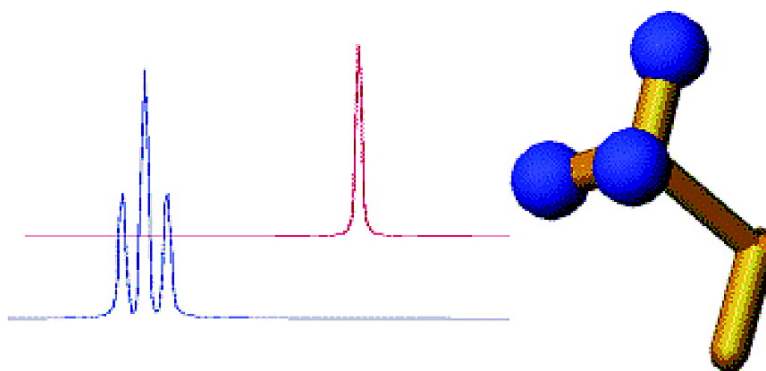Communication

Observation of Individual Transitions in Magnetically Equivalent Spin Systems

Konstantin Pervushin, and Beat Vgeli

More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

Observation of Individual Transitions in Magnetically Equivalent Spin Systems

Konstantin Pervushin* and Beat Vögeli

Laboratory of Physical Chemistry, Swiss Federal Institute of Technology, ETH Hönggerberg, CH-8093 Zürich, Switzerland

Received March 23, 2003; E-mail: kopeko@phys.chem.ethz.ch

Individual transitions of magnetically equivalent spin systems such as methyl groups residing in tumbling molecules in isotropic solution cannot be observed as multiplet-split NMR lines. Construction of an NMR experiment overcoming this limitation and thus enabling direct and selective observation of individual ^{1}H transitions in methyl spin systems would bring a number of advantages. This would enable (1) TROSY-type resolution enhancement[1] in the directly acquired ^{1}H^{Methyl} dimension of multidimensional NMR experiments by constructive use of the interference between ^{1}H^{Methyl} chemical shift anisotropy (CSA) and ^{1}H^{Methyl}–^{1}H^{Methyl} dipole–dipole (DD) interactions,[2] (2) direct measurement of cross-correlation between Curie spin relaxation (CSR) and ^{1}H^{Methyl}–^{1}H^{Methyl} DD relaxation in paramagnetic proteins[3], from which the distance and orientation of the methyl group relative to the paramagnetic center can be calculated,[4] (3) measurement of the values and sign of residual ^{1}H^{Methyl}–^{1}H^{Methyl} dipolar couplings,[5] D_{HH}, in proteins weakly aligned by means of either an external medium,[7] the natural anisotropy of diamagnetic susceptibility[8] or an internal orienting device such as a paramagnetic center.[9] The absence of scalar splitting in methyl groups eliminates the need to acquire an otherwise indispensable reference spectrum in isotropic phase or in the diamagnetic state of the protein as it the case for D_{CH} measurements in methyl groups.[6] In the current communication we describe such an experiment and demonstrate its use with two proteins weakly aligned by means of either Pf1 phages or a naturally present paramagnetic heme group.

The presented technique allows spin-state selection in CH$_3$ groups, which is not the case in previous methods. In the work of Sibille et al.[5b] spin-state selection is achieved in CH$_2$D methyl groups, and in the work of Kaikkonen et al.,[5a] which applies to CH$_3$ groups, the ^{1}H–^{1}H coupling is measured from an antiphase splitting. The acquisition of the resolved ^{1}H–^{1}H antiphase spectrum is crucial for the successful measurements of D_{HH}. If D_{HH} values are smaller than the ^{1}H^{Methyl} line-width, the negative and positive ^{1}H–^{1}H multiplet components in the antiphase spectrum largely cancel each other. This might result in strongly biased values of D_{HH} or the total absence of spectrum as it is the case of the magnetically equivalent ^{1}H methyl protons. The pulse sequence of Figure 1 was designed to alleviate the need to acquire the resolved ^{1}H–^{1}H antiphase spectrum by directly polarizing chosen individual components of the generally degenerate (under conditions of isotropic tumbling) ^{1}H^{Methyl} triplet. The individual components are not postacqusitionally reconstructed from inphase and antiphase-type of subspectra[5] but rather are selectively polarized and directly observed as positive spectral intensity using a single application of the pulse sequence. Thus, the very small values of D_{HH} can be measured.

Considering evolution only under the large $^1J_{HC} = 125$ Hz couplings in CH$_3$ groups, at the time point a one obtains the density operator $\sigma_a = (2S_xI_{1z} + 2S_xI_{2z} + 2S_xI_{3z}) \cos(\omega_S t_1)$, where S and I
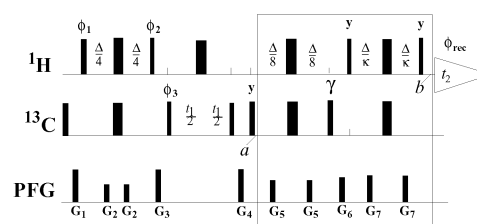

Figure 1. Experimental scheme of the 2D [^{13}C,^{1}H$^{\alpha\alpha}$]^{Methyl} and [^{13}C,^{1}H$^{\beta\beta}$]^{Methyl} HSQC experiments. The radio frequency pulses on ^{13}C and ^{1}H are applied at 20 and 4.8 ppm, respectively. Narrow and wide black bars indicate nonselective 90° and 180° pulses, respectively. The line marked PFG indicates the duration and strength of pulsed magnetic field gradients applied along the z-axis: G_1: 800 μs, 80 G/cm, G_2: 800 μs, 20 G/cm, G_3: 1 ms, −90 G/cm, G_4: 1.2 ms, 80 G/cm, G_5: 400 μs, 55 G/cm, G_6: 1 ms, 65 G/cm and G_7: 400 μs, 70 G/cm. The delay $\Delta = 1/(^1J_{CH})$ is set to 2 ms. The factor $\kappa = 6.577$. The phases are defined as follows: $\phi_1 = \{x, -x\}$, $\phi_2 = \{2y, 2(-y)\}$, $\phi_3 = \{4x, 4(-x)\}$ and $\phi_{rec} = \{y, -y, -y, y, -y, y, y, -y\}$. The phase γ is set to 150° and 30° to obtain the 2D [^{13}C,^{1}H$^{\alpha\alpha}$]^{Methyl} and [^{13}C,^{1}H$^{\beta\beta}$]^{Methyl} HSQC spectrum, respectively. Quadrature detection in the ^{13}C(t_1) dimension is achieved by the States-TPPI method applied to the phase ϕ_3.

stand for the ^{13}C and ^{1}H spins, respectively. The effect of the subsequent unitary transformations of the density operator during the pulse sequence element enclosed in the box (Figure 1) can be analyzed by calculating the projections of the density operators at time points a and b, $\langle\sigma_a|\sigma_b\rangle$.[10] For this purpose, the program POMA[11] modified to account for the effects of pulsed magnetic field gradients was used. As target operators we choose the single-transition operators $I_{1i}^{\alpha\alpha} = I_{1i}(E/2 + I_{2z})(E/2 + I_{3z})$, $I_{1i}^{\beta\beta} = I_{1i}(E/2 - I_{2z})(E/2 - I_{3z})$ defining two "outer" transitions and $I_{1i}^{\alpha\beta} = I_{1i}(E/2 + I_{2z})(E/2 - I_{3z})$ and $I_{1i}^{\beta\alpha} = I_{1i}(E/2 - I_{2z})(E/2 + I_{3z})$ defining two "central" transitions, where $i = x,y$. Because all three methyl protons are equivalent, a complete description of the ^{1}H triplet includes summation over the single-transition operators with indices 1, 2, and 3, where the relevant operators can be derived by circular permutation of their indices. For all values of the phase γ and factor κ (Figure 1) the "x"-projections $\langle 2S_xI_{1z}|I_{1x}^{,ij}\rangle$, $i,j = \alpha,\beta$ vanish and, thus, will not be considered further. Numerical analysis of the "y"-projection $\langle 2S_xI_{1z}|I_{1y}^{\alpha\beta} + I_{1y}^{\beta\alpha}\rangle$ as a function of γ and κ shows that at $\kappa = 6.577$ the central transitions are not polarized with any choice of γ. Calculating $\langle 2S_xI_{1z}|I_{1y}^{\alpha\alpha}\rangle(\gamma)|_{\kappa=6.577}$ and $\langle 2S_xI_{1z}|I_{1y}^{\beta\beta}\rangle(\gamma)|_{\kappa=6.577}$ as a function of γ (Figure 2) shows that at $\gamma = 150°$ and 30°, the ^{13}C antiphase magnetization evolving during the t_1 period is transferred exclusively to the $I_{1y}^{\alpha\alpha}$ and $I_{1y}^{\beta\beta}$ operators, respectively. Thus, in the absence of relaxation and the effects of passive couplings the experimental scheme of Figure 1 exclusively polarizes a chosen individual transition in a degenerate multiplet of magnetically equivalent ^{1}H. The element in the box in Figure 1 represents a new type of polarization transfer scheme, where the manifold of transitions of an insensitive spin is funneled exclusively to a selected transition of a system of sensitive spins, enabling its direct spectral
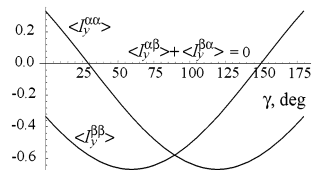
Figure 2. Amplitudes of projections of the $2S_xI_{1z}$ operator to the single-transition operators $\langle I_y^{ij}\rangle = \langle 2S_xI_{1z}|I_y^{ij}\rangle$ where $i,j = \alpha,\beta$ as a function of the phase γ of the ^{13}C 90° pulse.

observation even in the case of the multiplet degeneracy. The experimental scheme of Figure 1 can be run using the constant-time ^{13}C chemical shift evolution when the higher spectral resolution along the ^{13}C dimension is needed.

For molecules weakly aligned in the magnetic field a new RDC term in the Hamiltonian, namely $H = -D_{HH}\Sigma I_{zi}I_{zj}$, where $i < j$ and $i,j = \{1,2,3\}$, should be considered, where D_{HH} is the amplitude of the residual dipolar couplings.[5] The RDC Hamiltonian term does not interchange individual transitions of the ^{1}H multiplet. Therefore, the evolution of σ_{acq} during the acquisition time can be interpreted as a spectral separation of the $I^{\alpha\alpha}$ and $I^{\beta\beta}$ lines by $2D_{HH}$. Since these spectral transitions are observed in different experiments, the sign of D_{HH} can be inferred from the relative shifts of the $I^{\alpha\alpha}$ and $I^{\beta\beta}$ components.

The effect of the RDC Hamiltonian on the quality of selection of individual components is estimated by numerical evaluation of the density operator throughout the pulse sequence of Figure 1 using the program NMRSIM (Bruker). In simulations of the 2D $[^{13}$C,^{1}H$^{\alpha\alpha}]^{Methyl}$ HSQC with D_{HH} in the range of $0-20$ Hz and $D_{HC} = 1/2.3D_{HH}$, the $I^{\alpha\alpha}$ component was selected, and spectral intensities at the central and $I^{\beta\beta}$ components were monitored. At $D_{HH} = 20$ Hz, the intensity of the central and $\beta\beta$ transitions are 15% of the intensity of the selected component. Thus, we conclude that the proposed experiment should exhibit a good tolerance for the presence of RDC and passive scalar coupling interactions described by similar Hamiltonians.

The performance of the experiments of Figure 1 is demonstrated with two proteins, the diamagnetic heme chaperone apo-CcmE[12] weakly aligned in the presence of Pf1 phages,[13] and its paramagnetic, heme group-containing counterpart, holo-CcmE, complexed with KCN. The sensitivity (S/N) of 2D $[^{13}$C,^{1}H$^{\alpha\alpha}]^{Methyl}$ and $[^{13}$C,^{1}H$^{\beta\beta}]^{Methyl}$ HSQC spectra measured with both proteins is on average a factor of 0.5 smaller to that observed in the regular $[^{13}$C,^{1}H] HSQC (a theoretical attenuation factor is 0.58 without considering relaxation effects). At the achieved maximal $S/N = 20$ no cross-talk between $I^{\alpha\alpha}$ and $I^{\beta\beta}$ components of the ^{1}H triplet was detected in the spectra. The values and signs of D_{HH} can be easily extracted from the 1D ^{1}H slices taken through the corresponding peaks in the two spectra. These values are reported in the form of histograms in Figure 1s in Supporting Information. By repeating the measurements the statistical variation of the obtained values of D_{HH} was estimated to be in the range of 0.4 Hz. It should be noted that for holo-CcmE the proposed experiment provided values of D_{HH} which are significantly smaller than the line width of ^{1}H resonances, so that alternative methods designed to detect the individual transitions in the form of the ^{1}H antiphase magnetization would fail.[5] This fact bears special importance for paramagnetic proteins where ^{1}H lines are typically broadened by dipolar interactions with electronic spin. Very attractive is the absence of the need for a diamagnetic form as a reference state, which might be difficult or impossible to prepare as is the case for CcmE.[12]
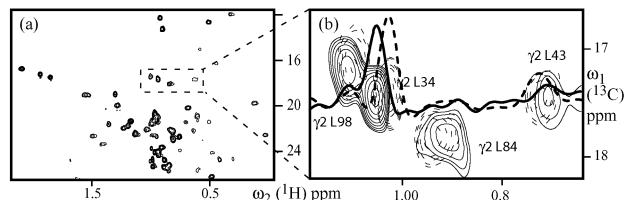

Figure 3. (a) 2D $[^{13}$C,^{1}H$^{\alpha\alpha}]^{Methyl}$ HSQC spectrum and (b) an expansion showing $[^{13}$C,^{1}H$^{\alpha\alpha}]^{Methyl}$ (solid lines) and $[^{13}$C,^{1}H$^{\beta\beta}]^{Methyl}$ (dashed lines) HSQC spectra of the 121 amino acid fragment of uniformly ^{13}C,^{15}N-labeled apo-CcmE weakly oriented in the magnetic field by addition of 20 mg/mL Pf1 phages measured with the experimental scheme of Figure 1. Sample conditions were 0.1 mM apo-CcmE in 20 mM Tris buffer, pH = 7, $T = 293$ K. For each spectrum $1024(t_2)$, $45(t_1)$ complex points were recorded with 64 scans per increment and 1 s recycle delay resulting in 1.2 h of measuring time.

For many cross-peaks stemming from the rigid core of the CcmE proteins, significantly different line-width and concomitant spectral intensities were observed (for example compare $\gamma2$ Leu 34 and Leu 43, Figure 3, a and b). Due to the relatively large ^{1}H^{Methyl} CSA values of $\Delta\sigma = 10$ ppm,[4] smaller line-width is expected for the $I^{\beta\beta}$ component of the ^{1}H triplet at high polarizing magnetic fields, thus making the 2D $[^{13}$C,^{1}H$^{\beta\beta}]^{Methyl}$ HSQC an attractive building block for TROSY-type spectroscopy with methyl groups.

Supporting Information Available: Figure 1s containing histograms of D_{HH} values of apo-CcmE weakly oriented by Pf1 phages and paramagnetic holo-CcmeE and Figure 2s of a comparison of $[^{13}$C,^{1}H$^{\alpha\alpha/\beta\beta}]^{Methyl}$ HSQC with regular $[^{13}$C,^{1}H]-HSQC (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Pervushin, K.; Riek, R.; Wider, G.; Wuthrich, K. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 12366–12371.
(2) Muller, N.; Bodenhausen, G. *J. Chem. Phys.* **1993**, *98*, 6062–6069.
(3) (a) Bertini, I.; Luchinat, C.; Tarchi, D. *Chem. Phys. Lett.* **1993**, *203*, 445–449. (b) Qin, J.; Delaglio, F.; Lamar, G. N.; Bax, A. *J. Magn. Reson. Ser. B* **1993**, *102*, 332–336. (c) Ghose, R.; Prestegard, J. H. *J. Magn. Reson.* **1997**, *128*, 138–143. (d) Boisbouvier, J.; Gans, P.; Blackledge, M.; Brutscher, B.; Marion, D. *J. Am. Chem. Soc.* **1999**, *121*, 7700–7701.
(4) (a) Mandal, P. K.; Madhu, P. K.; Muller, N. *Chem. Phys. Lett.* **2000**, *320*, 269–276. (b) Madhu, P. K.; Mandal, P. K.; Muller, N. *J. Magn. Reson.* **2002**, *155*, 29–38.
(5) (a) Kaikkonen, A.; Otting, G. *J. Am. Chem. Soc.* **2001**, *123*, 1770–1771. (b) Sibille, N.; Bersch, B.; Coves, J.; Blackledge, M.; Brutscher, B. *J. Am. Chem. Soc.* **2002**, *124*, 14616–14625.
(6) Kontaxis, G.; Bax, A. *J. Biomol. NMR* **2001**, *20*, 77–82.
(7) (a) Tolman, J. R.; Flanagan, J. M.; Kennedy, M. A.; Prestegard, J. H. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 9279–9283. (b) Tjandra, N.; Bax, A. *Science* **1997**, *278*, 1111–1114. (c) Choy, W. Y.; Tollinger, M.; Mueller, G. A.; Kay, L. E. *J. Biomol. NMR* **2001**, *21*, 31–40.
(8) (a) Bothnerby, A. A.; Gayathri, C.; Vanzijl, P. C. M.; Maclean, C. *J. Magn. Reson.* **1984**, *56*, 456–462. (b) Bothnerby, A. A.; Dadok, J.; Mishra, P. K.; Vanzijl, P. C. M. *J. Am. Chem. Soc.* **1987**, *109*, 4180–4184.
(9) (a) Tolman, J. R.; Flanagan, J. M.; Kennedy, M. A.; Prestegard, J. H. *Nat. Struct. Biol.* **1997**, *4*, 292–297. (b) Barbieri, R.; Bertini, I.; Cavallaro, G.; Lee, Y. M.; Luchinat, C.; Rosato, A. *J. Am. Chem. Soc.* **2002**, *124*, 5581–5587.
(10) Glaser, S. J.; Schulte-Herbruggen, T.; Sieveking, M.; Schedletzky, O.; Nielsen, N. C.; Sorensen, O. W.; Griesinger, C. *Science* **1998**, *280*, 421–424.
(11) Guntert, P.; Schaefer, N.; Otting, G.; Wuthrich, K. *J. Magn. Reson., Ser. A* **1993**, *101*, 103–105.
(12) Enggist, E.; Thony-Meyer, L.; Guntert, P.; Pervushin, K. *Structure* 2002.
(13) Hansen, M. R.; Mueller, L.; Pardi, A. *Nat. Struct. Biol.* **1998**, *5*, 1065–1074.

JA035288H