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Article

Detection of C′,C$^{\alpha}$ correlations in proteins using a new time- and sensitivity-optimal experiment

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Abstract

Sensitivity- and time-optimal experiment, called COCAINE (CO-CA In- and aNtiphase spectra with sensitivity Enhancement), is proposed to correlate chemical shifts of ^{13}C′ and ^{13}C$^{\alpha}$ spins in proteins. A comparison of the sensitivity and duration of the experiment with the corresponding theoretical unitary bounds shows that the COCAINE experiment achieves maximum possible transfer efficiency in the shortest possible time, and in this sense the sequence is optimal. Compared to the standard HSQC, the COCAINE experiment delivers a 2.7-fold gain in sensitivity. This newly proposed experiment can be used for assignment of backbone resonances in large deuterated proteins effectively bridging ^{13}C′ and ^{13}C$^{\alpha}$ resonances in adjacent amino acids. Due to the spin-state selection employed, the COCAINE experiment can also be used for efficient measurements of one-bond couplings (e.g. scalar and residual dipolar couplings) in any two-spin system (e.g. the N/H in the backbone of protein).

Introduction

Recently developed transverse relaxation-optimized spectroscopy (TROSY) (Pervushin et al., 1997) has opened a new avenue for investigating large macromolecule using NMR spectroscopy, since the sensitivity of spectra is dramatically increased due to the reduction of transverse relaxation (Pervushin et al., 1998). Further reduction can be obtained by uniform or partial replacement of non-labile protons with deuterons (Grzesiek et al., 1993; LeMaster, 1994; Yamazaki et al., 1994; Shan et al., 1996; Gardner and Kay, 1998; Salzmann et al., 1998). The sequential backbone resonance assignment of large proteins has been achieved with the usage of TROSY-type triple-resonance experiments (Mulder et al., 2000; Salzmann et al., 1999a, b ; Yang and Kay, 1999) together with the deuteration of non-labile protons. Among these experiments, TROSY-type HNCOCA and HNCACO experiments are used for matching intra-residual cross-peaks of the HNCA experiment with the strong sequential cross peaks derived from the HNCO experiment. However, their application has been limited to low field spectrometers by rapid ^{13}C′ transverse relaxation, due to the large ^{13}C′ chemical shift anisotropy (CSA).

A recently proposed 3D MQ-HACACO experiment (Pervushin and Eletsky, 2003) gives similar information to the TROSY-type HNCOCA and HNCACO experiments. Moreover, the experiment is very favourable in terms of relaxation, since the period involving transverse magnetization is

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minimized by a double- constant-time evolution period (Swapna et al., 1997), in which the $^{1}H^{\alpha}$—$^{13}C^{\alpha}$ multiple quantum coherences (Grzesiek et al., 1995a, b ; Swapna et al., 1997; Xia et al., 2000) are used simultaneously to record $^{1}H^{\alpha}$ and $^{13}C^{\alpha}$ chemical shifts and to achieve the $^{1}H^{\alpha}$ magnetization transfer to the $^{13}C'$ spins for acquisition (Serber et al., 2000, 2001). In addition, the acquisition on ^{13}C keeps the pulse sequence shorter than the HNCOCA and HNCACO experiments by omitting several polarization transfer periods for the magnetization transfer from $^{13}C'$ to ^{1}H, which result in a significant signal loss (Braun et al., 2003). However, the 3D MQ-HACACO experiment requires at least partial reprotonation at H^{α} positions, which can cause sensitivity loss due to the relaxation originating from the H^{α} (Grzesiek et al., 1993; Pervushin et al., 1997).

Here we propose a sensitivity enhanced, time-optimal experiment called COCAINE (CO-CA In- and aNtiphase spectra with sensitivity Enhancement) experiment to correlate chemical shifts of $^{13}C'$ and $^{13}C^{\alpha}$ spins, which is applicable to the sequential backbone assignments of fully or partially deuterated proteins. In the framework of this new experiment both Boltzmann reservoirs of thermal equilibrium magnetizations of $^{13}C'$ and $^{13}C^{\alpha}$ spins are constructively utilized (Pervushin et al., 1998). A comparison of the sensitivity and duration of the experiment with the corresponding theoretical unitary boundaries imposed by quantum spin dynamics shows that in the absence of relaxation maximum possible polarization transfer is achieved in the shortest possible transfer time. The performance of the experiment is compared with the standard HSQC approach and is shown to deliver sensitivity gains of factor 2.7 without an increase in the duration of the experiment. The COCAINE experiment can also be used for efficient measurements of $^{13}C'$ and $^{13}C^{\alpha}$ one bond couplings (e.g. scalar and residual dipolar couplings), due to the spin state selection employed (Andersson et al., 1998; Cordier et al., 1999; Lerche et al., 1999; Sørensen et al., 1999). The proposed principle underlying the COCAINE experiment can also be extended to time-optimal detection of H/N and H/C correlations with water suppression by a WATERGATE sequence (Piotto et al., 1992) before the acquisition and by presaturation, respectively.

Results and discussion

Unitary bounds and time-optimality of polarization transfer for the spin-state selection in the COCAINE experiment

The efficiency of polarization transfer experiments can be assessed by comparison with fundamental bounds imposed by unitary spin dynamics using available quantum control Hamiltonians (Glaser et al., 1998; Khaneja et al., 2003a, b). The unitary bound value, $b_{\max}$, defines the largest projection of an arbitrary source operator A on a target operator C achievable by a unitary propagator U (Glaser et al., 1998),

$$b_{\max} = \max(\mathrm{Tr}\{U\ A\ U^{\dagger}C\}/\mathrm{Tr}\{C^{\dagger}C\}) \qquad (1)$$

The unitary bound value can be determined numerically for arbitrary initial and target operators (Glaser et al., 1998). This number represents an important benchmark for construction and evaluation of NMR experiments utilizing non-selective rf-irradiation and heteronuclear J coupling described by H_{rf} and H_J Hamiltonians, respectively. The second parameter, which should be considered for an optimal experiment, is the minimal time, $\Theta_{\min}$, needed to transfer magnetization between these states (Untidt et al., 1998; Untidt et al., 1999; Khaneja et al., 2001, 2003a, b ; Schulte-Herbruggen et al., 2001; Reiss et al., 2002; Skinner et al., 2003).The polarization transfer (between the time points b and c in Figure 1) for the spin-state selection in the COCAINE experiment using the magnetization originating from spin I consists of two independent pathways that are schematically represented by:

$$\begin{aligned}\sqrt{2}I_zS^{-} &\rightarrow b(U^{\mathrm{COCAINE}})(-1/2)I^{-}(E+2S_z)\\ &= b(U^{\mathrm{COCAINE}})(-1/2)I^{-}S^{\alpha}, \qquad (2.1)\end{aligned}$$

$$\begin{aligned}\sqrt{2}I_zS^{+} &\rightarrow b(U^{\mathrm{COCAINE}})1/2I^{-}(E-2S_z)\\ &= b(U^{\mathrm{OCAINE}})1/2I^{-}S^{\beta}, \qquad (2.2)\end{aligned}$$

where the normalized operators $\sqrt{2}I_zS^{-}$, $\sqrt{2}I_zS^{+}$, $1/2I^{-}S^{\alpha}$ and $1/2I^{-}S^{\beta}$ are used. Analysis shows that for both pathways of Equations (2) $b(U^{\mathrm{COCAINE}}) = b_{\max} = \sqrt{2}$ indicating that in the absence of relaxation the maximum possible sensitivity for this type of polarization transfer is

actually achieved in the experiment. The magnetization originating from spin S can be described by changing I_z to I_x or I_y in the Equations (2.1) and (2.2).

The COCAINE experiment accomplishes the polarization transfer in $\tau = 1/(2J_{IS})$ seconds, corresponding to the shortest possible time. Indeed, the transfer of Equation (2.1) consists of two pathways $2I_zS_x \rightarrow -2I_xS_z$ and $2I_zS_y \rightarrow -I_y$. The first pathway can be implemented using just rf-Hamiltonians in a negligibly short amount of time. The second pathway represents the so called anti-phase transfer between hermitian operators with the maximal theoretical efficiency of $b_{theor}(t) = \sin(\pi J_{IS}t)$ for the transfer time $t \leq 1/(2J_{IS})$ and 1 for $t > 1/(2J_{IS})$ (the formal proof along the lines of Theorem 3 of Reiss et al. (Reiss et al., 2002) is presented in Supporting materials). Thus, the minimal time required to produce the transfer of Equation (2.1) is $t_{min} = 1/(2J_{IS})$, which is indeed achieved experimentally. The same bound for minimal transfer time is applicable for the pathway of Equation (2.2) as well as for the pathways starting on spin *S*. Thus, the COCAINE experiment attains both theoretical benchmarks (maximum transfer and minimal time) and by those measures is optimal.

In- and anti-phase [^{13}C,$^{13}C^{\alpha}$]-correlation experiment

Figure 1 shows the pulse scheme of the COCAINE experiment, utilizing both Boltzmann steady-state magnetizations of *I* and *S* spins. For simplicity, we consider first the evolution of the density operator originating from the *I* spin. For the downfield component of *S* spin doublet, the first and second phase cycling steps are required. The steady-state magnetization of the spin *I* is transferred to the spin *S* and the density operator σ^I at the time point *a* in Figure 1 (the first step in the phase cycle in Figure 1) may be described by:

$$\sigma^I(a) = I_zS^+ + I_zS^- . \quad (3)$$

After the frequency-labeling period of spin *S* (time point *b* in Figure 1), the density operator is given by:

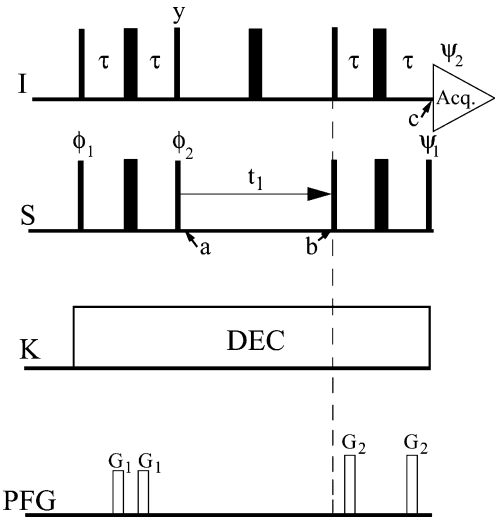

Figure 1. Pulse scheme of the COCAINE experiment. The rows marked *I*, *S*, and *K* represent the rf-channels. Narrow and wide bars stand for 90 ° and 180 ° pulses, respectively. All phases are set to {x,} unless indicated otherwise. For the $^{13}C^{\alpha}$-$^{13}C'$ correlation spectra, the $^{13}C'$, $^{13}C^{\alpha}$ and ^{2}H spins were represented by *I*, *S*, and *K*, respectively. The $^{13}C'$, $^{13}C^{\alpha}$ and ^{2}H carrier frequencies are set to 174, 55 and 3.2 ppm, respectively. ^{2}H decoupling is performed with the WALTZ pulse sequence at $\gamma B_1 = 2.5$ kHz. The delay τ corresponds to $1/(4^1J_{IS})$ (4.7 ms for $^{13}C^{\alpha}$-$^{13}C'$ correlation). The line marked PFG (pulsed field gradient) indicates the magnetic field gradients applied along the *z*-axis: G_1, amplitude = 19 G/cm, duration = 0.5 ms; G_2, 50 G/cm, 0.9 ms. The spectra of upfield and downfield components of doublets are obtained in the interleaved manner using the following phases: for the downfield $\Phi_1 = \{y,-x,-y,x,\}$, $\Phi_2 = \{y,x,-y,-x,\}$ $\psi_1 = \{-y,-y,-y,-y,\}$ ψ_2 (receiver) = $\{y,-x,-y,x\}$; and for the upfield $\Phi_1 = \{y,-x,-y,x\}$, $\Phi_2 = \{y,x,-y,-x\}$, $\psi_1 = \{y,y,y,y\}$, ψ_2 (receiver) = $\{y,-x,-y,x\}$, respectively. The phase-sensitivity in the t_1 dimension is obtained by recording a second FID for each t_1 value using for the downfield $\Phi_1 = \{-y,-x,y,x\}$, $\Phi_2 = \{y,-x,-y,x\}$, $\psi_1 = \{y,y,y,y\}$, ψ_2 (receiver) = $\{-y,x,y,-x\}$; for the upfield $\Phi_1 = \{-y,-x,y,x\}$, $\Phi_2 = \{y,-x,-y,x\}$, $\psi_1 = \{-y,-y,-y,-y\}$, ψ_2 (receiver) = $\{-y,x,y,-x\}$, respectively. The data is processed by the method described by Kay et al. (Kay et al., 1992).

$$\sigma^I(b) = -\exp(-\mathrm{i}\omega_s t_1) I_z S^+ - \exp(\mathrm{i}\omega_s t_1) I_z S^- . \quad (4)$$

The subsequent polarization transfer step (points *b* to *c*) transforms the operators $I_z S^+$ and $I_z S^-$ to the operators $I^- S^\alpha$ and $I^- S^\beta$, respectively, resulting in the density operator at the time point *c*:

$$\sigma^I(c) = -1/2i \ \exp(-\mathrm{i}\omega_s t_1) I^- S^\alpha + 1/2i \ \exp(\mathrm{i}\omega_s t_1) I^- S^\beta . \quad (5)$$

The downfield component of the doublet is achieved by changing the sign of $I^- S^\beta$ (the second step in the phase cycle in Figure 1).

The evolution of the density operator σ^S originating from the steady-state magnetization of the spin *S* is similar to the evolution of σ^I. At the time points *a*, *b* and *c* the density operators are given by:

$$\sigma^S(a) = -iI_x S^+ + iI_x S^- , \quad (6.1)$$

$$\sigma^S(b) = -\exp(-i\omega_s t_1) I_x S^+ + \exp(i\omega_s t_1) I_x S^- , \quad (6.2)$$

$$\sigma^S(c) = -1/2i \exp(-i\omega_s t_1) I^- S^\alpha - 1/2i \exp(i\omega_s t_1) I^- S^\beta . \quad (6.3)$$

In analogy with the evolution of σ^I, the downfield component of the doublet is achieved by changing the sign of $I^- S^\beta$ (the second step in the phase cycle in Figure 1).

The COCAINE type-correlation spectra contain individual components of doublets in the directly acquired dimension with two-fold gain in sensitivity due to the utilization of both Boltzman steady-state magnetizations, compared to the HSQC spectrum. A further $\sqrt{2}$ gain in sensitivity is achieved by the fact that all terms present during t_1 evolution period lead to observable magnetization during the acquisition. Thus, the total theoretical gain of COCAINE amounts to $2\sqrt{2}$ of the intensity of the corresponding *S*-coupled HSQC.

The validity of the COCAINE approach is shown for a sample of uniformly ^{2}H, ^{13}C, ^{15}N-labeled ubiquitin in 95% ^1H_2O/5% ^2H_2O at pH 7.3 and 20 °C. Figure 2 compares a region of the HSQC spectrum with the COCAINE experiment recorded using the pulse scheme of Figure 1. The suppression of the second doublet component was better than 93% in all cases. The unsuppressed

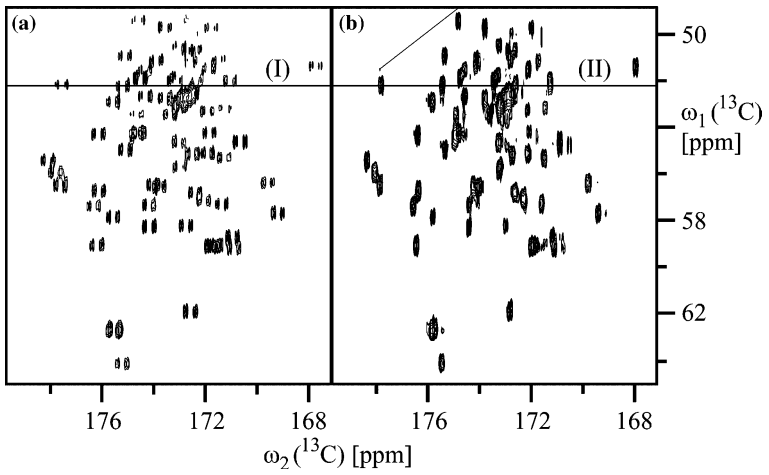

Figure 2. Contour plots of (a) the $^{13}C^\alpha$-coupled [$^{13}C'$, $^{13}C^\alpha$]-HSQC without the decoupling during t_2 and (b) the COCAINE experiment acquired with a uniformly ^{2}H, ^{13}C, ^{15}N-labeled ubiquitin in 95% ^1H_2O/5% ^2H_2O at pH 7.3 and 20 °C, using a DUX probe, which is optimized for the ^{13}C detection, in a Bruker Avance spectrometer operating at the ^{1}H frequency of 600 MHz. In order to record both spectra (the $^{13}C^\alpha$-coupled [$^{13}C'$, $^{13}C^\alpha$]-HSQC and the COCAINE), $t_{1max} = 19.8$ ms, $t_{2max} = 100$ ms and 90 ° ($\gamma B_1 = 2.5$ kHz) and 180 ° ($\gamma B_1 = 3.3$ kHz) selective pulses with Gaussian lineshape truncated at 5% were used. The overall suppression of second doublet component is better than 93%. The resulting spectrum of the COCAINE experiment shows the downfield component of each doublet. Comparison of two spectra illustrates the simplification achieved by the COCAINE spectrum due to the elimination of $^{13}C^\alpha$ splitting. The solid lines mark 1D slices (I) and (II), used for the sensitivity comparison in Figure 3.

second doublet component may be due to the ^{13}C pulse imperfection on the selectivity, variable relaxation of the multiquantum coherence in the $^{13}C^{\alpha}$ pathway, the $^{13}C^{\alpha}$–$^{13}C^{\beta}$ couplings, and/or the difference in the longitudinal relaxation of $^{13}C^{\alpha}$ and $^{13}C'$spins (Pervushin and Eletsky, 2003) (Figure 2). Thus, accurate values of homonuclear one-bond couplings can be obtained from the difference in the $^{13}C'$ frequencies for pairs of doublet components. The observed sensitivity gains of between 2.4 and 2.8 differ slightly from the theoretical calculation (Figure 3). These variations may be due to variable relaxation of the multiquantum coherence in the $^{13}C^{\alpha}$ pathway, the $^{13}C^{\alpha}$–$^{13}C^{\beta}$ couplings, and/or the difference in the longitudinal relaxation of $^{13}C^{\alpha}$ and $^{13}C'$spins (Pervushin and Eletsky, 2003). Nontheless, the average sensitivity gain of 2.7 in the COCAINE experiment, compared to the HSQC experiment, is in excellent agreement with the theoretical value of $2\sqrt{2}$.

Since the COCAINE experiment utilizes spin-state selection, it can also be used for efficient measurements of one-bond couplings (e.g. scalar and residual dipolar couplings) in any two-spin system (e.g. the N/H in the backbone of protein). However, the water suppression (i.e. a WATERGATE sequence (Piotto et al., 1992) before the acquisition is necessary for proton detection. It is worth noting that the suppression of the second doublet component was better than 97% in [^{15}N,^{1}H]-COCAINE experiment and the sensitivity gain, compared with the method suggested by Lerche et al. (Lerche et al., 1999), depends on the relative size of ^{15}N steady-state magnetization to ^{1}H steady-state magnetization.

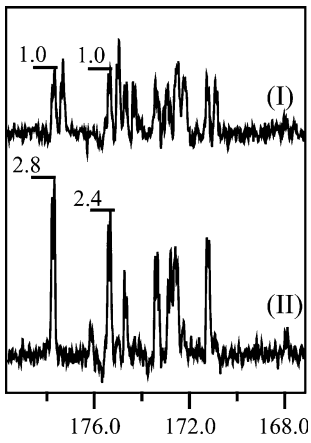

Figure 3. 1D slices along the $^{13}C^{\alpha}$ of (I) $^{13}C^{\alpha}$-coupled [$^{13}C'$, $^{13}C^{\alpha}$]-HSQC and (II) downfield doublet components of the COCAINE spectra of Figure 2. The relative intensities of selected peaks in (I) are quantitatively compared with the corresponding peaks in (II). This shows that the sensitivity gains are lying in between 2.4 and 2.8. Nonetheless, the average sensitivity gain of the COCAINE spectra is 2.7 in good agreement with the theoretical sensitivity gain of 2.8.

Conclusions

We have demonstrated that the sensitivity is significantly increased by the COCAINE experiment, compared to the corresponding HSQC and that in fact it achieves the theoretical maximum transfer. Furthermore, the COCAINE experiment simplifies the spectra in ^{13}C-observed NMR spectroscopy by eliminating the $^{13}C^{\alpha}$ splitting without any special processing (Serber et al., 2001; Pervushin and Eletsky, 2003). The COCAINE experiment can also be processed to isolate the two doublet components, allowing for its use in measurement of one-bond couplings (scalar and residual dipolar couplings) to obtain structural constraints. Using the newly proposed experiment, the backbone assignment of large proteins can be achieved by connecting information from the most sensitive triple-resonance experiments (TROSY-type HNCA and HNCO).

Supporting materials

Maximum anti-phase transfer between hermitian operators, available in electronic format at http://dx.doi.org/10.1007/s10858-005-2361-4.

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