NOTE

Contribution of Proton Exchange to the Oxygen-17 Nuclear Magnetic Resonance Transverse Relaxation Rate in Water and Starch-Water Systems[1]

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ABSTRACT

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The contribution of proton exchange to the oxygen-17 nuclear magnetic resonance (NMR) transverse relaxation rate (R_2/sec) in water and corn starch-water systems as a function of pH was investigated. Proton exchange broadening was observed for both systems to be maximal at neutral pH. Two methods of decreasing or eliminating the broadening were also investigated: use of D_2O as a solvent and use of proton decoupling in the ^{17}O NMR R_2 experiments. The use of proton decoupling efficiently reduces the broadening effects and thus is the recommended method when investigating water mobility by ^{17}O NMR.

The properties of water in biological systems have been intensively studied in recent years. One of the most successful techniques to investigate the mobility properties of water is nuclear magnetic resonance (NMR) spectroscopy. NMR provides a rapid, sensitive, direct, and most importantly a noninvasive, non-destructive determination of both the quantity of water present as well as its dynamic characteristics (Berendsen 1975).

For studying water mobility by NMR relaxation techniques, three accessible nuclei can be probed: proton (^1H), deuterium (^2H), and oxygen-17 (^{17}O). Compared with ^1H and ^2H magnetic relaxation, which have been more extensively studied, ^{17}O relaxation has several advantages (Halle et al 1981). Two major advantages of consequence here are 1) the ^{17}O nucleus, because it is a quadrupolar nucleus (spin $= -5/2$) relaxes without a contribution from cross-relaxation, a potential problem found with the ^1H nucleus, and 2) the ^{17}O relaxation can be made to be much less influenced by chemical exchange of protons, which is a serious problem with ^1H and ^2H relaxation. A detailed discussion of these concerns is presented in Richardson and Steinberg (1987). Because of these concerns, recent interest in measuring water mobility has focused on the use of ^{17}O NMR.

One concern with using ^{17}O NMR is that ^{17}O nuclear relaxation is influenced by proton exchange, through scalar spin-spin coupling between the ^1H and ^{17}O nuclei (Glasel 1972). In a nonviscous liquid, and when proton exchange rates are made to be very small, this spin-spin coupling of oxygen to protons results in the resolution of the spectrum into a triplet of ^{17}O peaks; more generally, however, proton exchange is rapid enough to manifest itself as extensive broadening of the existing ^{17}O peak(s). The extent of this broadening is influenced by the rate of proton exchange in water, which in turn is influenced by pH. It was found that only the transverse relaxation time, T_2, is affected by this coupling process, whereas the longitudinal relaxation time, T_1, is unaffected (Glasel 1972; Halle and Karlstrom 1983a,b).

^1H-^{17}O coupling has been investigated mainly as a means for obtaining the rate constants for proton exchange in pure water and aqueous solutions of acetate buffer and poly(methacrylic acid) (Meiboom 1961; Luz and Meiboom 1963; Rabideau and Hecht 1967; Schriever and Leyte 1977; Halle Karlstrom 1983a,b).

Halle and Piculell (1982) investigated the water ^{17}O magnetic relaxation, including the effect of proton exchange broadening in pure water and in solutions of poly(acrylic acid) and poly(meth-acrylic acid) as a function of pH. Their results show extensive broadening of the ^{17}O resonance at and near neutral pH for all three systems. However, the extent of broadening for the two polyelectrolyte solutions was significantly less than that obtained for pure water. Relatively little research has been done regarding the exchange contribution of the ^1H-^{17}O scalar coupling to the ^{17}O NMR transverse relaxation time (T_2) when it is being used as a measure of water mobility in biological systems.

Because of the increased focus on using ^{17}O NMR to determine the mobility properties of water in biological systems, it is important not only to quantitate the ^{17}O scalar spin-spin coupling effects (broadening effects) but to decrease or eliminate them so as to accurately determine the motional contribution to the T_2 relaxation time. The coupling effect was decreased by using deuterium oxide (D_2O) as a solvent (Halle et al 1981, Lioutas 1984) or by operating in a suitable pH range and was eliminated by the use of proton decoupling in water, acetone, and methyl formate (Earl and Niederberger 1977).

The objectives of this research were to 1) estimate the proton exchange contribution to ^{17}O relaxation rates in water and corn starch-water systems as a function of pH, and 2) to investigate the use of deuterium oxide (as the solvent) and ^1H decoupling as methods of decreasing or eliminating proton exchange broadening in this system.

MATERIALS AND METHODS

Materials

The corn starch was commercial grade, Argo brand (Best Foods, Englewood Cliffs, NJ). Moisture content was 8.5% as determined by vacuum oven method (AOAC 1980) using 60° C and 29.8 in. Hg vacuum for 24 hr. Protein content (N × 6.25) was determined by the macro-Kjeldahl method (AOAC 1980) to be 0.31%. The water was distilled and the deuterium oxide was 99.8% D_2O (Sigma Chemical Co., St. Louis, MO). The DCl and NaOD used were also obtained from Sigma Chemical Co.

Sample Preparation

The corn starch was dried in a vacuum oven at 60° C, 29.8 in. Hg for 8 hr. The corn starch-H_2O suspension was then prepared by hand mixing 40 g of dry starch with 60 g H_2O at room temperature for 2.5 min. The starch was dried before adding the H_2O to parallel the procedure required for preparing the corn starch-D_2O suspension. The corn starch for the D_2O suspension was first vacuum dried and exchanged twice with D_2O at 4° C (first exchange 16 hr, second 8 hr). The exchange was made to replace the exchangeable protons on the starch with deuterons. This starch was then vacuum dried. The corn starch-D_2O suspension was then prepared by hand mixing 40 g of dry exchanged starch with 60 g of D_2O at room temperature for 2.5 min. After preparation, both the 40% corn starch-H_2O and corn starch-D_2O suspensions were divided into three parts: A, for decreasing pH or pD ^{17}O NMR measurements;

B, for increasing pH or pD ^{17}O NMR measurements; and C, for correcting for concentration effects (i.e., dilution effects due to the addition of H_2O or D_2O from the added acid or base) in samples A and B above.

The samples of H_2O and D_2O without corn starch were divided in two parts: A, for decreasing pH; and B, for increasing pH ^{17}O NMR measurements (as A and B above). No dilution sample (as in sample C above) was needed for the H_2O or D_2O only measurements.

The pH for all samples (H_2O and D_2O with and without starch) was varied between 2 and 12 by adding either 0.01 or 0.1 M HCl (or DCl) to sample types A, 0.01 or 0.1 M NaOH (or NaOD) to sample types B, and distilled water (or D_2O) to sample C. The pH was measured before and after each NMR measurement with either an Orion Research pH meter (model 601A) or an Orion pH/ISE meter, model 720 (Orion Research, Cambridge, MA) at 20°C. If before and after pH varied slightly, an average was taken. The pH, which was measured for the D_2O containing samples in this experiment, can be converted to pD by the following equation: pD = pH + 0.45 (Covington et al 1968).

All pH determinations were made in duplicate.

NMR Measurements

A laboratory-assembled MSL-250 multinuclear NMR spectrometer operating at 33.9 MHz ^{17}O NMR resonance frequency was used. A low frequency multinuclear 12-mm probe was used. Single-pulse ^{17}O NMR experiments were done with and without proton decoupling in duplicate at 20°C. The 90° ^{17}O pulse width of 100 μsec and a recycling time of 0.21 sec were used. A WALTZ-16 decoupling sequence (Derome 1987) was used for proton decoupling. The proton decoupling frequency was 250.2 MHz with a 90° proton pulse of 250 μsec and decoupling power of 0.8 watts. The number of scans for each measurement was 2,000 total obtained in four blocks of 500 and the samples were spun at 15 ± 2 Hz. The spectral width was ±10,000 Hz, and the spectra were retained in an 8K point array using a Nicolet NMC-1280 computer system.

The line shape of each spectrum was analyzed using the NMC-1280 data reduction routine (Nicolet 1982). This program evaluated the line shapes by least squares fit to the equation for a Lorentzian peak. Good agreement, as evaluated by standard deviation calculations, was obtained between the experimental and fitted peaks (height, width at half-height, and frequency for fitted peaks were all within 95% confidence level of those of the experimental peak). Therefore, the transverse relaxation rate, R_2/sec, was calculated by the following (Dwek 1973):

$$R_2/\sec = \pi\Delta\nu_{obs} = 1/T_2 \qquad (1)$$

where $\Delta\nu_{obs}$ is the line width at half-height for each spectrum. The relaxation rates R_2 calculated for the samples containing corn starch were corrected for dilution effects from the added acid or base used to adjust the pH. The dilution correction procedure used was done as follows. A "dilution blank" was run (sample C described above) where small increments of H_2O (or D_2O) were added to the sample (in place of the acid or base) and the R_2 measured. These R_2 values were then plotted against their corresponding calculated concentration (g dry starch/g H_2O or D_2O). A linear relationship was obtained for samples containing H_2O (correlation coefficient [r] = 0.989; slope = 148.41 and intercept = 305.46) and D_2O (r = 0.999; slope = 153.12 and intercept = 253.70). These slopes were then used to correct R_2 for the dilution effects in samples A and B containing corn starch (described above) by subtraction.

RESULTS AND DISCUSSION

The results for ^{17}O NMR transverse relaxation rates (R_2/sec) in H_2O, with and without ^{1}H decoupling, and in D_2O as a function of pH (2–12) are shown in Figure 1.

The ^{17}O R_2 data for H_2O obtained without decoupling show the characteristic maximum in R_2 around neutral pH (Glasel 1972). In the pH range 5.5–8.5, the proton exchange rate is slow and the contribution due to proton exchange broadening is largest, reaching a maximum of 203/sec (360–157/sec) at pH 7.2 (Fig. 1). As the pH either decreases (pH <5.5) or increases (pH >8.5), the proton exchange rate increases, and the effect of the proton exchange broadening becomes small to negligible. The average R_2 at these pHs was approximately 157/sec (Fig. 1).

Halle and co-workers (Halle and Piculell 1982; Halle and Karlstrom 1983a,b), investigating prototropic charge migration in water (at 28.4°C), found a relation of ^{17}O R_2 to pH similar to the one reported here. They reported a maximum R_2 of 361/sec (at pH 7.2) and a minimum of 130/sec. They also derived both a theoretical (from the extended Bloch equations) and approximate equation for proton exchange phenomena in water, salt, and polyelectrolyte solutions.

From this ^{17}O R_2 data for H_2O without decoupling, it is evident that in order to use the ^{17}O NMR relaxation measurements to investigate water mobility, it is vital to account for, decrease, or eliminate the contribution to R_2 due to spin-spin coupling effect. To date, several researchers have simply decreased the pH of their systems or elected to work with low pH systems (Tait et al 1972, Halle et al 1981, Richardson et al 1987) to avoid the coupling effect.

The D_2O ^{17}O R_2 data also show a maximum around neutral pH. Similar to the H_2O R_2 data, this increase is due to ^{2}H-^{17}O spin-spin coupling. However, the magnitude of the ^{2}H-^{17}O spin-spin coupling constant, J_{OD}, is much smaller than that for ^{1}H-^{17}O: $J_{OH}/J_{OD} = 6.514$ (Halle and Karlstrom 1983a). Thus, the exchange contribution to the ^{17}O R_2 for D_2O ($R_2 = 244$/sec) is much smaller than for H_2O, reaching a maximum at pH 7.1, only 28.0/sec larger than the average R_2 (216/sec) obtained for D_2O at the extreme pH (Fig. 1). Therefore, using D_2O as a solvent should significantly decrease the exchange effect. Similar ^{17}O R_2 values for D_2O at pH values ranging from 2 to 12 (at 27.7°C) were obtained by Halle and Karlstrom (1983a).

The ^{17}O R_2 data for H_2O obtained with proton decoupling showed no change with pH. The effect of decoupling the ^{1}H from the ^{17}O nuclei is to completely remove the proton exchange broadening effect. The average R_2 over the entire pH range was 156/sec. As expected, this value is similar to the average R_2 value obtained for the H_2O without decoupling R_2 at the extreme pH (157/sec). Earl and Niederberger (1977), investigating ^{1}H-^{17}O coupling in water, acetone-water, and methyl formate, reported a decrease in the ^{17}O line width ($\Delta\nu$ obs) of water from 76 Hz at pH 7.3 (at 18°C) to 55 Hz when the ^{17}O measurement was done with decoupling. This corresponds to a decrease in R_2 from 239 to 173/sec.

As demonstrated in H_2O, using the instrumental technique of proton decoupling eliminates the effect of the ^{1}H-^{17}O coupling, yielding R_2 relaxation rate values that can be used to investigate the mobility of water in aqueous systems.
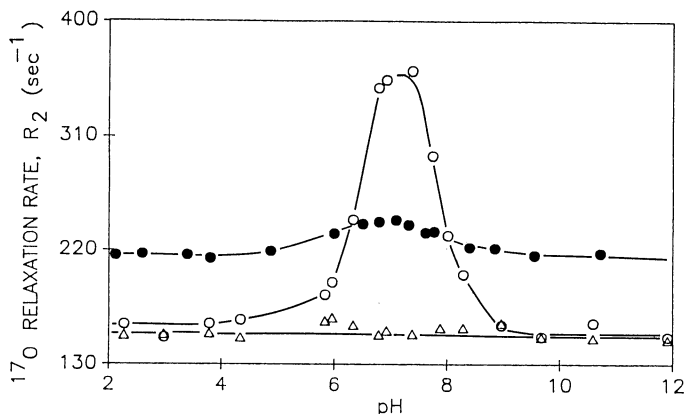

Fig. 1. Oxygen-17 nuclear magnetic resonance transverse relaxation rate (R_2/sec) in H_2O without proton decoupling (o), in D_2O (•), and in H_2O with proton decoupling (Δ) as a function of pH (at 20°C).

The effect of proton exchange broadening on the ^{17}O NMR R_2 in a starch-H_2O system as a function of pH (2–12) and the use of D_2O (as a solvent) and proton decoupling as methods of decreasing or eliminating it were also investigated (Fig. 2).

The ^{17}O R_2 data for corn starch-H_2O without decoupling show an increase in R_2 around neutral pH (pH 5.5 to 8.5) similar to the H_2O alone. The average R_2 value of the acidic (pH <5.5) and basic (pH >8.5) ranges was 315/sec, and the maximum was 515/sec at pH 7.2, a difference of 200/sec, similar to that found for H_2O only (203/sec). Thus, the exchange contribution is not affected by the presence of starch, an unchanged polymer. Halle and Piculell (1982), studying water-proton exchange in polymer solutions using ^{17}O NMR, found that the addition of even small amounts of either poly(acrylic acid) or poly(methacrylic acid) drastically reduces the maximum broadening of the ^{17}O absorption curve (i.e., R_2 of 361/sec for H_2O [at pH 7.2] and R_2 of 200/sec for the polyelectrolyte solutions [at pH 7.4]). They attributed this decrease in broadening to the introduction of additional catalytic mechanisms that are much more effective for water-proton exchange than those operating in bulk water around neutral pH. Based on their hypothesis, it appears that corn starch did not significantly alter the water-proton exchange from that of bulk water.

The D_2O ^{17}O R_2 data for the starch-D_2O system (Fig. 2) showed an increase in R_2 around neutral pH as previously observed for the D_2O alone. The basic pH showed a slightly higher average R_2 (425.5/sec) above those for the acidic pH (387.9/sec). Averaging these R_2, the difference from the maximum R_2 (457/sec at pH 7.4) was 38.0/sec compared to 28.0/sec for D_2O alone.

The ^{17}O R_2 data for corn starch-H_2O with decoupling show the smallest amount of change with pH. Because of the scatter in the data, it is difficult to say whether there is any change with pH. However, qualitatively a very small increase in R_2 is observed from pH 5.5 to 8.5. Two explanations are proposed for this slight increase in R_2: 1) the proton decoupling was not completely effective in eliminating the coupling effect (e.g., insufficient decoupling power or decoupling frequency not optimized), or 2) the slight increase in R_2 reflects the true pH effect on the mobility of the water in the starch-H_2O system. In order to eliminate the first explanation, the proton decoupling parameters were checked and found to be optimized. Thus, the second explanation appears to be valid.

CONCLUSION

Use of ^{17}O as the nucleus of choice for monitoring water mobility in biological systems has been advocated by several researchers (Halle et al 1981, Halle and Piculell 1982, Lioutas 1984
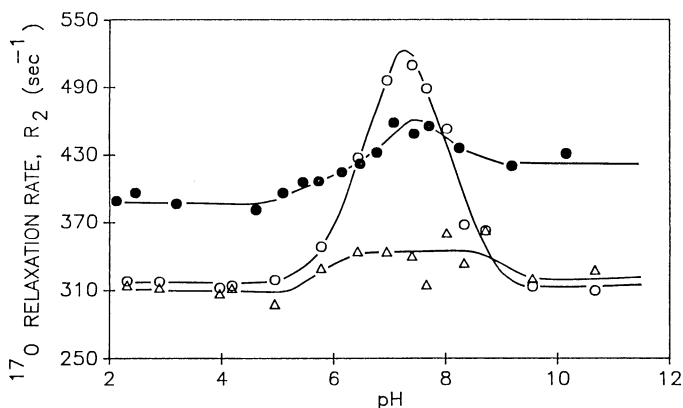
Richardson et al 1987). However, the sensitivity and the magnitude of the ^{17}O transverse relaxation rates to proton exchange broadening effects (via pH) must first be either accounted for, decreased, or eliminated before using relaxation data to deduce solvent (water) mobility information. Two methods were investigated here for the corn starch-water system: use of D_2O as the solvent or use of proton decoupling. Consistent with previous observations, using D_2O as the solvent decreased the exchange broadening by reducing the spin-spin coupling constant sixfold from that for H_2O. Using low-power proton decoupling eliminated the proton exchange broadening effects and thus is the recommended method.

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Fig. 2. Oxygen-17 nuclear magnetic resonance transverse relaxation rate (R_2/sec) in corn starch-H_2O without proton decoupling (o), in corn starch-D_2O (●), and in corn starch with proton decoupling (Δ) as a function of pH (at 20° C).