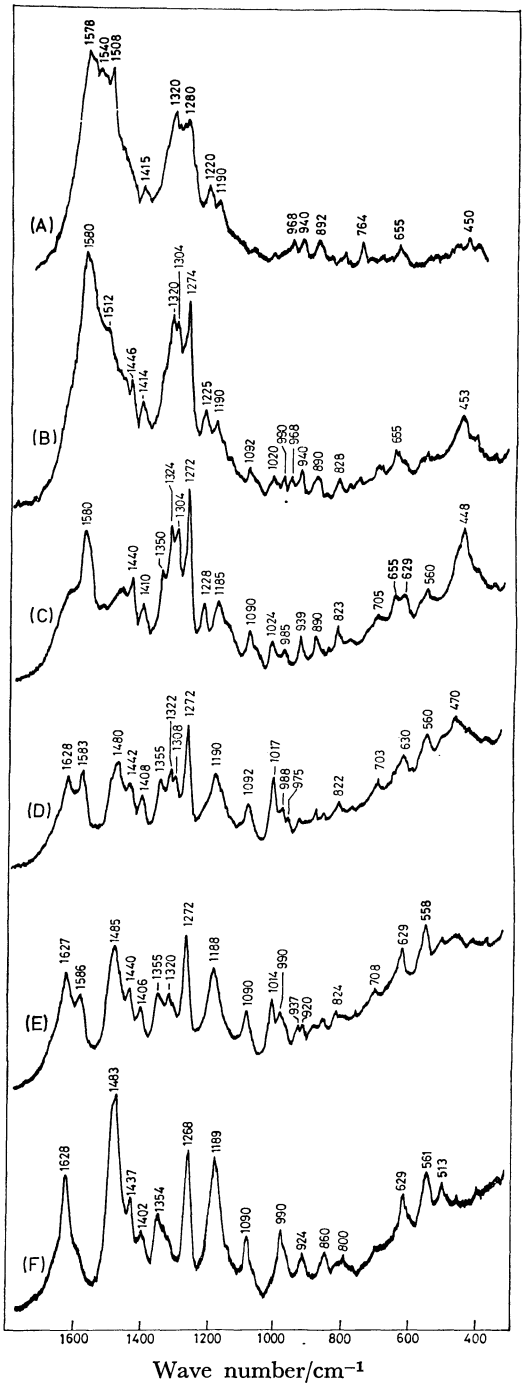


Fig. 2. Raman spectra of Cu(II)-L-histidine(1:2) in aqueous solutions (0.2 M) at a room-temperature: (A) pH=11.12, (B) 9.52, (C) 7.23, (D), 4.55, (E) 3.72, (F) 2.48.

ring (1580 and 1272 cm^{-1}, *etc.*). As already mentioned, the free imidazolyl group of L-histidine in a basic aqueous solution (Fig. 1(C)) gives rise to the doublets near 1580, 1270, and 990 cm^{-1}. The singlet bands observed at 1580 and 1272 cm^{-1} in Fig. 2(C) indicates that the imidazolyl group of the species MA_2 takes either one of the two tautomeric forms shown is Scheme 1. The strong band at 448 cm^{-1} in Fig. 2(C) can be ascribed to one of Cu(II)-ligand stretching vibrations because the free L-histidine with

the neutral imidazolyl group does not show any band in this region (Figs. 1(B) and (C)). This band shifts down to 432 cm^{-1} in D_2O (see the next section and Fig. 4(B)), suggesting that the 448 cm^{-1} band can be assigned to the Cu(II)–N stretching vibration of the bound amino groups. Generally two amino groups coordinated to the central Cu(II) ion with cis position show two Cu–N stretching bands (symmetric and asymmetric) while the amino groups occupying the trans position give only one Cu–N symmetric stretching vibration. This is supported by the observations that *trans*-Cu(II)(glycine)$_2 \cdot 2H_2O$ gives rise to only one Cu(II)–N stretching mode at 462 cm^{-1} while *cis*-Cu(II)(glycine)$_2 \cdot H_2O$ shows two Cu(II)–N stretching bands at 477 cm^{-1} (asymmetric) and 455 cm^{-1} (symmetric).[25] In Fig. 2(C) the only one Cu(II)–N stretching band appears at 448 cm^{-1} indicating that the trans position to the central Cu(II) ion is taken by the bound amino groups. The band at 560 cm^{-1} in Fig. 2(C), the intensity of which is a little weaker than that of the corresponding band in Fig. 2(D), can be assigned to the out-of-plane bending vibration of the bound carboxylato group. From the above-mentioned results it can be concluded that the coordination structure of the species MA_2 is similar to that proposed by Kruck and Sarkar,[15] in which two nitrogen atoms of the amino groups and two nitrogen atoms of the imidazole rings are bound to the Cu(II) ion in square-planar trans position and at least one, if not both, carboxyl group is bound in the axial position (Scheme 2 III).

Salama and Spiro have measured the Raman spectrum of octahedral Co(L-histidinato)$_2$ complex in H_2O with a UV excitation (363.8 nm).[5] They observed a band at 1574 cm^{-1}, which corresponds to the one observed at 1580 cm^{-1} in Fig. 2(C), and assigned it to the bound carboxylate mode. Cu(II)–histamine (1:2) in the aqueous solution with the pH values where the imidazolyl group of the ligand has the neutral form[26] and Co(4MeImH)$_6^{2+}$ complex[6] also show a well-defined peak in the region from 1590 to 1570 cm^{-1}. These results exclude the assignment made by Salama and Spiro and confirm that the 1580-cm^{-1} band in Fig. 2(C) are due to the C=C stretching vibration of the bound imidazolyl group.

At pH 9.52 (Fig. 2(B)) the 1580-cm^{-1} band is remarkably enhanced with a shoulder at 1512 cm^{-1} and, as the pH value is increased further (pH 11.12, Fig. 2(A)), a broad band centered around 1540 cm^{-1} begins to appear. The Raman spectrum of histidine with the imidazolide anion gives rise to the C=C stretching vibration of the group at 1527 cm^{-1}, which corresponds to the 1540-cm^{-1} band in Fig. 2(A). The shift to the higher frequency ($\Delta\nu$=13 cm^{-1}) can be ascribed to the chelation of the anionic group in the Cu(II)–L-histidine(1:2) system. Salama and Spiro[5] also measured the Raman spectrum of tetrahedral Co(L-histidinato)$_2^{2-}$ complex and observed the scattering peak at 1545 cm^{-1}, to which they assigned the anti-symmetric mode of the free carboxylate anion. The frequency of 1545 cm^{-1} is too low to be assigned to the free carboxylate mode because the infrared spectra of L-histidine and Cu(II)–L-histidine(1:2) in

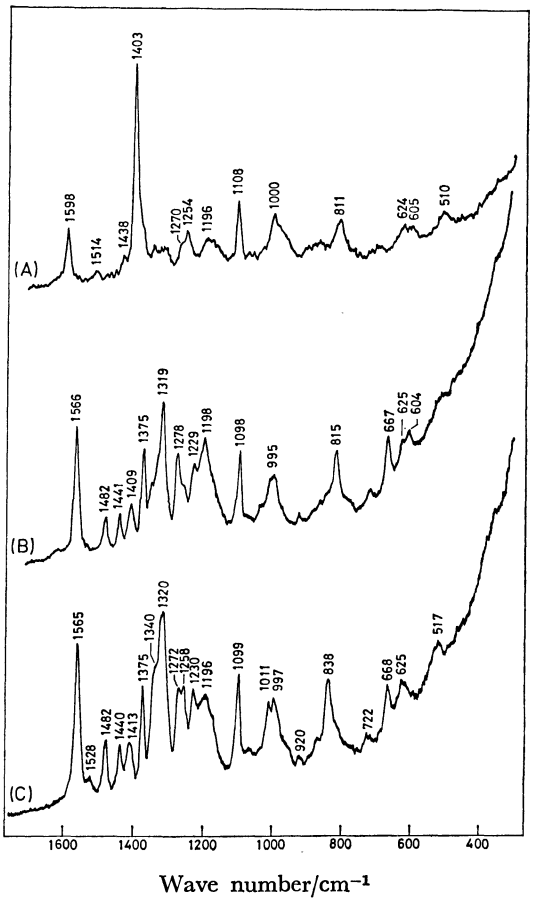


Fig. 3. Raman spectra of L-histidine-d_{3-5} in D_2O (*ca.* 10 wt%) at a room-temperature: (A) pD=2.80, (B) 8.34, (C) 13.20.

D_2O solutions show the free and the bound carboxylate mode at 1578 and 1590 cm^{-1}, respectively.[15] The appearance of the 1540-cm^{-1} band in Fig. 2(A) is accompanied by the shift and disappearance of several bands in Fig. 2(C). The bands observed at 1272 and 985 cm^{-1} in Fig. 2(C) shift to 1280 and 968 cm^{-1}, respectively. The 1090- and 1024-cm^{-1} bands in Fig. 2(C) almost disappear in Fig. 2(A). These results can be ascribed to the change in the chelation structure caused by the deprotonation of the imidazolyl group. Above pH 8, however, the Cu(II)–L-histidine(1:2) system becomes unstable (sometimes we observed a slight precipitation just above pH 9 presumably due to the copper(II) hydroxide formation) and we could not make a detailed analysis of the Raman spectra of Cu(II)–L-histidine (1:2) in which all the imidazolyl groups have the anionic form.

Raman Spectra of L-*Histidine*-d_{3-5} *and Cu(II)*–L-*Histidine*-d_{3-5} *(1:2) in* D_2O. Figure 3 shows the Raman spectra of L-histidine-d_{3-5} in D_2O at the pD values of 2.80(A), 8.34(B), and 13.20(C). The spectrum observed at pD 2.80 shows the bands at 1598, 1403, 1254, and 1000 cm^{-1}. They correspond to the 1627-, 1484-, 1264-, and 994-cm^{-1} bands in Fig. 1(A) and are assigned to the C=C stretching, the N–D in-plane bending, the ring breathing, and the C–H in-plane bending vibrations of the imidazolyl group,

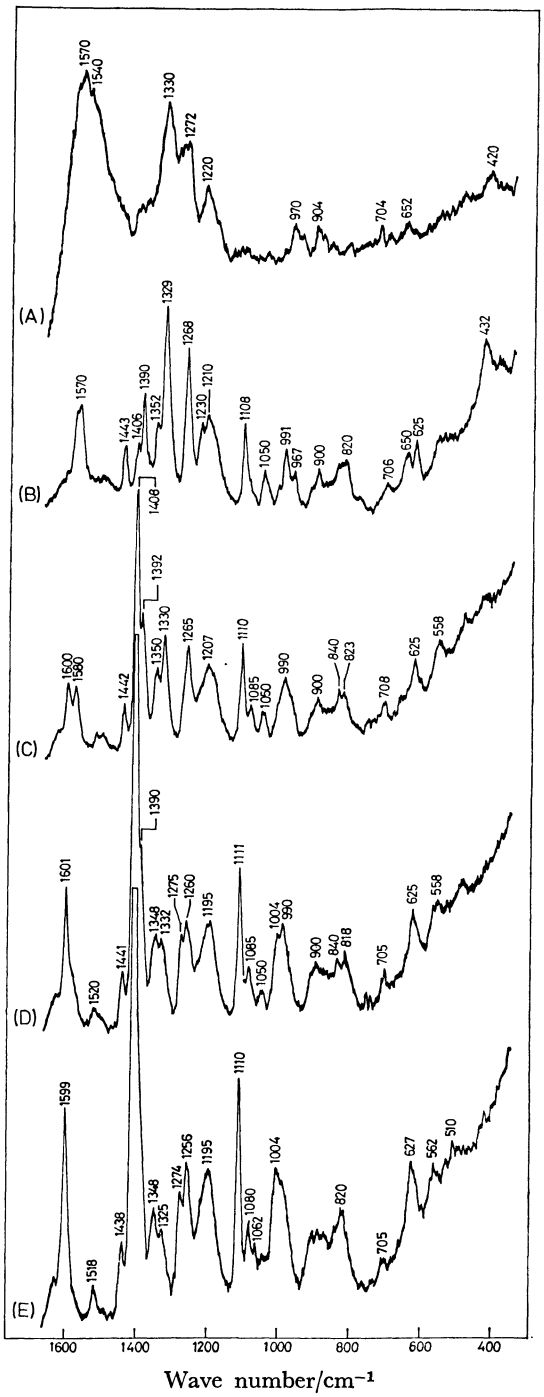


Fig. 4. Raman spectra of Cu(II)-L-histidine-d_{3-5} (1:2) in D_2O at a room-temperature: (A) pD=10.6, (B) 7.7, (C) 4.4, (D) 3.3, (E) 2.5.

respectively. At pD 8.34, where the neutral imidazolyl group favors the tautomeric form I, the spectrum (Fig. 3(B)) gives rise to the bands at 1566, 1278, and 995 cm^{-1} which are due to the C=C stretching, the breathing and the C–H in-plane bending modes of the neutral imidazolyl group with the tautomeric form I. On raising the pD value to 13.2, where both the tautomers I and II coexist, the Raman spectrum (Fig. 3(C)) shows the 1258- and 1011-cm^{-1} bands ascribable to the tautomer II in addition to the 1272- and 997-cm^{-1} bands due to the tautomer I. In the D_2O solution the tautomer I has the C=C stretching vibration with the frequency virtually identical with that of the tautomer II, giving rise to a sharp and strong band at 1565 cm^{-1} in Fig. 3(C).

Figures 4(A)—(E) show the Raman spectra of Cu(II)-L-histidine-d_{3-5}(1:2) in D_2O measured at pD values from 2.5 to 10.6. The bands at 1599, 1408, 1256, 1110, and 1004 cm^{-1} in Fig. 4(E) correspond to those at 1598, 1403, 1254, 1108, and 1000 cm^{-1} in Fig. 3(A), all of which are assigned to a protonated imidazolium cation. The out-of-plane bending vibration of the bound carboxylato group is observed at about 560 cm^{-1} in Fig. 4(E). Therefore, the Raman spectrum shown in Fig. 4(E) also confirms that the glycine-like binding occurs in MHA^{2+} which is the major species at pD 2.5.

In Fig. 4(D) the 1601- and 1408 cm^{-1} bands decrease their intensities, and there appear a shoulder band near 1580 cm^{-1} and a band at 990 cm^{-1}. The latter two bands can be ascribed to the bound neutral imidazolyl group. This is consistent with the result of the pH titration study[10] which brought the conclusion that in the pH region just above 3 there exist appreciable amounts of multiple species consisting of MHA^{2+} (Scheme 2 Ib), MHA_2^+ (Scheme 2 II) and MA^+ in which an L-histidine molecule is bound to Cu(II) as a terdentate anion.

At pD 4.4 (Fig. 4(C)), the intensity of the C=C stretching vibration observed at 1600 cm^{-1} (ascribed to the protonated imidazolium cation) is almost the same as that of the 1580-cm^{-1} band (assignable to the bound neutral imidazolyl group). This is consistent with the mixed-type structure (Scheme 2 II) proposed to MHA_2^+ which is the major species at pD 4.4.

The spectrum observed at pD 7.7 (Fig. 4(B)) also confirms the structure (Scheme 2 III) which was proposed to MA_2, the major species at pD 7.7. First, well-defined bands at 1570, 1268, and 991 cm^{-1} indicate that the species contains the neutral imidazolyl group with the tautomeric form I and the N(3) atom is bound to the Cu(II) ion. Second, the band near 560 cm^{-1} shows that in the species MA_2 at least one carboxylato group is bound to the central ion. Third, the sharp band at 432 cm^{-1} can be ascribed to the symmetric Cu(II)–N stretching vibration of the bound amino groups occupying the trans position to the Cu(II) ion. In Fig. 4(B) we cannot find the band which corresponds to the sharp band at 1482 cm^{-1} in Fig. 3(C). L-Histidine in a basic aqueous solution (Fig. 1(C)) also gives rise to a sharp band at 1485 cm^{-1} which reduces its intensity appreciably or almost disappears in Fig. 2(C). The 1482-cm^{-1} band in Fig. 3(C) (and the 1485 cm^{-1} in Fig. 1(C)) is presumably ascribed to one of the imidazole ring vibrations,[27] to which the N(3)=C(2) stretching mode contributes. On coordination of the imidazolyl group *via.* the N(3) atom, this band either shifts or decreases its intensity. The 1375-cm^{-1} band observed for the free ligand in D_2O (Fig. 3(C)) shifts to 1390 cm^{-1} in Fig. 4(B). The frequency shift can also be used to monitor the chelation of the imidazolyl group in D_2O.

In a higher pD region (Fig. 4(A)), there appears

Table 1. Obsd Raman frequencies of L-His and Cu(II)-L-His(1:2) system (1600—300 cm^{-1})

L-Histidine monomer						Cu(II)-L-Histidine(1:2) system						Assignments
H_2A^+		HA		A^-		MHA^{2+}		MHA_2^+		MA_2		
H_2O	D_2O	H_2O	D_2O	H_2O	D_2O	H_2O	D_2O	H_2O	D_2O	H_2O	D_2O	
1627	1598			1585[b]		1628	1599	1628	1600			R(ν(C=C))
		1570	1566	1570[a]	1565			1583	1580	1580	1570	
1484	1403	1491	1482	1485	1482	1483	1408	1480	1408			δ(N-H or N-D)+R
1432	1438	1441	1441	1438	1440	1437	1438	1442	1442	1440	1443	$\delta(CH_2)$
1408		1409	1409		1413	1402		1408		1410	1406	$\nu(CO_2)$
			1375		1375				1392		1390	R+δ(N-D)
1357		1352		1352		1354	1348	1355	1350	1350	1352	
1333		1318	1319	1320	1320		1325	1322	1330	1324	1329	R+$\delta(C_\alpha$-H)
	1270						1270	1308		1304		
1264	1254	1282[a] 1267[b]	1278	1285[a] 1262[b]	1272[a] 1258[b]	1268	1256	1272	1265	1272	1268	R(ring breathing)
		1231	1229	1230	1230					1228	1230	
	1196		1198		1196		1195		1207		1210	R, δ(N-H)
1190	1108	1151	1098	1160	1099	1189	1110	1190	1110	1185	1108	ν(NCN)+δ(N-H)
1090		1101 1087				1090	1080	1092	1085	1090		ν(C-H)
1076		1063					1062	1017	1050	1024	1050	$\nu(C_\alpha$-N)
994	1000	1007[b] 988[a]	995	1004[b] 988[a]	1011[b] 997[a]	990	1004	988	990	985	991	$\delta(C_{2,5}$-H)
		960						975			967	$r(CH_2)$
				935						939		
920				920	920	924			900	890	900	
855		853		862	838	860			840			π(N-H), ν(C-C)
810	811	810	815	818		800	820	822	823	823	820	
702		710			722		705	703	708	705	706	$\delta(CO_2)$
		660	667	660	668					655	650	ring deform.
630	624	621		620		629	627	630	625	629	625	+ν(C-C)
						561	562	560	558	560		$\pi(CO_2)$
518	510	520		520	517	513	510					$r(CO_2)$
										448	432	ν(Cu-N)
351		358		356								

Abbreviations: R, ring stretching; ν, stretching; δ, bending; π, out-of-plane bending; r, rocking. a) Assigned to Tautomer I. b) Assigned to Tautomer II.

a strong and broad band around 1540 cm^{-1}, giving a large background in the frequency range from 1600 to 1500 cm^{-1}. This band corresponds to the one at 1540 cm^{-1} in Fig. 2(A) and it can be assigned to the C=C stretching vibration of the bound imidazolide anion. As is the case with the Raman spectrum observed for the H_2O solution at a high pH value (Fig. 2(A)), the spectral features of Fig. 4(A) are different from those of Fig. 4(B) in several respects. The bands at 1268, 991, and 432 cm^{-1} in Fig. 4(B) shift to 1272, 970, and 420 cm^{-1} in Fig. 4(A), respectively. The bands observed at 1108 and 1050 cm^{-1} in Fig. 4(B) almost disappear in Fig. 4(A). Although these results clearly reflect the change in the coordination mode of the sample, the clear-cut explanation to these changes based on the assumed species (*i.e.*, $MH_{-1}A_2^-$, $MH_{-1}A$, and $M_2H_{-2}A_2$) could not be made because of the same reason given to the H_2O solution with a high pH value.[15] Sometimes the sample forms a dark-colored precipitation above pD 8.

Conclusions

The frequencies and tentative assignments of the Raman bands observed for L-histidine and Cu(II)-L-histidine(1:2) in H_2O and L-histidine-d_{3-5} and Cu(II)-L-histidine-d_{3-5}(1:2) in D_2O are listed in Table 1. As mentioned above, the C=C stretching and the breathing vibrations of the free and bound imidazolyl groups, the out-of-plane bending vibration of the bound carboxylato group, and the Cu(II)-N stretching vibrations of the bound amino groups can be used to determine the chelation structures of the species taken by the Cu(II)-L-histidine(1:2) system. The following are the conclusions. (1) The species MHA^{2+}, the major one below pH 3, assumes the structure shown in Scheme 2 Ib. The results obtained from the Raman spectra are incompatible with the structure shown in Scheme 2 Ia, which has been one of the most popular structures proposed to MHA^{2+}. (2) The Raman spectra measured at pH 4.55 and pD 4.4 (Figs. 2(D) and 4(C)) correspond quite well to the mixed-type chelation structure shown in Scheme 2 II, which is proposed to MHA_2^+, the major species in the pH region from 4 to 4.5. (3) From the Raman spectra measured at pH 7.23 and pD 7.7, where the species MA_2 is present almost to the extent of 100%,

it is concluded that the species assumes the chelation structure shown in Scheme 2 III. The intensity of out-of-plane bending vibration of the bound carboxylato group observed at 560 cm^{-1} in Fig. 2(C) is smaller than that of the corresponding band in Fig. 2(D), which suggests that one of the carboxylato groups exists in the equilibrium between the bound and free states.

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