Communications to the Editor

Selective Population Transfer (SPT) in Silicon-29 Fourier Transform Nuclear Magnetic Resonance Spectroscopy

Sir:

Recently it has been shown that selective population transfer (SPT), performed so as to approach selective population inversion (SPI), is a powerful technique in ^{13}C FT NMR spectroscopy for sensitivity enhancement[1] and for the assignment and determination of the relative signs of coupling constants.[2,3] Prompted by the increasing interest in natural abundance ^{29}Si FT NMR studies and the problem of negative nuclear Overhauser enhancements (NOE) for $^{29}Si\text{-}\{^1H\}$,[4] we have investigated applications of SPT to this low natural abundance (4.7%) nucleus.

The SPT technique is in principle equivalent to the transitory selective irradiation (TSI) experiments performed in CW NMR.[5] A weak irradiating H_2 field (e.g., $\gamma_H H_2/2\pi$ = 0.1-0.5 Hz) is selectively applied to one of the ^1H transitions in the $^{29}Si\text{-}^1H$ satellite spectrum for τ seconds, fulfilling the condition $\gamma_H H_2 \tau = \pi$ (i.e., τ = 1-5 sec), prior to excitation of the ^{29}Si resonances by a strong H_1 rf pulse and sampling of the free induction decay. Provided τ is chosen so that $\tau \ll T_1$, where T_1 is the shortest spin-lattice relaxation time for the connected ^{29}Si and ^1H transitions, the SPT π pulse causes complete inversion of the corresponding energy level populations.

Application of the SPT technique to the degenerate ^1H transitions in first-order AX_n (A = ^{29}Si, X = ^1H) spin systems may be especially useful for sensitivity enhancement in proton coupled ^{29}Si FT spectra for two reasons: (i) the equilibrium population differences between energy levels for ^1H transitions are larger than those for ^{29}Si by a factor $|\gamma_H/\gamma_{Si}| = 5$; (ii) SPT occurs between levels separated by several units of the magnetic quantum number. Calculated relative intensities for the ^{29}Si transitions in AX_n (n = 0-6) spin systems after a SPT π pulse has been applied are shown in Figure 1. The calculations were based on subenergy level diagrams as in the case of ^{13}C,[1] assuming complete population inversion (i.e., neglecting any population changes due to ^{29}Si and/or ^1H relaxation during the SPT π pulse) and using $|\gamma_H/\gamma_{Si}| = 5$. However, for organosilicon compounds with short ^{29}Si and/or ^1H T_1's, partial relaxation during the SPT π pulse may cause reduced intensity enhancements; this especially holds in cases where the SPT π pulse must be applied very selectively (long τ's).

The ^{29}Si FT spectra[6] of $(CH_3)_4Si$ (TMS) in Figure 2a illustrate the sensitivity enhancement obtained for this AX_{12} spin system. The theoretical relative intensities for the 13 ^{29}Si transitions may be obtained by extension[1] of the triangle in Figure 1 as: −59:−588:−2574:−6380:−9405:−7128: 924:8712:10,395:6820:2706:612:61. However, maximum enhancements were not achieved experimentally mainly because the spin system was not allowed to relax fully between each pulse sequence.[6] Suppression of the intensity enhancement due to relaxation during the SPT π pulse is probably negligible since T_1 (^{29}Si) = 19 sec[4] and T_1 (^1H) = 16.7 sec[7] for TMS at 25°.

No. of eqv. protons (n)							
0	1						
1	-4	6					
2	-9	2	11				
3	-14	-12	18	16			
4	-19	-36	6	44	21		
5	-24	-70	-40	60	80	26	
6	-29	-114	-135	20	165	126	31

Figure 1. Relative intensities in the A spectrum of AX_n (A = ^{29}Si and X = ^1H, n = 0-6) spin systems after a selective SPT π pulse is applied to one of the X transitions (see text).

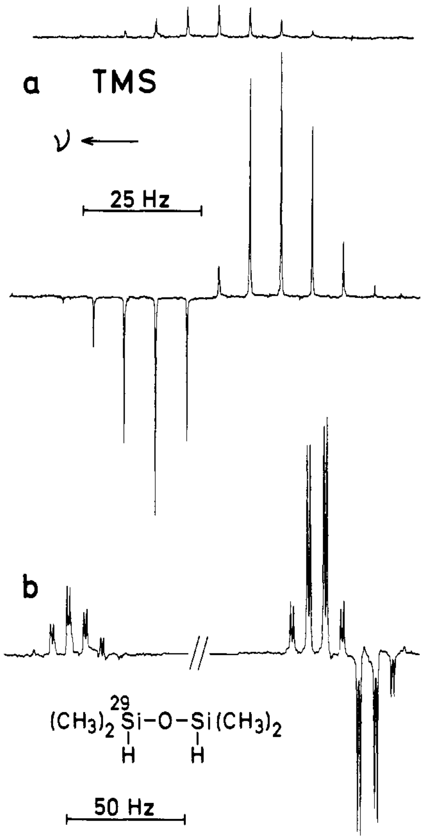

Figure 2. ^{29}Si FT NMR spectra of TMS (a) and TMDS (b): (a) upper part, normal coupled spectrum ($^2J_{Si\text{-}H}$ = 6.62 Hz); lower part, SPT spectrum obtained after a SPT π pulse has been applied to the high frequency line in the ^1H doublet spectrum, τ = 1.3 sec, $\gamma H_2/2\pi$ = 0.38 Hz (for both spectra acquisition time, 16 sec; transients, 100; and otherwise identical experimental conditions); (b) relative sign determination from the SPT spectrum of TMDS ($|^1J_{Si\text{-}H}|$ = 205.4 Hz, $|^2J_{Si\text{-}H}|$ = 7.12 Hz, and $|^3J_{Si\text{-}H}|$ = 1.26 Hz), see text, τ = 1.3 sec, $\gamma H_2/2\pi$ = 0.38 Hz (acquisition time, 4 sec; and transients, 300).

The use of the SPT method for determinations of relative signs of coupling constants is demonstrated for $[(CH_3)_2SiH]_2O$ (TMDS) in Figure 2b. Gated irradiation of the lowest frequency line in the $^{29}Si\text{-}CH_3$ satellites in the proton spectrum (doublet of doublets, $|^2J_{Si\text{-}H}|$ = 7.12 Hz and $|^3J_{H\text{-}H}|$ = 2.8 Hz[8]), i.e. corresponding to a definite spin state for the directly bonded Si-H proton, gives rise to emission and enhanced absorption for the septet of doublets at low frequency within the large $^1J_{Si\text{-}H}$ doublet of the ^{29}Si spectrum (see Figure 2b). The same effect is observed in the high-frequency component of the $^1J_{Si\text{-}H}$ doublet when the SPT π pulse is applied at 2.8 Hz ($^3J_{H\text{-}H}$) *higher* frequency.

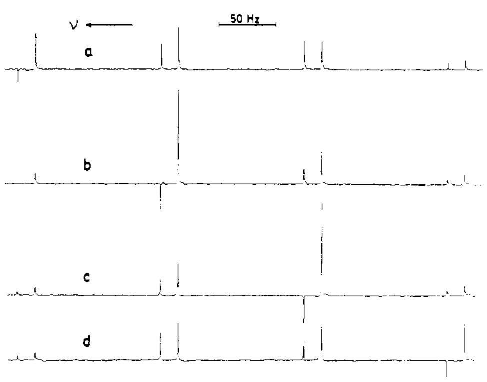

Figure 3. The 25.16-MHz ^{13}C FT SPT NMR spectra of CH_3SiHCl_2 ($^1J_{C-H} = +124.3$ Hz, $|^2J_{C-H}| = 15.0$ Hz) obtained after SPT π pulses have been applied to the four lines in the high-frequency quartet of the ^{13}C-^1H satellite doublet of quartets ($|^2J_{C-H}| = 15.0$ Hz, $|^3J_{H-H}| = 2.3$ Hz) for the proton directly bonded to silicon. Relative to the chemical shift of this proton the irradiation frequencies are: (a) +10.87 Hz, (b) +8.61 Hz, (c) +6.41 Hz, and (d) +4.10 Hz. From the spectra $^1J_{C-H} \times {}^3J_{H-H} > 0$ is obtained, i.e., $^3J_{H-H} > 0$.

This shows $^3K_{H-H} \times {}^1K_{Si-H} > 0$, where $K_{ij} = 4\pi^2 J_{ij}/h\gamma_i\gamma_j$ are the reduced coupling constants. Similarly, from SPT experiments performed in the proton spectrum for the one-bond ^{29}Si-^1H satellites, we obtain $^3K_{H-H} \times {}^2K_{Si-H} < 0$. Since $^1K_{Si-H}$ must be considered positive ($^1J_{Si-H} < 0$),[9] a positive sign is obtained for both $^3J_{H-H} = +2.8$ and $^2J_{Si-H} = +7.12$ Hz. These results are in agreement with the signs determined for the related couplings in CH_3SiHCl_2 from ^{13}C SPT spectra (Figure 3) and spin tickling experiments.[9]

The present results indicate that the SPT technique may prove useful in ^{29}Si FT NMR studies. This applies especially to systems where the NOE causes reduced intensities in ^{29}Si-$\{^1H\}$ spectra.

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Nuclear Magnetic Resonance Relaxation in Frozen Lysozyme Solutions

Sir:

Efforts to understand water in biological systems have been extensive. Nuclear magnetic resonance has been an important tool in this area because it potentially provides both structural and dynamic information.[1,2] Recent work in this laboratory[3] has demonstrated that the NMR relaxation of water in protein crystals breaks into several components, two of which have properties similar to those reported for water adsorbed on surfaces.[4] It was concluded that about 80% of the water protons associated with the protein surface in the crystal does not exchange rapidly with the bulk water while the remainder does. It is important to determine whether the properties of water associated with the protein surface observed in protein crystals are unique to the crystal phase or are characteristic of water-protein interactions in general.

Previous work in concentrated protein solutions has demonstrated that surface water components are too dilute to be resolved using present techniques.[2] The problem of the dominant free water signal may be eliminated by freezing a concentrated protein solution and studying the remaining nuclear magnetic resonance signal.[5]

NMR measurements were made at 30 MHz using a pulsed spectrometer built in this laboratory.[6] Temperature was controlled using a Varian variable temperature controller calibrated against a diode thermometer. Lysozyme used without further purification was purchased from Worthington Biochemical Corporation as the salt-free powder, and solutions were prepared using deionized water. Samples were frozen either in a refrigerator or directly in the NMR probe. T_1 was measured using a 180-90° pulse sequence and T_2 using the Gill-Meiboom[7] modification of the Carr-Purcell[8] pulse sequence. Signals were accumulated on a Varian C-1024 CAT.

The free induction decay amplitude in the frozen solution is a linear function of the protein concentration as shown in Figure 1. The NMR signal observed at 253°K is completely eliminated by exchanging the water with D_2O several times prior to freezing. This demonstrates that the observed signal is associated with the water protons or protein protons which exchange with the solvent. At 253°K the relaxation rates are independent of protein concentration up to 30% by weight.

The temperature dependence of the several relaxation components observed is shown in Figure 2. The proton T_1 relaxation is described by two relaxation times; 81% of the

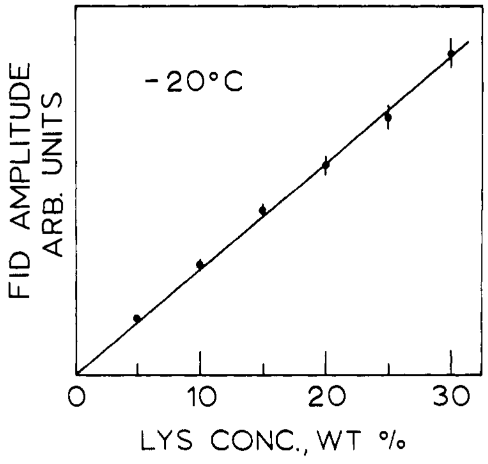

Figure 1. ^1H NMR free induction decay amplitude at 30 MHz as a function of lysozyme concentration in solutions measured at 253°K.