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Optimization of cross-polarization at low radiofrequency fields for sensitivity enhancement in solid-state NMR of membrane proteins reconstituted in magnetically aligned bicelles

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ABSTRACT

Solid-state NMR (ssNMR) of oriented membrane proteins (MPs) is capable of providing structural and dynamic information at nearly physiological conditions. However, NMR experiments performed on oriented membrane proteins generally suffer from low sensitivity. Moreover, utilization of high-power radiofrequency (RF) irradiations for magnetization transfer may give rise to sample heating, thereby decreasing the efficiency of conventional cross-polarization schemes.

Here we have optimized the recently developed repetitive cross-polarization (REP-CP) sequence (Tang et al., 2011) to further increase the magnetization transfer efficiency for membrane proteins reconstituted in magnetically aligned bicelles and compared its performance to single-contact Hartmann–Hahn cross-polarization (CP), CP-MOIST and the adiabatic transfer. It has been found that employing the REP-CP sequence at RF amplitudes of 19 kHz instead of the commonly used higher RF fields (>45 kHz) enhances the efficiency of REP-CP. An additional 30% signal can be obtained as compared to the previously published REP-CP, and 20% when compared to the re-optimized REP-CP at 50 kHz RF fields. Moreover, the ^{15}N signal gain of low-power REP-CP was found to be 40% over the adiabatic CP and up to 80% over CP-MOIST. Thus, the low-power REP-CP sequence surpasses all of the previous CP schemes in addition of having the tremendous advantage of reducing the RF powers by a factor of seven, thereby preserving the liquid-like bicelle sample. By contrast, in purely static (NAL crystal) and semi-rigid systems (Pf1 phage), the adiabatic CP was found to be more effective. Periodic oscillations of the intensity profile (distinct from the transient oscillations) as a function of the CP contact time and B_1 RF field strengths were observed during the REP-CP optimization with the oscillations becoming more pronounced with lower RF fields. Many-spin simulations were performed to explain the oscillations and their periodicity.

1. Introduction

Magnetically oriented bicelles [1,2] provide both a native-like environment for membrane proteins (MPs) and high degree of alignment, which narrows down the resonance lines. Therefore, MPs reconstituted in magnetically aligned bicelles can be analyzed by solid-state NMR methods at complete hydration, near-physiological temperatures and in a lipid-rich environment. As is mostly the case in solid-state NMR, the strong couplings among the protons make the detection of ^{1}H spins quite difficult, thus leaving the low-gamma spin detection (such as ^{15}N or ^{13}C) as the most practical option. However, NMR experiments involving the low-γ nuclei generally suffer from poor sensitivity due to their small gyromagnetic ratios, low natural abundance and dilution relative to the strongly coupled surrounding protons. In addition, the low protein-to-lipid ratios and limited sample volume (about two hundred of microliters or less) make the sensitivity issue particularly important. Therefore, increasing the signal-to-noise ratio is one of the main concerns for MP structure elucidation by solid-state NMR along with improving spectral resolution and shortening the overall experimental time of multidimensional experiments.

Following the groundbreaking work on cross-polarization (CP) [3] under the Hartmann–Hahn conditions [4], various schemes have been designed to further improve magnetization transfer to the dilute spins. Notable examples include: adiabatic CP [5,6], CP-MOIST [7,8], CP with phase inversion [9], ramp-CP [10], variable-amplitude CP [11], frequency-modulated CP [12], selective

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excitation RELOAD-CP [13], CP-COMPOZER [14] and, finally, the Multiple-Contact CP (MC-CP) or repetitive CP (REP-CP) [15–18]. Most of the above magnetization-enhancing sequences utilize a single CP contact; whereas it was recently demonstrated [16–18] that transferring the maximum possible magnetization from high- to low-gamma spins in one step (even of several milliseconds) is not feasible. This is mainly due to the thermodynamic limits caused by the finite ratio between the number-densities of the high- and low-gamma spins, Hartmann–Hahn mismatches caused by inhomogeneity of the radio-frequency (RF) fields, and short $T_{1\rho}$ spin-lock relaxation times. By contrast, the REP-CP sequence, which is based on a repetition of much shorter (hundreds of microseconds) CP-MOIST contacts separated by re-equilibration intervals to repolarize the proton bath, was shown to increase the signal-to-noise ratio by almost 100% as compared to the conventional CP scheme and by up to 45% as compared to CP-MOIST when applied to membrane proteins reconstituted in magnetically aligned bicelles [16]. Magnetization enhancement techniques based on the adiabatic transfer [19–21] also represent a potentially attractive alternative to the Hartmann–Hahn cross-polarization since the former is based on applying relatively low powers to achieve the polarization transfer between the two spin baths. However, the typical experimental enhancements are in the range of 30–140% as compared to CP [21–23]; whereas much greater gains are expected from the thermodynamic considerations [3]. This may be caused by the limited number of protons that predominantly contribute to the adiabatic transfer as well as the fundamental bounds on spin dynamics under unitary evolution as discussed by Sørensen and Levitt [24–26]. Furthermore, there is still a need to quantitatively assess and optimize the efficiency of the adiabatic transfer for the dynamic oriented membrane proteins reconstituted in fluid-like bilayer environments [27].

In the present work, we investigate and compare, both theoretically and experimentally, two sensitivity-enhancing pulse sequences when applied to rigid solids, semi-rigid, and dynamic protein systems. Namely, we employ the adiabatic CP sequence for an NAL crystal, Pf1 phage, and the dynamic Pf1 coat protein reconstituted in oriented DMPC/DHPC bicelles. We then focus on the optimization of the REP-CP sequence for Pf1 in bicelles at high B_1 RF field (50 kHz) and its applications at lower B_1 RF fields (as low as 12 kHz) in order to minimize the heating deposited into the sample, which may cause Hartmann–Hahn mismatches of the RF magnetic fields and affect the sample stability.

2. Spin dynamics simulations

Numerical solutions of the many-spin density matrix equation were obtained using in-house MATLAB scripts (Mathworks, Inc.). Spin coordinates from an ideal poly-alanine α-helix tilted of 30° with respect to the B_0 field were used with the dipolar couplings (DC) scaled by −0.4 to account for the perpendicular orientation and uniaxial rotation of the protein molecules in bicelles [28]. Up to twelve spins were utilized for the simulations.

During cross-polarization, the spin system evolves under the simplified Hamiltonian:

$$H = \omega_I I_x^{total} + \omega_S S_x^{total} + \sum_{i<j}^{N_I} a_{ij}\left[I_z^{(i)} I_z^{(j)} - \frac{1}{2}\left(I_x^{(i)} I_x^{(j)} + I_y^{(i)} I_y^{(j)}\right)\right] + \sum_{k=1}^{N_S}\sum_{l=1}^{N_I} b_{kl} S_z^{(k)} I_z^{(l)} \quad (1)$$

where ω_I and ω_S are the spin-locking amplitudes for the protons (I) and nitrogen spins (S), a_{ij} and b_{kl} are the homonuclear and heteronuclear dipolar coupling constants, respectively. Here, the chemical shift anisotropy is neglected when relatively high radiofrequency amplitudes are used. The initial magnetization of the system of $N = N_I + N_S$ spins is described by the normalized density matrix $\rho(0)$:

$$\rho(0) = \frac{I_x^{(initial)}}{2^{N-2}} \quad (2)$$

which is then evolved in time under the exponential Hamiltonian operator:

$$\langle S_x^{(k)} \rangle = Trace\left(S_x^{(k)} e^{-iHt} \rho(0) e^{iHt}\right) \quad (3)$$

The real part of $S_x(t)$ represents the build-up of the ^{15}N magnetization as a function of the contact time, t.

3. Experimental section

3.1. Materials

N-Acetyl-Leucine crystal (NAL). A single ^{15}N-labeled NAL crystal of 0.7 mg (prepared in-house) was used as a static model. It exhibits four sharp lines in the ^{15}N spectrum corresponding to four different N–H amide bond orientations in the unit cell.

Pf1 bacteriophage (Pf1 phage). A sample of 170 μL of uniformly ^{15}N-labeled Pf1 bacteriophage at 40 mg/ml was used as a semi-dynamic oriented model protein system (purchased from Hyglos GmbH, Regensburg, Germany, and later expressed in-house).

Pf1 coat protein reconstituted in a lipid bilayer (Pf1 in bicelles). About 4.5 mg of uniformly ^{15}N-labeled Pf1 coat protein obtained from the phage sample following the previous protocol [29] were reconstituted in 170 μL of DMPC/DHPC bicelles in 0.2 M HEPES buffer at pH = 7.0 containing 0.05% w/v of NaN_3 to prevent bacteria growth. The total w/v ratio was 28% for the lipids, the lipid molar ratio q was 3.2 and the lipid-to-protein ratio was about 60:1.

3.2. NMR spectroscopy

The NMR experiments were performed on a Bruker Avance II spectrometer operating at the ^1H Larmor frequency of 500 MHz and using the Topspin™ 2.0 software. The NMR samples were sealed in a 5 mm New Era™ glass tube and placed in a static triple resonance $^1H/^{15}N/^{31}P$ Bruker 5 mm round-coil E-free™ probe. The temperatures were set to 25 °C for the NAL crystal (ambient temperature), −4 °C for Pf1 phage (yielding the best phage alignment) and 37 °C for Pf1 in bicelles (optimal bicelle alignment as monitored by ^{31}P NMR linewidths). Due to the possible non-linearity of the high-power amplifiers, the various B_1 RF fields were experimentally determined by calibrating the 90° pulse lengths for the ^1H and ^{15}N channels yielding the values of 5 μs or 50 kHz B_1 field, 9.67 μs at 25 kHz, 13.3 μs at 19 kHz and 19.5 μs at 12 kHz. All other parameters of the pulse sequences used in this paper are summarized in the Supporting Information.

3.3. Data processing

All data were processed using Bruker Topspin 2.0 software. The NAL crystal spectra exhibit four well-resolved peaks, which allows one to directly compare the signal-to-noise ratios by measuring the noise level between 600 and 250 ppm. For the one-dimensional spectra of Pf1 phage and Pf1 coat protein in bicelles containing many overlapping peaks, the spectra were instead integrated from 300 to 0 ppm and from 150 to 50 ppm, respectively.

3.4. NMR pulse sequences

The adiabatic sequence (Fig. 1A) transfers the Zeeman order of the high-γ nuclei (^1H) to the heteronuclear dipolar order (used as