^{1}H Spin–Lattice Relaxation and Reduced Values for the ^{13}C Nuclear Overhauser Enhancement

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Abstract: ^{1}H spin–lattice relaxation data are presented for a 0.39% solution of PMA in D_2O, pD = 8.0. These data, as well as ^{13}C data available in the literature, are used to illustrate a method for eliminating the ambiguity present when interpreting the submaximal values of the ^{13}C heteronuclear Overhauser enhancement which are usually observed in macromolecular systems.

It is generally accepted that the dipolar mechanism is overwhelmingly dominant for the nuclear magnetic resonance (NMR) relaxation of ^{13}C nuclei which are covalently bonded to protons. The theoretical basis[1-3] for this acceptance rests on the relatively large ^{1}H magnetic moment and the inverse sixth power dependence of the dipolar relaxation rates on internuclear separation. However, situations of practical importance exist where the a priori assumption of dipolar relaxation for protonated carbons is not valid. The use of methyl ^{13}C probes of molecular motion is such a situation, and the utility of isotopically enriched ^{13}C methionyl methyl probes has recently been emphasized in protein systems.[4] It is well known[5] that the internally mobile methyl group in small molecules may exhibit appreciable spin–rotation interaction in addition to dipolar effects, and we have recently confirmed that such is the case for the methionyl methyl group in the oligopeptide tetragastrin.[6,7] The presence of three covalently bonded protons is, therefore, no guarantee that nondipolar interactions will be absent. Likewise, the relative flexibility of the side chains of large polypeptides and proteins implies that spin–rotation may also contribute to the relaxation of various side chain nuclei in these species.

Experimentally the dominance of dipolar ^{13}C relaxation due to covalently bonded protons may be sometimes confirmed by measurements of the heteronuclear Overhauser enhancement (NOE).[8] Such confirmation is possible for sufficiently small molecules, which are characterized by rapid overall tumbling, since nondipolar mechanisms result in NOE values which are reduced relative to the theoretical maximum of 3.0 expected on the basis of dipolar interactions.[3,8]

For macromolecules it is not possible to confirm the applicability of the dipolar relaxation formalism solely by measuring ^{13}C NOE factors. An ambiguity arises in the interpretation of reduced NOE's when the molecular motions determining the relaxation are not rapid compared to the Larmor frequencies of the resonant nuclei. Thus, in the presence of the reduced motional rates characteristic of macromolecular systems the NOE may be less than the maximum value of 3.0, even though a completely dipolar relaxation mechanism is operative. Nondipolar mechanisms would, of course, reduce the NOE even further. It has been pointed out[9] that such ambiguity cannot be resolved without independent estimates of the motional rates involved.

In view of the above difficulty in utilizing ^{13}C NOE measurements in macromolecular systems two approaches are generally taken. If questions of motional anisotropy and correlation time distributions can be ignored, the tenability of assuming 100% dipolar relaxation sometimes may be supported by the NOE values and relaxation rates observed in a given macromolecule for a variety of ^{13}C nuclei, preferably nonprotonated as well as protonated.[10,11] Alternatively one can assert a priori that a dipolar mechanism dominates, based on

extrapolation of data available for smaller molecules. Such extrapolations[12] are usually made on the basis of data[13,14] for molecules with molecular weights of a few hundred Daltons and thus should be made in studies of macromolecular systems only with caution and if a more preferable alternative approach is unavailable.

The problem of unambiguously interpreting submaximal ^{13}C NOE's is thus important in the quantitative understanding of relaxation effects in macromolecules, especially since additional evidence also exists[11,15,16] which calls into question the assumption that dipolar interactions with covalently bonded protons always overwhelmingly dominate the ^{13}C relaxation. Doubtless the assumption is often a good one, but as our knowledge of the causes and interpretation of nuclear relaxation in macromolecules becomes more detailed, it seems logical to examine all assumptions very carefully.

A readily available but presently unexploited method for clarifying the interpretation of ambiguously reduced NOE values involves the use of the ^{1}H spin–lattice relaxation time T_1. The method is discussed below and is illustrated with data on poly(methacrylic acid) (PMA).

Experimental Section

An aqueous PMA (mol wt = 16 000 D)[10] solution was prepared as 0.39% by weight in 100% D_2O, to which 10^{-4} M disodium ethylenediaminetetraacetate (EDTA) was added to suppress the effects of possible paramagnetic impurities. The acidity was adjusted to pD = 8.0 by the addition of LiOD. Dissolved oxygen was removed with three freeze–pump–thaw cycles after which the sample was sealed with a pressure cap, under nitrogen, in a 5 mm o.d. tube.

^{1}H spin–lattice relaxation measurements were performed at 100 MHz with the 180°–τ–90°–t pulse sequence on a JEOL PFT-100 Fourier transform spectrometer which has been described elsewhere.[6] A bandwidth of 2.00 kHz was employed with 8192 data points and 700 accumulated transients. The water resonance was suppressed[17] during relaxation measurements.

The ^{1}H T_1's determined for PMA in aqueous solution are given in Table I, along with ^{13}C data from our earlier study.[10] All relaxation behavior was characterized by single exponentials.

Discussion

The utility of experimental ^{1}H T_1 data in clarifying ambiguously reduced ^{13}C NOE values derives from an ability to predict ^{1}H T_1's from correlation times obtained from ^{13}C measurements.[6] The necessary dipolar formalism is available for predicting relaxation rates arising from internal reorientation about an axis which exhibits isotropic overall tumbling.[2] The effect of internal reorientation must be included since the methyl groups of PMA are known to be reorienting about their threefold symmetry axes.[10] Values of τ_R = 1.77 × 10^{-9} s (overall tumbling correlation time) and τ_G = 2.21 × 10^{-10} s (methyl reorientation correlation time) have been determined[10] from the ^{13}C relaxation data in Table I for a D_2O solution of

Table I. Spin–Lattice Relaxation Times for Aqueous Poly(methacrylic acid) Solution at pD = 8.0.

	Exptl ^{1}H T_1, s^{a,b}		Predicted ^{1}H T_1, s^c
–CH$_2$–	0.087 ± 0.004		0.094 ± 0.017
–CH$_3$	0.058 ± 0.002		0.064 ± 0.019

	Exptl ^{13}C T_1, s^{b,d}	Exptl ^{13}C NOE^d	Predicted ^{13}C T_1, s^c
–CH$_2$–	0.029 ± 0.003	1.8	
–CH$_3$	0.033 ± 0.006	2.7	
>C<	0.52 ± 0.08	2.0	0.67 ± 0.13

^{a} 0.39% PMA by weight, 30 °C. ^{b} ± figures denote approximate 95% confidence limits ($\approx$ two standard deviations) determined in a two-parameter nonlinear regression analysis, using an exponential decay function with preexponential factor. ^{c} ± figures denote *maximum* propagated error. ^{d} 4.0% PMA by weight, 26 °C, ref 10.

PMA (4.0% by weight) at 26 °C and pD = 8.0. Assuming only intramolecular interactions between tetrahedrally bonded protons and a ^{1}H–^{1}H distance[18] of 1.78 Å, these values for τ_R and τ_G may be used to predict[2] T_1 values for the methyl and methylene protons. These calculations are summarized in Table I, where they are compared with the measured values for a 0.39% solution. The agreement indicated is within experimental error, and the fact that the calculated T_1's are slightly larger than the measured values may be attributed to the slight temperature difference. The overall molecular tumbling at 26 °C is somewhat to the low-temperature side of the ^{1}H T_1 minimum. Thus, the experimental values for ^{1}H T_1's measured at 30 °C would be expected to be slightly smaller than those calculated for 26 °C.

The previous calculation illustrates the useful point that ^{1}H and ^{13}C relaxation data may be interconverted via the dipolar formalism. Moreover, the example shows that because of this fact ^{1}H data may be used, under certain circumstances, to eliminate the ambiguity which arises in ^{13}C studies when NOE factors may be lowered from their maximum dipolar values by reduced motional rates as well as by nondipolar mechanisms. The exact nature of the circumstances in which it is appropriate to use ^{1}H data for such a purpose depends on the details of the relaxation mechanism. In the case where the dipolar mechanism is governed by a single correlation time τ_R it is necessary that τ_R correspond to the high-temperature side of the ^{13}C T_1 minimum and low-temperature side of the ^{1}H T_1 minimum. The required condition is $0.16 \leqslant \omega_C\tau_R \leqslant 0.79$. Using the frequency range available with the various readily available NMR spectrometers the range covered is 3.8×10^{-10} s $< \tau_R < 8.4 \times 10^{-9}$ s. This corresponds to globular polymers in the molecular weight range ~1 000 to 20 000 D. The restriction exists because nondipolar relaxation may occur in both the ^{13}C and the ^{1}H mechanisms. For τ_R outside the specified restricted range the influence of nondipolar contributions on both ^{1}H and ^{13}C relaxation is in the same direction.

Since the specific case of PMA satisfies the above restriction on $\omega_C\tau_R$, the results in Table I confirm our earlier inference[10] that the operative relaxation mechanism is 100% dipolar even though reduced NOE's were observed. Moreover, the suc-

cessful prediction of the ^{1}H T_1's indicates that dipolar interactions with noncovalently bonded protons[11] do not contribute to the relaxation of the methylene and methyl carbons.

The above calculation involved concentrations differing by a factor of 10 for the ^{1}H and ^{13}C studies. The results in Table I indicate that the ^{1}H studies were conducted at a sufficiently low concentration that intermolecular dipolar interactions did not contribute to the relaxation. Furthermore, the fact that ^{13}C studies on a 4.0% solution may be used to predict accurately the ^{1}H relaxation of a 0.39% solution indicates that concentration-dependent effects of association between polymer molecules are absent. Such association, if it occurs, apparently does not alter the segmental motions which are known to bring about the relaxation of this polyelectrolyte in basic aqueous solution.[10]

It should be noted in conclusion that the use of ^{1}H relaxation data as an aid in evaluating submaximal ^{13}C NOE's in macromolecular systems has been illustrated in the present example for protonated carbons. For nonprotonated carbons a similar technique may also be useful if correlation times estimates are available from relaxation data on protonated carbons, if the location of nearest neighbor protons is known from structural considerations, and if the neglect of motional anisotropy and a correlation time distribution may be justified. With these restrictions in mind the ^{13}C T_1 for the nonprotonated carbon is calculated and compared with the measured value. For example, assuming values of 1.09 and 1.54 Å for the C–H and C–C distances,[19] respectively, and tetrahedral bond angles, the values of τ_R and τ_G given above may be used to predict[3] the quaternary ^{13}C T_1 and its maximum propagated error, taking into account interactions with the adjacent methylene and methyl protons. The agreement with the experimental value indicated in Table I is within experimental error, the deviation reflecting our lack of complete conformational information.

References and Notes

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