Measurement of Overhauser Effects in Magnetic Resonance of Proteins by Synchronous Nutation

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The measurement of cross-relaxation rates (Overhauser effects) has become an essential technique for structural studies by nuclear magnetic resonance (NMR) in solution.[1] Unfortunately, such measurements are often impaired by spin-diffusion effects, which occur when the magnetization does not simply migrate from spin A to X directly, but via some other spins in the vicinity.[2,3] Spin diffusion effects can be suppressed with our "synchronous nutation" method.[4] In this communication, we report the first nontrivial application of this method: the measurement of cross relaxation between different, nonneighboring amino acids in a protein.

The experiment is basically very simple. After the two resonances of interest have been chosen and their chemical shifts Ω_A and Ω_X identified, the radio frequency (rf) carrier is set halfway at $\omega_{rf} = {}^1/_2(\Omega_A + \Omega_X)$. A string of rectangular 2π pulses with alternating phases is then applied, $[(2\pi)_x(2\pi)_{-x}]_n$, with each 2π pulse having a duration $\Delta\tau_m$, so that rotary echoes are generated at multiples of $2\Delta\tau_m$. The waveform of the entire string of 2π pulses is multiplied with $\cos \omega_a t$, where $\omega_a = {}^1/_2(\Omega_A - \Omega_X)$. This audio modulation[5] causes the spectrum of the rf irradiation to split up into two sidebands which coincide with the two shifts Ω_A and Ω_X. To minimize interference effects between the two sidebands,[4] one must chose an rf amplitude (for each sideband) of $\omega_1 = (\Omega_A - \Omega_X)/k$; the integer k may be chosen to give a convenient rf amplitude, typically in the range 30–90 Hz. The string of 2π pulses is applied either to a system in equilibrium (described by a density operator $\sum = I_z^A + I_z^X$) or to a system where one of the magnetization vectors has been inverted selectively by a Q^3 Gaussian cascade[6] to prepare a state $\Delta = I_z^A - I_z^X$. At the end of the nutation period, a selective 270° Gaussian pulse[7] converts I_z^A into I_x^A. In crowded spectra, I_x^A can be transferred to observable magnetization I_x^M of a scalar-coupled neighbor M, using a homonuclear Hartmann–Hahn transfer.[5]

Figure 1 shows the outcome for a pair of spins in basic pancreatic trypsine inhibitor (BPTI),[8-10] where A = $H^{\alpha(Tyr\text{-}23)}$ and X = $H^{\alpha(Cys\text{-}30)}$. It is obvious that the difference mode Δ decays faster than the sum mode $\sum$. This may be interpreted qualitatively to mean that the cross-relaxation rate σ_{AX} does not vanish. A quantitative interpretation calls for an independent measurement[11,12] of the longitudinal and transverse self-relaxation rates $\rho = 1/T_1$ and $\rho' = 1/T_2$ of each site. If these parameters are known, only σ_{AX} remains to be adjusted. Scalar couplings between the irradiated spins lead to additional modulations[4] but do not preclude the determination of σ_{AX} if a numerical simulation of the trajectories is performed. The cross-relaxation rate constant between $H^{\alpha(Tyr\text{-}23)}$ and $H^{\alpha(Cys\text{-}30)}$ was estimated to be $\sigma_{AX} = 4\ s^{-1}$ which, if we assume a correlation time $\tau_c = 4$ ns, corresponds to a distance $r_{AX} = 195$ pm, in reasonable agreement with the 218 pm measured by diffraction,[8,9] and compatible with the upper limit of 500 pm estimated by Wagner et al.[10] Our method gives a measure of σ_{AX} which, unlike other methods, is not affected by cross-relaxation rates σ_{AM} or $\sigma_{M'X}$ to other spins.[4]

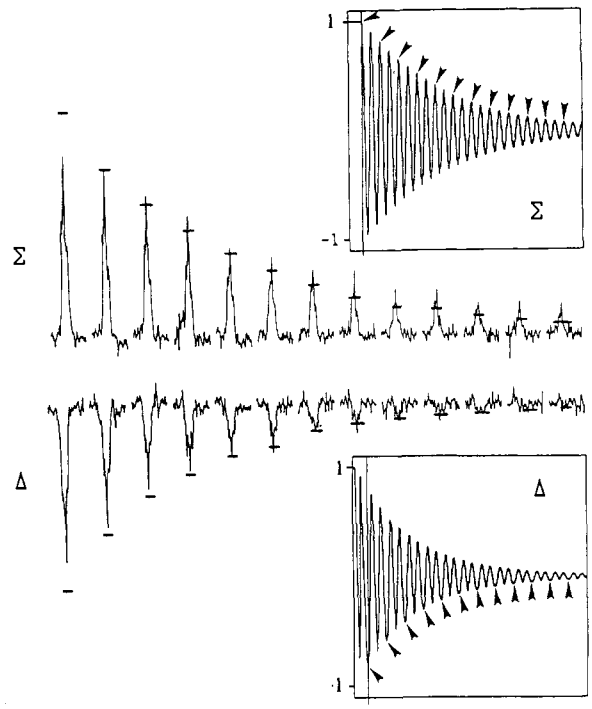

Figure 1. Time dependence of the magnetization I_z^A of $H^{\alpha(Cys\text{-}30)}$ in basic pancreatic trypsine inhibitor (BPTI), as a function of the duration of synchronous nutation of $H^{\alpha(Tyr\text{-}23)}$ and $H^{\alpha(Cys\text{-}30)}$, which resonate at 4.32 and 5.57 ppm, respectively. The magnetization has been transferred from $H^{\alpha(Cys\text{-}30)}$ to $H^{\beta(Cys\text{-}30)}$ via $J_{\alpha\beta} \approx 12$ Hz through a homonuclear Hartmann–Hahn (HOHAHA) transfer of 82 ms duration. The labels $\sum$ and Δ correspond to initial conditions where the longitudinal magnetization vectors of $H^{\alpha(Tyr\text{-}23)}$ and $H^{\alpha(Cys\text{-}30)}$ are initially parallel and antiparallel, respectively. The corresponding simulations (insets) show the trajectories of I_z^A, with arrows to emphasize rotary echoes at multiples of $2\Delta\tau_m = 26.66$ ms, where the magnetization is sampled. For the Δ mode, the trajectory preceding the vertical line corresponds to inversion by a Q^3 cascade. The simulations are based on self-relaxation rates (determined in separate experiments) $\rho(H^{\alpha(Cys\text{-}30)}) = 3.3\ s^{-1}$, $\rho'(H^{\alpha(Cys\text{-}30)}) = 11.3\ s^{-1}$, $\rho(H^{\alpha(Tyr\text{-}23)}) = 4.6\ s^{-1}$, and $\rho'(H^{\alpha(Tyr\text{-}23)}) = 17.6\ s^{-1}$. The best fit is obtained by assuming that the cross-relaxation rate constant σ_{AX} between $H^{\alpha(Tyr\text{-}23)}$ and $H^{\alpha(Cys\text{-}30)}$ is $4\ s^{-1}$. The calculated amplitudes of the rotary echoes are indicated by horizontal bars on the experimental spectra. The chemical shift difference is $\Delta\Omega/2\pi = [\Omega^{\alpha(Tyr\text{-}23)} - \Omega^{\alpha(Cys\text{-}30)}]/2\pi = 375$ Hz. The rf amplitude was $\omega_1/2\pi \approx 75$ Hz, so that $\omega_1/\Delta\Omega \approx 1/5$ to minimize interference between the rf sidebands. Rotary echoes were generated by switching the rf phase at intervals $\Delta\tau_m = 2\pi/\omega_1 = 13.33$ ms. The overall nutation interval τ_m was varied between 0 and 319.92 ms (12th rotary echo). For each value of τ_m, 256 scans were accumulated (about 13 min per multiplet) with a Bruker MSL 300 spectrometer equipped with a selective excitation unit.

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Registry No. BPTI, 9087-70-1; Tyr, 60-18-4; Cys, 52-90-4.

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