

# JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

© Copyright, 1982, by the American Chemical Society

VOLUME 104, NUMBER 21

OCTOBER 20, 1982

## Use of the Inept Polarization Transfer Method for $^{15}\text{N}$ NMR Relaxation Study of Linear Peptides

Dominique Marion, Christiane Garbay-Jaureguiberry, and Bernard P. Roques\*

Contribution from the Département de Chimie Organique, ERA 613 du CNRS et SC 21 de l'INSERM, U.E.R. des Sciences Pharmaceutiques et Biologiques, 75006 Paris, France. Received November 30, 1981

**Abstract:** A new utilization of the cross-polarization INEPT sequence for  $^{15}\text{N}$  spin-lattice relaxation studies is reported. The INEPT sequence is used as a preparation pulse, allowing one to observe the magnetization difference recovery within one multiplet. When 90–95%  $^{15}\text{N}$  enriched peptide models Tyr-\*Gly-\*Gly-\*Phe and Boc-Tyr-\*Gly-\*Gly-\*Phe-OCH<sub>3</sub> are used, the results obtained by this method were compared with the conventional  $T_1$  relaxation times. In this way, sharp differences in the relaxation mechanisms are discerned. The "antisymmetric" relaxation times are shown to depend on both proton-relaxation mechanisms such as  $^1\text{H}$ - $^1\text{H}$  dipolar contribution and proton-exchange processes. Besides the  $^{15}\text{N}$  signal enhancement brought about by the INEPT, the present method provides conformational information, particularly for free linear peptides where intramolecular exchange processes related to folded conformations may be easily seen.

Despite its great potential in conformational studies of biological materials,  $^{15}\text{N}$  NMR spectroscopy still remains a scarcely used method due to its low sensitivity.<sup>1</sup> Three main factors contribute to the low sensitivity of  $^{15}\text{N}$  NMR: (i) a low natural abundance (0.37%), (ii) a low resonance frequency, and (iii) a negative magnetogyric ratio. In previous reports<sup>1–4</sup> we have used  $^{15}\text{N}$ -enriched peptide models to alleviate the first limiting factor and described conformational and dynamic investigations of these molecules. The two later factors can be canceled by using a new pulse sequence named INEPT by Freeman et al.<sup>5–7</sup> (Insensitive Nuclei Enhanced by Polarization Transfer). Indeed, this allows an enhancement of the NMR signal of nuclei with a low magnetogyric ratio by the transfer of polarization from protons scalar coupled to the weak nucleus. Moreover, in the case of nuclei with a negative magnetogyric ratio such as  $^{15}\text{N}$ , this technique shows a significant improvement over the nuclear Overhauser enhancement (NOE). Indeed  $^1\text{H}$  broad-band decoupling can result in signal cancellation due to incomplete NOE arising from slow molecular motion or nondipolar relaxation contributions. In this respect,  $^{15}\text{N}$ -enriched peptides such as Tyr-\*Gly-\*Gly-\*Phe (I) and Boc-Tyr-\*Gly-\*Gly-\*Phe-OCH<sub>3</sub> (II) provide appropriate probes for testing the potentiality and the limitations of the INEPT

sequence in  $^{15}\text{N}$  NMR, particularly for molecular dynamics investigations. Thus, we have measured the  $^{15}\text{N}$  spin-lattice relaxation times after a slightly modified INEPT sequence for spin preparation and compared the results obtained for the two peptide models with conventional  $^{15}\text{N}$   $T_1$ 's. Our results reveal that these two kinds of relaxation times do not originate from the same mechanisms and that, in the free linear peptide, the second amino acid exhibits an original behavior when an INEPT spin preparation is used.

### Materials and Methods

Tyr-\*Gly-\*Gly-\*Phe and Boc-Tyr-\*Gly-\*Gly-\*Phe-OCH<sub>3</sub> were synthesized by the liquid-phase method; 90–95%  $^{15}\text{N}$ -enriched amino acids were purchased from CEA (France). Details on their preparation are reported elsewhere.<sup>4</sup> The peptides were dissolved in H<sub>2</sub>O and freeze-dried. Me<sub>2</sub>SO-*d*<sub>6</sub> samples (1.8-mL solution at 0.1 M concentration with a small amount of EDTA for removing paramagnetic impurities) were prepared in a 10-mm o.d. tube and degassed by 3 freeze-pump-thaw cycles under high vacuum before the tube was sealed.

$^{15}\text{N}$  spectra were recorded on a Bruker WH 270 spectrometer equipped with an ASPECT 2000 computer (new pulse programmer) and with a 10-mm  $^{15}\text{N}$  probe operating at 27.4 MHz. Temperature was controlled to within  $\pm 1$  °C.

The two excitation sequences used respectively for the two types of  $T_1$  measurements are shown in Figure 1: it is seen that the two methods differ from each other by the  $^{15}\text{N}$  preparation pulse and the presence (or absence) of  $^1\text{H}$  irradiation during the evolution period  $\tau_b$ . The conventional  $T_1$  measurements (Figure 1A) are performed by using the Freeman-Hill inversion recovery method<sup>8</sup>  $180^\circ\text{-}\tau_b\text{-}90^\circ$  with continuous  $^1\text{H}$  broad-band decoupling.

(1) Levy, G. C.; Lichter, L. L. In  $^{15}\text{N}$  NMR Spectroscopy; John Wiley & Sons: New York, 1979.

(2) Garbay-Jaureguiberry, C.; Baudet, J.; Florentin, D.; Roques, B. P. *FEBS Lett.* **1980**, *115*, 315–318.

(3) Marion, D.; Garbay-Jaureguiberry, C.; Roques, B. P. *Biochem. Biophys. Res. Commun.* **1981**, *101*, 711–718.

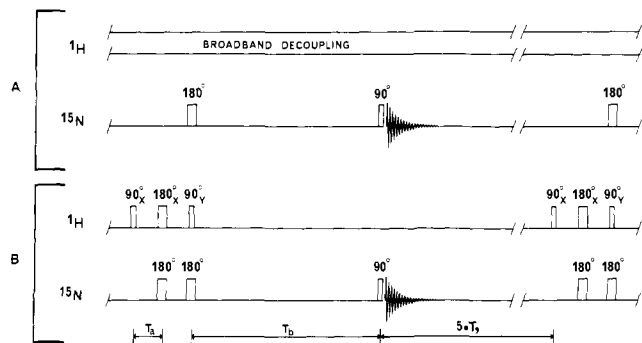
(4) Garbay-Jaureguiberry, C.; Marion, D.; Fellion, E.; Roques, B. P. *Int. J. Pept. Protein Res.*, in press.

(5) Morris, G. A.; Freeman, R. *J. Am. Chem. Soc.* **1979**, *101*, 560–562.

(6) Morris, G. A. *J. Am. Chem. Soc.* **1980**, *102*, 428–429.

(7) Pegg, D. T.; Doddrell, D. M.; Brooks, W. A.; Bendal, M. R. *J. Magn. Reson.* **1981**, *44*, 32–40.

(8) Freeman, R.; Hill, H. D. W. *J. Chem. Phys.* **1971**, *51*, 3140–3141.



**Figure 1.** Pulse sequence used for relaxation measurements: (A) symmetric  $T_1$  (Freeman-Hill method), (B) antisymmetric  $T_1$  (slightly modified INEPT sequence);  $^{15}\text{N}$   $180^\circ$  pulse =  $42 \mu\text{s}$ ,  $^1\text{H}$   $180^\circ$  pulse =  $400 \mu\text{s}$ .

Thus, the proton-decoupled  $^{15}\text{N}$  signal is related to the total nitrogen magnetization. The second kind of  $T_1$  is obtained by using an excitation sequence based on the spin-echo polarization transfer method INEPT described by Morris and Freeman.<sup>5</sup> The original INEPT sequence, which creates a  $^{15}\text{N}$  magnetization in the  $x$ - $y$  plane by a  $^{15}\text{N}$ -modulated proton spin-echo, was altered by converting the  $^{15}\text{N}$   $90^\circ$  pulse into a  $180^\circ$  pulse without any other modification. This modified sequence allows one to flip the nitrogen spins along the  $z$  axis for spin-lattice relaxation investigation (Figure 1B).<sup>9</sup>

After the INEPT excitation, one component of the  $^{15}\text{N}$  doublet is enhanced whereas the other is enhanced but inverted. Therefore, our modified pulse excitation allows one to observe the recovery (along the  $z$  axis) of the magnetization difference between the two components of the doublet for various  $\tau_b$  (Figure 2).

As this observable parameter is antisymmetric with respect to line position,<sup>10</sup> the derived  $T_1$  will be named later "antisymmetric  $T_1$ " ( $T_1^a$ ) as opposed to the conventional "symmetric  $T_1$ " ( $T_1^s$ ). About 100 scans for conventional spectra and 50 for enhanced ones were accumulated, insuring a satisfactory signal-to-noise ratio.  $T_1$  values were obtained from 13 different  $\tau_b$  values by a three-parameter ( $M$  (initial),  $M$  (final),  $T_1$ ) nonlinear iterative fitting algorithm.<sup>11</sup> Due to the much shorter  $T_1^a$  for Gly<sup>2</sup> with respect to the other residues in the free peptide (I), two experiments with different sets of  $\tau_b$  values were performed for this compound.

## Results and Discussion

As remarked by Morris and Freeman,<sup>5</sup> the INEPT method is not based on relaxation phenomena, unlike the  $^{15}\text{N}$ - $^1\text{H}$  NOE. However, this sequence relies on the excitation of spin-echoes with fixed delay  $\tau_a$  equal to  $(1/4J_{\text{NH}})$ . As a result, the 10% dispersion of the  $^1J_{\text{N-H}}$  coupling constants within each peptide<sup>4</sup> prevents us from obtaining the maximal sensitivity improvement for all the signals in one single experiment. Nevertheless, at 310 K almost 60% of the predicted enhancement ( $\gamma(^1\text{H})/\gamma(^{15}\text{N}) = -10$ ) is obtained for all the resonances except for Gly<sup>2</sup> in the free peptide (only 30%). Moreover, an increase in temperature leads to a dramatic loss of polarization transfer for this later signal, even for the suitable delay.<sup>12</sup>

From Table I, one observes that the  $^{15}\text{N}$  antisymmetric  $T_1^a$  values (INEPT sequence) are shorter than the symmetric  $T_1^s$  ones (conventional ones). On the other hand, the  $T_1^a$  measurements illustrate the particular behavior of the Gly<sup>2</sup> signal of the free peptide (I), i.e., a very short  $T_1^a$ , as compared to all the other  $^{15}\text{N}$  resonances. It is noteworthy that the conventional  $T_1$  do not evidence such anomalous behavior.

(9) The authors are pleased to acknowledge Dr. Freeman for his suggestion that the removal of the second  $^{15}\text{N}$   $180^\circ$  pulse should have virtually no effect on the results but should reduce effects of the pulse imperfections.

(10) Werbelow, L. G.; Grant, D. M. *Adv. Magn. Reson.* **1979**, *9*, 189-299 and references cited herein.

(11) Marquardt, D. W. *Chem. Eng. Prog.* **1959**, *55*, 65-73.

(12) Marion, D.; Garbay-Jaureguiberry, C.; Roques, B. P. *J. Magn. Reson.*, in press.

**Table I.**  $^{15}\text{N}$  Relaxation Times and Conformational Parameters for Tyr- $^*$ Gly- $^*$ Gly- $^*$ Phe and Boc-Tyr- $^*$ Gly- $^*$ Gly- $^*$ Phe-OCH<sub>3</sub><sup>a</sup>

|                  | Tyr- $^*$ Gly- $^*$ Gly- $^*$ Phe (I) |             |                   |                         | Boc-Tyr- $^*$ Gly- $^*$ Gly- $^*$ Phe-OCH <sub>3</sub> (II) |             |                   |                         |
|------------------|---------------------------------------|-------------|-------------------|-------------------------|---|-------------|-------------------|-------------------------|
|                  | $T_1^s$ , s                           | $T_1^a$ , s | $R = T_1^a/T_1^s$ | $\langle r \rangle$ , Å | $T_1^s$ , s   | $T_1^a$ , s | $R = T_1^a/T_1^s$ | $\langle r \rangle$ , Å |
| Gly <sup>2</sup> | 0.98                                  | 0.028       | 0.028             | 2.04                    | 1.33  | 0.54        | 0.40              | 2.23                    |
| Gly <sup>3</sup> | 0.98                                  | 0.34        | 0.34              | 1.94                    | 1.40  | 0.61        | 0.43              | 2.15                    |
| Phe <sup>4</sup> | 0.87                                  | 0.22        | 0.25              | 2.78                    | 1.61  | 0.78        | 0.47              | 2.29                    |

<sup>a</sup>  $T_1^s$  and  $T_1^a$  measured at 310 K in Me<sub>2</sub>SO-*d*<sub>6</sub> (0.1 M) are the symmetric and antisymmetric spin-lattice relaxation times as defined in the text.  $\langle r \rangle = (\sum r_{ij}^{-6})^{-1/6}$  is the distance of a single proton which would relax the amide proton as all the neighboring  $\alpha$ -ones. In the free peptide (I), the  $\langle r \rangle$  distances were computed from the  $\beta_{\text{II}}$ ' folded conformation,<sup>4,15</sup> whereas in the protected derivative (II), the  $\langle r \rangle$  distances correspond to the direct average of the three staggered conformers around the C $_{\alpha}$ -N bond.

The differences between symmetric and antisymmetric  $T_1^a$  can be essentially related to three different features: (i) contrary to the symmetric  $T_1^s$ , where the total magnetization recovery rate is measured, the antisymmetric  $T_1^a$  are derived from the recovery rate of the magnetization difference within each doublet; (ii) the initial  $^1\text{H}$  and  $^{15}\text{N}$  magnetizations after the preparation pulse are different in the two kinds of experiments; and (iii)  $^1\text{H}$  nuclei are saturated during symmetric  $T_1$  measurements whereas the protons evolve freely during antisymmetric  $T_1$  measurements.

In order to analyze the  $^{15}\text{N}$  relaxation data, each amide nitrogen is considered as an independent unit. Due to the small value of the  $^2J_{\text{N-H}}$  and  $^3J_{\text{N-H}}$ ,<sup>4</sup> it is not possible to observe separate lines within each component of the  $^{15}\text{N}$  doublets arising from  $^1J$  nor to excite them selectively. Therefore the N-H pairs can be considered for relaxation investigation as AX spin systems, according to the mathematical formalism of Werbelow and Grant.<sup>10</sup> Relaxations due to the non-amide protons are introduced as external contributions.

Three independent observable parameters can be used in each AX spin system (A =  $^{15}\text{N}$ ; X =  $^1\text{H}$ ): the total magnetization of the nitrogen,  $M_{\text{N}}$ , related to the symmetric  $T_1^s$ , that of the proton,  $M_{\text{H}}$ , and the intensity difference within each doublet  $M_{\text{NH}}$  related to the antisymmetric  $T_1^a$ .

The time evolution after the spin preparation is determined by the equation

$$\frac{d}{dt} \begin{bmatrix} M_{\text{N}} \\ M_{\text{H}} \\ M_{\text{NH}} \end{bmatrix} = \begin{bmatrix} \rho_{\text{NH}} + \rho_{\text{N}} & \sigma_{\text{NH}} & 0 \\ \sigma_{\text{HN}} & \rho_{\text{HN}} + \rho_{\text{H}} & 0 \\ 0 & 0 & \Delta_{\text{NH}} + \rho_{\text{N}} + \rho_{\text{H}} \end{bmatrix} \begin{bmatrix} M_{\text{N}} \\ M_{\text{H}} \\ M_{\text{NH}} \end{bmatrix} \quad (1)$$

where

$$\rho_{ij} = \xi_{ij} [1/3 J_{ij}(\omega_i - \omega_j) + J_{ij}(\omega_i) + 2J_{ij}(\omega_i + \omega_j)] \quad (2)$$

$$\sigma_{ij} = \xi_{ij} [2J_{ij}(\omega_i + \omega_j) - 1/3 J_{ij}(\omega_i - \omega_j)] \quad (3)$$

$$\Delta_{ij} = \xi_{ij} [J_{ij}(\omega_i) + J_{ij}(\omega_j)] \quad (4)$$

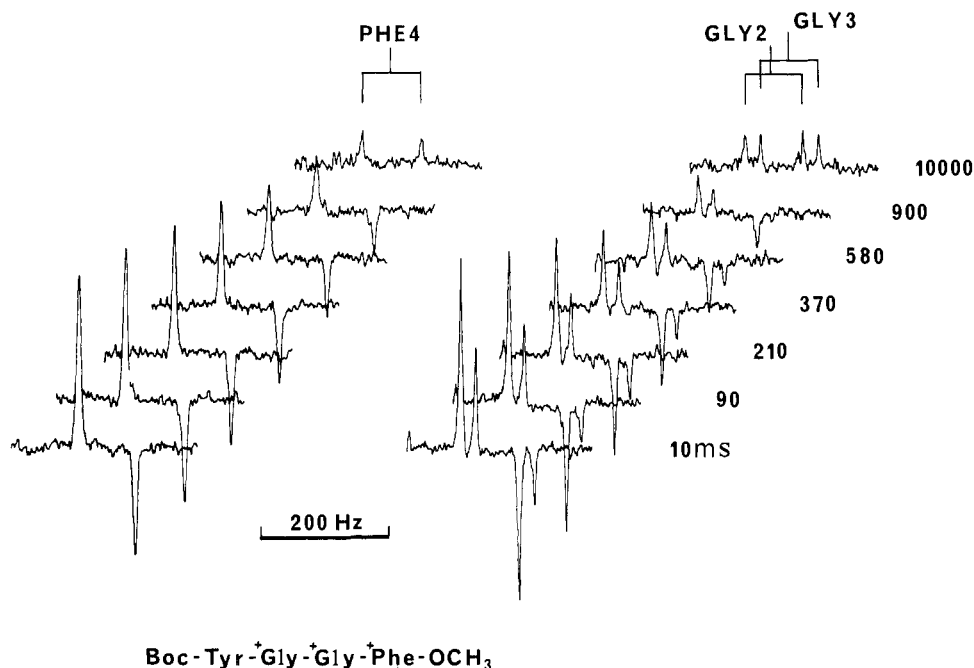
with  $i, j = \text{N, H}$ .  $J_{ij}(\omega)$  is the dipolar spectral density function at  $\omega$  frequency, and  $\xi_{ij} = 3/20 \gamma_i^2 \gamma_j^2 \hbar^2 r_{ij}^{-6}$ .

The terms  $\rho_{\text{N}}$  and  $\rho_{\text{H}}$  are the relaxation contributions which do not arise from the dipolar N-H system, i.e., dipolar relaxation with other nuclei as  $\alpha$  protons, scalar relaxation, and exchange processes.

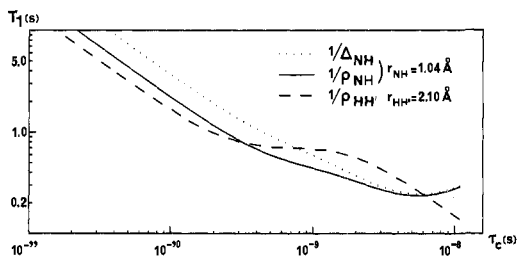
Since there is no coupling coefficient between the magnetization difference within each doublet,  $M_{\text{NH}}$ , and the total magnetization,  $M_{\text{N}}$ , the term  $M_{\text{NH}}$  achieves equilibrium ( $M_{\text{NH}}^{\text{equil}} = 0$ ) with the rate:

$$1/T_1^a = \Delta_{\text{NH}} + \rho_{\text{N}} + \rho_{\text{H}} \quad (5)$$

On the other hand, the rate of saturation by  $^1\text{H}$  broad-band decoupling in the symmetric  $T_1^s$  experiment is much larger than the natural rates of relaxation. Therefore, the coupling term with



**Figure 2.** Antisymmetric  $T_1$  measurement for Boc-Tyr-<sup>15</sup>Gly-<sup>15</sup>Gly-<sup>15</sup>Phe-OCH<sub>3</sub> in Me<sub>2</sub>SO (0.1 M) at 310 K (50 scans).



**Figure 3.** Relaxation parameters as a function of effective correlation time at an external magnetic field of 6.3 T (<sup>1</sup>H, 270 MHz; <sup>15</sup>N, 27.4 MHz).

$M_H$  vanishes and the recovery rate of  $M_N$  may be simply written as

$$1/T_1^s = \rho_{NH} + \rho_N \quad (6)$$

The  $\rho_N$  term includes two contributions: the scalar relaxation mechanism induced by amide proton exchange<sup>13,14</sup> and the dipolar relaxation due to non-amide protons in the surrounding area. It was previously pointed out that in heteronuclear spin systems, the scalar relaxation is not efficient for spin-lattice relaxation due to the wide difference in the Larmor frequencies.<sup>14</sup> On the other hand, as the distance between the nitrogen and the non-amide protons is at least twice the N-H bond length, their dipolar contributions to <sup>15</sup>N  $T_1$  are negligible, as compared to the  $\rho_{NH}$  term. Accordingly, the  $\rho_N$  term can be removed from eq 5 and 6.

The variations of  $\Delta_{NH}^{-1}$  and  $\rho_{NH}^{-1}$  (related to relaxation times) are plotted in Figure 3 vs. the correlation time  $\tau_c$  for an isotropic molecular motion.<sup>10</sup> It is informative to note that the relaxation time ratio  $\Delta_{NH}^{-1}/\rho_{NH}^{-1}$  decreases from 5:3 in the motionally narrowed conditions to 1 for slower molecular movement. Thus, if the proton relaxation contribution  $\rho_H$  is not taken into account in eq 5, the experimental ratio  $R = T_1^a/T_1^s$  is expected to fall within these limits. However, the results of Table I ( $0.028 < R < 0.47$ ) do not fall within the above range. This clearly shows the efficiency of the amide proton relaxation term  $\rho_H$  for the <sup>15</sup>N antisymmetric relaxation. At this stage, the  $\rho_H$  term, which does not include the proton-nitrogen dipolar interaction, can be at-

tributed to two mechanisms: the dipolar interaction with the non-amide protons of the surrounding area and the exchange processes encountered by the amide proton itself.

The efficiency of the dipole-dipole relaxation between amide proton and  $\alpha$ -ones is of the same order of magnitude for all amino acids since it depends, above all, on the internuclear distance modulated by the  $\psi$  and  $\phi$  dihedral angles. It can be evaluated from the structure of the peptide.

As we previously determined the conformations of Tyr-<sup>15</sup>Gly-<sup>15</sup>Gly-<sup>15</sup>Phe (I) and Boc-Tyr-<sup>15</sup>Gly-<sup>15</sup>Gly-<sup>15</sup>Phe-OCH<sub>3</sub> (II)—a 1-4  $\beta_{II}$  turn for the free peptide (I)<sup>15</sup> with a hydrogen bond between the CO of Tyr<sup>1</sup> and the NH of Phe<sup>4</sup> and a random form for the protected peptide (II)<sup>2</sup>—these conformations were used for computing the internuclear distances between each amide proton and the neighboring protons which relax it. In all cases, due to conformational restrictions, the distance between the amide proton and the  $\alpha$ -ones is greater than 2.1 Å. Furthermore, protons other than  $\alpha$ -ones remain far enough from the <sup>1</sup>H amide to be neglected. In order to estimate the relaxation efficiency in a simpler manner, the protons which relax the amide one may be replaced by a single proton at an equivalent distance  $\langle r \rangle$ , calculated as  $\langle r \rangle = (\sum r_{ij}^{-6})^{-1/6}$ , so that it induces similar relaxation (see Table I for further details). The large  $\langle r \rangle$  value reported for the Phe<sup>4</sup> residue of the free peptide (I) stems from the existence in its preferential conformation of a head-to-tail interaction between the terminal NH<sub>3</sub><sup>+</sup> and COO<sup>-</sup> parts, which brings the amide proton of Phe<sup>4</sup> far from any H $_{\alpha}$  spin. It should be pointed out that the self dipolar relaxation ( $\rho_{HH}$ ) of two protons 2.1 Å apart is always greater than 0.1 s for  $\tau_c$  smaller than 10<sup>-9</sup> s (see Figure 3). Therefore, the very small relaxation time  $T_1^a$  of Gly<sup>2</sup> <sup>15</sup>N in the free peptide ( $T_1^a = 0.028$  s) originates from a mechanism other than dipolar interaction. This feature agrees with a larger line width of <sup>15</sup>N as well as <sup>1</sup>H Gly<sup>2</sup> resonances and a marked temperature dependence of the <sup>15</sup>N chemical shift, as opposed to the other amide signals of the peptide. These results were previously interpreted in terms of proton exchange between the terminal ammonium group and the Gly<sup>2</sup> amide proton in the free tetrapeptide.

The presence of a Boc group instead of NH<sub>3</sub><sup>+</sup> in the protected peptide (II) obviously prevents the exchange and actually no one resonance exhibits anomalous behavior. In that way, the poor efficiency of the polarization transfer and the short  $T_1^a$  of the Gly<sup>2</sup>

(13) Solomon, J.; Bloembergen, N. *J. Chem. Phys.* **1956**, *25*, 261-265.  
 (14) Leipert, T. K.; Noggle, J. H. *J. Am. Chem. Soc.* **1975**, *97*, 269-272.

(15) Fourniè-Zaluski, M. C.; Prangè, T.; Pascard, C.; Roques, B. P. *Biochem. Biophys. Res. Commun.* **1977**, *79*, 1199-1206.

nitrogen of the free peptide (I) can be safely ascribed to the exchange of the NH proton with the  $\text{NH}_3^+$  group because of their close proximity. Similar behavior can be expected for any NH located in a sufficient spatial proximity with the  $\text{NH}_3^+$  group. The smaller  $T_1^a$  value for  $\text{Phe}^4$   $^{15}\text{N}$  in the free peptide could be explained in that way. Indeed, as shown above ( $r$ ) = 2.78 Å for  $\text{Phe}^4$  in the free peptide is larger than that for all the other residues while it exhibits the second smaller  $T_1^a$  value. This apparent discrepancy may be attributed therefore to an exchange process between  $\text{Phe}^4$  NH and the terminal ammonium group. Such a result supports the previously proposed structure<sup>15</sup>—a 1-4 turn that brings into proximity the  $\text{NH}_3^+$  group and  $\text{Phe}^4$  NH.

The influence of exchange phenomena on  $^{15}\text{N}$  relaxation time  $T_1^a$  may be analyzed in the following manner: using the Bloch equations modified to include exchange effects, Allerhand and Gutowsky<sup>16</sup> have extensively investigated the decay of the echo amplitude in the Carr–Purcell sequence and this treatment could be directly applied to this  $^{15}\text{N}$  modulated proton spin-echo experiment. Nevertheless, without any calculation one may notice that the loss of the spin coherence during the polarization transfer is correlated to  $^1\text{H}$  line broadening, which implies a relative mismatch of the echo delay for a large part of the amide protons. The dephasing due to this line broadening is not refocused by the echo sequence. Likewise, an analytical relationship can be derived for calculating the magnetization evolution after the INEPT excitation with the Bloch formalism. However, the small  $T_1^a$  of  $\text{Gly}^2$  in the free peptide (I) can be interpreted as follows: Since the INEPT sequence results in a selective inversion of only one of the two amide proton doublet signals, the spin-state populations are different for protons coupled to upwards  $^{15}\text{N}$  spin and for those coupled to downwards spin. Therefore an exchange with a common set of spins (those of  $\text{NH}_3^+$ ) leads to a loss of specificity of each doublet signal and hence effectively reduces the  $^{15}\text{N}$ -induced

magnetization as we observed in the case of  $\text{Tyr}^*\text{-Gly}^*\text{-Gly}^*\text{-Phe}$ .

In 1980 Avent and Freeman<sup>17</sup> proposed a method for obtaining antisymmetric relaxation times. The two essential differences with the experimental scheme proposed here are (i) the use of a pulse sequence without any  $90^\circ$  phase shift in the  $^1\text{H}$  excitation and (ii) the introduction of refocusing delays to prevent mutual cancellation of the antiphase component of the spin multiplet during the  $^1\text{H}$  noise-decoupled acquisition. Furthermore, their method implies two-dimensional Fourier-transform techniques, requiring rather time-consuming data gathering. This obviously extends the application of this technique to the study of large molecules of biological interest. However, in the case of rather small molecules, our method remains appropriate, because it considerably reduces the number of collected spectra.

In conclusion, the great sensitivity gain of the INEPT sequence is very attractive for relaxation studies which require long instrumental time. This paper demonstrates the usefulness of the cross-polarization method in conformational studies of peptides but also its limitations related to chemical exchange processes. The relaxation times obtained by using this method are not sensitive to the same features as the conventional ones: proton–proton dipolar relaxation and proton-exchange processes are the dominant  $^{15}\text{N}$  relaxation mechanisms. At least the distance dependence of the exchange processes between  $\text{NH}_3^+$  and NH protons within one peptide makes this sequence suitable for conformational studies of nonprotected linear peptides.

**Acknowledgment.** We would like to thank Miss A. Bouju for typing the manuscript. Financial support by the Centre National à la Recherche Scientifique, the Institut National de la Santé et de la Recherche Médicale, and the Université René Descartes is gratefully acknowledged.

Registry No. I, 60254-82-2; II, 64829-35-2.

(16) Allerhand, A.; Gutowsky, H. S. *J. Chem. Phys.* 1964, 41, 2115–2120; 1965, 42, 1587–1592.

(17) Avent, A. G.; Freeman, R. *J. Magn. Reson.* 1980, 39, 169–174.

## Effect of Electron Correlation on Theoretical Equilibrium Geometries. 2. Comparison of Third-Order Perturbation and Configuration Interaction Results with Experiment

Douglas J. DeFrees, Krishnan Raghavachari, H. Bernhard Schlegel, and John A. Pople\*

Contribution from the Department of Chemistry, Carnegie-Mellon University, Pittsburgh, Pennsylvania 15213. Received October 13, 1981

**Abstract:** Theoretical MP3/6-31G\* (third-order Møller–Plesset, 6-31G\* basis) and CID/6-31G\* (configuration interaction with double substitutions, 6-31G\* basis) equilibrium geometries have been obtained for a large set of one- and two-heavy-atom molecules containing only first-row elements and for which experimental structural data are available. Both theoretical techniques lead to good agreement with experiment, a majority of the calculated lengths and angles lying within the experimental error range. Systematic bond length deficiencies previously noted at the Hartree–Fock (HF/6-31G\*) and second-order Møller–Plesset (MP2/6-31G\*) levels are largely removed. Mean absolute differences between MP3/6-31G\* theory and experiment are 0.008 Å for bond lengths and  $1.3^\circ$  for bond angles. The MP3/6-31G\* and CID/6-31G\* methods give comparable results for equilibrium geometries.

Even with quite large basis sets, Hartree–Fock (HF) (single configuration) molecular orbital theory leads to equilibrium structures that show systematic deviations from the best experimental results.<sup>1,2</sup> For a basis such as 6-31G\* (split-valence or

double- $\zeta$  plus polarization functions on non-hydrogen atoms<sup>3</sup>), Hartree–Fock bond lengths are usually too short, particularly for

(1) Hariharan, P. C.; Pople, J. A. *Mol. Phys.* 1974, 27, 209.