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NITROGEN-15 NUCLEAR MAGNETIC RESONANCE OF HISTIDINE. THE EFFECT OF pH

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Natural abundance ^{15}N nmr spectra of L-histidine, and τ- and π-methylhistidines were measured. The signal assignments to the τ- and π-nitrogen could be made by comparison of these spectra. From the pH titration of the ^{15}N chemical shifts, it is directly proved that the τ-H tautomer is predominant in a basic solution.

Biologically important molecules usually contain nitrogen atoms. They are generally located at the sites which directly interact with other groups or molecules, for example, those of the purine and pyrimidine bases of nucleic acids, flavins as coenzymes and the imidazole groups at the active sites of enzymes. Nuclear magnetic resonance (nmr) is useful to elucidate these interactions. So far, however, ^{1}H and ^{13}C magnetic resonances have been mainly used for this purpose. ^{1}H and ^{13}C nuclei are usually located more remotely from the interaction sites, so that ^{1}H or ^{13}C nmr monitors indirectly the effect of the interaction on these nuclei. In contrast, nitrogen nmr may be expected to detect these interactions directly, and would be more sensitive and specific in probing them. However, ^{14}N, the predominant isotope (99.64 %), is not useful for this purpose, because it possesses a quadrupole moment which results in nmr line broadening. On the other hand, the ^{15}N isotope is very low in its abundance (0.36%), even though it gives sharp resonance lines. Recent developments in instrumentation, especially pulsed Fourier transform methods, have largely overcome this problem of low sensitivity, and we can observe ^{15}N nmr in its natural abundance for solutions of high concentration.

Histidyl residues often exist as important parts of proteins. For example, the imidazole nitrogen binds to substrates through hydrogen bonding, or complexes with transition metal ions. These interactions have been studied by ^{1}H and ^{13}C nmr, and have provided significant information about the imidazole binding site.[1] ^{15}N nmr may provide more direct and useful information. In this communication we shall report the effect of pH on the ^{15}N magnetic resonances of histidine in aqueous solutions which provide direct evidence on the tautomeric structure.

EXPERIMENTAL

The ^{15}N nmr spectra were obtained on a JEOL PFT-100 spectrometer operating in

the pulsed-Fourier transform mode at 10.14 MHz, with complete proton decoupling. D of solvent D_2O was used for the internal lock. The resolution was 1.2 Hz. Chemical shifts were referred to ^{15}N-enriched nitrate anion of aqueous $NH_4{}^{15}NO_3$ contained in a coaxial 3-mm tube inserted into a 10-mm sample tube.

Monohydrochloride salt of histidine was of commercial grade. τ-Methyl- and π-methyl-L-histidines were obtained from Sigma Chemical Co. About 250 mg of the sample was dissolved in 1.5 ml of H_2O, and HCl and NaOH were used for the adjustment of pH. Spectra were obtained after 20,000 - 60,000 pulses repeated every 2 sec.

SIGNAL ASSIGNMENT

Fig. 1 illustrates the ^{15}N nmr spectrum of histidine at pH 3.8. The inversion of the signals in the figure is due to the negative nuclear Overhauser effect (NOE) by the irradiated protons. The ^{15}N chemical shifts of histidine presented in Table 1 agree approximately with those of histidine methyl ester in acidic solution,[2] and correspond well to the ^{14}N shifts of histidine reported by Richards and Thomas.[3]

The higher field signal at 333.8_5 ppm is assignable to the nitrogen of the α-amino group by comparison with the signals of other amino acids.[4] The assignments of the two low field resonance signals at 198.1_5 and 201.0_5 ppm have not yet been made. Those assignments are expected to be accomplished by comparison of the spectrum of histidine with those of τ- and π-methylhistidines. Since the ^{15}N signal of methylated nitrogen cannot be enhanced by NOE due to the directly attached proton, obviously only the signal of the π-nitrogen can be observed in the ^{15}N nmr spectrum of τ-methylhistidine, and vice versa. Consequently, the ^{15}N resonance signal of histidine at 198.1_5 ppm is assigned to the π-nitrogen and that at 201.0_5 ppm to the τ-nitrogen (Table 1). The ^{15}N chemical shift of the τ-nitrogen of histidine does not correspond well to that of π-methylhistidine. This may be due to the difference in electronic structures of the two materials. ^{13}C nmr studies of histidine and its methylated derivatives indicated that

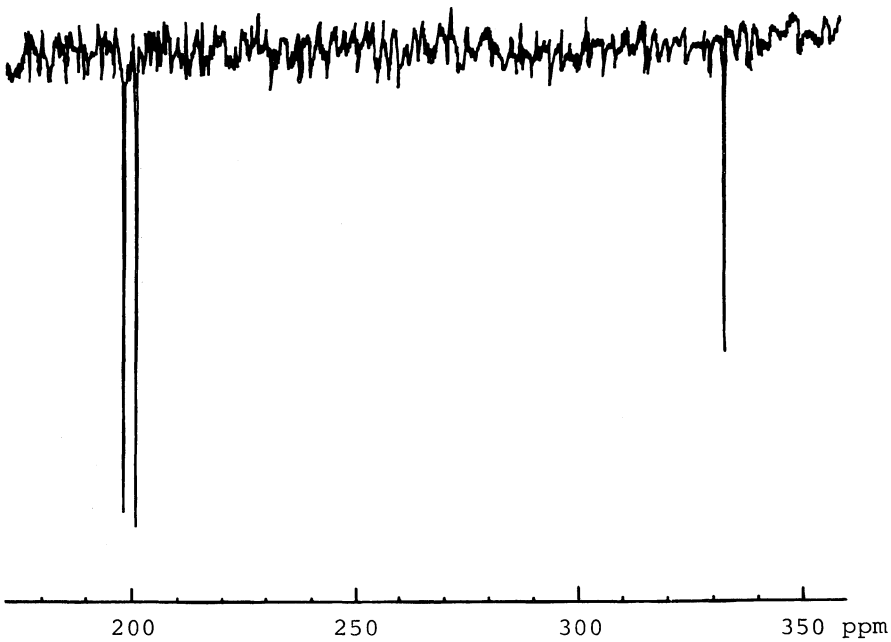

Fig. 1. Natural abundance, proton-decoupled ^{15}N nmr spectrum of histidine monohydrochloride at pH 3.8. The spectrum is the result of 22,000 pulses repeated every 2 sec.

the ^{13}C chemical shift-pH profile of τ-methylhistidine is identical to that of histidine, whereas that of π-methylhistidine is not.[5] This fact supports the above assignments, which are summarized in Table 1.

Table 1. ^{15}N-chemical shifts of L-histidine and its derivatives [a]

compound	π	τ	amino
L-Histidine	198.1_5	201.0_5	333.8_5
L-τ-Methylhistidine	198.9		333.7
L-π-Methylhistidine		203.4_5	333.5_5

a) ppm relative to external $NH_4{}^{15}NO_3$.

pH TITRATION OF ^{15}N CHEMICAL SHIFTS

The imidazole ring of histidine in basic solutions can exist in two tautomeric structures, i.e., the τ-H tautomer and the π-H tautomer illustrated in Fig. 2. By the ^{13}C nmr study of histidine, it was proposed that the τ-H tautomer is the predominant form of the imidazole ring of histidine in basic solution. This is confirmed by the ^{15}N chemical shift-pH profiles of histidine. As the pH is raised, the ^{15}N resonance signal of the π-nitrogen shifts extensively down field, by about 40 ppm and decreases in intensity, then ultimately vanishes into the baseline noise at pH 8.1 (Fig. 3), probably due to deprotonation of the nitrogen. On the other hand, the τ-nitrogen signal shifts down field by only 4 ppm, and also decreases its intensity by a factor of three with the increase of pH from 3.8 to 8.1. From this evidence it is apparent that the τ-H tautomer is predominant in basic solutions. The ^{15}N signal of the $\alpha\text{-}NH_3^+$ group of glycine is known to shift upfield about 12 ppm with increasing pH to 13.6 due to the deprotonation of the amino group.[6] It is reasonable that the ^{15}N signal of $\alpha\text{-}NH_3^+$ of histidine does not shift so greatly in the pH region examined.

It has been proposed that the ratio of τ-H tautomer to π-H tautomer is 4:1 from the result of ^{13}C nmr.[5] This ratio between two co-existing tautomers of histidine in alkaline solution is expected to be more accurately determined by ^{15}N nmr. However the ratio could not be determined by the present ^{15}N nmr data for the following reasons. The measurement of the natural abundance ^{15}N nmr of histidine at high pH was difficult, i.e., even the accumulation

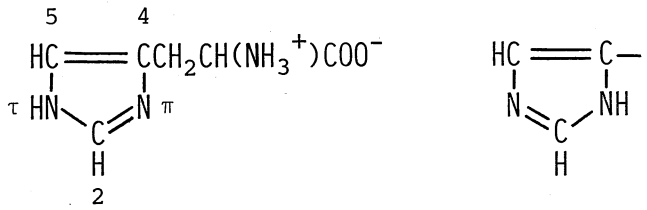

Fig. 2. Two tautomers of histidine in basic solutions, τ-H tautomer (left) and π-H tautomer (right). The imidazole ring is numbered according to the nomenclature by IUPAC and IUPAC-IUB commission (Biochemistry 14, 449 (1975)).

of data for 24 hours did not provide a spectrum with sufficient signal to noise ratio, and we could not see weak signals in the spectrum. In addition, the ratio of the intensity of the τ-nitrogen signal and the π-nitrogen may not be identical to the abundancy ratio of the tautomers because the former also depends on the relaxation time of the ^{15}N nuclei and the lifetime of their respective protons. Detailed studies of these exchanges and of the NOE of ^{15}N signals using ^{15}N-enriched histidine as the sample will be discussed elsewhere.

In the present study we found that the signals of the τ- and π-nitrogen in the imidazole ring could be observed separately and therefore in principle the strength of hydrogen bonding of the N-H groups could be correlated to the chemical shifts and NOE of the ^{15}N signals. Thus the present results indicate that the ^{15}N nmr technique may be applicable to the analysis of the state of the histidyl residues in proteins, particularly of those residues involved in the active sites of enzymes. To realize this goal, however, it will be necessary to prepare protein samples which incorporate the ^{15}N-enriched histidyl residue.

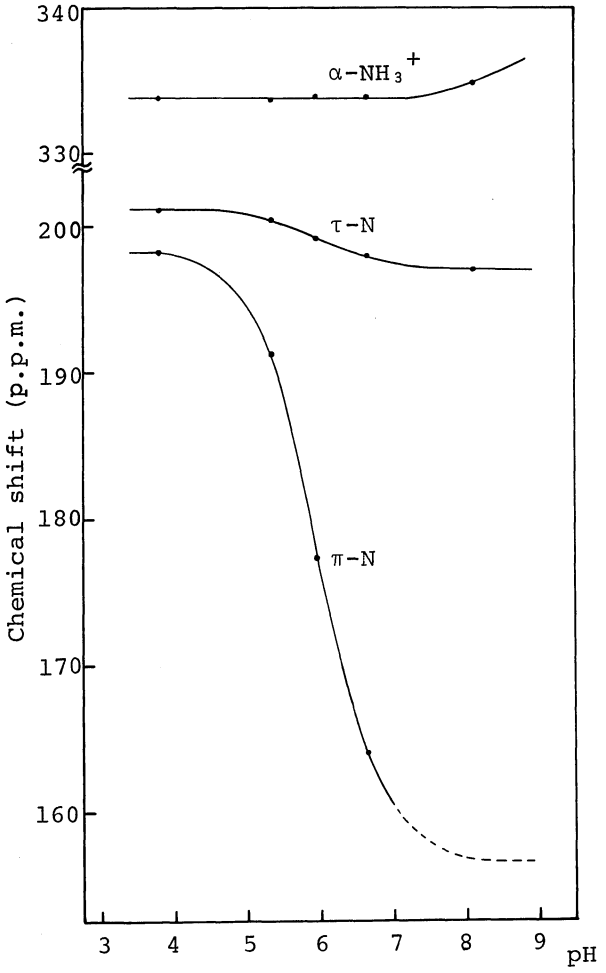

Fig. 3. ^{15}N chemical shifts of histidine as a function of pH. Chemical shifts are reported in ppm upfield from external $^{15}NO_3^-$.

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