MQ-PGSTE: A new multi-quantum STE-based PGSE NMR sequence

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ABSTRACT

A new multi-quantum stimulated echo based pulsed gradient spin-echo sequence, MQ-PGSTE, has been developed for measuring translational diffusion. The new sequence provides a higher signal-to-noise ratio than the (Hahn spin-echo based) MAXY-D sequence at long diffusion times, and thus potentially affords better diffusion measurements on macromolecule samples. Based on multi-quantum coherence encoding, the MQ-PGSTE sequence needs considerably lower gradient strengths for diffusion characterization compared to standard single quantum pulsed gradient spin-echo sequences. By using the new sequence, the diffusion coefficient of L-[3-^{13}C]-alanine was found to be $8.1 \pm 0.1 \times 10^{-10}$ m^2 s^{-1}, which is in line with the value obtained by the use of the standard stimulated echo based pulsed gradient spin-echo sequence.

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1. Introduction

For the standard Hahn spin-echo and stimulated echo (STE) based pulsed gradient spin-echo (PGSE) sequences [1,2], the normalized spin-echo attenuation is described by

$$E = \exp\left(\gamma_{\text{eff}}^2 D g^2 \delta^2 (\Delta - \delta/3)\right), \tag{1}$$

where γ_{eff} is the effective gyromagnetic ratio, D is the translational diffusion coefficient, g is the strength of the applied pulsed magnetic field gradients, δ is the duration of the pulsed gradients and Δ is the diffusion time (i.e., the timescale of the diffusion measurement). To achieve accurate diffusion determinations, a series of PGSE experiments need to be performed and a maximum attenuation of $\geqslant 70\%$ (i.e., less than 30% of the original signal intensity remaining) is normally required. However, typical high resolution NMR probes have limited gradient strength (e.g., $g_{\text{max}} = 0.6$ T m^{-1}) and it is therefore challenging to achieve the attenuation required for the diffusion determination on samples containing macromolecules and macromolecular aggregates due to their relatively short transverse and (possibly) longitudinal relaxation times and slow diffusion (e.g., $D < 10^{-11}$ m^2 s^{-1}). For example, for the standard Hahn spin-echo PGSE sequence with $\delta = 5$ ms, $\Delta = 200$ ms, and a typical maximum gradient strength of 0.6 T m^{-1}, an attenuation of ~70% can be achieved for the diffusing species with $D = 1 \times 10^{-11}$ m^2 s^{-1}; however, many macromolecular aggregates have smaller diffusion coefficients and extremely short transverse relaxation time so it becomes impossible to accurately determine the diffusion of these species without significant signal loss owing to the use of long diffusion times. It is desirable therefore to develop new methods that facilitate diffusion measurements on these slowly diffusing species.

Based on the attenuation term with the effective gyromagnetic ratio (i.e., Eq. (1)), there are two common ways to increase the attenuation for the same gradient parameters by manipulating spins: (i) Enhancing γ_{eff} by using multi-quantum (MQ) coherences (e.g., [3,4]) (e.g., $\gamma_{\text{eff}} = 3\gamma_{^1\text{H}} + \gamma_{^{13}\text{C}}$ for the quadruple quantum (QQ) coherence of $8^1\text{H}_x{}^1\text{H}_y{}^1\text{H}_y{}^{13}\text{C}_x$ while $\gamma_{\text{eff}} = \gamma_{^1\text{H}}$ for $^1\text{H}_x$); (ii) Using long T_1 species (e.g., $^{15}\text{N}_z$) in STE-based PGSE experiments to extend Δ (e.g., [5]). In this study, we focus on the enhancement of γ_{eff} by the use of heteronuclear MQ coherences.

Homonuclear MQ PGSE experiments were first developed in the 1980's for the study of anisotropic diffusion in various liquid crystals due to their abilities to enhance γ_{eff} and remove dipolar couplings [6,7]. Whereas, heteronuclear MQ PGSE experiments were mainly developed for the selection of $^1\text{H}-^{15}\text{N}$ MQ coherences in the diffusion measurements on proteins (e.g., [8,9]).

The core parts of the heteronuclear MQ PGSE sequences are the MQ coherence generation units. One of the simplest and most effective MQ coherence generation units is the maximum-quantum proton spectroscopy (MAXY) unit [10], $90_x^\circ(I) - 1/(2\,J_{\text{IS}}) - 180_x^\circ(I)\,90_x^\circ(S) - 1/(2\,J_{\text{IS}}) - 90_x^\circ(I)$, which is a modified version of the DEPT [11] sequence, where I stands for ^1H, S stands for ^{13}C, and J_{IS} is the $^{13}\text{C}-^1\text{H}$ J-coupling constant. The evolution of product operators for SI_n (where $n = 1$ for $^{13}\text{C}^1\text{H}$, $n = 2$ for $^{13}\text{C}^1\text{H}_2$, and $n = 3$ for $^{13}\text{C}^1\text{H}_3$) during the MAXY unit is [10]

$$
\begin{aligned}
SI_n \quad : \; & +I_z \xrightarrow{90_x^\circ(I)} -I_y \xrightarrow{\pi J_{\text{IS}}\tau(2I_zS_z)} +2I_xS_z \xrightarrow{180_x^\circ(I)90_x^\circ(S)} -2I_xS_y \\
& \xrightarrow{\pi J_{\text{IS}}\tau(2I_zS_z)} -2I_xS_y \xrightarrow{90_x^\circ(I)} \begin{array}{ll} -2I_xS_y & n=1 \\ +4\,I_xI_zS_x & -4I_xI_yS_x & n=2 \\ +8I_xI_zI_zS_y & +8I_xI_yI_yS_y & n=3. \end{array}
\end{aligned} \tag{2}
$$

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As shown in Scheme (2), the MAXY unit generates the maximum-quantum coherences (i.e., double quantum (DQ) coherence of $^{13}C^1H$, triple quantum (TQ) coherence of $^{13}C^1H_2$, and QQ coherence of $^{13}C^1H_3$) and therefore the maximum enhancement of γ_{eff}.

By combining the MAXY unit with the standard Hahn spin-echo based PGSE sequence, Liu et al. [12] and Luo et al. [13] developed the MAXY-D sequence (Fig. 1A) for MQ PGSE experiments. However, because the MAXY-D sequence is based on the Hahn spin-echo sequence [14], it suffers significant signal loss caused by transverse relaxation when long diffusion times (e.g., 500 ms) are employed. In order to minimize the signal loss due to transverse relaxation, which is normally very significant in the diffusion measurements of macromolecules and macromolecular aggregates, an STE-based equivalent of the MAXY-D sequence, MQ-PGSTE (Fig. 1B), was developed by combining the MAXY unit with the standard STE-based PGSE sequence. The new sequence was tested on a sample containing L-[3-^{13}C]-alanine.

2. Materials and methods

L-[3-^{13}C]-alanine (ISOTEC, Matheson, Miamisburg) 4.3 mg was dissolved in 5 mL ^2H_2O (ISOTEC, Matheson, Miamisburg) to give a 9.6 mM L-[3-^{13}C]-alanine solution. 0.3 ml of this solution was dispensed into a magnetic susceptibility-matched (to ^2H_2O) NMR tube (BMS-003, Shigemi, Tokyo).

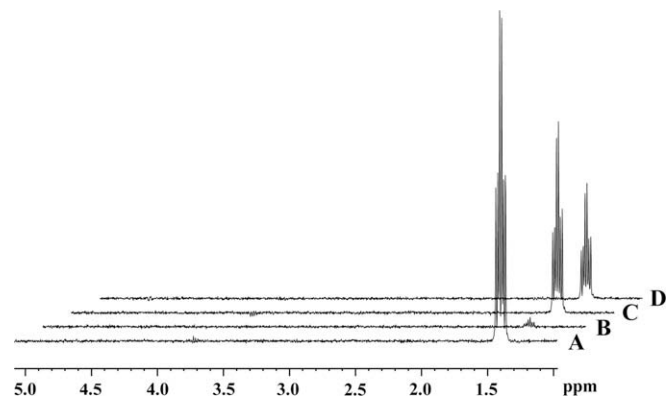^1H PGSE NMR spectra were acquired on a Bruker Avance 500 spectrometer (Karlsruhe, Germany) at 500 MHz using a TXI high resolution probe equipped with a gradient coil at 25 °C. ^{13}C CW decoupling was used during acquisition. Typical acquisition parameters were: spectral width 7 kHz; digitized into 20 K data points; $\pi/2$ pulse length 8–9 μs for ^1H and 19–20 μs for ^{13}C. Diffusion measurements were performed by linearly incrementing g_1 from 0.02 to 0.22 T m^{-1}.

Fig. 2. The ^1H MAXY-D L-[3-^{13}C]-alanine spectra with Δ = 50 ms (A) and 500 ms (B) and the ^1H MQ-PGSTE L-[3-^{13}C]-alanine spectra with Δ = 50 ms (C) and 500 ms (D). g_1 = 0.007 T m^{-1}, g_3/g_2 = 13/4 for selecting the QQ coherence from $^{13}C^1H_3$, and number-of-scans = 64.
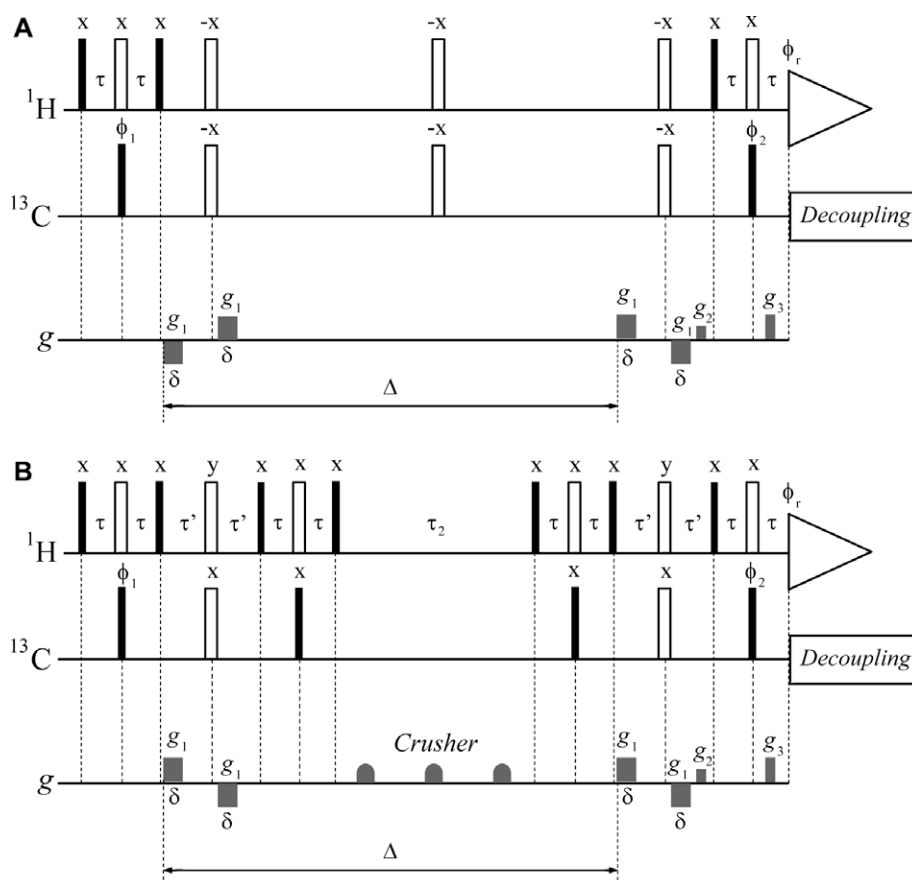

Fig. 1. The MAXY-D (A) and MQ-PGSTE (B) sequences. Black bars, hollow bars, and grey rectangles represent $\pi/2$, π, and gradient pulses, respectively; g_1 is used for diffusion determination and g_2 and g_3 are used for MQ coherence selection with $g_3/g_2 = (\gamma_{13C} + 3\gamma_{1H})/\gamma_{1H} = 13/4$ for $^{13}C^1H_3$, $g_3/g_2 = (\gamma_{13C} + 2\gamma_{1H})/\gamma_{1H} = 9/4$ for $^{13}C^1H_2$, and $g_3/g_2 = (\gamma_{13C} + \gamma_{1H})/\gamma_{1H} = 5/4$ for $^{13}C^1H$. $\tau = 1/(2 J_{13C^1H})$ and τ' is long enough to let eddy currents dissipate. For the MAXY-D sequence, $\phi_1 = x, -x, \phi_2 = x, x, -x, -x$, and $\phi_r = -y, y, y, -y$; for the MQ-PGSTE sequence, $\phi_1 = x, -x, \phi_2 = x, x, -x, -x$, and $\phi_r = -y, y, y, -y$.
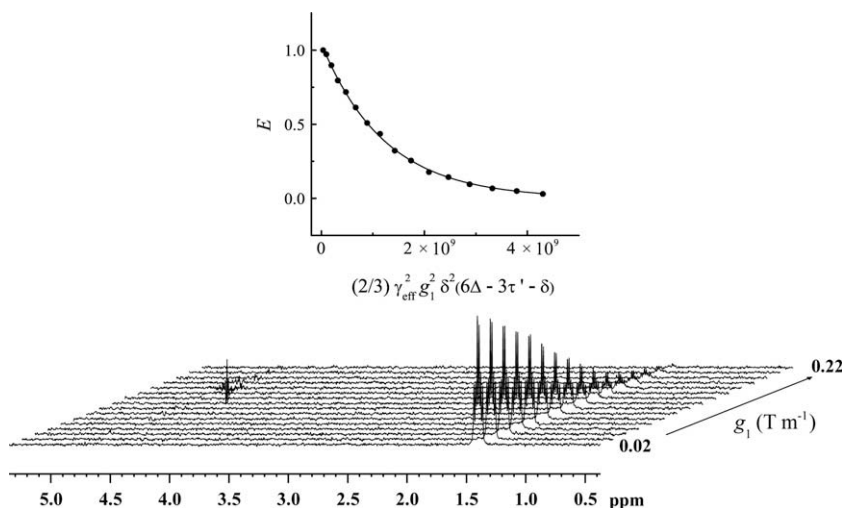
Fig. 3. The ^{1}H MQ-PGSTE L-[3-^{13}C]-alanine spectra with Δ = 30 ms, g_3/g_2 = 13/4, number-of-scans = 16, and different g_1 values. The diffusion decays calculated from the spectra presented are also shown. A small residual HDO signal is evident in some of the spectra and is likely the result of imperfect coherence selection.

Maple 12 (Maplesoft, Waterloo) was used for the Stejskal and Tanner analysis [1] of the new sequence. Origin 8 (OriginLab, Northampton, MA) was used for all diffusion data analysis.

3. Results and discussion

For the MQ-PGSTE sequence (Fig. 1B), the evolution of the product operators [15] after the MAXY unit may be written as

$$
\begin{array}{llll}
SI_1 & : -2I_xS_y & \xrightarrow{g_1-180^\circ_x(I)180^\circ_x(S)-g_1} -2I_xS_y & \xrightarrow{90^\circ_x(I)} -2I_xS_y \\
SI_2 & : -4I_xI_yS_x & +4I_xI_yS_x & +4I_xI_zS_x \\
SI_3 & : +8I_xI_yI_yS_y & +8I_xI_yI_yS_y & +8I_xI_zI_zS_y
\end{array}
$$

$$
\begin{array}{llll}
\xrightarrow{\pi J_{IS}\tau(2I_zS_z)} & \xrightarrow{180^\circ_x(I)90^\circ_x(S)} & \xrightarrow{\pi J_{IS}\tau(2I_zS_z)} & \xrightarrow{90^\circ_x(I)} -I_z \xrightarrow{90^\circ_x(I)} +I_y \\
& & & +I_z \quad\quad -I_y \\
& & & -I_z \quad\quad +I_y
\end{array}
$$

$$
\begin{array}{llll}
\xrightarrow{\pi J_{IS}\tau(2I_zS_z)} & \xrightarrow{180^\circ_x(I)90^\circ_x(S)} & \xrightarrow{\pi J_{IS}\tau(2I_zS_z)} & \xrightarrow{90^\circ_x(I)} +2I_xS_y \\
& & & -4I_yI_yS_x \\
& & & -8I_xI_yI_yS_y
\end{array}
$$

$$
\xrightarrow{g_1-180^\circ_y(I)180^\circ_x(S)-g_1-g_2} \xrightarrow{90^\circ_x(I)-\pi J_{IS}\tau(2I_zS_z)-180^\circ_x(I)90^\circ_x(S)-\pi J_{IS}\tau(2I_zS_z)-g_3} \begin{array}{l} +I_y \\ +I_y \\ +I_y. \end{array}
$$

$$(3)$$

As shown in Scheme (3), MQ terms are generated after the application of the MAXY unit. The $8I_xI_yI_yS_y$ term is dephased by the application of g_1 gradients and only one of the dephased MQ terms is selected and transformed into I_z, which is not susceptible to transverse relaxation, by the use of another MAXY unit, that is the MQ-to-SQ transformation of the dephased MQ term causes signal loss. After the storage period (i.e., τ_2 period), I_z is transformed into dephased $8I_xI_yI_yS_y$, rephased by the application of g_1 gradients, and then transformed into (measurable) I_y.

To demonstrate the S/N ratio enhancement of the MQ-PGSTE sequence, the MAXY-D and MQ-PGSTE experiments were performed with a relatively short diffusion time (i.e., 50 ms) and a relatively long diffusion time (i.e., 500 ms) (Fig. 2). As shown in Fig. 2A and

Fig. 2C, at short diffusion times, the MQ-PGSTE sequence provided a lower S/N ratio than the MAXY-D sequence due to the signal loss by using a stimulated echo and more MQ-to-SQ transformations; as shown in Fig. 2B and D, however, at long diffusion times, the MQ-PGSTE sequence provided a much higher S/N ratio than the MAXY-D sequence due to its much lower susceptibility to transverse relaxation. Therefore, the MQ-PGSTE sequence is preferable for studying macromolecules and macromolecular aggregates, which usually have very short transverse relaxation times. Furthermore, the HDO resonance at 4.8 ppm was completely removed, indicating that the new sequence affords solvent suppression.

The echo attenuation for the MQ-PGSTE sequence can be described by

$$
E = \exp\left(-\frac{2}{3}\gamma_{\text{eff}}^2 g_1^2 \delta^2 D(6\Delta - 3\tau' - \delta)\right), \tag{4}
$$

where γ_{eff} = $\gamma_{^1\text{H}}$ + $\gamma_{^{13}\text{C}}$, 2 $\gamma_{^1\text{H}}$ + $\gamma_{^{13}\text{C}}$, and 3 $\gamma_{^1\text{H}}$ + $\gamma_{^{13}\text{C}}$ for ^{13}C^{1}H, ^{13}C^{1}H$_2$, and ^{13}C^{1}H$_3$, respectively.

A MQ-PGSTE diffusion experiment was performed on the L-[3-^{13}C]-alanine sample (Fig. 3) and the obtained diffusion coefficient (i.e., 8.1 ± 0.1 × 10^{-10} m^2 s^{-1}) is in line with the diffusion coefficient (7.9 ± 0.1 × 10^{-10} m^2 s^{-1}) measured using the standard STE-based PGSE sequence.

4. Conclusions

Due to its lower susceptibility to transverse relaxation, the MQ-PGSTE sequence provides a higher signal-to-noise ratio than the MAXY-D sequence at long diffusion times, and is thus suitable for better diffusion measurements on macromolecule samples. However, both MQ-PGSTE and MAXY-D sequences suffer from significant signal loss due to the MQ-to-SQ transformations of the dephased MQ coherences. We are currently investigating improved MQ-to-SQ transformation strategies.

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