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Differential Rates of Proton Exchange for the Guanidinium Nitrogens of L-Arginine Determined by Natural-Abundance Nitrogen-15 Nuclear Magnetic Resonance Spectroscopy[1]

ISSA YAVARI AND JOHN D. ROBERTS
Gates and Crellin Laboratories of Chemistry
California Institute of Technology, Pasadena, California 91125

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Differential rates of N-H proton-exchange reactions have been determined for the guanidinium nitrogens of L-arginine, 1, in the pH range of 0.5 to 11.5 by natural-abundance nitrogen-15 NMR spectroscopy. Base-catalyzed N-H proton exchange of the -NH- group is found to be two times faster than for the guanidino-NH_2 groups. The results can be rationalized by consideration of the contributions of various valence-bond structures to the resonance hybrid of 1.

Charge and proton transfers through hydrogen bonding are believed to be important to the catalytic efficiencies of enzymes (1). The positively charged guanidinium group of arginyl residues has been shown to be present at the active sites of several enzymes (2,3), and chemical modification of these arginines leads to loss of the enzymatic activity (3). Two recent papers (4,5) have described the use of nitrogen-15 nuclear magnetic resonance (^{15}N NMR) spectroscopy to determine the pH dependence of proton transfers for the guanidino-NH_2 groups of L-arginine, 1, enriched in ^{15}N to the extent of 95% at these nitrogens. Although these studies have shown that base-catalyzed

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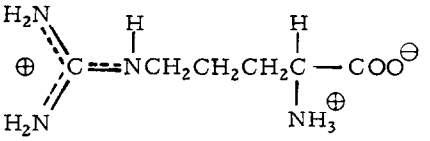
proton exchange for the guanidino-NH_2 groups become fast on the NMR time scale above pH 7, no information was reported regarding exchange involving the guanidinium -NH- nitrogens. We have now determined the differential rates of proton transfers for the -NH_2 and the -NH- groups of the guanidinium moiety of 1 by natural-abundance ^{15}N NMR spectroscopy.



L-Arginine, 1

Proton-coupled ^{15}N NMR spectra of L-arginine were taken over the pH range of 0.5 to 11.5 at 25°C, and typical spectra are shown in Fig. 1. The chemical shifts, upfield from external nitric acid, and peak assignments (4-7) are given in Table I. At pH 8 or above, the ^{15}N spectrum shows three sharp singlet resonances for the three different types of nitrogens in 1, because of rapid N-H proton exchange. Below pH 8, however, the two guanidinium resonances broaden, but not equally. At pH 6.6, there is a very broad band for the guanidino-NH_2 groups and a broad, but distinct, doublet for the -NH- group (Fig. 1d). At pH 6 (close to the isoelectric point), a broad 1:2:1 triplet and a broad 1:1 doublet are observed for the -NH_2 and -NH- groups, respectively. A rather sharp doublet ($^1J_{^{15}N-^1H}$ = 92 Hz) and a sharp triplet ($^1J_{^{15}N-^1H}$ = 91 Hz) are found below pH 5. The $^{15}N-^1H$ spin-spin splittings are still visible even at a pH value of 0.5.

The ^{15}N resonance of the α-NH_2 group of 1 remains sharp from pH 5 to 11.5. Below pH 5, however, this resonance appreciably broadens because of the slow N-H proton exchange rate (8).

The observed line broadenings of the ^{15}N spectra of the guanidinium nitrogens of 1 over the pH interval of 6 to 8 could be well simulated by line-shape calculations, using an effective line width (9) of 6 Hz and the

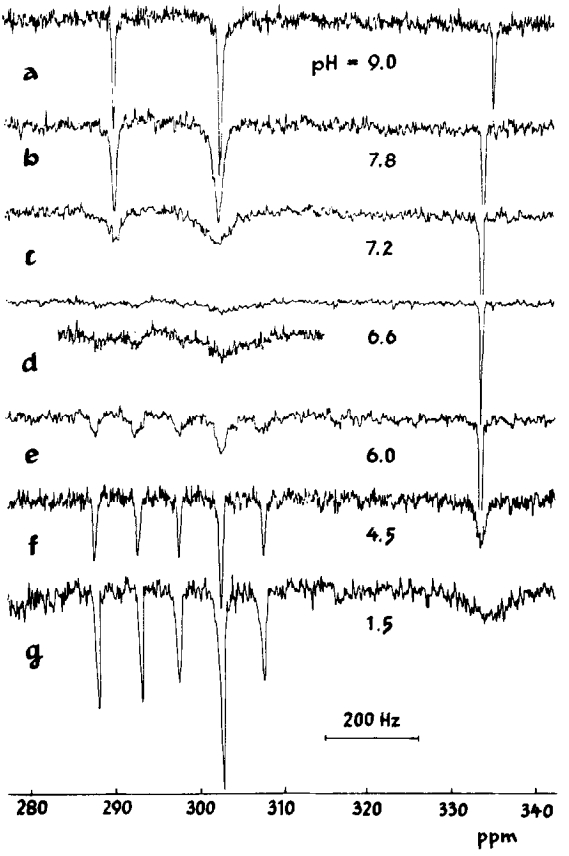


Figure 1. Illustrative natural-abundance, 18.25-MHz ^{15}N NMR spectra of 1.5 M aqueous solution of L-arginine at 25°C with gated ^{1}H noise decoupling: a, 610 transients; b, 1240 transients; c, 2510 transients; d, 2460 transients; e, 3121 transients; f, 1280 transients; g, 2612 transients.

experimentally determined $^{1}J_{^{15}N-^{1}H}$'s. These calculations show that the rate process which causes collapse of the guanidino-NH_2 triplet is about twice that for the -NH- doublet (10).

Recent theoretical calculations (11) on arginine and positively charged arginine suggest that there should be a much higher positive charge on the guanidino-NH_2 nitrogens than on the -NH- nitrogens. As a result, and also because the -NH- proton is expected to be less accessible to attack by bases for steric reasons, one would expect a substantially lower rate of base-

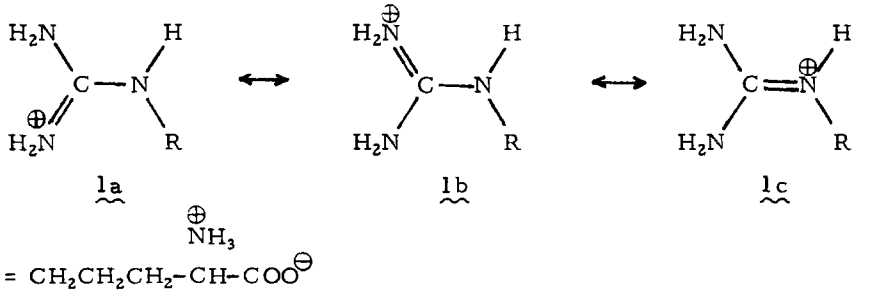
TABLE I

Nitrogen-15 Resonances[a] in L-Arginine

pH	-NH-	Guanidino-NH_2	α-NH_2
11.5	289.7	303.7	342.5
7.8	290.2	302.7	333.9
6.0	290.2	302.7	333.7
3.5	290.3	302.7	333.8
1.5	290.4	302.4	334.3

[a]In parts per million upfield from external 98% ^{15}N-enriched nitric acid in D_2O.

catalyzed proton exchange for the guanidino-NH- group compared to the guanidino-NH_2 groups. However, the spectra in Fig. 1 clearly show that this is not the case. The larger proton-exchange rate found for the -NH- group is most easily accounted for on the basis that the contribution of the valence-bond structure 1c to the resonance hybrid of arginine is in fact more important than those of 1a and 1b for the simple reason that it corresponds to a more highly substituted carbon-nitrogen double bond.



In this connection, it is interesting that the guanidino group of sulfaguanidine has been found (12) to have the structure $R-SO_2N=C(NH_2)_2$, again corresponding to the higher level of possible C=N substitution.

EXPERIMENTAL SECTION

L-Arginine and its mono hydrochloride were obtained from Sigma and Matheson, respectively. "Ultra-pure" sodium hydroxide (30%, Ventron) and

hydrochloric acid (30%, Ventron) were diluted with doubly distilled water to five molar. None of the samples used for ^{15}N NMR measurements were buffered; the pH was adjusted in the NMR sample tubes, using small amounts of 5 M NaOH or 5 M HCl. To reduce paramagnetic impurities, the solutions were shaken with Chelex-100 (Bio-Rad) according to the procedure of Irving and Lapidot (13). The pH values of each sample were measured with a Radiometer PHM-26C pH meter at 25°C by direct insertion of the combined glass reference electrode into the NMR sample tube, before and after taking each spectrum. In no case did the pH of the sample change by more than 0.1 unit during the 1-5 h required for taking the spectra.

The natural-abundance ^{15}N NMR spectra were obtained at 18.25 MHz with a Bruker WH-180 pulse spectrometer (14), using 25-mm o.d. spinning sample tubes containing about 20 ml of solution. A 5-mm concentric tube containing a 1.0-M solution of 98% ^{15}N-enriched nitric acid in D_2O provided both the external reference standard and the field-frequency lock. The spectra were taken with a 45° pulse angle, 4 K data points, 2400-Hz spectrum width, and a pulse interval of 4 s. To take advantage of the Overhauser effect, but still retain the N-H splittings, the protons in the sample were decoupled by a gating technique at a power of 4 W. The sample temperatures were maintained at about 25°C and were checked by direct insertion of a thermometer into the NMR sample tube before and after taking each spectrum.

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8. The α-NH_2 resonance sharpens with proton noise-decoupling below pH 5.

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10. The two guanidine-NH_2 groups of 1 appear to be magnetically equivalent over the pH range of 0.5 to 11.5. This would be the case if rotation about the partial carbon-nitrogen double bonds suggested by the several valence-bond structures of 1 is rapid on the NMR time scale.

    If these rotations are indeed rapid, then potentially diastereotopic "inside" and "outside" protons of the two guanidino-NH_2 groups should also appear to be equivalent and thus only the overall rate of proton exchange is measured for these groups.

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