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Nitrogen-15 Spin–Lattice Relaxation Times of Amino Acids in *Neurospora crassa* as a Probe of Intracellular Environment†

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ABSTRACT: The nitrogen-15 spin–lattice relaxation time, T_1, and the nuclear Overhauser enhancement (NOE) have been measured for intracellular glutamine, alanine, and arginine in intact *Neurospora crassa* mycelia to probe their various intracellular environments. The relaxations of $^{15}N_\gamma$ of glutamine, $^{15}N_\alpha$ of alanine, and $^{15}N_{\omega,\omega'}$ of arginine in *N. crassa* were found, on the basis of their NOE values, to be predominantly the result of ^{15}N–H dipolar relaxation. These relaxations are therefore related to the microviscosities of the various environments and associations of the respective molecules with other cellular components that act to increase the effective molecular sizes. For $^{15}N_\gamma$ of glutamine in the cytoplasm, the intracellular T_1 (4.1 s) was only slightly shorter than that in the culture medium (4.9 s). This indicates that the microviscosity of the cytoplasm surrounding the glutamine molecules is not much greater than 1.3 cP. By contrast, for $^{15}N_{\omega,\omega'}$ of arginine, which is sequestered in vacuoles containing polyphosphates, the intracellular T_1 (1.1 s) was only one-fourth of that in the medium (4.6 s). In model systems, the T_1 of $^{15}N_{\omega,\omega'}$ in a 1 M aqueous solution of arginine containing 0.2 M pentaphosphate was 0.95 s, whereas in an isoviscous (2.8 cP) solution without pentaphosphate, the T_1 was 1.8 s. These results suggest either that the vacuolar viscosity is substantially above 2.8 cP or that the ω,ω'-nitrogens of vacuolar arginine are associated with a polyanion, possibly polyphosphate. The implications of these results for the properties of the vacuolar interior are discussed in relation to the mechanism of amino acid compartmentation.

In many eucaryotic microorganisms, intracellular amino acids are not randomly distributed in the cell but are localized within subcellular organelles (Davis, 1975). In *Neurospora crassa*, the bulk of basic amino acids such as arginine and ornithine is compartmentalized in vacuoles (Weiss, 1973). Compartmentation plays an important role in controlling arginine metabolism; it permits the accumulation of large pools of arginine without induction of the respective catabolic enzymes or degradation by preexisting enzymes.

Several hypotheses have been proposed for the mechanism by which amino acids are sequestered in the vacuoles. One possibility is that an intracellular active-transport system moves amino acids across the vacuolar membrane and maintains the resultant concentration gradient. Alternatively, it has been suggested that basic amino acids become associated with polyanions such as polyphosphates in the vacuole (Durr et al., 1979; Urrestarazu et al., 1977). Recent studies suggest that the mechanism of compartmentation is more complex. Metabolic energy is required for the movement of arginine across the membrane, but not for retention within the vacuole (Drainas & Weiss, 1982). *N. crassa* grown in low-phosphate medium do not accumulate polyphosphate but continue to sequester arginine in the vacuoles (Cramer et al., 1980). It is possible that an active-transport system moves basic amino acids into the vacuole, but the vacuolar interior has properties which enables it to retain these amino acids. To test this hypothesis, we have investigated the physical properties of vacuolar arginine and its possible association with polyanions in living *N. crassa* mycelia.

High-resolution nuclear magnetic resonance (NMR) spectroscopy is a useful technique for studying the dynamic properties of cellular components in intact organisms. ^{15}N NMR spectroscopy has been used to probe the dynamic properties of cell wall components of bacteria [Lapidot & Irving, 1979a; see Lapidot & Irving (1979b) and references cited therein]. Recently, ^{15}N spectra of intracellular amino acids in suspensions of intact *N. crassa* mycelia have been obtained (Legerton et al., 1981) and shown to provide useful information on their in vivo metabolism (Kanamori et al., 1982). Well-resolved ^{15}N resonances were observed for both cytosolic amino acids such as glutamine and alanine and vacuolar amino acids such as arginine and lysine. These results open the way to utilizing ^{15}N NMR to probe the intracellular environments of amino acids through measurements of the spin–lattice relaxation time, T_1, and nuclear Overhauser enhancements (NOE) of their ^{15}N nuclei.

The spin–lattice relaxation time, T_1, of a ^{15}N nucleus of a molecule can be a sensitive probe of its correlation time, τ_c, which is a measure of its rotational freedom. Among the various mechanisms that contribute to the relaxation of a ^{15}N nucleus, ^{15}N–^1H dipolar interaction is ofter dominant for the protonated nitrogens of amino acids. In mobile liquids where

$$\tau_{c,\mathrm{eff}}^2(\omega_N + \omega_H)^2 \ll 1 \quad (1)$$

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holds, the dipolar contribution to T_1, $T_{1,dd}$, of a ^{15}N nucleus can be determined by measuring its nuclear Overhauser enhancement and by using

$$T_{1,dd} = T_{1,obsd}\frac{NOE_{max}}{NOE_{obsd}} \quad (2)$$

where $NOE_{max} = -3.93$. Assuming isotropic reorientation

$$1/T_{1,dd} \propto \tau_c \quad (3)$$

τ_c is given to a first approximation by

$$\tau_c = \frac{4\pi\eta a^3}{3kT}$$

where η is the viscosity of the liquid, a is the radius of the molecule (assumed to be spherical), T is the absolute temperature, and k is Boltzmann's constant. Hence, T_1 of a ^{15}N nucleus of an amino acid in intact *N. crassa* is expected to be related to its molecular mobility which is a function of the microviscosity of its intracellular environment as well as of interactions of the molecule with other cellular components that act to increase its effective radius and restrict its rotational freedom.

We report here the T_1 and NOE values of [$^{15}N_\gamma$]glutamine, [$^{15}N_\alpha$]alanine, and [$^{15}N_{\alpha,\delta,\omega,\omega'}$]arginine in intact *N. crassa* and the information thus provided on their intracellular environments.

Experimental Procedures

Strains, Media, and Growth. *Neurospora crassa* strains LA1 (wild type, 74A) and LA6 (*aga*, allele UM906) were from the collection of one of the authors (R.L.W.). The mutant strain, LA6, lacks arginase and cannot degrade arginine. The culture medium was Vogel's minimal medium supplemented with 1.5% sucrose (Vogel, 1964). Nitrogen-free medium is the same medium without ammonium nitrate.

Cultures were inoculated with an aqueous suspension of washed conidia to a final concentration of 1×10^7 conidia/mL. Conidia were germinated in 1-L baffled flasks containing 500 mL of minimal medium, with aeration provided by shaking at room temperature for 12–14 h until growth of the culture was logarithmic as measured by culture turbidity. Where specified, cycloheximide [4-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]-2,6-piperidinedione] was added to the culture medium to a final concentration of 20 μg/mL to prevent the incorporation of ^{15}N-enriched amino acids into proteins (Legerton et al., 1981).

For preparation of mycelia containing intracellular [$^{15}N_\gamma$]glutamine, strain LA1 was grown in minimal medium, transferred to nitrogen-free medium for 3 h, and then, after addition of cycloheximide, transferred to a medium containing 0.2% $^{15}NH_4Cl$. After sufficient intracellular [$^{15}N_\gamma$]glutamine was biosynthesized (6 h), the mycelia were collected by filtration and washed extensively with water. For preparation of mycelia containing intracellular [$^{15}N_\alpha$]alanine, the growth conditions were the same as above except for the addition of L-methionine DL-sulfoximine (an inhibitor of glutamine synthetase) to a final concentration of 5 mM to the $^{15}NH_4Cl$ medium to optimize the biosynthesis of alanine (Kanamori et al., 1982).

For preparation of mycelia containing ^{15}N-enriched L-arginine, strain LA6 was grown in 500 mL of minimal medium, and after addition of cycloheximide, 200 mg of L-[$^{15}N_{\omega,\omega'}$]-arginine or 80 mg of L-[$^{15}N_{\alpha,\delta,\omega,\omega'}$]arginine was added to the culture medium. After approximately 90% of the [^{15}N]arginine had been incorporated (2–4 h), the mycelia were collected by filtration and washed extensively with water.

Chemicals. The ^{15}N-substituted ammonium chloride (95% enriched in ^{15}N) was purchased from MSD Isotopes. L-[$^{15}N_\gamma$]Glutamine, DL-[$^{15}N_\alpha$]alanine, and L-[$^{15}N_{\omega,\omega'}$]arginine used for extracellular T_1 and NOE measurements were purchased from MSD Isotopes, Bio-Rad Laboratories, and KOR Isotopes, respectively. Uniformly ^{15}N enriched arginine (L-[$^{15}N_{\alpha,\delta,\omega,\omega'}$]arginine) was isolated from *N. crassa* as described below. Sodium phosphate glass (average chain length 5.5) was purchased from Sigma Chemical Co. and treated with Chelex to remove paramagnetic ions.

Isolation of [$^{15}N_{\alpha,\delta,\omega,\omega'}$]*Arginine.* Wild-type *N. crassa* was grown in 4 L of culture medium containing 0.25% $^{15}NH_4Cl$ as the sole nitrogen source. After 3–4 days of growth, the mycelia (27 g wet weight) were harvested by filtration and suspended in 400 mL of 6 N HCl at 110 °C for 48 h for the hydrolysis of cellular proteins. Hydrolysates were filtered through Whatman 1 filter paper. Then 0.5 g of Norit was added to the filtrate, and the suspension was heated to boiling for 5–10 min, cooled, and filtered, first through Whatman 1 and then through type HA Millipore filters (0.45-μm pore size) to remove any residual particulate matter.

The filtrate was evaporated to dryness and resuspended in 20 mL of 0.116 M sodium citrate at pH 5.3. This solution was added in 2-mL portions to 0.6 cm × 4 cm columns of Dowex AG50W-X8 (Na^+ form) equilibrated with the same buffer. An additional 6 mL of buffer was then run through. The first 8 mL of eluate contained most of the acidic and neutral amino acids; the second 8 mL of eluate contained lysine and ornithine; the third 8 mL of eluate contained histidine. The arginine left on the column was eluted with 8 mL of 0.2 N NaOH. The basic amino acid solution was acidified with 0.1 N HCl and applied to a 0.6 × 8 cm AG50W-X8 (H^+ form) column equilibrated with 8 mL of 0.1 N HCl. The columns were then washed with 8 mL of 0.1 N HCl and 16 mL of 1.5 N HCl to desalt the samples and the amino acids finally eluted with 8 mL of 6 N HCl, after which they were evaporated to dryness.

NMR Measurements. The mycelial suspensions for T_1 and NOE measurements were prepared by suspending 3.4 g (wet weight) of washed mycelia in enough nitrogen medium to make 18 mL of mycelial suspension in a 25-mm diameter NMR tube. The samples for the T_1 and NOE measurements of amino acids in the medium were prepared by dissolving [$^{15}N_\gamma$]glutamine, DL-[$^{15}N_\alpha$]alanine, or L-[$^{15}N_{\alpha,\delta,\omega,\omega'}$]arginine at 20–40 mM concentration in Vogel's nitrogen-free medium. Arginine–sodium pentaphosphate solutions were prepared by dissolving L-arginine (1 M) and sodium pentaphosphate (0.2 M) in water. Amino acid–glycerol solutions having viscosities of 2.8 cP were prepared by adding glycerol to aqueous solutions of L-arginine (1 M) and L-[$^{15}N_\gamma$]glutamine (70 mM) to give final concentrations (v/v) of 10% and 20%, respectively. The sample temperatures were maintained at 10 ± 2 °C unless specified otherwise.

The ^{15}N spectra for T_1 and NOE measurements were obtained with a Bruker WH-180 spectrometer operating at 18.25 MHz. An additional T_1 measurement was performed on a Bruker WM-500 spectrometer operating at 50.65 MHz to investigate the magnetic-field dependence of T_1.

The spin–lattice relaxation times, T_1, were measured by the fast inversion–recovery method (Canet et al., 1975), using the pulse sequence $(180°-\tau-90°-T)_n$ with $T = 4$ s. The T_1 values were calculated by the semilogarithmic method and/or the exponential fit method (Sass & Ziessow, 1977). The T_1 values calculated by the two methods agree to within 4%.

Table I: T_1 and NOE Values of ^{15}N Nuclei of Intracellular Glutamine, Alanine, and Arginine in *N. crassa* Compared to Those of the Amino Acids in the Culture Medium and in Model Systems[a]

amino acid		viscosity (cP)	T_1 (s)			NOE		
glutamine N_γ:	intracellular		4.05			−3.9		
	medium	1.3	4.85			−3.8		
	+glycerol	2.8	2.5			−3.9		
alanine N_α:	intracellular		5.1			−3.6		
	medium	1.3	7.8			−3.9		
			N_δ	$N_{\omega,\omega'}$	N_α	N_δ	$N_{\omega,\omega'}$	N_α
arginine:	intracellular		1.2	1.1	1.3	−1.5	−3.6	−1.7
	medium	1.3	8.6	4.6 (4.25)[b]	5.4	−3.6	−3.9	−3.9
arginine + pentaphosphate		2.8	1.8	0.95 (0.96)[b]	1.8	−2.4	−3.7	−3.9
arginine + glycerol		2.8	3.3	1.8 (1.86)[b]	3.1	−2.4	−3.6	−3.9
arginine + glycerol (at 10 °C and 50.65 MHz)		2.8	2.7		2.5			
arginine + glycerol (at 50 °C and 18.25 MHz)						−3.5	−3.4	−2.0

[a] All measurements were obtained with a Bruker WH-180 spectrometer operating at 18.25 MHz with a sample temperature of 10 °C unless specified otherwise. The pH is 5.85 in all model systems as well as in the medium in which *N. crassa* was suspended. [b] Values in parentheses are those obtained with L-[$^{15}N_{\omega,\omega'}$]arginine, or in a previous experiment.

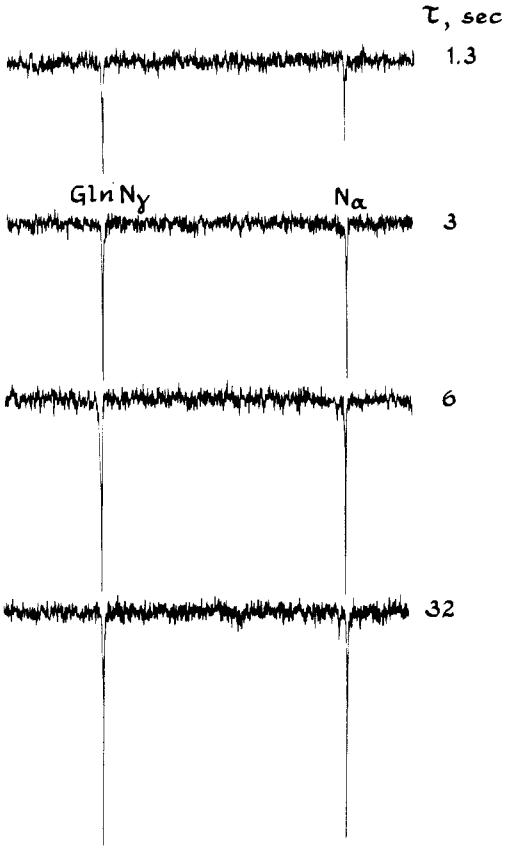

FIGURE 1: Representative proton-decoupled ^{15}N spectra of intracellular [^{15}N]glutamine in cultures of intact *N. crassa* mycelia from which the signal intensity, S_τ, was measured as a function of τ for T_1 measurements. T_1 was measured by the fast inversion–recovery method by using the pulse sequence $(180°-\tau-90°-T)_n$, with τ = 1.3, 2, 3, 4.5, 6, and 32 s. Each spectrum represents an accumulation of 100 scans.

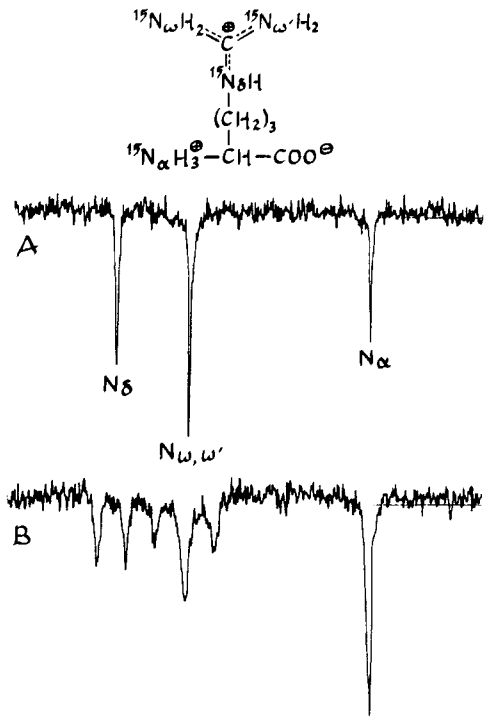

FIGURE 2: ^{15}N spectra of intracellular L-[$^{15}N_{\alpha,\delta,\omega,\omega'}$]arginine in intact *N. crassa* mycelia obtained at 18.25 MHz. (A) Typical proton-decoupled spectrum for T_1 measurement; (B) proton-coupled spectrum showing a doublet for N_δ and a triplet for $N_{\omega,\omega'}$.

The nuclear Overhauser enhancement was calculated from the ratio of the ^{15}N resonance intensity obtained with continuous proton decoupling to that obtained with gated proton decoupling.

To check the viability of mycelia during the NMR experiments (typically 3–4 h), we compared the doubling time and the ability to take up arginine from the medium in mycelia before, and after, exposure to a similar environment. No change was observed in either cellular property. We conclude that the mycelia are fully viable under these conditions.

The solution viscosities were measured with an Ostwald viscometer at 10 °C.

Results and Discussion

Figure 1 shows some representative ^{15}N spectra of intracellular [^{15}N]glutamine in *N. crassa* from which the signal intensities, S_τ, were measured as a function of τ for the T_1 measurements. A typical ^{15}N spectrum of intracellular [$^{15}N_{\alpha,\delta,\omega,\omega'}$]arginine in *N. crassa* is shown in Figure 2. Figure 3 shows the plots of ln $(S_\infty - S_\tau)$ against τ for $N_{\omega,\omega'}$ of intracellular arginine. Its T_1 value was calculated from the slope of the computer-fitted least-squares line through the plots. Table I summarizes the T_1 and NOE values of $^{15}N_\gamma$ of glutamine, $^{15}N_\alpha$ of alanine, and $^{15}N_\alpha$, $^{15}N_\delta$, and $^{15}N_{\omega,\omega'}$ of arginine in intact *N. crassa* mycelia (intracellular) compared to those for the amino acids dissolved in the culture medium (extracellular).

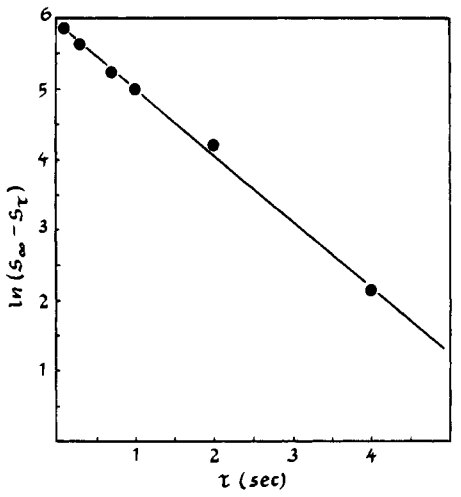

FIGURE 3: Plots of ln ($S_\infty - S_\tau$) against τ for the T_1 measurement of $N_{\omega,\omega'}$ of arginine in *N. crassa*. S_τ represents the signal intensity at each τ value, and S_∞ is the signal intensity at τ = 32 s. A T_1 of 1.1 s was obtained from the slope of the least-squares line through the plots.

For $^{15}N_\gamma$ of glutamine, $^{15}N_\alpha$ of alanine, and $^{15}N_{\omega,\omega'}$ of arginine, the NOE_{obsd} values are 90–99% of NOE_{max} for ^{15}N (−3.93) for both the intracellular and the extracellular amino acids, indicating that $T_{1,obsd}$ is predominantly the result of dipolar relaxation. Thus, the observed T_1 values are expected to be related to the microviscosities of their various environments and possible association with other cellular components that increase their effective molecular size.

Significantly, for $^{15}N_\gamma$ of glutamine, which is localized predominantly in the cytoplasm (Vaughn, 1980), the intracellular T_1 (4.05 s) is only slightly shorter than that of the amino acid in the culture medium (4.85 s). This suggests that the microviscosity of the cytoplasm is not much greater than that of the culture medium (1.3 cP). It is much less than 2.8 cP because the T_1 is observed to decrease to 2.5 s in a medium of this viscosity (Table I). For $^{15}N_\alpha$ of alanine, the bulk of which is found in the cytoplasm (Vaughn, 1980), the intracellular T_1 is approximately 65% of the extracellular value. By contrast, the intracellular T_1 (1.1 s) for $^{15}N_{\omega,\omega'}$ of arginine is reduced to one-fourth of the extracellular T_1 (4.6 s). The bulk of the intracellular pool of arginine is sequestered in vacuoles (Weiss, 1973). Thus, the shorter T_1 of intracellular arginine can reasonably be ascribed to a higher microviscosity within the vacuole and/or association of arginine with other cellular components which restrict its rotational freedom. The subcellular fraction representing the vacuole contains large quantities of polyphosphates as well as arginine (Vaughn, 1980). It has been suggested that the positively charged guanidino groups of arginine are associated with the negatively charged polyphosphates in the vacuole. The estimated concentration of basic amino acids in the vacuole is approximately 1 M, and that of polyphosphate is equivalent to approximately 1 M monophosphate units (Cramer et al., 1980; Martinoia et al., 1979).

To test the effect of such an environment, we measured the T_1 for a 1 M solution of arginine in the presence of 0.2 M sodium pentaphosphate (arginine:monophosphate ratio of 1:1) as a simple model for the intravacuolar environment. The $^{15}N_{\omega,\omega'}$ of arginine in this solution was found to have a T_1 of 0.95 s, a value quite close to the T_1 of 1.1 s observed for intracellular arginine in *N. crassa*. The short T_1 of arginine in the presence of pentaphosphate compared to that of free arginine in the culture medium could be due to increased viscosity (2.8 vs. 1.3 cP, see Table I) and/or interactions with the negatively charged pentaphosphates. The effect of a viscosity of 2.8 cP on the T_1 of $^{15}N_{\omega,\omega'}$ was separately assessed by measuring T_1 of $^{15}N_{\omega,\omega'}$ in an aqueous solution of arginine made isoviscous by addition of glycerol. The resulting T_1 was 1.8 s. Thus, viscosity can only partially account for the short T_1 of arginine in the presence of pentaphosphate, and interaction with the pentaphosphate is likely to be associated with the further decrease to 0.95 s.

From these results, we conclude either that the vacuolar viscosity must be substantially above 2.8 cP, a value considerably higher than in the cytoplasm, or else that the ω,ω'-nitrogens of intracellular arginine are highly associated with polyanions (e.g., polyphosphates) in the vacuole.

For N_α and N_δ of arginine, similar effects of different in vitro environments on their T_1 values were observed (Table I). The fact that T_1 of the α-amino nitrogen is also shorter in the presence of pentaphosphate than in the isoviscous arginine–glycerol solution suggests that the interaction with the polyphosphate in the model system may be occurring through both the guanidino and the α-amino groups. However, the T_1 values of N_α and N_δ of *intracellular* arginine cannot be used to extract information about their environments because these nitrogens, unlike $N_{\omega,\omega'}$, have small NOE values, which suggests that mechanisms other than ^{15}N–H dipolar interaction may be contributing to their relaxation. One possibility is that small amounts of paramagnetic ions in the vacuoles may affect the relaxation of the α-amino nitrogen.

The NOE of N_δ decreases with increasing viscosity (Table I). Mechanisms other than ^{15}N–H dipolar interaction that can contribute to its relaxation are scalar interactions, spin rotation, and chemical-shift anisotropy (Farrar & Becker, 1971; Levy & Lichter, 1979). The contribution of scalar interactions to T_1 is significant only when the rate of either the chemical exchange or the relaxation of the proton attached to N_δ is much greater than the $^{15}N_\delta$–H coupling (93 Hz) and the coupled nuclei have close Larmor frequencies. However, this is not the case for arginine under the conditions used here because $\omega_{^{15}N} \ll \omega_{^1H}$ and $^{15}N_\delta$–H coupling is clearly observed in the proton-coupled ^{15}N spectra (Figure 2B). If spin rotation were important and

$$1/T_{1,obsd} = 1/T_{1,dd} + 1/T_{1,SR}$$

where $T_{1,SR}$ is relaxation due to spin rotation, then

$$NOE_{max}/NOE_{obsd} = 1 + T_{1,dd}/T_{1,SR}$$

Because $T_{1,dd}$ increases with temperature and $T_{1,SR}$ decreases with temperature, NOE_{obsd} is expected to decrease with temperature. However, comparison of the NOE values of N_δ in arginine–glycerol solution at 10 and 50 °C (Table I) showed that the NOE of N_δ increases with temperature. This indicates that spin rotation does not make a significant contribution to the relaxation of N_δ. It is possible that the decrease in NOE of N_α with temperature would arise from the presence of trace paramagnetic ions whose effect on the relaxation time of the α-amino group increases with temperature.

If chemical-shift anisotropy (CSA) is contributing to the relaxation of N_δ, the T_1 of N_δ is expected to decrease with increasing magnetic-field strength, H_0, because

$$1/T_{1,CSA} \propto H_0^2(\sigma_\parallel - \sigma_\perp)^2\tau_c$$

where $\sigma_\parallel$ and $\sigma_\perp$ refer to the shielding parallel and perpendicular, respectively, to the symmetry axis. The T_1 value of N_δ in arginine–glycerol solution is 3.3 s at 18.25 MHz and 2.7 s at 50.65 MHz. The 20% difference between these T_1

values is too large to be attributed solely to differences in sample temperatures and suggests at least some contribution to the relaxation arising from chemical-shift anisotropy. The chemical-shift anisotropy contribution cannot, however, be more than a few percent because the difference in T_1 values is 20% and $1/T_{1,CS}$ has a quadratic dependence on field strength.

The T_1 of $^{15}N_{\omega,\omega'}$ in arginine–glycerol solution was not determined at 50.65 MHz because the resonance broadens as N_ω and $N_{\omega'}$ become chemically nonequivalent as the result of hindered rotation around the N_δ=C bond. At 10 °C, this broadening occurs only when the spectrum is measured at 50.65 MHz, where the chemical-shift difference between N_ω and $N_{\omega'}$ (~90 Hz) is of the same order of magnitude as the reciprocal of the lifetime before rotation about the N_δ=C bond. In all T_1 experiments at 18.25 MHz, the $N_{\omega,\omega'}$ resonances were sharp with line widths of approximately 10 Hz (see Figure 2A).

In summary, the results indicate that, while the interpretation of the T_1 values of N_α and N_δ of arginine must take into account the contribution of nondipolar relaxation mechanisms, the T_1 values of $N_{\omega,\omega'}$ of arginine, N_γ of glutamine, and N_α of alanine, which are predominantly the result of dipolar relaxation, provide useful information about the microviscosities of their various environments and the association with other cellular components. Furthermore, the results suggest either that the vacuolar viscosity is substantially larger than that of the cytoplasm or that the ω,ω'-nitrogens of intracellular arginine are highly associated with polyanions (e.g., polyphosphates). The possible role of polyphosphates in binding vacuolar arginine can be examined by determining the effect of growth on limited phosphate on the T_1 value of $N_{\omega,\omega'}$ of intracellular arginine. If the T_1 is short in the absence of polyphosphate, this will suggest a highly viscous vacuolar environment or an association of arginine with other polyanions. This study is now under way.

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Studies of the Binding Interactions of *cis*-Diamminedichloroplatinum(II) with Amines and Nucleosides by Nitrogen-15 Nuclear Magnetic Resonance†

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ABSTRACT: The ^{15}N chemical shifts and couplings have been measured for several platinum(II)–amine complexes. The ^{15}N chemical shift changes found on coordination of azine-type nitrogens to platinum appear to be related to those that occur on protonation of the same nitrogens. Both the ^{15}N chemical shifts and the one-bond ^{15}N–^{195}Pt coupling constants depend on the nature of the cis and trans ligands. *cis*-$[Pt(NH_3)_2Cl_2]$ ("*cis*-platinum") forms a complex with cytidine through N3. Guanosine becomes bound to platinum of *cis*-$[Pt(NH_3)_2Cl_2]$ at N7 and at least one other nitrogen. Adenosine appears to bind *cis*-$[Pt(NH_3)_2Cl_2]$ to at least some degree through N1, N3, N7, and the 6′ $-NH_2$.

There has been much interest in the chemistry and biochemistry of platinum complexes since the discovery of the antitumor activity of *cis*-$[Pt(NH_3)_2Cl_2)$, "*cis*-platinum", by Rosenberg and co-workers (Rosenberg et al., 1969). This substance has proven to be an especially effective agent for treatment of cancer of genitourinary origin (Cleare & Hydes, 1980). It appears to act by combining with specific nitrogens of the nucleotide bases of deoxyribonucleic acid, DNA (Roberts & Thomson, 1979).

Platination of the DNA purine and pyrimidine bases has been studied previously by ultraviolet (Mansy et al., 1973) and Raman (Chu et al., 1977, 1978) spectroscopies, ^1H (Chu et al., 1977, 1978; Kong & Theophanides, 1974a,b, 1975) and ^{13}C NMR[1] spectroscopies (Chu et al., 1977; Lim & Martin,

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[1] Abbreviations: NMR, nuclear magnetic resonance; an, aniline; py, pyridine; EDTA, ethylenediaminetetraacetic acid; Cyt, cytidine; Guo, guanosine.

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