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^{15}N FT NMR SPECTRA OF AMINO ACIDS IN NATURAL ABUNDANCE. pH DEPENDENCE OF ^{15}N CHEMICAL SHIFTS FOR L-ARGININE

Teruo Suzuki,* Takeo Yamaguchi and Mamoru Imanari
JEOL LTD., AID Application Laboratories, Nakagami 1418
Akishima, Tokyo, 196 Japan

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Natural-abundance ^{15}N NMR spectra of amino acid methyl esters in strongly acid solutions under proton-decoupled conditions have recently been reported,[1] and ^{15}N NMR spectra of several ^{15}N-enriched amino acids in aqueous solutions of varying pH have been studied.[2,3]

We have determined the natural-abundance ^{15}N FT NMR spectra of eight amino acids[4] to investigate the influence of pH on those for L-arginine. All ^{15}N NMR signals for the amino acids were assigned by complete proton-decoupling and proton-undecoupling techniques, and by comparing with ^{15}N chemical shifts among their homologous compounds. The ^{15}N chemical shifts thus obtained for the eight amino acids in acidic media are shown in Table 1. The ^{15}N signals for α-amino groups in these compounds appear at the upfield side of 330 - 338 ppm from that for ^{15}N-enriched HNO_3. The ^{15}N signals for ω- and ω'-amino, and ε-imino groups in L-arginine were assigned from the result that the ^{15}N signal for the ε-imino group was split into a doublet (J_{NH} = 80 Hz) by the attached proton under proton undecoupled conditions, and those for both ω- and ω'-amino groups into triplets (J_{NH} = 80 Hz). Also, both ω- and ω'-amino groups were found to be magnetically equivalent because their ^{15}N signals coincide and can not be separated from each other in a pH range from 2.1 to 11.8. The present result agreed approximately with that obtained by Pregosin et al.[1] The ^{15}N signal due to the ω-amino group in L-lysine was observed at an upfield

Table 1. ^{15}N Chemical shifts[a] of several amino acids

Compound	α-Amino	ω-Amino	ε-Imino	pH
DL-Valine	335.9			0.3
L-Serine	335.9			2.4
L-Cysteine	330.0			0.0
L-Asparagine	330.0	260.0		0.8
L-Glutamine	331.6	259.8		1.0
L-Lysine	337.8	330.8		2.6
L-Arginine	330.6	299.3(ω, ω')	286.8	2.1
L-Citrulline	332.8	295.9	283.6	0.1

a) In ppm upfield from external HNO_3, ±0.2 ppm.

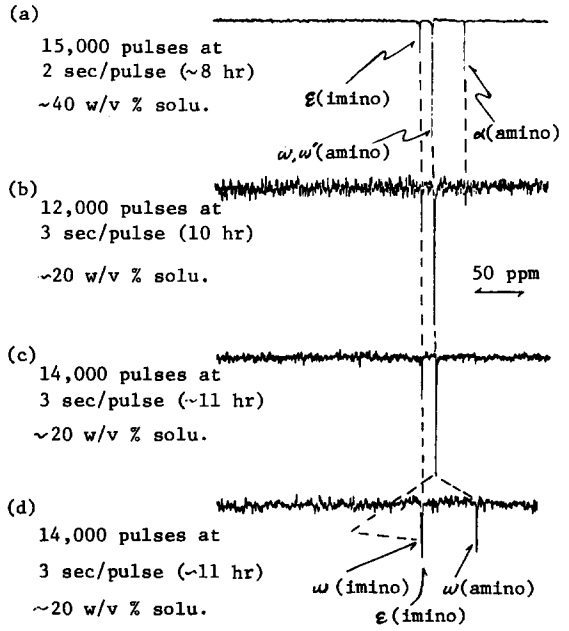


Fig. 1 Proton-decoupled natural-abundance ^{15}N FT NMR spectra of L-arginine in H_2O as (a) dication (pH ∿ 2.1), (b) zwitterion (pH ∿ 5.8), (c) zwitterion (pH ∿ 11.8), and (d) anion (pH ∿ 13.5)

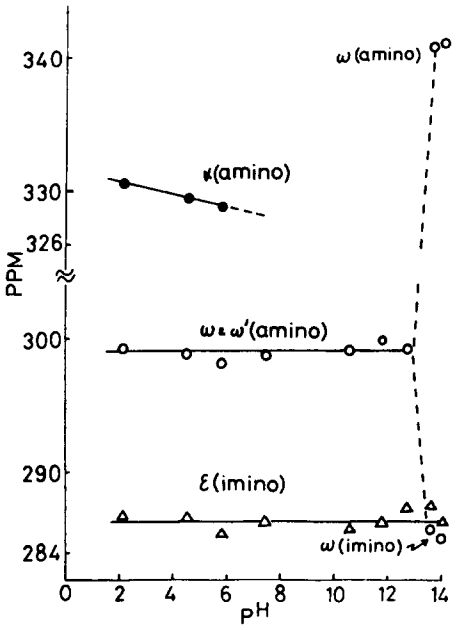


Fig. 2 ^{15}N Chemical shifts of L-arginine as a function of pH

(330.8 ppm) like as those for α-amino groups in other compounds studied (see Table 1). On the other hand, ^{15}N signals due to the ω-amino groups in L-asparagine and L-glutamine show an about 70 ppm lower field shift than that in L-lysine owing to the inductive effect (-I effect) of carbonyl groups in their molecules.

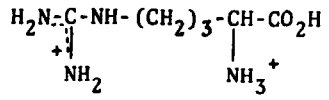
Figures 1 and 2 show the proton-decoupled ^{15}N FT NMR spectra of L-arginine in natural abundance and the pH dependence of ^{15}N chemical shifts, respectively.

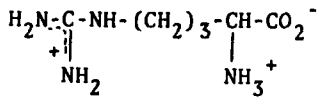
The pH dependence of the ^{15}N chemical shift for the α-amino group in L-arginine agrees well with that reported for the α-amino group in ^{15}N-enriched glycine.[2] The ^{15}N chemical shift for the α-amino group was found to decrease gradually up to pH 5.8; above pH 5.8, the ^{15}N signal becomes gradually broader and disappears in the baseline noise because of the scaler interaction between ^{15}N and ^{1}H.[2] On the other hand, the ^{15}N chemical shift for the ε-imino group is least influenced by pH. Also, the single ^{15}N signal for the ω- and ω'-amino groups is not sensitive in a pH range from 2.1 to 11.8. However, at pH's higher than 13.5, this signal is clearly separated into two signals; their intensities correspond roughly to the number of nitrogen atoms responsible for the separated ^{15}N signals (Fig. 1-d). This fact may be explained as follows: The ω-imino signal caused by direct electronic changes from $\overset{+}{N}H_2$ to =NH was newly observed as a sharp signal at a lower field (285.1 ppm). The other ω-amino signal ($-NH_2$) appears also at an upfield (341.3 ppm), contract to the disappearance of the α-amino signal owing to direct electronic changes from $-\overset{+}{N}H_3$ to $-NH_2$ in strongly basic media.

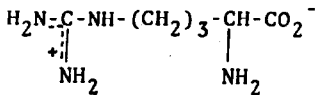
As regards configurations of an L-arginine molecule, the L-arginine dication, Arg^{++}, is known to prevail as the principal species below pH 2.1 by analogy with the formation of the L-arginine methyl ester dication in the range of pH 0.5 to 2.0.[3] From the fact that three values, pK(COOH)=2.01, pK(NH_3^+)=9.04 and pK(Guan)=12.48 had been obtained for L-arginine thermodynamically,[5] the two zwitterions, $Arg^{\pm+}$ and $Arg^{\pm}$, are determined to be the dominant ionic forms of L-arginine at pH 5.8 and pH 11.8, respectively. Above pH 13.5, the anion, Arg^-, prevails because ^{15}N signals for non-charged nitrogen atoms in =NH

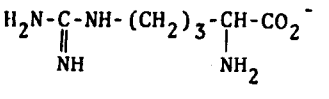
and $-NH_2$ appear, and because the pH value of 13.5 may correspond approximately to the pK(Guan) value of 12.48.[5)]

It has become clear in the present study that the configuration of L-arginine in aqueous solutions of varying pH can be described at least in the four forms of ionization shown below:


Dication (pH ∿ 2.1)


Zwitterion (pH ∿ 5.8)
with positively charged α-, ω- and ω'-amines.


Zwitterion (pH ∿ 11.8)
with positively charged ω- and ω'-amines.


Anion (pH ≳ 13.5)

REFERENCES AND NOTES

1) P. S. Pregosin, E. W. Randall and A. I. White, Chem. Comm. 1602 (1971)

2) R. A. Cooper, R. L. Lichter and J. D. Roberts, J. Amer. Chem. Soc. 95, 3724 (1973)

3) J. A. Sogn, W. A. Gibbons and E. W. Randall, Biochemistry 12, 2100 (1973)

4) The ^{15}N NMR spectra were obtained on a JEOL PS-100/PFT-100 spectrometer system operating at 10 MHz with internal ^{19}F locking. The internal ^{19}F locking signal was obtained on C_6F_6 contained in a 3-mm capillary inserted into a 10-mm tube. All ^{15}N chemical shifts were referred to ^{15}N-enriched nitric acid as an external reference. Amino acids for NMR use were purchased from Tokyo Kasei Co., Ltd. A series of sample was prepared in 20 ∿ 40 w/v % aqueous solutions and made available by adjusting the pH with HCl and NaOH. The samples were held in a 10-mm O.D. tube and their spectra were run at room temperature.

5) C. L. A. Schmidt, P. L. Kirk and W. K. Appleman, J. Biol. Chem. 88, 285 (1930)