proposed that its fluorescence rise and decay curve is interpreted by three-exponential fitting and not by a two-exponential one.[56] This might raise a question on the applicability of Scheme I to the present systems. However, it should be remembered that an advantage of the transient absorption spectral measurement is to identify the main transient species and its dynamic range is not as large as that of the single photon counting one. Actually, the monomer $S_n \leftarrow S_1$ absorption band was not detected in the DCzP and m-DCzPe spectra at long delay times. This indicates that the monomer component that does not form the sandwich excimer is a minor contribution in transient spectral analysis. It is considered that the present analysis is correct as a first approximation.

In contrast to the above DCzP and m-DCzPe systems, the triplet formation yield of r-DCzPe in THF is slightly higher than that of EtCz. This result means that the triplet yield from the second excimer is not large, since the concentration of this excimer is higher than that of the monomer fluorescence state. This difference in behavior from the sandwich excimer is consistent with the fact that the $S_n \leftarrow S_1$ spectrum and the lifetime of the second excimer are almost equal to those of EtCz, respectively.

Although the same triplet absorption spectrum was observed for the present dicarbazolyl compounds, their decay time depends on the geometrical configuration. Since the intermolecular interactions between the triplet states and between the triplet and ground states are involved to some extent, the fact that the lifetime of the DCzB triplet is larger than that of the EtCz triplet is acceptable in view of the more rapid diffusional motion of the latter. On the other hand, the decay of DCzP and m-DCzPe is faster than that of EtCz, suggesting an additional deactivation process. This tendency is more pronounced in the case of r-DCzPe. Therefore this intersystem crossing to the ground state is considered to be sensitive to the geometrical configuration. The intramolecular interaction between the triplet- and ground-state carbazolyl groups induces the deactivation to the ground state, which is more efficient in the partial overlap structure than in the sandwich structure.

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Model Compounds for Polymer Photophysics. We have studied the excited-state dynamics of poly(*N*-vinylcarbazole) and related polymers in solution and in film by using pico- and nanosecond laser photolysis methods.[27-32,57] The excitation and the positive charge in the polymer have been considered to be stabilized by taking dimer forms, which indicates the importance of the detailed investigations on carbazole excimers and dimer ions. From this viewpoint the diastereoisomeric model compounds with different geometrical configurations are considered to be appropriate models,[40] since isotactic and syndiotactic sequences result in the formation of two types of excimers. The present work has offered such information on the excited and ionic states of dicarbazolyl compounds with specific geometrical structures. The electronic structure and dynamics of the excited states and ion radicals of poly(*N*-vinylcarbazole) can be elucidated by comparing their absorption spectral data with the present ones. A distribution of these excimers and dimer ions might determine the properties of polymers, or a new state not identified as a dimer might be produced in the polymer. In the latter case a new electronic process and reactivity, not present in the monomer and oligomer system, will be expected. Along this line we are performing absorption spectral studies on polymers in the excited and ionic states, which will be published shortly.

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Proton Nuclear Magnetic Relaxation of ^{15}N-Labeled Nucleic Acids via Dipolar Coupling and Chemical Shift Anisotropy

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Abstract: We have observed a differential in the relaxation rates of the imino proton NMR doublet of 3-^{15}N-substituted 2′,3′,5′-tri-*O*-benzoyluridine. The differential is ascribed to interference between dipole–dipole and chemical shift anisotropy mechanisms of relaxation. A value of 5.7 ppm is derived for the proton chemical shift anisotropy, using a theory described in the Appendix, and due to M. Goldman (manuscript in preparation). Differential broadening of ^{15}N-substituted transfer ribonucleic acid by the same mechanism is shown to be quantitatively compatible with published observations. We conclude that these observations do not provide strong evidence of base-pair tautomerism.

Changing the conditions of magnetic resonance experiments may modify the relative importance of various relaxation mechanisms. Thus the use of large magnetic fields enhances the effects of chemical shift anisotropy, and the exploration of large molecules with long tumbling times reveals broadening mechanisms whose contribution in small molecules might go unnoticed.

Consider for instance a proton bound to ^{15}N. The spectrum is a doublet with a splitting of ca. 90 Hz due to scalar coupling. The amplitude of the dipolar field of ^{15}N at the proton site is *d*

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$= \mu/(4\pi\epsilon_0 c^2 r^3)$ where $\mu = \hbar\gamma S$ is the magnetic moment of ^{15}N and r is the nitrogen–proton distance. For $r = 10^{-10}$ m, this amplitude is 1.4×10^{-4} T.

The chemical shift anisotropy (CSA) of an NH proton could be of the order of 10 ppm. In a field of six teslas this corresponds to 0.6×10^{-4} T, an appreciable fraction of the dipolar amplitude d.

Both the dipolar and the CSA interactions are modulated by molecular rotation, and both therefore contribute to transverse and longitudinal relaxation.[1]

Moreover, they do not contribute independently. The fluctuating field at the proton site is the sum of the fields due to the two causes. The square of the fluctuating field (which comes in the computation of the relaxation rate) will then include cross-terms, and these will not cancel out in the time average because CSA has components that transform under rotation like the dipolar interaction (a second rank, traceless, symmetric tensor).[2-4]

The contribution of the cross-terms takes opposite values for the two orientations of the ^{15}N spin. Therefore the relaxation rates are expected to differ for the two lines of the proton doublet. Similarly, relaxation differentials should also occur in the ^{15}N doublet. Such effects are also expected in a ^{13}C–H group. They have been reported in the fluorine spectrum of =CFH groups.[2]

The proton NMR spectra of ^{15}N-substituted transfer ribonucleic acids (tRNA) exhibit conspicuously different line widths for the two lines of ^{15}N-linked imino proton doublets.[5-7] It has been proposed[5,6] that this is due to tautomerism of the hydrogen-bonded base pairs, which would cause the imino proton to jump from one base to the other. An alternative explanation, suggested by A. G. Redfield, is also considered in ref 6: the differential broadening could be due to the CSA/dipolar cross-terms mentioned above. In order to study this problem, we have measured nuclear magnetic relaxation in a model system. Our results support the alternative explanation.

This explanation is now favored by the authors of ref 6, on the basis of other observations (personal communication of E. Kaun and H. Rüterjans to M. Guéron).

Requirements for a Model System

In order to measure the dipolar and CSA contributions to T_1 and T_2, other contributions should be as small as possible. This requires a molecule where the ^{15}N–H group is not too close to other protons. The solvent should be devoid of paramagnetic species.

Proton exchange must also be avoided. If it occurs between two molecules of the observed species, it will transfer longitudinal magnetization between the two lines of the doublet and thus tend to equalize their T_1s. Exchange with other species (water) will give a contribution to T_1^{-1}. Both types of exchange will contribute to T_2^{-1} (line broadening).

In order to distinguish the cross-term effects from others due to tautomerism, hydrogen bonding should be avoided as far as possible. At the least, one should avoid systems where imino–imido hydrogen bonding is possible, since this is the type found in Watson–Crick base pairs, for which tautomerism has been invoked.

Lastly, the chosen molecule should be available in ^{15}N-substituted form.

The system retained here is a deuteriochloroform solution of 2′,3′,5′-tri-*O*-benzoyl(3-^{15}N)uridine, abbreviated as TOBU.

Materials and Methods

TOBU was prepared as described from bis-trimethylsilyl(3-^{15}N)uracil and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose[8,9] and was used as provided without further purification. The samples were dissolved in deuteriochloroform which had been dried and neutralized by passage over a mixed column of magnesium sulfate and potassium carbonate. The solutions were freed of oxygen by bubbling nitrogen inside the NMR tube. TOBU concentration is 5.5 mM/L unless specified otherwise.

Except as mentioned, the NMR measurements were made at 276 MHz on a home-built spectrometer.[10] Complementary measurements at 500 MHz were made on a Bruker instrument. Longitudinal relaxation was studied by the inversion-recovery method, which is well adapted to differential measurements. The excitation pulse frequency was in the middle of the ^{15}N proton doublet. For better precision, the surface, rather than peak height, of each line was measured.

Line-width differentials were small and could not be measured precisely. They were evaluated after spectral narrowing by sine multiplication.[11,12]

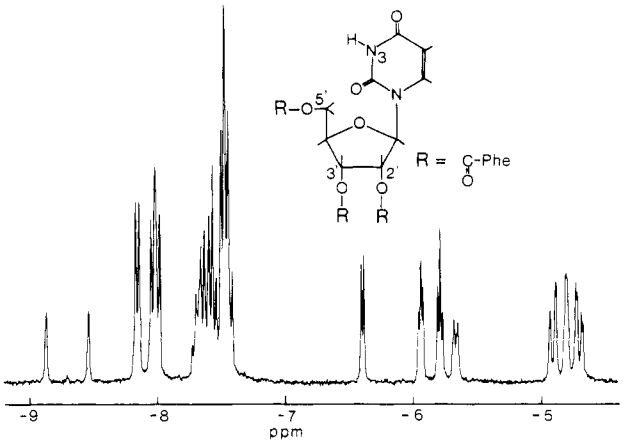

Figure 1. 276-MHz proton NMR spectrum of 3-^{15}N-TOBU in deuteriochloroform. The two lines at lowest field form the imino proton doublet, with the characteristic 90-Hz splitting. The weak line between them is the corresponding singlet from traces of 3-^{14}N-TOBU. Concentration is 5.5 mM; $T = -15$ °C.

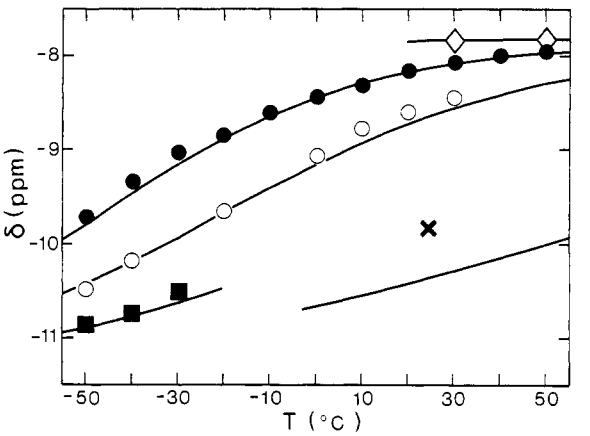

Figure 2. Chemical shift of the TOBU imino proton for various temperatures and concentrations: ◇, 0.43 mM; ●, 5.5 mM; ○, 21 mM; ■, 112 mM; X, 0.5 M (ref 12). Solid lines are a theoretical fit for a monomer–dimer equilibrium, using the parameters given in the text; $\delta_{monomer} = -7.8$; $\delta_{dimer} = -11.3$.

Results

1. Spectral Shifts, Aggregation, Relaxation. Our spectra (Figure 1) conform to earlier publications[13,14] except for the previously unreported shift of the ^{15}NH proton doublet with temperature and concentration (Figure 2). Conditions which

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Table I. Relaxation Rates and Line Widths of the 3-^{15}N-TOBU Imino Proton Doublet

T, °C	% dimer[b]	R_1 (s^{-1}) low field	R_1 (s^{-1}) high field	$\delta R_1/R_1$	Δ (Hz)[a] low field	Δ (Hz)[a] high field	$\delta\Delta$	$\delta\Delta/\delta R_1$
30	8	0.488	0.438	0.11 ± 0.02	3.8	3.75	0.05 ± 0.05	1
−10	24	0.970	0.870	0.11 ± 0.02	6.2	6.16	0.04 ± 0.05	0.4
−10[c]	24	0.520	0.442	0.16 ± 0.04	6	5.8	0.2 ± 0.15	2.5
−15	27	1.07	0.96	0.11 ± 0.02	5.24	5.18	0.06 ± 0.05	0.55
−50	45	1.33	1.26	0.05 ± 0.03	11.7	11.3	0.4 ± 0.1	5.8

[a] The full line width at half-height Δ includes contributions from resolved and unresolved J couplings to other protons. Exchange broadening from the monomer-dimer equilibrium may also be significant, particularly at −50 °C. [b] Number of TOBU molecules participating in dimers; computed with the parameters given in the text. TOBU concentration is 5 mM. [c] This measurement at 500 MHz, all others at 276 MHz.

promote aggregation lead to a down-field shift, as expected if the associated form is a hydrogen-bonded complex. In Figure 2 the data are fitted to chemical shifts of −7.8 ppm for the monomer and −11.3 ppm for a dimeric complex; the association enthalpy and entropy are −24.9 kJ/M and −(19/300) kJ/M. The predicted shift for a concentration of 0.5 mol/L at 25 °C is −10.3 ppm, as compared to the reported value of −9.84 ppm.[14] The difference may be due to stacking of dimers, a phenomenon that will be mentioned again below.

An obvious model for the TOBU dimer is that of two molecules bound by two N−H···O hydrogen bonds between the uracil moieties. The same model is used in the interpretation of an NMR and infrared study of 5′-*O*-acetyl-2′-3′-isopropylideneuridine[15] and in an infrared study of uracil.[16] The thermodynamical parameters were close to those reported here.

The chemical shifts of the imino protons of TOBU in the monomer and dimer forms may also be compared to those of pseudouridine and uridine in tRNA. Unbonded imino protons of pseudouridine and uridine have been assigned in yeast $tRNA^{Asp}$ [17] and yeast $tRNA^{Phe}$.[18,19] The H-bonded imino protons of the (pseudouridine 13–uridine 22) base pair in yeast $tRNA^{Val}_I$ have also been assigned.[20] In all cases the chemical shifts fall between −10.5 and −11.55 ppm, close to the shift for the TOBU dimer. This is expected in the case of the pseudouridine–uridine base pair. For the "unbonded" imino protons, the explanation must lie in hydrogen bonding to water. Indeed, the imino proton of uridine-2′-phosphate is observed at ca. −11.2 ppm in H_2O at pH 3 (E. Kaun and H. Rütejans, personal communication). The uridine imino proton in Me_2SO (a hydrogen-bond acceptor) has the same chemical shift.[21] This is in contrast to the −7.8-ppm chemical shift of uridine derivatives in chloroform.

Results of relaxation and width measurements of the two lines of the ^{15}N−H doublet are shown in Table I. Differences between the two lines are discussed below. As for the average values and their variations vs. temperature, concentration, and magnetic field, they are reasonably well explained in a model that incorporates the proportion of dimers derived from the chemical shift (Figure 2 and second column of Table I), and the viscosity of chloroform.

In the case of the dimer, one must take into account not only the proton–nitrogen dipolar coupling but also the coupling between imino protons, which given the ratio of ten between the proton and nitrogen gyromagnetic ratios and the intermolecular distances (N−H = 0.1 nm, proton–proton distance ≃ 0.25 nm) is a fraction 0.64 of the NH coupling.

For short correlation times, the contributions to the longitudinal relaxation rate go as coupling squared, so that the proton–proton contribution is 40% of the NH contribution. However, as the correlation time becomes long compared to $1/\omega_I$, the contribution of the proton–proton coupling increases (Appendix, section 5), since mutual flip of two protons requires much less energy than flip of one proton, with or without a nitrogen flip—in other words, spin diffusion sets in. In this context one notes the increase in the longitudinal relaxation rate at low temperature, which may be due to the contribution of the proton–proton coupling in large, slow-tumbling, transient aggregates.

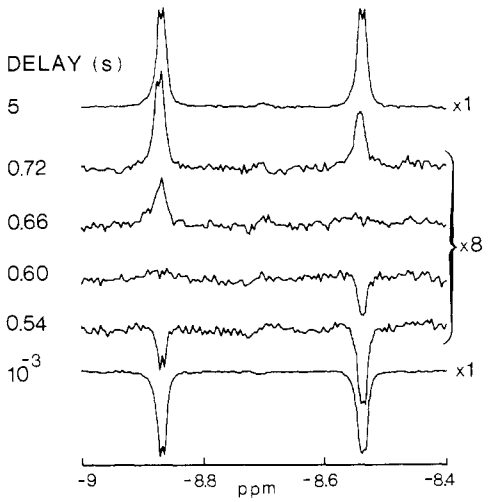

Figure 3. Inversion-recovery measurement of longitudinal relaxation, showing the different rates for the two components of the imino doublet. The relative differential in the relaxation rate is (11 ± 2)%. The splittings of each line are due to J couplings with the other protons of uracil. A broad line that relaxes quickly is discernible under each line of the doublet. It is ascribed to large aggregates. The fraction of molecules in the dimer form is 27%.

The comparison of longitudinal relaxation rates at 276 and 500 MHz (T = −10 °C) gives a rotational correlation time in the range of 5×10^{-10} s.

The line widths reported in Table I are difficult to interpret because of unresolved J couplings and spurious broadening by temperature fluctuations (up to 10 Hz/°C, see Figure 2). Monomer–dimer exchange broadening may also be significant, in particular at low temperatures.

2. Relaxation Differentials. Differences in the longitudinal relaxation of the two lines of the ^{15}N3−H doublet were observed in all conditions of concentration and temperature. An example is shown in Figure 3, where the difference in relaxation rates δR_1 is 0.11 s^{-1} and corresponds to 11% of the average rate. At −50 °C, the δR_1 is unchanged, but the average rate R_1 has increased. This is in agreement with the assumption made above of transient aggregates wherein proton–proton relaxation (spin diffusion) would be more efficient because of the longer correlation time.

At higher frequencies also, the relative contribution of proton–proton relaxation must increase. This could explain why the relative differential in longitudinal relaxation increases by less than a factor of 2 between 276 and 500 MHz.

The R_2 differential (and the corresponding width differential $\delta\Delta$, full width at half height, $=\delta R_2/\pi$) is much harder to measure

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because of the other broadening factors mentioned above. The low-field peak is broader in TOBU, as it is in tRNA. For very short correlation times one expects δR_2 to be two thirds of δR_1 (hence $\delta\Delta/\delta R_1 \simeq 0.2$), whereas δR_2 becomes much larger than δR_1 when the correlation time is large (eq A9 and A10). Values of $\delta\Delta/\delta R_1$ are given in the last column of Table I. They are in the range that is expected.

Discussion

In this discussion, we shall use the experimental results described above to evaluate the chemical shift anisotropy of TOBU.

Using this value, we shall compute the differential broadenings expected in ^{15}N-tRNA and show that they are as large as those observed.[5-7] Hence the observed differentials do not provide evidence for tautomerism.

The discussion will use elements of relaxation theory that are summarized in the Appendix and are developed in a separate paper.[22] Qualitatively, we point out that for small molecules the longitudinal and transverse relaxation differentials are comparable and the former is much easier to demonstrate. In large molecules the relative longitudinal differential is strongly reduced by the proton–proton contribution. By contrast the relative transverse differential is up to three times larger than that in small molecules.

1. Chemical Shift Anisotropy of TOBU. The previous discussion shows that the relaxation differentials of TOBU observed in various conditions are consistent. The relative differential of the TOBU monomer longitudinal relaxation is about 11% at 276 MHz. The absolute value of the CSA, $\Delta\sigma = \sigma_{\parallel} - \sigma_{\perp}$, is then (eq A8 of the Appendix):

$$|\Delta\sigma| = 5.7 \text{ ppm}$$

This result is valid if the CSA is axial and oriented along NH. Otherwise the CSA needed for explaining the data is larger.

For comparison, the CSA of CH protons may be around 6 ppm, and it is in the range of 10 ppm for OH protons. Hydrogen bonding (O—H···O) gives values of the CSA in the range of 20 to 30 ppm.[23,24]

The data of Table I do not indicate large changes in the CSA upon hydrogen bonding, since there is no obvious effect of dimer formation on the relaxation differentials. This is somewhat surprising since the average value of the chemical shift is changed, and also in view of the effect of hydrogen bonding in the O—H···O case. On the other hand, the difference in CSA between O—H···O and N—H···O hydrogen bonds may be related to the property that the former are often stronger than the latter, featuring a shorter H···O distance and a larger enthalpy.[25]

2. Differential Broadening in ^{15}N-tRNA. What effects would be expected in ^{15}N-tRNA from a CSA of 5.7 ppm? The relaxation effect of the interaction is a function of the correlation time for molecular motion, which is of course different in TOBU and tRNA. For the purpose of comparison, the *relative differentials* are most appropriate since they depend weakly on the correlation time. As shown in the Appendix (eq A5 vs. A6), the relative differential broadening Y_2 in tRNA should be up to (10/3) times larger than the relative differential of the longitudinal relaxation Y_1 of the TOBU monomer in the same field. At 400 MHz, a value of Y_2 as large as (11/100) × (400/276) × (10/3) = 53% may then be expected, if proton–proton couplings are neglected, and if the CSA is the same in the tRNA base pairs as in the U monomer.

In the case of long correlation times, proton–proton couplings drastically reduce the relative differential of longitudinal relaxation. They also reduce somewhat the relative differential broadening. The magnitude of this effect depends on the distribution of protons near the imino proton being studied. In a Watson–Crick AU pair, the imino proton is about 0.24 nm from an adenine amino proton and 0.3 nm from the adenine C2 proton.[26] In a GC pair, it is 0.22 nm from a G amino proton and 0.25 nm from a C amino proton.[27] Considering all the protons in the neighborhood of an imino proton,[28] one finds that for different base pairs of tRNA, the proton contribution to transverse relaxation could be typically from 0.6 to 1.5 times that of nitrogen. This would reduce the relative differential from 53% to values in the range of 20 to 33%. Since the closest protons are exchangeable, we would expect the relative differential to increase upon partial solvent deuteration.

Large values of Y_2 have indeed been observed. For instance, the thio-uracil N3H of the 8–14 tertiary base pair in *E. coli* $tRNA_I^{Val}$ (−14.8 ppm) has a relative broadening Y_2 of 45% at 500 MHz (Figure 4 of ref 6). The theoretical value corresponding to a CSA of 5.7 ppm is 66% if interactions with other protons are ignored. These interactions may be estimated by using[28] the crystallographic structure of yeast $tRNA^{Phe}$, and they reduce Y_2 to 40%, in good agreement with the experimental value.

One may also consider the −13.8-ppm peak in *E. coli* $tRNA^{fMet}$. As measured from Figure 5 of ref 6, the relative differential broadening at 20 °C and 400 MHz is in the range of 50% to 65%, and varies somewhat with temperature (see also the data of Figure 4, ref 5 and 7).

In the framework of our interpretation, the large value observed at 20 °C implies either that the contribution of neighboring protons to the line width is small or that the chemical shift anisotropy is larger by a factor of 1.5 to 2 than that of TOBU. One may point out that the −13.8-ppm peak is presently assigned not to a uracil but to the hymine imino proton from the T54-A58 base pair (ref 29; also Coffino and Reid, personal communication to J. L. Leroy).

The variation of the relative differential broadening with temperature may be due to a change in the proton–proton dipole coupling caused by a variation of interproton distances. This could occur because of structural changes, for which a spectral shift provides independent evidence.

3. Related Considerations. The well-resolved peak at −10.75 ppm in *E. coli* $tRNA^{Val}_1$ exhibits differential broadening in the ^{15}N-substituted species.[6] This peak is assigned to N_1H of pseudo-uracil 55.[30] If the crystalline structure of yeast $tRNA^{Phe}$ is assumed, this proton is not hydrogen bonded, so that the differential broadening cannot be due to base-pair tautomerism.

The interpretation of differential broadening in terms of tautomerism implies that each imino proton resonance is accompanied by a less intense satellite located typically 1 ppm upfield. With the proposed kinetics, the satellite would be broad but not necessarily undetectable. Furthermore, it would saturate together with the main line and this could give anomalies in NOE experiments. Such phenomena, which would occur both with ^{14}N and ^{15}N, have not been reported.

The sign of the differential broadening (broader line at low field) is the same for TOBU and for all tRNA peaks. It is the one expected if the CSA tensor is approximately axial along the NH bond with $\Delta\sigma$ positive (the usual case for C–H and O–H bonds[24]) and if the J coupling is the product of a positive electronic term (the general case for N–H) by the (negative) nuclear magnetic moment of ^{15}N.[31,32]

The situation is the same for ^{13}CH bonds, except that the nuclear magnetic moment is positive. Hence the CSA–dipolar interference should lead to asymetric ^{13}CH proton doublets with the broader line located upfield. Such spectra have been observed

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in the past, for instance in the proton spectrum of the H-2^{13}C group of histidine in α-lytic protease.[33] Tautomerism is excluded in such a case.

Conclusion

In conclusion, we have observed a relative differential of 11% in the longitudinal relaxation of the ^{15}NH proton of TOBU and shown that it can be explained by a 5.7-ppm anisotropy of the chemical shift. The corresponding differential broadening is difficult to observe in TOBU, but is predicted to be large and easily visible in ^{15}N-tRNA. The predictions are in rather good agreement with prior observations on ^{15}N-tRNA. These observations do not therefore provide good evidence for base-pair tautomerism. At the least, the anisotropy of the chemical shift should be taken into account in their interpretation.

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Appendix

Differential Broadening by Interference between CSA and Dipole–Dipole Interactions. The complete analysis of this problem is presented in a separate paper.[22] A simple presentation of the results is attempted here.

1. Transverse Relaxation. The relaxation rate $R_2 = 1/T_2$ of a proton (spin I) due to dipole–dipole coupling to ^{15}N (spin S) is[1]

$$R_2 = D\tau_c(4 + 3/(1 + \omega_I^2\tau_c^2) + 1/(1 + (\omega_I - \omega_S)^2\tau_c^2) + 6/(1 + \omega_S^2\tau_c^2)^2\tau_c^2) \quad \text{(A1)}$$

The resonance frequencies are $\omega/2\pi$, and τ_c is the rotational correlation time of the NH vector $\vec{r}$. We have

$$D = (1/15)\gamma_I^2\gamma_S^2\hbar^2S(S + 1)/(4\pi\epsilon_0c^2r^3)^2$$

with γ equal to the gyromagnetic ratio and $4\pi\epsilon_0c^2 = 10^7$ in MKS units. For ^{15}N and H 0.1 nm apart, we find

$$D = 2.56 \times 10^8 \text{ s}^{-1}$$

In (A1) the first and second terms originate in the operators I_ZS_Z and $I_\pm S_Z$ which do not change the longitudinal orientation of the nitrogen spin. It is these terms that couple with the CSA, by simple addition of the corresponding magnetic fields.

The relaxation rate due to CSA is

$$R_2 = C\tau_c(4 + 3/(1 + \omega_I^2\tau_c^2)) \quad \text{(A2)}$$

where C is equal to $(1/45)\omega_I^2(\Delta\sigma)^2$, and the CSA tensor is assumed to be axial.

Let us set $\alpha^2 = C/D$. If the CSA axis is in the NH direction, the relaxation rates of the proton doublet lines are

$$R_{2\pm} = D\tau_c(4(1 \pm \alpha)^2 + 3(1 \pm \alpha)^2/(1 + \omega_I^2\tau_c^2) + 1/(1 + (\omega_I - \omega_S)^2\tau_c^2) + 6/(1 + \omega_S^2\tau_c^2) + 6/(1 + (\omega_I + \omega_S)^2\tau_c^2)) \quad \text{(A3)}$$

For an angle θ between the CSA axis and the NH direction, each interference term (linear in α) should be multiplied by $(3\cos^2\theta - 1)/2$.

Numerical Values. For $\omega_I/2\pi = 500$ MHz, we find that $C = D$ (α=1) if $\Delta\sigma = 34.2$ ppm; at 276 MHz, the $\Delta\sigma$ for $\alpha = 1$ would be 62 ppm.

2. Relative Differential in Transverse Relaxation. Taking into account that $\omega_S \simeq \omega_I/10$, we consider three cases: (a) $\omega_I\tau_c$, $\omega_S\tau_c \ll 1$. Then, by (A3) we have

$$Y_2 = \frac{(R_{2+} - R_{2-})}{1/2(R_{2+} + R_{2-})} \simeq \frac{28\alpha}{7(1 + \alpha^2) + 13} \simeq \frac{1.4\alpha}{1 + 0.35\alpha^2} \quad \text{(A4)}$$

(b) $\omega_S\tau_c = 1$, $\omega_I\tau_c \ll 1$

$$Y_2 \simeq \frac{16\alpha}{4(1 + \alpha^2) + 3} \simeq \frac{2.3\alpha}{1 + 0.57\alpha^2} \quad \text{(A5)}$$

(c) $1 \ll \omega_s\tau_c$, $\omega_I\tau_c$

$$Y_2 \simeq \frac{16\alpha}{4(1 + \alpha^2)} \simeq \frac{4\alpha}{1 + \alpha^2} \quad \text{(A6)}$$

3. Longitudinal Relaxation. For a two-spin system, longitudinal relaxation after inversion of the magnetization of one spin is not exponential. There are two relaxation parameters ρ and σ for each spin. For spin I, we have[1]

$$\rho = D\tau_c(6/(1 + \omega_I^2\tau_c^2) + 2/(1 + (\omega_I - \omega_S)^2\tau_c^2) + 12/(1 + (\omega_I + \omega_S)^2\tau_c^2))$$

$$\sigma = (I(I + 1)/S(S + 1))D\tau_c(-2/(1 + (\omega_I - \omega_S)^2\tau_c^2) + 12/(1 + (\omega_I + \omega_S)^2\tau_c^2)) \quad \text{(A7)}$$

When one includes CSA, the only term affected is the first term of ρ, which is multiplied as before by $(1 \pm \alpha)^2$. The relative differential in ρ is independent of τ_c in the present case where $\omega_S \ll \omega_I$:

$$Y_1 \simeq \frac{24\alpha}{6(1 + \alpha^2) + 14} = \frac{1.2\alpha}{1 + 0.3\alpha^2} \quad \text{(A8)}$$

In the experiments reported here, the longitudinal relaxation was characterized by the time when proton magnetization becomes zero, following inversion of the proton spins. The relative differential of this time is quite close to the relative differential Y_1.

4. Absolute Differentials. From (A3) we have

$$R_{2+} - R_{2-} = D\tau_c(16\alpha + 12\alpha/(1 + \omega_I^2\tau_c^2)) \quad \text{(A9)}$$

$$\rho_+ - \rho_- = D\tau_c[24\alpha/(1 + \omega_I^2\tau_c^2)] \quad \text{(A10)}$$

5. The Effect of Neighbor Protons for Large Correlation Times. Equations A1 and A7 are also valid when S is a proton rather than a nitrogen nucleus. When S is a proton, one may ignore the difference between ω_S and ω_I. The proton contribution to R_2 is therefore 5/4 that of nitrogen for a given coupling. The contributions to R_1 and R_2 are equal for proton–proton coupling, and they increase proportionally to τ_c, whereas the contribution to R_1 of nitrogen–proton coupling varies as $1/\tau_c$.

6. Application to the Comparison of TOBU and tRNA. The correlation time of tRNA may be estimated from (A1). A line width of 15 Hz (half-width at half-height) corresponds to $R_2 = 94$ s^{-1}. In an A–U Watson–Crick base pair the U imino proton is 0.1 nm from the nitrogen and 0.25 nm from the nearest proton. Consideration of the interproton distances shows that the sum of the squares of the couplings of the imino proton to neighboring protons is comparable to the square of the NH dipolar coupling. Assuming that the contribution of the NH coupling to R_2 is 47 s^{-1}, one obtains from (A1) $\tau_c \simeq 4.4 \times 10^{-8}$ s. The value of τ_c is so large that only the first term in (A1) is significant for the ^{15}N–H interaction. Hence the proper relation for Y_2 in tRNA is (A6).

The rotational correlation time just derived may be compared to that, τ_R, for a molecule with a Stokes radius of 3 nm: $\tau_R = 4\pi\eta a^3/3kT = 2.8 \times 10^{-8}$ s^{-1}, where the value of η for water (10^{-3} MKS) has been used.[1]

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