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RAMAN SCATTERING STUDY ON COORDINATION SITE OF IMIDAZOLE GROUP IN ITS METAL COMPLEX

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Raman spectra of cobalt(II)-(4-methylimidazole) and nickel(II)-(L-histidine) complexes were analyzed and it was found that the C=C stretching, breathing, and C-H in-plane bending vibrations of the imidazole group clearly reflect the coordination site, the N(1) atom or the N(3) atom, of this group to the central metal ion.

Histidine residue is one of the most common ligands in the active site of metalloproteins. It is therefore biologically important to elucidate the site of its coordination to a metal ion. In this letter, we report the Raman spectra of cobalt(II)-(4-methylimidazole) and nickel(II)-(L-histidine) complexes. Through the analysis of the spectra it is possible to determine which nitrogen atom of the imidazole group, N(1) or N(3), (N(1) is the nitrogen atom which is the farther from the C_γ atom of histidine and N(3) is the other nitrogen) coordinates to the central metal ion in these complexes. The result suggests that the ligand vibrations, if they can be observed in the Raman spectra of metalloproteins, will reveal the coordination geometries of these proteins.

A pink crystalline sample of hexakis(4-methylimidazole)cobalt(II)chloride (abbreviated to $Co(4MeImH)_6Cl_2$) was synthesized as reported by Dash and Pujari[1]. Bis(L-histidino)nickel(II)monohydrate (abbreviated to $Ni(L\text{-}His)_2\cdot H_2O$) was prepared as a purple crystal following the procedure reported by Fraser *et al.*[2]. Raman spectra were recorded by using a JEOL 400D spectrophotometer equipped with a Spectra Physics Model 164 argon ion laser. The 514.5 nm line was used as an excitation source. All the Raman spectra of metal complexes were measured on polycrystalline samples mixed with KCl as a dispersion carrier, the weight fraction of the samples being in the range from 10 to 20 %. The Raman spectra of Co(II)-imidazole and Co(II)-histidine complexes were already measured by Yoshida *et al.*[3] and Salama and Spiro[4]. They reported that it is preferable to use resonance enhancement by UV excitation to observe the ligand vibrations. With the excitation wavelength adopted in this paper (514.5 nm), we could obtain the Raman spectra of high quality, which consist mainly of the ligand vibration modes.

Figures 1(A), (B), and (C) show the Raman spectra of $Co(4MeImH)_6Cl_2$ in solid state, 4-methylimidazole (abbreviated to 4MeImH) in solid state, and 4MeImH in a basic aqueous solution, respectively. In a neutral or basic aqueous solution of 4MeImH, the imidazole group has been known to exist in tautomeric

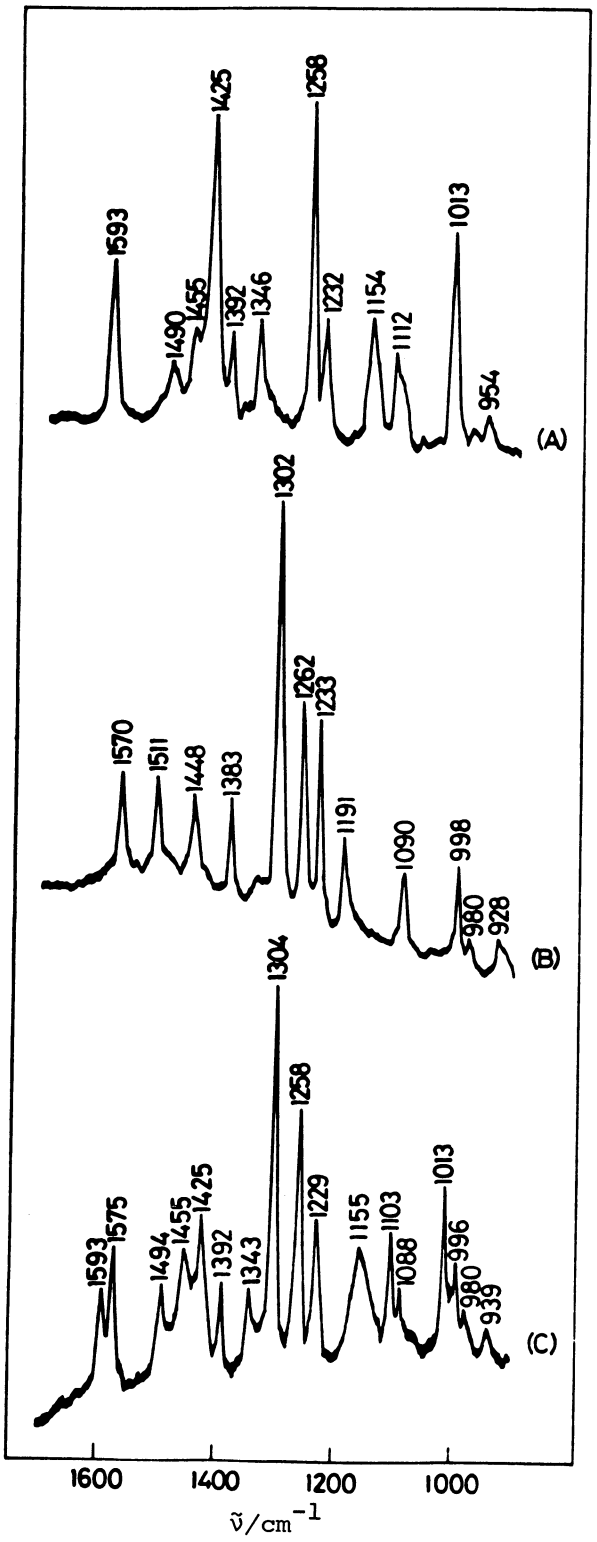

Fig. 1. Raman spectra of (A) $Co(4MeImH)_6Cl_2$ in solid state, (B) 4MeImH in solid state, and (C) 4MeImH in H_2O (pH 10.98, 25°C).

equilibrium between the N(1) protonated form and the N(3) protonated one[5-8]. Ashikawa and Itoh[9-10] analyzed the Raman spectra of 4MeImH in basic aqueous solutions and concluded that the 1575, 1304, and 996cm^{-1} peaks in Fig. 1(C) arise from the N(1) protonated tautomer and the 1593, 1258, and 1013cm^{-1} peaks from the N(3) protonated one. In the spectrum of $Co(4MeImH)_6Cl_2$ (Fig. 1(A)), the scattering peaks from the N(3) protonated tautomer are observed at 1593, 1258, and 1013 cm^{-1}, while the peaks from the N(1) protonated one cannot be found. On the other hand, 4MeImH in solid state (Fig. 1(B)) gives rise to only the 1570, 1302, and 998 cm^{-1} peaks assignable to the N(1) protonated tautomer. The 1262cm^{-1} peak in Fig. 1(B) is due to the C-H in-plane bending mode[11], which overlaps with the 1258cm^{-1} peak characteristic of the N(3) protonated tautomer in Fig. 1(C). These results indicate that the N(3) protonated tautomer is assumed by the imidazole groups in $Co(4MeImH)_6Cl_2$ and the N(1) protonated one in the solid 4MeImH. We can now state that the coordination site in $Co(4MeImH)_6Cl_2$ is the N(1) atom, placing the methyl groups as far away as possible from the central cobalt(II) ion. This minimizes the steric hindrance between the ligands.

On the basis of the normal coordinate analysis made by Ashikawa and Itoh[10], the 1593cm^{-1} peak in Fig. 1(A) and the 1570cm^{-1} peak in Fig. 1(B) can be assigned to the C=C stretching mode of the imidazole group with the corresponding tautomeric form. The 1258cm^{-1} peak in Fig. 1(A) and the 1302cm^{-1} peak in Fig. 1(B) are due to the ring breathing vibration mode and the 1013 cm^{-1} peak in Fig. 1(A) and the 998cm^{-1} peak in Fig. 1(B) to the C-H in-plane bending mode. The 1425cm^{-1} peak in the spectra of $Co(4MeImH)_6Cl_2$ (Fig. 1(A)) and the free 4MeImH (Fig. 1(C)) cannot be observed for the solid 4MeImH (Fig. 1(B)),

indicating that the 1425cm^{-1} peak is characteristic of the N(3) protonated tautomer. The intensity of this peak, to which the N(3)-H in-plane bending mode mainly contributes, is remarkably enhanced for $Co(4MeImH)_6Cl_2$ (Fig. 1(A)). This may be due to the effect of complexation of the ligands to the cobalt ion.

Figures 2(A) and (B) show the Raman spectra of $Ni(L\text{-}His)\cdot H_2O$ and a basic aqueous solution of L-histidine (pH 11.02, 41°C), respectively. The doublets around 1580, 1270, and 1000cm^{-1} observed in Fig. 2(B) also reflect the tautomeric equilibrium of the neutral imidazole side chains[9-10]. The 1570, 1285, and 985cm^{-1} components correspond to the N(1) protonated tautomer and the 1585, 1262, and 1006cm^{-1} components to the N(3) protonated one. Although the scattering peaks assignable to the ligand vibrations of $Ni(L\text{-}His)_2\cdot H_2O$ shift appreciably from the positions at which the corresponding peaks are observed for the free ligand (Fig. 2(B)), the singlet peaks observed at 1570 and 1272 cm^{-1} for the metal complex strongly suggest that the imidazole side chain ligands take only one tautomeric form. Fraser *et al.*[2] determined the crystal structure of $Ni(L\text{-}His)_2\cdot H_2O$ by X-ray diffraction and concluded that the nickel atom is octahedrally coordinated by the N(3) imidazole nitrogen, the amino nitrogen, and the carboxyl oxygen of each histidine. Therefore, the 1570 and 1272cm^{-1} peaks can be assigned to the C=C stretching and breathing vibration mode of the imidazole groups with the N(1) protonated tautomeric form. The 1574 and 1276cm^{-1} peaks are observed also in the Raman spectrum of octahedral $Co(L\text{-}His)_2$ in H_2O[4]. The 1008cm^{-1} peak observed in Fig. 2(A) is considered to be a new band which appears on complexation. The Raman spectrum of $Zn(L\text{-}His)_2\cdot 2H_2O$ shows the corresponding peak at

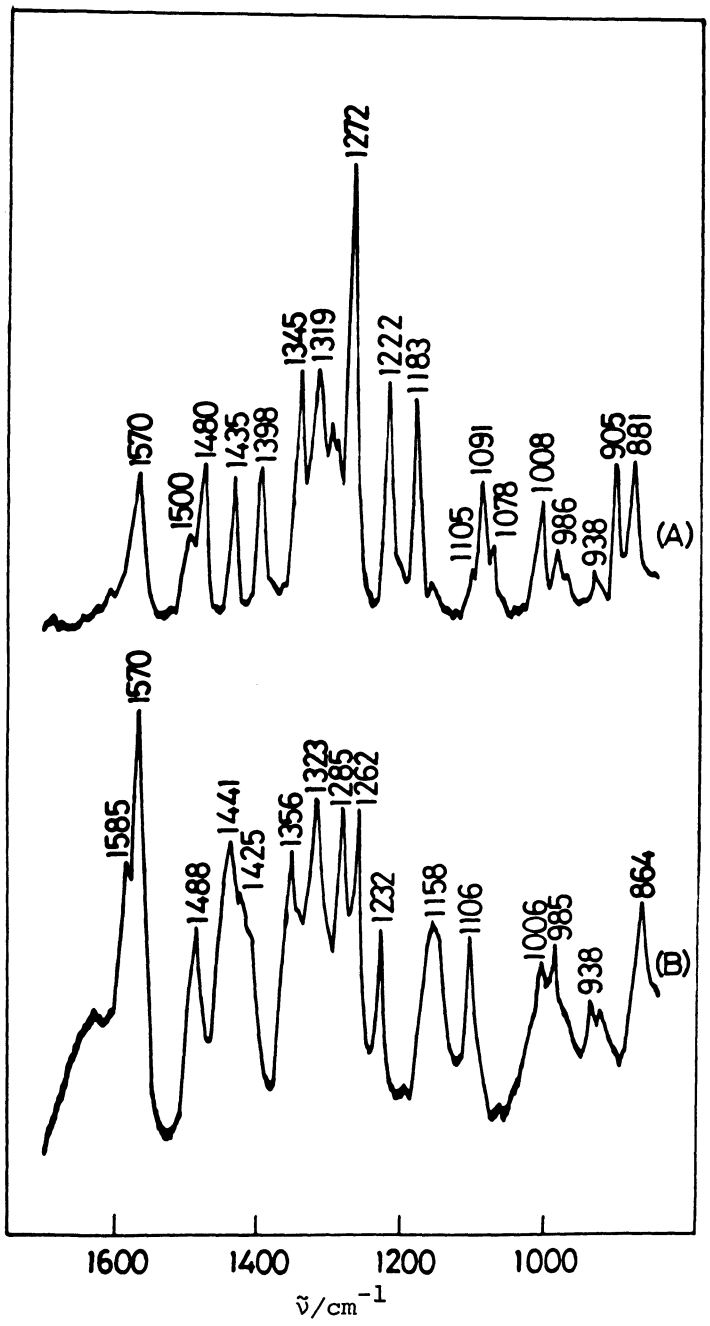

Fig. 2. Raman spectra of (A) $Ni(L\text{-}His)_2\cdot H_2O$ in solid state and (B) L-histidine in H_2O (pH 11.02, 41°C)

1020 cm^{-1}.

Thus, the analysis of the imidazole ring vibrations except for the C-H in-plane bending mode is very effective for determining the coordination site of the imidazole group in its metal complex. We believe that this technique is valid for studying the coordination structure of metalloproteins containing L-histidine residues as the ligands.

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