Cross Relaxation and Exchange in Poly(acrylamide) Hydrogel Studied through ^{1}H MASS NMR and 2D Nuclear Overhauser Enhancement Spectroscopy[†]

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ABSTRACT: A combination of two-dimensional (2D) NMR techniques, namely, (^{1}H–^{1}H) NOESY, and ROESY, has been used for the first time to study cross-relaxation and exchange phenomena in a poly(acrylamide) hydrogel. Intramolecular cross-relaxation interactions are detected for the main-chain protons and are seen to arise from polymer mobility occurring in the long correlation regime ($\omega_0\tau_c \gg 1$). Temperature-dependent studies show chemical exchange, direct and relayed cross-relaxation pathways for the magnetization transfer. For amide protons, cross relaxation dominates over chemical exchange at room temperature and below. Intermolecular exchange and cross-relaxation processes are identified for hydrated water, and evidence has been gathered for interacting water molecules with a relatively long reorientational correlation time. The 2D data also point to the most probable location of water close to the carbonyl group.

Introduction

Polymeric gels, especially those responding to stimuli, such as temperature, pH, light, electric field, solvent, etc., are gaining importance due to the potential application of these materials.[1] Most of the studies reported in the literature focus on macroscopic observations, but in recent years microscopic studies on gels have begun to appear.[2–5] The present paper is a step forward in the sense that for the first time a combination of two-dimensional (2D) NMR techniques, such as (^{1}H–^{1}H) NOESY[6] and ROESY,[7] will be applied to study the dynamical behavior of a hydrogel.

The significant retardation of Brownian motion and the predominance of local chain motions in hydrogels permit a direct application of two-dimensional Overhauser spectroscopy under magic-angle sample spinning (MASS) conditions. The enhanced polymer mobility due to the presence of a diluent[2a] causes various NMR spin interactions to be effectively averaged in a polymeric gel. Sample rotation at the "magic angle" is sought to remove the residual line broadening and to reveal chemically distinct ^{1}H resonances in the repeat unit. The ultimate signal resolution achieved in the MASS spectra is found to be adequate for 2D spectral analysis with minimal interference from spinning side bands. Thus, 2D Overhauser enhancement spectroscopy can be readily applied under MASS to polymeric gels,[8] such as poly(acrylamide) studied here, to investigate cross-relaxation and chemical exchange phenomena.

Experimental Section

The 2D experiments were carried out on a Bruker MSL-300 FT-NMR spectrometer at 300.13 MHz for ^{1}H. NOESY and ROESY pulse sequences were designed to include the TPPI procedure[9] so that the absolute signs of 2D cross peaks were distinguished. Multiple quantum effects were eliminated by proper phase cycling, and *J* cross-peaks were eliminated by randomly varying the mixing period. The $\pi/2$ pulse length was typically 15 μs. A continuous spin-locking radio-frequency field of 16.7 kHz was used for ROESY. MASS was kept at around 2.6 kHz. Temperature variation studies (278–343 K) used the control of spinner bearing air in conjunction with a Bruker BVT-1000 temperature controller (accuracy ±1 K).

Poly(acrylamide) was synthesized using known procedures,[10] and the percentage of cross-linker, *N,N'*-methylenebisacrylamide, was 6.0%. Gels were prepared by the direct addition of known amounts of deionized distilled water and subsequent equilibration for several days.

Results and Discussion

In order to distinguish magnetization transfer through dipole–dipole relaxation and chemical exchange, NOESY and ROESY have been used in parallel in this study. NOESY data alone cannot distinguish these processes since, in the long correlation limit, which is invariably obeyed in hydrogels,[11] both cross relaxation and chemical exchange lead to the same sign for the cross peaks. Their effects are therefore additive for the NOESY cross peak intensity and are not separated from a single measurement. In the ROESY experiment, on the other hand, they contribute differently to the magnetization transfer because of their opposite sign.[12]

For the poly(acrylamide) hydrogel, intramolecular H_E–H_Z exchange occurs due to C–N bond rotation. There is an additional amide to water exchange as well, although in a hydrogel containing less number of water molecules the relative importance of this latter process remains to be explored. The increased polymer mobility in the gel phase also caters to an efficient cross relaxation between dipolar-coupled protons, resulting in significant NOE's in the slow motion regime. A comparison of NOESY and ROESY therefore provides a basis for identifying the exchange and cross-relaxation pathways in the hydrogel.

Cross Relaxation and Exchange between Polymer Protons. We show in Figure 1 the NOESY contour plot of 1.5 g/g (water/polymer) gel at 298 K. Connectivities are established over the entire network of polymer and water protons. Despite the appearance of a strong water ridge parallel to F_1, the polymer to water cross peaks are unmistakably revealed along F_2. Further, short- and long-range interactions are delineated by an optimal choice of mixing time (100 ms).

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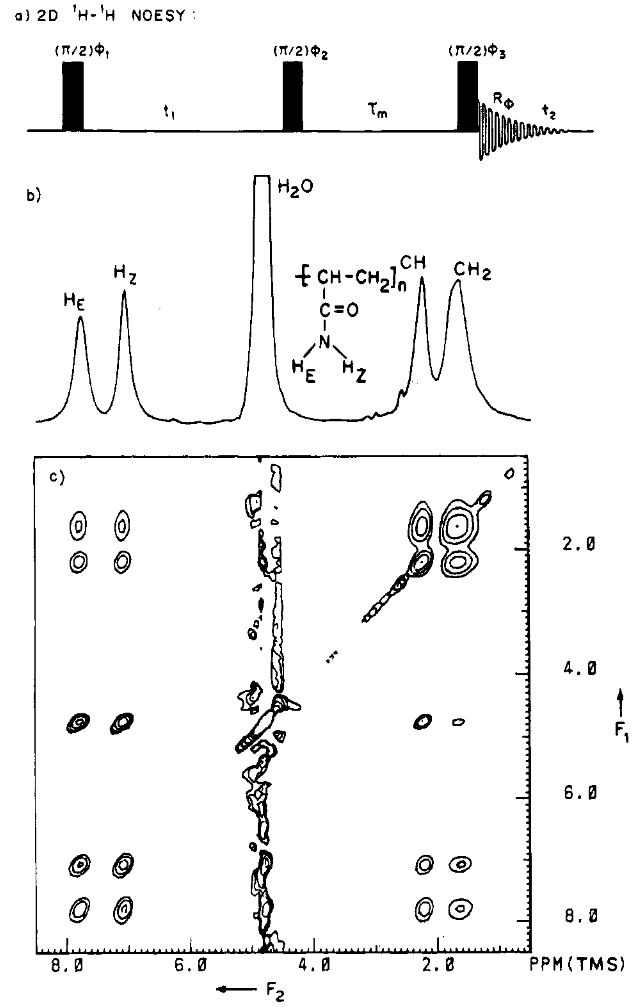

Figure 1. Phase-sensitive homonuclear ^1H−^1H NOESY contour plot of poly(acrylamide) gel hydrated to 1.5 g/g (water/polymer). The pulse sequence used is shown in a. τ_m = 100 ms. Sixteen transients with a recycle delay of 2 s were coadded in each of the 256 experiments in the t_1 domain with increments of 104.1 μs using 512 data points. The transmitter radio-frequency phases (ϕ_n) and the receiver phases (R_ϕ) are cycled to provide quad detection along both axes and pure absorption phase in four quadrants. Spectrum at the top (b) shows F_2 projection. The strong water signal gives a ridge parallel to the F_1 axis. Here all the diagonal and cross peaks appear with positive absorption.

The phase-sensitive 2D experiments lead to positive cross peaks for the nonexchangeable protons, implying the occurrence of main-chain mobility in the long correlation ($\omega_0\tau_c \gg 1$) or the so-called "spin diffusion" regime. The sign of these cross peaks remains unaltered in a 5 g/g gel, although their intensities are greatly attenuated. We reckon that increased hydration levels drive the motional dynamics of the polymeric gel to shorter correlation times and decrease the resultant NOE.

The relative importance of cross relaxation and chemical exchange to the cross-peak development of H_E and H_Z was studied in a 1.5 g/g gel by temperature-dependent phase-sensitive ROESY experiments (Figures 2 and 3). Intense negative cross peaks at 278 and 298 K (Figure 3a,b) readily identify the dominance of cross relaxation over chemical exchange for the amide protons. This may be contrasted with the room temperature solution phase studies where NOE effects are usually neglected and only chemical exchange effects are considered.[13] Chemical exchange effects assume an important role only at higher temperatures (Figure 3d). The exchange rate is, however, smaller than the chemical shift difference between H_E and H_Z as these proton resonances are clearly resolved in the spectrum.

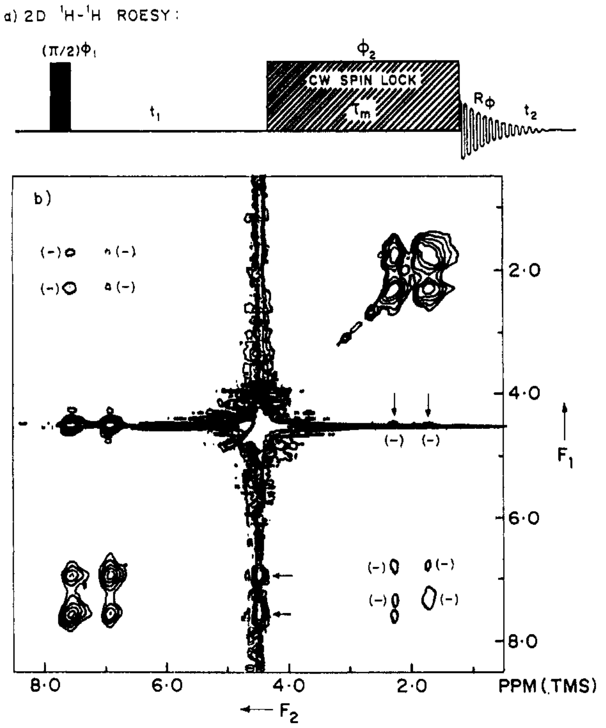

Figure 2. Phase-sensitive homonuclear ^1H−^1H ROESY contour plot of poly(acrylamide) gel hydrated to 1.5 g/g in water (T = 343 K). The pulse sequence used is shown in a. τ_m = 20 ms. A total of 650 experiments were collected with a t_1 increment of 104.1 μs. For each experiment 24 transients were coadded with a recycle delay of 1 s using 1K data points. The transmitter radio-frequency phases (ϕ_n) and the receiver phases (R_ϕ) are cycled to provide quad detection along both axes and a pure absorption phase in four quadrants. Contours marked with a negative sign depict negative cross peaks. Others and all diagonal peaks show positive absorption. Arrows on the strong water ridge indicate cross peaks between water and backbone CH and CH_2 protons.

The ROESY data (298 K) also show a "three spin effect"[14] between H_E, H_Z, and CH. This feature is not observed in the corresponding NOESY experiment as both direct and relayed pathways lead to positive cross peaks in the slow motion regime. The dipolar cross-relaxation pathway $H_Z \rightarrow H_E \rightarrow CH$ and the reverse pathway $CH \rightarrow H_E \rightarrow H_Z$ are readily identified by the alternation of sign for the cross peaks while traversing from H_Z to CH or *vice versa*. While H_Z to H_E is a direct two-spin NOE due to cross relaxation between these strongly interacting protons, the magnetization transfer to CH essentially occurs through the intermediate spin H_E. Stereochemically, H_E is proximal to CH, further confirming our signal assignments. However, at elevated temperatures, the distinction between H_E and H_Z is progressively lost on the relaxation time scale due to rapid H_E−H_Z exchange and a relay through amide protons to CH is no more distinguishable (Figure 3d). The observation of negative cross peaks (Figure 3c,d) for CH and CH_2 with the amide protons, determined at higher temperature, suggests direct cross relaxation rather than a relayed one.

Intermolecular Exchange and Cross Relaxation between Polymer and Water Protons. The polymer to water NOESY correlations occur with a positive sign and are weaker than those between the polymer protons

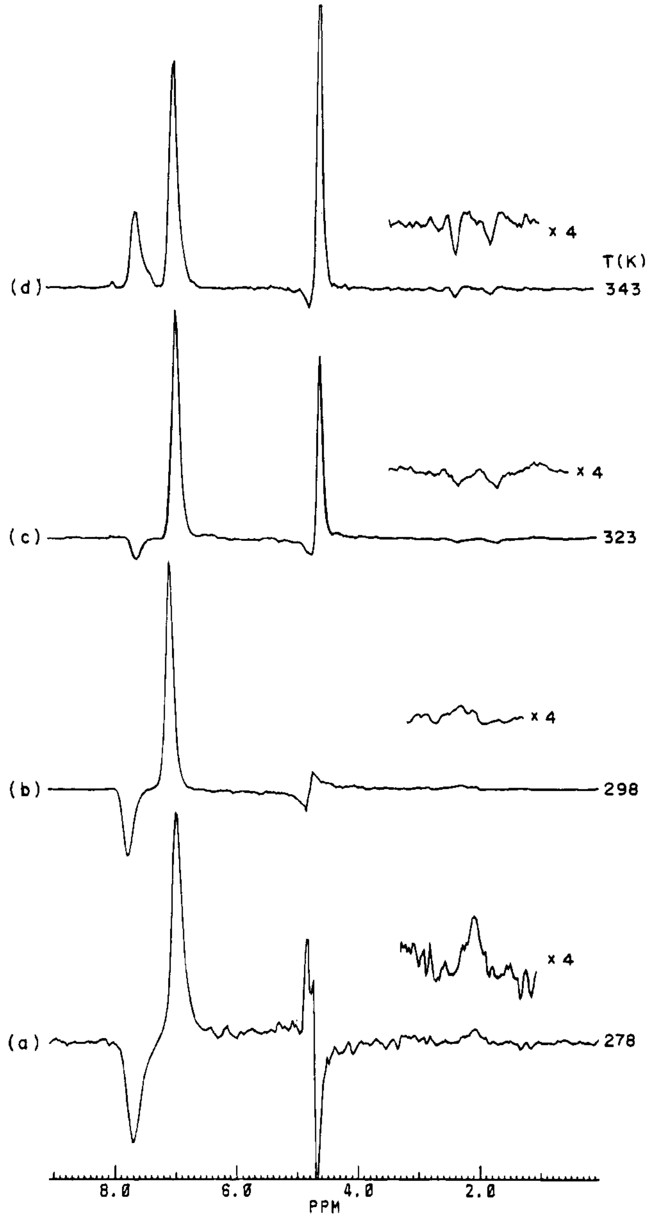

Figure 3. 1D cross sections parallel to the F_2 axis taken from the 2D ROESY data set passing through the H_Z resonance at indicated temperatures in 1.5 g/g poly(acrylamide) gel. The dispersive water signals in a and b arise due to a strong ridge parallel to F_1. These are less severe in c and d as the cross peaks develop with strong intensity.

(Figure 1). The water to amide interaction is more intense than the corresponding one to the polymer backbone. In the 1.5 g/g hydrogel, both NOESY and ROESY cross peaks are positive and they grow in intensity at higher temperatures. This at once identifies the dominant mechanism for magnetization transfer to be chemical exchange. Previous solution NMR studies on amides have shown that magnetization transfer due to chemical exchange occurs with widely differing rates and is strongly dependent on the pH of the medium.[13,15] In the 1.5 g/g hydrogel at neutral pH, there are about six water molecules on the average per repeat unit. It appears that this is a large enough number to facilitate the exchange we observe.

We also observe that, in the initial rate region of the NOESY cross peak buildup as a function of mixing time, the water to H_Z cross-peak intensity is significantly greater than that of the corresponding one to H_E. If chemical exchange is the only mechanism, this would imply a facile exchange of water with H_Z than with H_E. In our case, cross relaxation between water and amide protons cannot be neglected as water to backbone NOE is clearly detected in both NOESY and ROESY. In such a case, the addition of an NOE contribution to the exchange should be considered. If the rates of exchange of water with H_E and H_Z are assumed to be equal, the observation of differential cross peak intensity would imply a differential NOE development in the $\omega_0\tau_c \gg 1$ regime for the amide to water cross-relaxation interaction. Independent transient $^1H-^{13}C$ NOE measurements in this hydrogel have unambiguously shown a direct cross relaxation between water and the amide carbonyl.[16] Considering the fact that H_Z is closer to carbonyl, one can envisage a stronger interaction of H_Z with water, thus enhancing the cross-peak intensity for this interaction. This is indeed observed. The 2D data thus point to the most probable location of water close to the carbonyl group, in agreement with an *ab initio* study.[17]

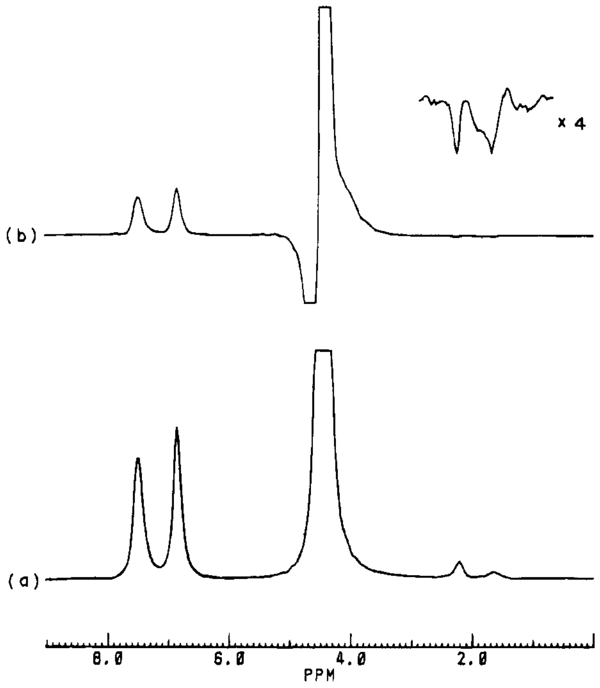

Figure 4. 1D cross sections parallel to the F_2 axis taken from the NOESY (a) and ROESY (b) data set (T = 343 K) passing through the water resonance in 1.5 g/g poly(acrylamide) gel. The dispersive water signal in b is due to a strong ridge parallel to F_2.

It is interesting to note that the intermolecular cross relaxation between the nonexchangeable polymer protons and water protons leads to positive NOESY and negative ROESY cross peaks (Figure 4). Recent studies on protein hydration[18] have shown that the intermolecular cross relaxation between interior water and the protein protons results in a positive NOESY cross peak. Wüthrich and co-workers have observed negative NOESY cross peaks between the surface water and protein protons.[19] In general, the structure of water can be described by a two-site exchange model, namely, that the water exchanges rapidly between a bound and free environment. The intermolecular dipolar relaxation interaction between the macromolecular protons and the bound water results in a cross-peak development. The sign of the cross peak is completely determined by mobility of the bound water which can arise from exchange between bound and free water (residence time), reorientational motion of bound water (reorientational correlation time), or both. For macromolecular solutions, the NOE's between the water molecules and the macromolecular protons are governed by the residence time of water.[19a] In polymeric gels at low levels of hydration most of the water may be considered to be bound. Our observations of positive NOE in the 1.5 g/g gel therefore point to the presence of tightly bound water with a relatively long reorientational correlation time, τ_r. Considering the frequency of our measurements (300 MHz), we estimate τ_r to be longer than 0.6 ns.

Conclusions

Proton MASS NMR in combination with 2D NOESY and ROESY techniques provides an elegant method for studying polymer–polymer and polymer–water interactions in cross-linked gels. This has been demonstrated in water-swollen poly(acrylamide). The nonexchangeable protons give rise to NOESY cross peaks in the spin diffusion regime, while for the exchangeable amide protons H_E and H_Z, direct cross-relaxation effects dominate over chemical exchange at 323 K and below. Both direct and relayed magnetization transfer pathways have been identified in the present study. The hydrated water is seen to interact with the polymer protons of the main chain through NOE and with the side chain mainly through exchange. Our studies also point toward the presence of bound water in the vicinity of the side-chain carbonyl with a relatively long reorientational correlation time.

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