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RAMAN SCATTERING STUDY ON TAUTOMERISM OF L-HISTIDINE

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The Raman spectra of L-histidine in basic aqueous solutions show the scattering peaks clearly assignable to the 1-N and 3-N protonated forms of the imidazole ring. From the measurement of the spectra as a function of temperature, it was concluded that the 1-N protonated form is energetically more stable than the 3-N protonated form and ΔH is 1.0 ± 0.3kcal/mol.

The imidazole side chain of L-histidine in its neutral form has been known to exist in the two tautomeric forms depicted in Fig.1. This tautomerism has been studied by means of ^{13}C[1], ^{15}N[2-3], and ^{1}H[4] NMR spectroscopy. Recently we analyzed the Raman spectra of L-histidine and copolypeptides containing L-histidine residues. Considering the spectral change due to the deuterium substitution and the pH variation of the samples, we made the assignment of the scattering peaks from the imidazole side chain.[5] In the course of this study we observed that the Raman spectra of L-histidine in aqueous solutions with the pH value larger than the pK value of the first ionization of the imidazole side chain (5.91[6]) clearly reflect the existence of the two tautomers shown in Fig.1. We call the 1-N protonated form as tautomer I and the 3-N protonated form as tautomer II. In this paper we show the Raman spectra of alkaline solutions of L-histidine and 4-methylimidazole and describe how the Raman scattering peaks from the imidazole ring indicate the existence of the tautomeric equilibrium. The enthalpy differences between the two tautomeric forms of L-histidine and 4-metylimidazole are calculated from the temperature dependence of the Raman spectra.

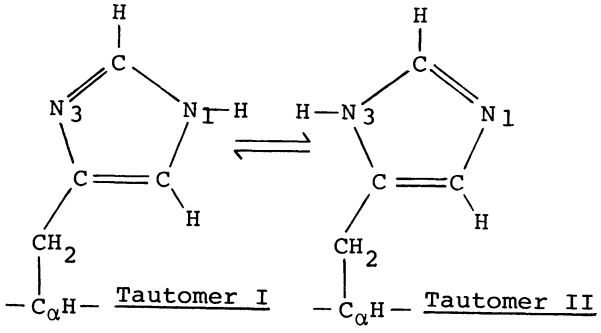

Fig. 1. Two tautomeric forms of the neutral imidazole side chain of L-histidine.

Raman spectra were recorded by using a JEOL JRS-400D spectrophotometer equipped with a Spectra Physics Model 164 argon-ion laser. The 514.5 nm line (200-700mW) was used as an excitation source. The sample cell was thermostatted to $\pm$ 1°C at a desired temperature using a Shimadzu Model TB-95 circulating bath in combination with a brass mantle which jacketted the cell. L-Histidine monohydrochloride salt was purchased from Kanto Chemical Co. Inc. and was purified by recrystallization from water-ethanol. 4-Methylimidazole was obtained from Tokyo Kasei Kogyo Co. Ltd. and was purified

by passage through active charcoal before use. The pH values of sample solutions were adjusted by adding 2-5 N NaOH solution.

Figure 2 shows the Raman spectra of L-histidine aqueous solutions (ca. 10 wt%) with the pH values of 7.20 and 11.80. The pK value of the amino group is reported to be 8.98.[6] As the pK value of the second ionization, which corresponds to the deprotonation of the neutral imidazole group to the anionic form, has not so far been reported in literatures, we can only conclude that this value is not appreciably different from the value reported for imidazole, i.e., 14.44.[7] On the basis of these results, it can be considered that both of the samples which give the spectra Fig. 2 (A) and (B) have the neutral imidazole group and that the sample of Fig.2(A) is in the NH_3^+ form and that of Fig.2(B) in the NH_2 form. Comparing these spectra with those of copolypeptides containing the L-histidine residues, we can conclude that the 1572, 1285, and 991 cm^{-1} peaks in Fig.2(A) arise from the ring vibrations of the imidazole group.[5] In addition to these peaks Fig.2(B) shows the peaks at 1585, 1262, and 1004 cm^{-1}. The latter three peaks cannot be ascribed to the nonprotonated amino group because the Raman spectra of glycine do not show any change in these frequency regions with protonation of the amino group.[8] Therefore, it can be concluded that the 1585, 1262, and 1004 cm^{-1} peaks in Fig.2(B) arise from the imidazole ring vibrations.

Tanokura et al. extensively analyzed the ^1H NMR titration curves of

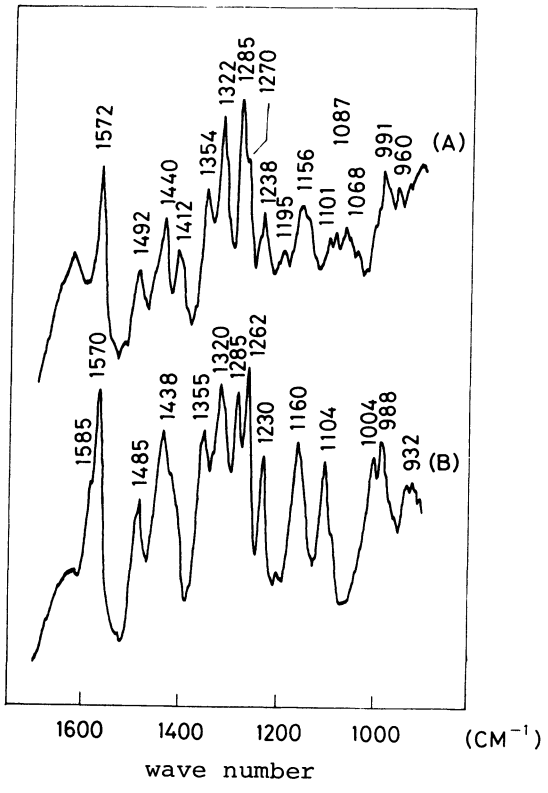

Fig. 2. Raman spectra of L-histidine (10 wt% in H_2O at a room temperature). (A) pH 7.20; (B) pH 11.80.

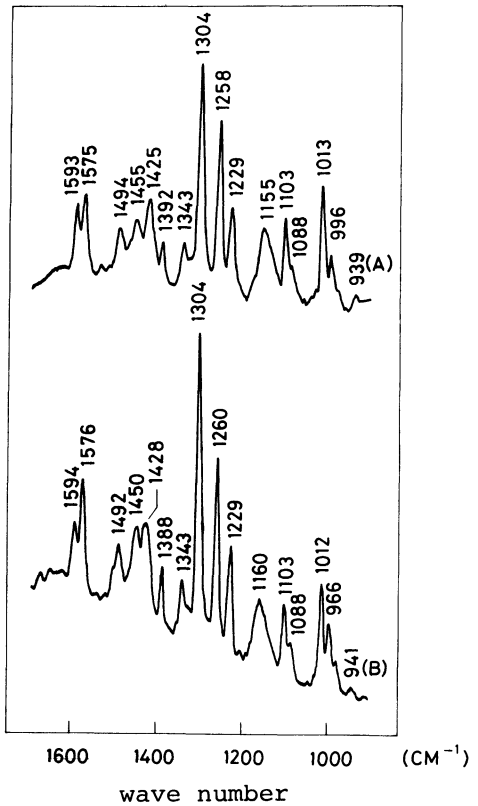

Fig. 3. Raman spectra of 4-methylimidazole (10 wt% in H_2O at pH 10.84). (A) 80°C; (B) 3°C.

L-histidine and its N^{im}-methyl derivatives and concluded that the existence ratio of the tautomer I to II of the imidazole side chain of L-histidine in its neutral form is 8:2 at the pH range where the amino group is in the NH_3^+ form and the ratio is 5.3:4.7 where the amino group is in the NH_2 form. On the other hand, from the ^{15}N NMR investigation Blomberg et al.[3] reported that the neutral imidazole ring of L-histidine with the NH_3^+ form shows the tautomeric equilibrium with the mole ratio of 8.8:1.2 (I:II) while the equilibrium shifts to 8:2 for L-histidine with the NH_2 form. Although the different sets of the mole ratios of the tautomer I to II have been reported for L-histidines with the NH_3^+ and NH_2 forms, it seems certain that the equilibrium shifts to the tautomer I with protonation of the α-amino group. On the basis of these results, it can be concluded that the imidazole ring vibration peaks at 1570, 1285, and 988 cm^{-1} in Fig.2(B) arise from the tautomer I and the peaks at 1585, 1262, and 1004 cm^{-1} in Fig.2(B) from the tautomer II.

Figure 3 shows the Raman spectra of the aqueous solutions of 4-methylimidazole with the pH value of 10.84. As the imidazole group of this compound has the pK value around 7.7 for the first ionization[9], the sample takes the neutral form at the pH value of 10.84. The 1575, 1304, and 996 cm^{-1} peaks in Fig.3(A) correspond very well to the peaks at 1570, 1285, and 988 cm^{-1} observed for the tautomer I of L-histidine (Fig.2(B)) and the peaks at 1593, 1285, and 1013 cm^{-1} in Fig.3(A) to the 1585, 1262, and 1004 cm^{-1} observed for the tautomer II of L-histidine. This result verifies that 4-methylimidazole also exist in the tautomeric equilibrium in aqueous solutions with the pH value larger than 7.7.

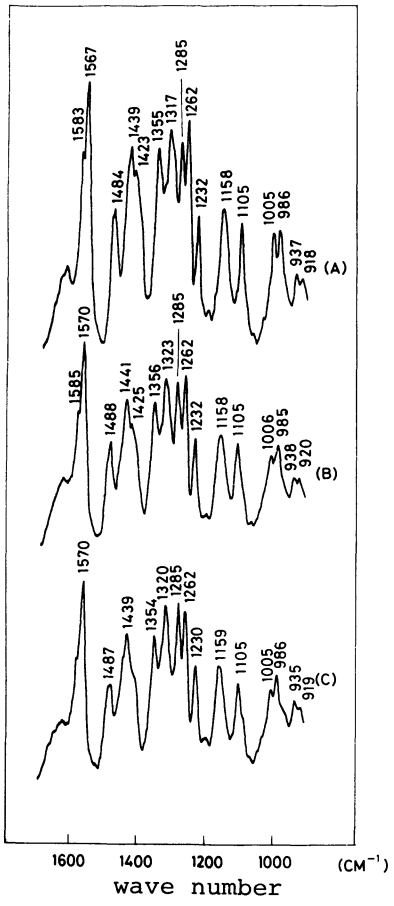

Fig. 4. Temperature dependence of Raman spectra of L-histidine (10 wt% in H_2O at pH 11.02). (A) 63°C; (B) 41°C; (C) 3°C.

Figure 4 shows the Raman spectra of the L-histidine aqueous solution with the pH value of 11.02 at 3, 41, and 63°C. The intensity ratios, I(1585)/I(1570) (the ratio of the peak intensity of the 1585 cm^{-1} peak to that of the 1570 cm^{-1} peak), I(1262)/I(1285), and I(1005)/I(986), increase as the temperature is raised. This result indicates that the tautomer I is energetically more stable than the tautomer II. The logarithm of I(1262)/I(1285) is plotted against 1/T in Fig.5, which allows us to obtain the enthalpy difference between the two tautomers of L-histidine with the NH_2 form. The calculated value was 1.0± 0.3 kcal/mol. As Fig.3 shows, the relative intensities of the peaks from the tautomer II of 4-methylimidazole (the 1593, 1258, and 1013 cm^{-1} peaks) to those of the tautomer I (the 1575, 1304, and 996 cm^{-1} peaks) increase as the temperature is raised. We measured the

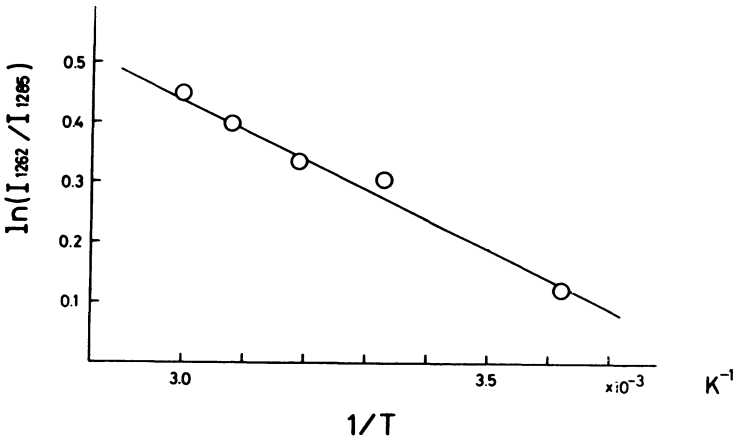

Fig. 5. Plot of the relative intensity $\ln(I_{1262}/I_{1285})$ vs. 1/T.

intensity ratio, I(1258)/I(1304), as a function of temperature and obtained the enthalpy difference, 0.4±0.1 kcal/mol for this compound. Assuming that the entropy difference between the tautomers of L-histidine is zero, we can calculate from the enthalpy difference (about 1.0 kcal/mol) the population ratio of the tautomer I to II to be about 8:2 at 25°C. This ratio is very similar to the value proposed by Blomberg et al.[3] for L-histidine with the NH_2 form of the α-amino group. The enthalpy difference of L-histidine is smaller than that of 4-metylimidazole by about 0.5 kcal/mol. In the basic solution (pH=11.02) the NH_2 group of L-histidine may be hydrogen-bonded to the unprotonated nitrogen (3-N) of the tautomer I (see Fig.1). This interaction can be considered to stabilize the tautomer I, which is one of the possible reasons for the enthalpy difference of L-histidine being larger than that of 4-methylimidazole.

In the serine proteases, the hydrogen bonded bridge of aspartic acid 102, histidine 57, and serine 195 forms the active site and the tautomerism of histidine 57 has been considered to play a crucial role in the proton-transfer mechanism.[10] This hypothesis assumes that the imino proton of histidine 57 easily alternates its protonation site between the 1-N and 3-N positions of the imidazole ring. The enthalpy difference between the tautomer I and II obtained for L-histidine and 4-methylimidazole is appreciably smaller than the energy of the hydrogen-bond interaction and is of the order of thermal energy. This result is consistent with the above-mentioned assumption.

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