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Communication

Solvent-localized NMR spectroscopy using the distant dipolar field: A method for NMR separations with a single gradient

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Abstract

Solvent-localized NMR (SOLO) is a new method which allows the separation of NMR spectra of substances dissolved in different solvents. It uses the selective HOMOGENIZED pulse sequence to produce a two-dimensional NMR spectrum resulting from intermolecular zero-quantum coherences in one distinct solvent. The detected signal is locally refocused by the action of the distant dipolar field, which is created by a frequency selective pulse only in regions containing the selected solvent. The prerequisites are that the different solvents have sufficiently different chemical shifts to be excited separately and that compartments with different solvents are spatially separated by more than the typical diffusion distance. Here, the method is demonstrated for the solvents water and DMSO on a length scale of 0.5 mm. Because signal in the spectra is refocused locally, SOLO is insensitive to variations in the magnetic field which may result from inhomogeneities or structures in the sample. This makes applications in strongly structured samples possible. SOLO is the first method that achieves localization of NMR signal with a single gradient pulse. Therefore, it can be used in conventional NMR spectrometers with one-axis gradient systems and lends itself to a wide range of applications including in vivo NMR.

Keywords: Distant dipolar field; Intermolecular multiple-quantum coherences; Localized NMR spectroscopy; Separation; In vivo

1. Introduction

Recent progress in instrumentation, such as the development of cryo-probes or microcoils, has significantly increased the sensitivity of NMR spectroscopy. Small amounts of substances can be detected, which allows for coupling with other spectroscopic techniques. Combinations with liquid chromatography (LC), mass spectrometry, or circular dichroism spectroscopy have strongly enhanced the analytical power of NMR spectroscopy. Rather long experimental times, especially during separation processes, have triggered the development of: (a) probes containing several radiofrequency (RF) resonators to process several samples at a time [1–4] and (b) methods for simultaneous investigation of several samples in one probe [5,6]. Dedicated multiplex-probes offer optimized sensitivity for different capillaries, but have to be bought or constructed as additional equipment to conventional NMR spectrometers. Multiplex methods, using chemical shift imaging to separate spectra from different samples in one probe, can easily be implemented on conventional spectrometers with three-axis gradients. In addition, this method can be used to separate spectra from compartments in structured samples. However, long experimental times are required and the filling factor of the probe may be low in a particular setup with few capillaries running through one probe.

This communication describes a new method for separating NMR spectra of substances that are dissolved in different solvents. Since only a single gradient pulse is

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required, the method can be used in conventional NMR spectrometers with single-axis gradients. The only prerequisites are that the different solvents have a sufficiently different chemical shift to be excited selectively and that they are spatially separated on a mesoscopic scale.

2. Theoretical background

Solvent-localized (SOLO) NMR spectroscopy is a 2D method based on the effect of the distant dipolar field (DDF) that originates from the magnetization of the sample itself. At high magnetic field strength the DDF can lead to unexpected effects, such as multiple spin echoes [7,8], or additional cross peaks in two-dimensional NMR spectra [9]. These effects can be explained by not negligible non-linear terms in the Bloch equations [7] or differently, in a quantum mechanical picture, by intermolecular multiple-quantum coherences [10,11]. The underlying principle is that the DDF locally refocuses transverse magnetization leading to detectable signal [12]. In particular, two 2D pulse sequences, HOMOGENIZED (homogeneity enhancement by intermolecular zero-quantum echo detection) [13] and IDEAL (intermolecular dipolar interaction enhanced all lines) [14], have been presented which exploit the local nature of the refocusing processes, and thus can yield spectra with narrow line shapes in inhomogeneous magnetic fields. The local reach of the action of the DDF can, in these experiments, be adjusted by a correlation gradient of the strength G and duration T that spatially modulates the magnetization. Typically, the correlation distance is defined as $d_C = \pi/\gamma GT$, with γ being the gyromagnetic ratio. The pulse sequence for the SOLO experiment (Fig. 1) is a variant of HOMOGENIZED, using a frequency selective second RF-pulse, similar to experiments presented previously that make use of the selective pulse for solvent suppression purposes [15,16]. These experiments rely on the principle that the second pulse creates an effective DDF which locally refocuses magnetization, leading to detectable signal. In SOLO, this pulse is applied frequency selectively, creating an effective DDF only in a volume where the solvent spins are excited. Thus, signal is only detected from regions inside the probe that contains the excited solvent. The spectrum displays lines from all solutes present at sufficiently high concentrations in these regions.

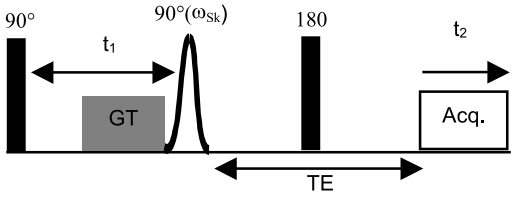

Fig. 1. 2D pulse sequence used for SOLO. Black bars indicate non-selective RF-pulses. Second pulse is frequency selective for ω_{Sk}. Gray box indicates correlation gradient along z direction.

SOLO can be described in the extended product operator formalism proposed by Warren and co-workers [10,11,17]. There, to describe DDF effects, the equilibrium density matrix is extended to second order terms. To describe signal evolution in SOLO, it is convenient to assume a probe with two separate compartments, one containing solvent A and solute a, the other solvent B and solute b. For simplicity, all substances are assumed to have only one resonance corresponding to the spin operators S^A, S^B, I^a, and I^b, respectively. In HOMOGENIZED-like sequences, only zero-quantum coherences contribute to detectable signal [13]. Coherences across the compartments can be made negligible by proper choice of the correlation gradient [18]. Thus, the only terms in the equilibrium density matrix that will contribute to detectable signal in the end are $S_z^A I_z^a$ and $S_z^B I_z^b$. For a selective pulse on solvent A, signal evolution for solute a in SOLO is identical to conventional HOMOGENIZED

$$S_z^A I_z^a \xrightarrow{90^\circ} S^{A+} I^{a-} \xrightarrow{90^\circ(\mathrm{sel},\omega_A)} S_z^A I^{a-} \xrightarrow{\mathrm{DDF}} I^{a-}. \quad (1)$$

The first pulse produces zero-quantum coherences which are converted into two-spin one-quantum operators by the second, selective pulse. These are converted into observable magnetization by the action of the DDF. In the 2D spectrum, a peak is observed at the frequencies $(\omega_a - \omega_A, \omega_a)$ [13]. In the second compartment, signal evolution is given by

$$S_z^B I_z^b \xrightarrow{90^\circ} S^{B+} I^{b-} \xrightarrow{90^\circ(\mathrm{sel},\omega_A)} S^{B+} I^{b-} \xrightarrow{\mathrm{DDF}} \text{non-observable}. \quad (2)$$

The pulse produces zero-quantum coherences, but these are not converted by the second pulse selective on solvent A. No observable magnetization is refocused.

Summarizing for both compartments, only solute signals will be produced from the compartment where the solvent is excited by the second pulse—spectra from both solutes can be separated by choice of the irradiation frequency of the second pulse. The simplified example with one resonance per solute can be readily expanded to a number of solutes with more than one line each. Mutual effects of the solvent spins can be neglected, as has been pointed out previously [16]. SOLO can also be performed with a larger number of solvents, in principle, only limited by the fact that each has to be excited selectively. The spatial resolution, meaning the minimum distance of two compartments that can be separated, is limited by diffusion attenuation. The spatial modulation of the DDF is essential for the refocusing of observable magnetization. If the modulation distance approaches the mean diffusion distance the DDF becomes blurred and no magnetization will be refocused [19].

3. Experimental details

Experiments were performed on a Bruker Avance 750 widebore spectrometer equipped with imaging gradients and a 5 mm bird cage resonator. For the experiments, only the z-gradient was used. To minimize radiation damping, the correlation gradient was split into two parts [13]. A 0.5 ms (sine-shaped) pulse was applied immediately after the first RF-pulse and a 1 ms (sine-shaped) pulse immediately prior to the second RF-pulse. Both gradient pulses had a maximum strength of 400 mT/m resulting in a correlation distance of 0.05 mm. For the selective RF-pulse, a gauss-shape with a duration of 4 ms was used. TE was set to 280 ms and the relaxation delay to 5 s. No phase cycling was done. 4096 points were acquired along the direct dimension and 64 or 1024 along the indirect dimension. Zero-filling by a factor of two was applied in the indirect dimension.

Two phantom samples were used (Fig. 2). Phantom 1 consisted of a 5 mm NMR tube that was filled with a 2 M solution of glutamate in H_2O and contained a PE tube with 1.0/0.5 mm outer/inner diameter filled with DMSO/ethanol (9:1). The PE tube was passed six times through the 5 mm tube (Fig. 2A). Phantom 2 was identical to phantom 1, except that the glutamate solution contained additional contaminations and the PE tube was passed only once through the 5 mm tube (Fig. 2B).

4. Results and discussion

To demonstrate the feasibility of SOLO, experiments were performed with phantom sample 1. Fig. 3A displays a conventional 1D NMR spectrum of the phantom. Peaks for both solvents and both solutes were observed. Line widths of more than 25 Hz are due to the structure of the sample and the use of an imaging resonator. For the SOLO experiment, d_C was adjusted to 0.05 mm, to make the action of the DDF through the wall of the tubes small. Since both solvents have different chemical shifts, ω_{water} and ω_{DMSO}, the selective pulse on either frequency creates an effective DDF,

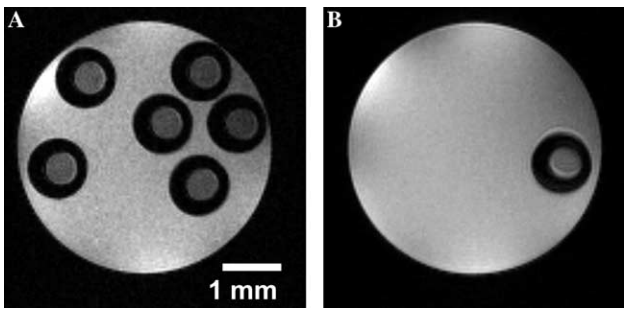

Fig. 2. Gradient echo images of the two phantom samples used: (A) phantom 1; (B) phantom 2, motion artifacts are observed because the lose end of the PE tube is not fixed.

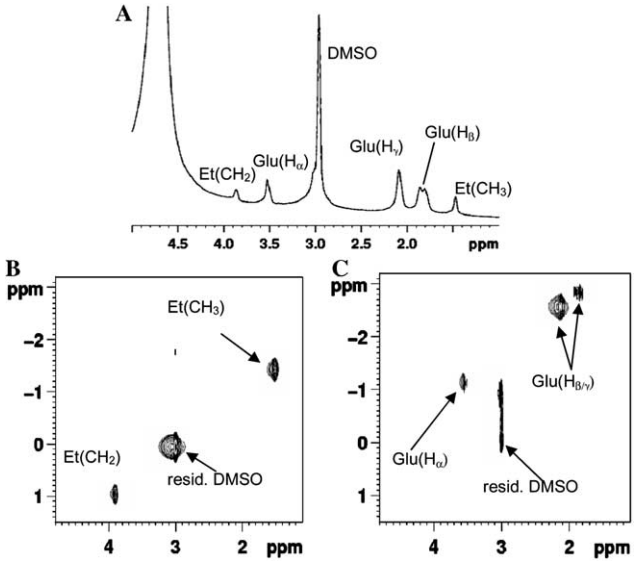

Fig. 3. (A) 1D NMR spectrum of phantom 1 showing peaks of both solvents and both solutes. Intensities of the solvent peaks reflect different volume fractions. (B) SOLO spectrum localized to the DMSO compartments showing ethanol peaks. (C) SOLO spectrum localized to the water compartments showing glutamate peaks. All spectra were arbitrarily referenced to DMSO at 3.0 ppm along the direct dimension; center frequency in f2 was set to 4.0 ppm for SOLO; 64 t1 increments with a spectral width of 7 ppm; 1 average.

and thus a signal only in the region with the respective solvent. For the second pulse selective on DMSO, only resonances at the ethanol frequencies were observed, but no resonances at the glutamate frequencies (Fig. 3B). For a selective pulse on water, only the glutamate spectrum was obtained (Fig. 3C). Each spectrum was obtained in 7 min with 64 t1 increments. This resulted in a resolution of more than 100 Hz along the indirect dimension which makes the peaks appear broad in the spectra. Both spectra show a DMSO peak at zero frequency along f1. This results from pulse imperfections that are unavoidable in this setup. The zero-quantum signal from unwanted solvents and solutes is suppressed, even when the volume fraction of the selected solvent is very small. This was demonstrated with phantom sample 2. In the 1D spectrum, ethanol peaks appear smaller than contaminations in the glutamate solution (Fig. 4A). A SOLO spectrum localized to the DMSO tube shows only ethanol peaks (Fig. 4B). In the corresponding spectrum localized to the water compartment glutamate signals and two cross peaks from the contamination were observed (data not shown), allowing for the assignment of the contaminations to the glutamate solution.

One major advantage of SOLO is that it has the property to yield narrow line widths along the indirect dimension, like all HOMOGENIZED-like experiments. Even in inhomogeneous magnetic fields, narrow-line spectra can be obtained [13–15]. Acquiring a larger number of t1 increments allows for resolution of the fine

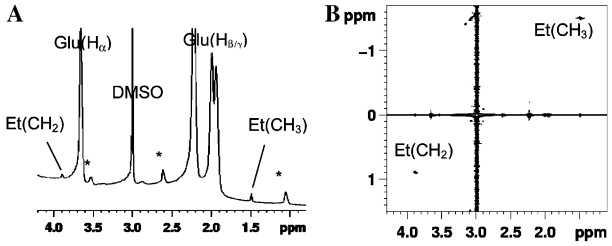

Fig. 4. Spectra recorded with phantom 2. Ethanol peaks are smaller than contaminations in the glutamate solution (asterisks) (A) 1D spectrum; (B) SOLO localized to the DMSO compartments showing ethanol peaks. Center frequency in f2 was set to 4.55 ppm for SOLO; 1024 t1 increments with a spectral width of 3.5 ppm.

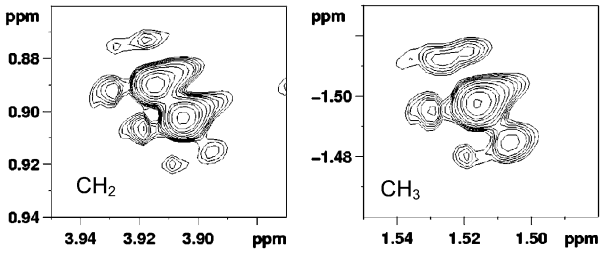

Fig. 5. Zoom from the spectrum shown in Fig. 4B. Fine structure of the ethanol peaks is resolved. Typical sheared multiplet structures are observed [14,20].

structure of the cross peaks (Fig. 5). In structured objects, global shimming is extremely problematic and local shimming procedures can only be preformed for one single voxel, requiring a voxel-localized detection technique. With submillimeter structures, the latter would require extensive averaging which leads to far longer experimental times than required for SOLO, because SOLO produces detectable signal from all compartments that contain the excited solute. In phantom 1, these are the six sections of the small tube that are within the sensitive region of the probe.

SOLO is not limited to a rod-like geometry as found in the phantoms. The solutes to be separated can, for example, be contained in compartments, large pores of rock or soil samples, or in large vesicles. The spatial resolution of this separation can be adjusted by the strength of the correlation gradient and is only limited by diffusion attenuation. Therefore, SOLO allows for localizations to smaller compartments than single voxel techniques which make use of three different gradient axes. Although SOLO is an inherently insensitive method compared to conventional NMR experiments, the fact that signal from all interesting compartments is added up makes it more sensitive than single voxel techniques for strongly structured samples.

A potential application is the simultaneous detection of several LC-capillaries running through one conventional probe. Depending on the solvents that are investigated, different pH values or solvent gradients shifted in time may provide a sufficient difference in chemical shift to use SOLO without using a dedicated probe with single radiofrequency coils for each capillary. SOLO may provide higher spectral resolution along the indirect dimension when shimming is problematic. But longer experimental times and the inherently low sensitivity that is further reduced by poor filling factors are likely to render applications in LC-coupling inefficient. The strengths of SOLO may be best exploited in applications with samples that are structured and compartmentalized on a submillimeter scale, such as, for example, rock or soil specimens. Potential applications in vivo may be the investigation of cultures of large cells, exploiting either intrinsic susceptibility effects or chemical shift agents to shift the intracellular from the extracellular water signal. Finally, SOLO may be used to select water-soluble metabolites in vivo and thus suppresses unwanted lipid signal.

In conclusion, SOLO represents the first method to obtain volume-localized NMR spectra with a single gradient pulse. Due to its insensitivity to inhomogeneities, shimming problems with several or structured objects in the probe will not occur. This greatly enhances the range of potential applications to samples inaccessible to conventional NMR spectroscopy.

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