and to minimize steric interaction of the methyl groups one may imagine the incoming monomer molecule to approach the oxazolinium ion from above the plane of the five-membered oxazolinium ring, with the plane of the monomer ring roughly parallel to it but twisted slightly clockwise to facilitate the formation of the new CH_2–N bond. However, from the fact that racemic monomer gives atactic polymer it is evident that there is an equally efficient pathway for the addition of L monomer to L ions. In this case we may imagine the same sort of approach of monomer to the ion but with the monomer ring turned over so as to avoid the methyl–methyl interactions. The polymerization of 4-methyl-2-oxazoline initiated by methyl iodide is thought to proceed via a covalent intermediate[17] and it will be interesting to see whether this results in any degree of stereoelectivity, unlike initiation by dimethyl sulfate.

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^{13}C Nuclear Spin–Lattice Relaxation and Nuclear Overhauser Enhancement in Aqueous Solutions of Poly(methacrylic acid)

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ABSTRACT: ^{13}C spin–lattice relaxation times (T_1) and nuclear Overhauser enhancement (NOE) factors have been measured for aqueous solutions of poly(methacrylic acid) (PMA). The T_1's are independent of pH, but the NOE's exhibit a definite pH dependence. Comparison of these data indicates that dipolar relaxation mechanisms dominate, and correlation times are calculated. The correlation times indicate that (1) the extended conformation of the polymer (high pH) is more motionally free than the random coil conformation (low pH); (2) the backbone motions are not in the high-temperature limit for either conformation; (3) segmental motions dominate the relaxation of backbone carbons in the extended conformation, but slower overall molecular tumbling may play a role in the relaxation of these carbons in the random coil conformation; (4) methyl reorientation is rapid enough in either conformation to increase the observed NOE. Linewidth and integrated intensities of ^2H NMR spectra have also been measured for aqueous solutions of perdeuterio-PMA. These results support and contribute to our interpretation of the ^{13}C data. Considerations for ^{13}C relaxation studies of biopolymers are discussed.

The application of ^{13}C NMR to macromolecular systems of biophysical interest is occurring at an accelerating rate. In particular relaxation studies involving Fourier transformed spectra are providing data which are useful in characterizing motional phenomena. To provide some assessment of the problems encountered in such studies a model system is useful as illustrated by Allerhand and Oldfield.[1]

Aqueous solutions of poly(methacrylic acid) (PMA) have several properties which make them attractive as model systems for ^{13}C relaxation studies of biopolymers. Many small proteins or enzymes of current biophysical interest have molecular weights of approximately 15000, and PMA can be easily polymerized from the monomer in this molecular weight range. In addition one of the important properties of biopolymers is the ability to undergo conformational changes. Aqueous PMA solutions are known to exhibit a conformational transition from what is evidently a spherical mass distribution (i.e., random coil) at low pH to an elongated form at high pH.[2] The solution properties of PMA are also favorable with respect to high solubility and availability of an aqueous theta solvent.[3] From the NMR standpoint the ^{13}C spectrum of PMA is known to be considerably simpler than that of biopolymers in the same molecular weight range, and complications arising from stereochemical effects have been analyzed.[4] Furthermore, the methyl groups attached to the PMA backbone provide an analogy to the side groups attached to the backbone of biopolymers. Such side groups may possess independent motional degrees of freedom quite different from those of the backbone. Finally the presence of both methylene and quaternary carbons on the backbone is useful in judging the extent to which nondipolar relaxation mechanisms are significant.

Acrylic polymers have figured prominently in the NMR literature on macromolecular systems with ^{13}C studies receiving considerable recent emphasis in stereochemical

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Figure 1. Natural-abundance, noise-decoupled ^{13}C spectrum of a 4.0% poly(methacrylic acid)–D_2O solution at 26°C and pD = 8.0. Number of scans = 40000. Repetition rate of 90° pulses = 0.66 sec.

studies.[4-9] The majority of polymer NMR relaxation studies, however, has been on solid polymers[10] with relatively fewer studies of polymer solutions.[11-13] Moreover, a large body of work on the ^{13}C relaxation of acrylic polymers has not evolved, and neither ^{1}H nor ^{13}C relaxation studies of aqueous PMA solutions have appeared.

In this publication we discuss ^{13}C spin–lattice relaxation times and nuclear Overhauser enhancement factors in aqueous PMA solutions. Our intent is to provide experimental background for additional work in biophysical systems and to call further[1,14] attention to the use of NOE factors in detecting conformational changes occurring in solutions of macromolecules.

Experimental Section

^{13}C NMR. The poly(methacrylic acid) (PMA) used in obtaining ^{13}C spectra was prepared by polymerization of the monomeric acid (Eastman Organic Chemicals) with ammonium persulfate/sodium thiosulfate as an initiator. A single fractionation of the polymeric material was carried out by means of precipitation from concentrated HCl. After exhaustive dialysis against distilled water the material was lyophilized and stored as a powder. The weight average molecular weight was determined via a sedimentation velocity experiment to be 16000. Equilibrium sedimentation revealed a slightly narrower than most probable molecular weight distribution. Sedimentation experiments were performed on a sample of 10.7 mg of PMA/ml of theta solvent (2×10^{-3} *M* HCl).[3] Molecular weights were calculated from the Svedberg equation and Stokes law using a partial specific volume[3] of 0.712 cm^3/g and a viscosity estimate of 0.93 cP based on measured values for a series of PMA solutions of various concentrations.

Separate solutions of PMA were prepared in 99.7% D_2O, including 10^{-3} *M* NaEDTA, to suppress the effects of paramagnetic impurities. The acidic solution was made up in the theta solvent, 2×10^{-3} *M* DCl, whereas the basic solution was obtained by the addition of LiOD. pH meter readings were corrected to pD values according to the procedure of Glasoe and Long.[15] Repeated freeze–pump–thaw cycles were used to remove dissolved oxygen, after which the samples were sealed with pressure caps, under nitrogen, in 10-mm o.d. tubes.

Natural abundance ^{13}C spectra were obtained at 25.15 MHz using a JEOL-PFT-100 Fourier transform spectrometer. A bandwidth of 6.25 kHz was used with 8192 data points. Temperature was controlled to ±1°C using the standard JEOL temperature controller. The standard 180°–τ–90°–t pulse sequence,[16] under the control of the JEOL auto-T_1 program and with proton noise decoupling, was employed for all measurements of T_1. The value of t used was at least 3.4 and usually more than 5 times the value of the measured T_1. T_1 values were determined via nonlinear regression analysis using a single exponential decay function (not its logarithm) to describe the approach of the magnetization to equilibrium. Magnetization was taken to be proportional to peak heights except where the breadth of the line necessitated that integrated intensities be used. The peak height output of the JEOL auto-T_1 program was used in the regression analysis since the T_1 determined from this output agreed within experimental error with that determined from peak intensities read manually from recorded spectra.

NOE factors were determined by two methods, the experimental error in these measurements being approximately ±10%. In method I the NOE was determined from integrated equilibrium spectra recorded in the presence and absence of proton noise decoupling. This method was used for the nonprotonated carbons in the basic solution and for all carbons in the acidic solution. In these measurements care was taken to keep the 90° pulse repetition rate *at least* five times the T_1 measured for the carbon under consideration. The number of scans used depended on the sample and the nature of the resonance under consideration and was the same for both the enhanced and nonenhanced spectra, being 1600, 6000, and 60000 respectively for the nonprotonated carbons of the basic solution, the nonprotonated carbons of the acidic solution, and the protonated carbons of the acidic solution. Figure 1 shows an example of the enhanced spectrum used for NOE measurement for the protonated carbons. Integrations were performed by cutting out and weighing a tracing of the recorded spectra. Tracings were made of all spectra (including noise) for at least two different horizontal magnifications and the results were averaged after being weighted by the appropriate factor. This procedure was used to ensure that the broader resonances were not being mis-integrated. Except for one deviation of ±9% such multiple tracings yielded areas agreeing within ±7% for all spectra used, nonenhanced as well as enhanced. Care was taken to use sufficient power at the 2.5 kHz noise bandwidth employed for these measurements. Decreasing the power by approximately a factor of 2 yielded integrated intensities agreeing within ±8% for the protonated carbons in the acidic solution.

In method II the NOE factor was determined via a decoupled but not enhanced spectrum obtained by gating the decoupling power on only for the time during which the free induction decay was being accumulated. This method was used for the protonated carbons in both the acidic and basic solutions. In these measurements the 90° pulse repetition rate was at least twenty times and the off-time of the decoupling power at least ten times the measured T_1 for the carbon under consideration. These conditions suffice for accurate NOE measurement via this method.[17] The number of scans used was 40000 and 60000 respectively for the basic and acidic solution. Integrations were performed as described for method I. The results of these measurements for the NOE of the protonated carbons in the acidic solution agreed with the values determined via method I to within ±10%.

^{2}H NMR. The perdeuterio-PMA was prepared as described above for the protonated material except that the perdeuteriomonomeric acid was used. Perdeuteriomethacrylic acid was prepared from 99.7% deuterioacetone-d_6 (Merck Sharpe and Dohme Inc.) which was converted to acetone cyanohydrin (70% yield) by condensation with sodium cyanide in sulfuric acid–D_2O.[18] The cyanohydrin-d_7 was hydrolyzed (30% yield) to methacrylamide with concentrated sulfuric acid in the presence of copper wire and flowers of sulfur to inhibit polymerization.[19] This product was further hydrolyzed to perdeuteriomethacrylic acid (90% yield) by refluxing with dilute sulfuric acid–D_2O in the presence of *p*-methoxyphenol to inhibit polymerization. Perdeuterio-PMA was prepared as described above for the protonated material. A weight average molecular weight of 16000 was determined from a sedimentation velocity experiment as described above. The partial specific volume used was 0.673 cm^3/g which reflects a correction for the increase of 5.8% in the molecular weight of the perdeuterio-monomer.

^{2}H spectra were obtained at 9.2 MHz on undegassed perdeuterio-PMA–H_2O solutions in a manner which has been described elsewhere.[20]

Results

The ^{13}C spectrum of a 4.0% (by weight) D_2O solution of PMA at 26°C and pD = 8.0 is shown in Figure 1. The assignments have been made on the basis of the relative magnitudes of the various T_1's, the greater width of the lines due to protonated vs. nonprotonated carbons in the absence of noise decoupling, and the fact that the methyl carbon resonance is expected to occur upfield from the other resonances. These assignments are consistent with those made by Schaefer[4] who has shown that the presence of two quaternary carbon lines can be related to heterotactic and syndiotactic triads. In acid solution, pD = 2.8, all of the lines are broadened, the two quaternary carbon lines are no longer resolvable, and two carboxyl lines are barely resolvable, all consistent with the observations of Schaefer.[4]

The ^{13}C spin–lattice relaxation times for a 4.0% PMA–D_2O solution at 26°C are shown in Table I except for the methylene carbon at pD = 2.8. The width of the methylene resonance at this pD and the attendent long accumulation

Table I
Spin–Lattice Relaxation Times and NOE's for Aqueous PMA–D_2O Solutions at 26° C

	–CO–	$-CH_2-$	–C–	CH_3
		PD = 2.8		
T_1, sec	1.77 ± 0.24		0.57 ± 0.06	0.032 ± 0.007
NOE	1.1[a]	1.1[a,b]	1.6[a]	2.1[a,b]
		PD = 8.0		
T_1, sec	1.49 ± 0.05	0.029 ± 0.003	0.52 ± 0.08[c]	0.033 ± 0.006
NOE	1.8[a]	1.8[b]	2.0[a]	2.7[b]

[a] Method I, see Experimental Section. [b] Method II, see Experimental Section. [c] Observed for both quaternary carbon lines.

time prohibited convenient measurement of T_1, and hence this datum was omitted since it is not crucial to the conclusions drawn in this publication. It should be noted that the T_1 given in Table I for the quaternary carbon was obtained for both quaternary resonances, confirming the lack of a dependence of T_1 on tacticity.[12] The ± figures denote approximate 95% confidence limits as determined by computer. It is evident from Table I that the T_1's agree to within experimental error for solutions with pD = 2.8 and 8.0. Furthermore, T_1's measured for a 8.0% PMA–D_2O solution at these same pD's agree within experimental error with the results shown in Table I, indicating an absence of concentration effects.

Also shown in Table I are the NOE's (maximum = 3.0) for all carbons. For lines where stereochemically induced splitting is observed the NOE given is an average since the splitting is not completely resolved. Contrary to the T_1 results a definite pD dependence was observed for the NOE. For each carbon the NOE was found to be less at pD = 2.8 than at pD = 8.0. It should also be noted that, with the exception of the methyl carbon at pD = 8.0, all NOE's at both pD's are less than the maximum of 3.0 expected for a dipolar relaxation mechanism in the high-temperature limit. Since this observation is true for the methylene carbon at both pD's and the methyl carbon at pD = 2.8, as well as for the nonprotonated carbons, it is a strong indication that motions are occurring which are not in the high-temperature limit.[21] The methyl carbon NOE's are observed to be larger than those for the other carbons in the same molecule. The difference is beyond experimental error, and its reality is further supported by two additional facts: (1) the difference is observed for *both* acidic and basic solutions, (2) a calculation (see below) of the effect of methyl reorientation predicts a result in excellent agreement with experiment.

The deuterium spectrum of a 5.0% (by weight) perdeuterio-PMA–H_2O solution at 30°C consisted of a single broad line with a pH independent line width. We assign this line to the methyl deuterons on the basis that the methylene deuterons are on the backbone, and hence their resonance is even broader and likely to be unobservable. The pH independence of the width of this line is shown in Figure 2 as is the fact that its integrated area exhibits a marked pH dependence. Such a marked pH dependence is interesting in view of the fact that the line width is independent of pH. The inference is that part of the methyl deuterium resonance exists as the observed line and part exists as a very broad, nonobservable line favored at low pH.

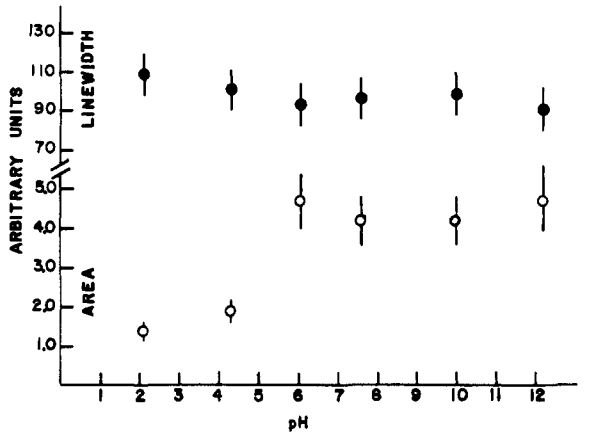

Figure 2. Line width (●) of and integrated area (○) under the ^2H resonance as a function of pH for a 5.0% perdeuteriopoly(methacrylic acid)–H_2O solution at 30°C.

Discussion

Theoretical Considerations. The determination of correlation times from NMR relaxation data has been discussed in the literature by a number of authors. The theoretical framework provided by Solomon[22] and Woessner[23] for dipolar relaxation has proved to be especially useful, the work of the former author for situations describable by a single correlation time and the latter for situations in which internal as well as overall molecular reorientation must be considered. This dipolar framework has been applied by Doddrell, Glushko, and Allerhand (DGA)[24] to spin–lattice (T_1) and spin–spin (T_2) relaxation times and nuclear Overhauser enhancement (NOE) factors in proton-decoupled ^{13}C spectra. The equations used in the calculations described below are given in detail by DGA and hence are only summarized here in eq 1. γ_C and γ_H are the gyromagnetic ratios of ^{13}C and ^1H, respectively, $\hbar$ is Planck's constant divided by 2π, r is the internuclear carbon–proton distance, and N is the number of protons directly bonded to carbon. The value and form of the functions χ, θ, and ϕ are dependent on the number and sizes of the correlation times required to describe the relaxation mechanism. Therefore, values of relaxation times and NOE's may be used with the aid of eq 1 to determine correlation times if suitable values for r and N are available.

$$T_1^{-1} = \left(\frac{\gamma_C^2\gamma_H^2\hbar^2N}{10r^6}\right)\chi$$
$$T_2^{-1} = \left(\frac{\gamma_C^2\gamma_H^2\hbar^2N}{20r^6}\right)(\chi + \theta) \qquad (1)$$
$$\mathrm{NOE} = 1 + \left(\frac{\gamma_H}{\gamma_C}\right)(\phi/\chi)$$

Basic Solution. We *assume* that a dipolar relaxation mechanism involving a single correlation time is sufficient to describe the motions of carbons on the main chain of the polymer molecule. This assumption may be insufficient for several reasons. Among them are the presence of anisotropic reorientations,[25] distributions of correlation times,[26] sig-

nificantly different segmental motions for each main chain carbon, and nondipolar interactions. In our case the above assumption provides a reasonable starting point, supported by the observation that the NOE's for the methylene (protonated) and quaternary (nonprotonated) main chain carbons are virtually identical with that of the carboxyl carbon, which is not on the main chain. If anisotropic reorientations or nondipolar interactions are present, they are present to the same degree for each of these very different carbons. Since this is an unlikely situation, a dipolar mechanism involving a single correlation time τ_R is indicated. Furthermore, it is seen below that any distribution of correlation times present must be a narrow one. Therefore, the T_1 of 0.029 sec measured for the methylene carbon may be converted graphically to a value for the correlation time via the expression for T_1^{-1} in eq 1 (see DGA eq 19) with values of $N = 2$ and $r = 1.09$ Å. Since the function χ is double valued, we obtain the result that $\omega_c\tau_R = 2.2$ or 0.18, where ω_c denotes the ^{13}C Larmor frequency. A choice between these two values may be made on the basis of the measured NOE of 1.8. This NOE value may be used graphically to calculate a value of $\omega_c\tau_R = 0.36$ from the NOE expression in eq 1 (see DGA eq 21). The possibility that $\omega_c\tau_R = 2.2$ may, therefore, be excluded with the result that backbone carbon motions at 26°C are only slightly to the high-temperature side of the T_1 minimum and definitely not in the extreme narrowing limit. It should be pointed out here that since the backbone carbon motions at 26°C are near the T_1 minimum, the discrepancy between $\omega_c\tau_R = 0.18$ and $\omega_c\tau_R = 0.36$ corresponds to a difference in T_1 values of only 15% which is near the experimental error for these measurements. A third estimate of $\omega_c\tau_R = 0.30$ is obtained for the main chain carbon correlation time from the NOE of 2.0 measured for the quaternary carbon. The calculation of this third estimate assumes a dipolar relaxation mechanism for the quaternary carbon even though it has no covalently bonded protons. Such an assumption is not unreasonable, however, in view of the nearly identical NOE's observed for the quaternary and methylene carbons. The average of the three estimaes obtained for $\omega_c\tau_R$ is 0.28 which is used below to calculate a correlation time for methyl group reorientation.

The value of $\omega_c\tau_R = 0.28$ yields a correlation time of $\tau_R = 1.8 \times 10^{-9}$ sec, which we ascribe to segmental motions of the polymer backbone since it is significantly smaller than the τ_R calculated from a hydrodynamic model[27] for overall molecular tumbling. This model predicts that τ_R is given by eq 2 where η is the viscosity and kT has its usual meaning. r is the radius of the isotropically tumbling sphere and may be approximated by the radius of gyration ($l\sqrt{n/3}$) for a freely rotating polymer chain with n monomer units and a carbon–carbon distance of l, subject to the restriction of tetrahedral angles.[28] A value of 3.1×10^{-9} sec is calculated for τ_R at 26°C from the measured molecular weight of 16000, a monomer molecular weight of 86, and a carbon–carbon distance of 1.53 Å, together with a viscosity estimate from data on a 4.6% PMA–H_2O solution. It should be noted that this calculated value is a lower limit since more realistic restrictions in addition to the tetrahedral angle restriction will raise the value calculated for r.

$$\tau_R = 4\pi r^3\eta/3kT \qquad (2)$$

Furthermore, chain molecules may be extended and subject to entanglements, with the result that actual overall rotation may be slower than predicted by eq 2.

Having thus obtained an estimate for the correlation time describing segmental motions, we may determine an average distance of separation between the quaternary carbon and the protons which relax it. The near identity of the methylene and quaternary carbon NOE's tends to indicate that the assumption of a dipolar relaxation mechanism for the quaternary carbon is justified. With such an assumption the measured T_1 of 0.52 sec may be used in eq 1 to determine an average separation of 1.6 Å. A similar calculation for the carboxyl carbon (but one which is much more likely to be in error from nondipolar effects such as spin–rotation interaction) yields a value of 1.9 Å. These distances are reasonable as can be seen from space filling models.

The reorientation of the methyl group of PMA requires at least two correlation times for its description. Reorientation is possible because of its attachment to a segmentally mobile main chain, and we use $\omega_c\tau_R = 0.28$ to describe this reorientation. Internal reorientation of the methyl group, however, is also possible about its threefold symmetry axis. This reorientation is describable by a correlation time τ_g. In order to calculate this correlation time graphically we make use of the measured T_1 value of 0.033 sec, together with $N = 3$, $r = 1.09$ Å, and $\omega_c\tau_R = 0.28$ in the expression for χ (see DGA eq 37). The result obtained is $\omega_c\tau_g = 0.035$, the internal motion of the methyl group being eight times faster than the backbone motions. It is this added degree of motional freedom which is responsible for the larger NOE observed for the methyl carbon as compared to the backbone carbons. Using $\omega_c\tau_R = 0.28$ and $\omega_c\tau_g = 0.035$, we may calculate the NOE expected for the methyl carbon via eq 1 (see DGA eq 39). The predicted value of 2.6 is in excellent agreement with the measured value of 2.7.

The effect of methyl reorientation in significantly raising the methyl NOE above that for the backbone carbons has not been observed in some solid glassy polymers[29] and in some solid polymers above the glass transition temperature.[30] This has been shown to be due to the averaging effect of a distribution of correlation times.[26] The presence of long correlation times in the distribution tends to reduce the NOE and thus counteracts the effect of rapid internal methyl reorientation which tends to increase it. On the basis of the NOE results any distribution of correlation times present in PMA–D_2O solutions appears to be much narrower or much less skewed toward large correlation times than that found in other polymer systems.

This conclusion concerning the distribution of correlation times, however, appears to conflict with the line width for either of the quaternary carbon lines in Figure 1. The measured width is much larger than expected on the basis of the correlation times calculated above. Using eq 1 (see DGA eq 19 and 20), a T_2/T_1 ratio may be obtained with which the measured T_1 may be used to calculate the line width according to $(\pi T_2)^{-1}$. The measured and calculated line widths (full width at half-height) are 8 and 1 Hz, respectively. Even after allowing a few hertz broadening due to filtering such a discrepancy is characteristic of systems which indeed may require a distribution of correlation times.[26] However, other explanations for the observed line broadening are possible such as incomplete motional narrowing.[30]

We suggest an additional possible explanation for the line width discrepancy (involving neither incomplete motional narrowing nor correlation time distributions) based on the ^2H NMR results in Figure 2. The data in Figure 2 indicate that the methyl ^2H resonance is comprised of two parts with pH determining the proportion of nuclei associated with each part. High pH favors the narrower, observed line responsible for the pH independent line width. Low pH favors a line which is broadened to the point of being unobservable. As pH is lowered, more ^2H nuclei contribute to the very broad, unobservable line with the result that

the integrated area under the observed line appears to decrease. We attribute these two parts of the ^2H resonance to different conformations of the polymer molecules since PMA–D_2O solutions are known to exhibit a pD dependent conformational change.[2,31] At high pD the polymer molecules are extended while at low pD they are isotropic light scatterers. The ^2H results, therefore, indicate that the extended form of the molecules contributes the observable ^2H line, while the isotropic light-scattering conformation contributes the very broad, unobservable line characterizable by a longer correlation time. It is important to note that in general both conformations coexist. It may be expected then that the line widths observed in ^{13}C spectra will be determined in part by how the polymer molecules are distributed between the two conformations, especially if the corresponding ^{13}C resonances have slightly different chemical shifts.

Acid Solution. Calculations identical with those described above for the basic solution may be performed for the acidic solution as well. We do not include the results of these calculations, however, as they are not meaningful for several reasons. First of all the width of the backbone methylene carbon line did not permit convenient measurement of T_1. Second the measured methylene carbon NOE of 1.1 is well within the limit where the NOE is no longer a sensitive function of the correlation time.[24] Finally there appears to be a difference between the NOE's observed for the quaternary and methylene carbons, which raises questions concerning the sufficiency of a single correlation time description of the backbone segmental motions. Because of these reasons a quantitative characterization of correlation times is not possible for the acidic PMA–D_2O solution. Nonetheless, some qualitative observations concerning the acidic PMA–D_2O solution may be made on the basis of the data in Table I.

The observed NOE's are lower for all carbons (protonated and nonprotonated) at pD = 2.8 than they are at pD = 8.0. This indicates that the molecular conformation existing in acidic solution is less motionally free than that existing in basic solution. A similar conclusion follows from the ^2H NMR results in Figure 2. The very broad line favored at low pH implies a conformation characterizable by a larger correlation time than that appropriate at high pH. Recognizing a ±10% error in NOE measurement, we may use the methylene carbon NOE of 1.1 to estimate graphically that $\omega_c\tau_R \geq 1.7$ (see DGA eq 21), which implies that $\tau_R \geq 11 \times 10^{-9}$ sec. Since our earlier calculation of a correlation time for overall molecular tumbling yielded $\tau_R \geq 3.1 \times 10^{-9}$ sec, the measured value for the random coil form of PMA may no longer correspond to segmental motions.

A further observation concerns the difference between the NOE's measured for the methylene (1.1) and quaternary (1.6) carbons.[32] Such a difference is unlikely to be due to nondipolar relaxation mechanisms since the effect of such mechanisms is to lower the NOE, and the quaternary carbon, having no covalently bonded protons, may be expected to be more sensitive to nondipolar effects than the methylene carbon. The difference in NOE's thus raises the possibility that motional anisotropy or a difference in segmental mobilities becomes apparent as the pH is lowered and a less motionally free conformation develops.

A final observation is that the effect of internal methyl reorientation in raising the NOE is present at pD = 2.8 as it is at pD = 8.0. Evidently the motional restrictions arising from the pH induced conformation change have not eliminated internal methyl reorientation.

Considerations for Studies of Biopolymers. The pH independence of T_1 for all of the carbons in PMA is attributed to the fact that the motions determining the relaxation are occurring near the T_1 minimum. These results, then, illustrate the insensitivity of the dipolar mechanism to changes which do not cause the motional rate to deviate grossly from that defined by the Larmor frequency. This has also been observed by Allerhand and Oldfield[1] in a less extreme example. T_1 measurements may, therefore, not be a sensitive indicator for changes of molecular motions in biopolymers. One approach which can be useful in such a situation is to redefine the Larmor frequency by changing the magnetic field. DGA have presented an informative discussion indicating that the advantages to be gained from working at high magnetic fields are limited. However, their conclusions were based on the signal-to-noise ratio as the determining criterion.[24] From the standpoint of improving the sensitivity of T_1 to conformational changes a change in magnetic field would be beneficial. Whether an increase or decrease in field strength (or either) is required depends on which carbon is used as a monitor. This follows since the advantage to be gained comes from moving to a point on the T_1 curve which is away from the minimum, and the shape of the minimum depends markedly on the number and relative sizes of the correlation times required to characterize the relaxation.[24]

A decision to alter the magnetic field must also take into account the effect the change will have on the NOE, especially when (as here) the NOE provides the main indicator of motional changes. As with T_1 the sensitivity of the NOE to motional changes is determined by the reference time scale established by the Larmor frequency.[24] The choice of reference time scale dictated by signal to noise considerations may conflict with that dictated by the phenomenon being investigated.

The results we have presented for PMA emphasize the utility of the NOE in detecting conformational transitions, as has been observed for polypeptides.[1,14] It is important to keep in mind, however, that use of the NOE is not straightforward in situations where less than a maximal NOE (3.0) is found. Difficulties in interpretation arise because a reduction in NOE may stem from nondipolar interactions as well as from motional effects on a completely dipolar relaxation mechanism. It is not possible to separate these two sources of reduced NOE's unless independent estimates are available for the correlation times involved.[12] Usually such estimates are not available, and it is argued that the ^{13}C relaxation is dominated by covalently bonded protons.[33] In situations where the NOE is decreased below its maximal value this argument reduces to an a priori statement, one which is based on the inverse sixth power dependence of the dipolar interaction on internuclear separation. However, some evidence is available[30,34,35] which does not support such an a priori claim even for carbons with directly bonded protons, both from the point of view of nondipolar contributions and the dipolar effects of nonbonded protons. The assumption of dipolar relaxation via covalently bonded protons has not been thoroughly evaluated in macromolecular systems.

From the standpoint of assessing nondipolar interactions the present study underscores the value of having both protonated and unprotonated carbons situated in various locations in the macromolecule. In particular it is useful to have both protonated and quaternary carbons on the backbone. Unfortunately in naturally occurring polypeptides of biophysical interest it is unlikely that a quaternary carbon will occur on the backbone.

Once correlation times have been obtained on the basis of dipolar interactions, the question of interpretation arises. In the present study we have determined that seg-

mental motions dominate the relaxation of the backbone carbons of PMA in its extended conformation, while slower overall molecular tumbling may play a role in the relaxation of these carbons in the random coil conformation. These findings are the converse of those for poly(γ-benzyl L-glutamate)[1] and the collagen peptide α1-CB2.[14] For these polypeptides it was found that the relaxation of the α carbons in the extended helical conformation was dominated by overall molecular tumbling, while rapid segmental motions dominated the relaxation in the random coil conformation. No doubt this difference between the polypeptides and PMA is structure related and reflects the significance of the peptide bond in determining the molecular motions which characterize a given polypeptide configuration. The present work thus indicates that comparisons of correlation times for polypeptides and nonpeptide containing models such as PMA may provide a useful experimental approach with which to elucidate the details of molecular motions observed in polypeptides.

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Short-Chain and Long-Chain Branching in Low-Density Polyethylene

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ABSTRACT: The branch content of two fractions of low-density polyethylene has been examined by ^{13}C NMR (at 25 MHz) and by limiting viscosity number (intrinsic viscosity) measurements. The ^{13}C spectra, interpreted with the aid of modified Grant–Paul chemical shift rules and the spectra of model copolymers, confirm that the principal type of short branch is trifunctional *n*-butyl (5–6 per 1000 CH_2) with smaller contents of *n*-amyl (ca. 2 per 1000 CH_2) and ethyl (ca. 1 per 1000 CH_2). A resonance at (32.1$_6$) ppm (from TMS), corresponding to the third carbon (C-3) from the branch end, provides a measure of branches longer than *n*-amyl, but does not at present distinguish such branches, presumably formed by intramolecular "backbiting", from the truly "long" branches, containing possibly many tens or hundreds of carbons and formed by intermolecular chain transfer to other polymer chains. If it is assumed that this resonance provides in fact a direct measure of the "long" branch content, "short" branches longer than *n*-amyl being of negligible probability, the results agree well with the long branch content estimated from the intrinsic viscosities of branched and linear polyethylene via the Zimm–Kilb *g* value. The long branch content thus deduced is ca. 0.8 per 1000 CH_2. For these samples, no marked dependence on molecular weight is observed for either the long- or short-branch frequencies.

Next to molecular weight and its distribution, branching is the most important structural variable influencing the properties of polymers and polymer solutions. Short-chain branching is well known to be particularly critical in its effects on the morphology and solid state properties of semicrystalline polymers such as polyethylene, while long-chain branching has a comparably profound effect on solution viscosity and melt rheology. It is therefore important to