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# Phase modulation in dipolar-coupled $A_2$ spin systems: effect of maximum state mixing in $^1H$ NMR in vivo

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**Abstract**

Coupling constants of nuclear spin systems can be determined from phase modulation of multiplet resonances. Strongly coupled systems such as citrate in prostatic tissue exhibit a more complex modulation than AX connectivities, because of substantial mixing of quantum states. An extreme limit is the coupling of $n$ isochronous spins ($A_n$ system). It is observable only for directly connected spins like the methylene protons of creatine and phosphocreatine which experience residual dipolar coupling in intact muscle tissue in vivo. We will demonstrate that phase modulation of this ''pseudo-strong'' system is quite simple compared to those of AB systems. Theory predicts that the spin-echo experiment yields conditions as in the case of weak interactions, in particular, the phase modulation depends linearly on the line splitting and the echo time.


*Keywords:* Phase modulation; Dipolar $A_2$ system; Creatine(phosphate); Residual dipolar coupling; Muscle tissue

## 1. Introduction

In one-dimensional NMR spectra, the dipolar coupling strength of isochronous spins systems manifests only in a line splitting. Alternatively, it can be determined from a series of PRESS (point-resolved spectroscopy) spectra acquired with different echo times, because the coupled system exhibits phase modulation, which can be observed in experiments where a series of 180° pulses is irradiated.

Dipolar $A_2$ systems are characterized by symmetrized eigenfunctions [1] which correspond to a state mixing that exceeds that of strongly coupled AB systems. It is well-known that scalar-coupled AB systems exhibit a complex phase modulation. Our purpose was to delineate the differences between the strong mixing for $J$-coupling and the extreme limit for dipolar-coupled systems. The latter are also called ''pseudo-strong'' systems because despite the strong mixing, the coupling represents a weak perturbation compared to the effect of the static field $B_0$ and the dependence of the energy levels on $B_0$ (displayed in Breit–Rabi diagrams) is simplified compared to systems with a slightly reduced mixing [1].

Spin–spin couplings observed in conventional in vivo NMR spectroscopy are always weak compared to the interaction of magnetic moments with the static magnetic field. The criterion to estimate the coupling strength of a spin system is the ratio $p = K/(\nu_1 - \nu_2)$ of coupling constant $K$ and chemical-shift difference $\nu_1 - \nu_2$ ($\nu_1 > \nu_2$). This quantity is also known as perturbation parameter; $K$ is either the scalar or dipolar coupling constant. Small $p$ means weak coupling which is characterized by the ''uncoupled base'' $|m_I,m_S\rangle$ and ''first-order'' spectra. This case is comparable to the

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Paschen-Back effect of the hydrogen hyperfine interaction in a strong external field (i.e., each spin is coupled individually to the external field). In contrast, large $p$ corresponds to conditions of the (internuclear) Zeeman effect which implies the formation of a total spin using the "coupled base" $|F,m_F\rangle$ (like the case of the $^1$H hyperfine interaction in zero-field experiments) and leads to more complex "second-order" spectra.

In vivo proton NMR spectroscopy in conventional whole-body MR scanners faces conditions where the Zeeman splitting caused by the external field is the dominant effect. However, because of the narrow range of chemical shifts of $^1$H and the low magnetic field ($B_0 = 1.5\,\mathrm{T}$), the chemical-shift difference ($\nu_1 - \nu_2$) can be small compared to the coupling constant and decoupling of the spin system by the external field is incomplete.

A biomolecule detected by in vivo $^1$H NMR, where this phenomenon of strong state mixing occurs, is citrate (Cit). The line splitting is about twice the chemical-shift difference at 1.5 T (15.1 Hz vs. 7.83 Hz, [2]). The quantum-mechanical properties of the $(AB)_2$ system as a model of the protons in Cit have been studied to explain the unconventional phase modulation observed for the two doublets [2,3]. It differs from the simple, i.e., linearly with echo time increasing phase modulation of weakly coupled resonances (e.g., the $AX_3$ system of lactate). The two intense resonances of the Cit multiplet exhibit a slower and more complex modulation than the two weaker ones.

The spin system we consider is that of the endogenous metabolite creatine (Cr; and phosphocreatine PCr) whose methylene protons ($\delta = 3.9\,\mathrm{ppm}$ relative to TMS at $\delta = 0\,\mathrm{ppm}$) are affected by residual dipolar couplings in living skeletal muscle [4]. The two isochronous protons form an $A_2$ system in the limit $p \to \infty$ with maximum state mixing. For this pseudo-strong interaction, the vanishing chemical-shift difference evokes a quasi unrealistic condition, i.e., the case of an infinite strong coupling.

After derivation of a suitable model for the $A_2$ phase modulation occuring during a PRESS pulse sequence, experimental data are presented that allow determination of the observable (P)Cr–$CH_2$ coupling constant. This is of particular interest in in vivo NMR spectroscopy since it is directly related to the degree of immobilization of this intramyocellular metabolite (see also [5]).

## 2. Theory

An appropriate set of eigenfunctions for a weakly coupled AX system (spins $I$, $S$) are the four product states $|m_I,m_S\rangle$. When the perturbation parameter increases, this eigenbase must be transformed by the following rotational matrix

$$\mathbf{U} = \begin{pmatrix} 1 & 0 & 0 & 0 \\ 0 & \cos\alpha & \sin\alpha & 0 \\ 0 & -\sin\alpha & \cos\alpha & 0 \\ 0 & 0 & 0 & 1 \end{pmatrix}, \tag{1}$$

which tilts the two-dimensional subspace with $m_I + m_S = 0$. The trigonometric functions can be interpreted as Clebsch-Gordan coefficients where the rotational angle $\alpha$ is characteristic for the spin system ($\alpha = \pi/4$ yields the well-known values for the case of the hydrogen hyperfine interaction). In particular, $\alpha$ is related to the perturbation parameter:

$$-\tan 2\alpha = \frac{J_{12}}{\nu_1 - \nu_2} \quad \text{for scalar coupling,} \tag{2}$$

$$2\tan 2\alpha = \frac{\Delta\nu}{\nu_1 - \nu_2} \quad \text{for dipolar coupling.} \tag{3}$$

The dipolar line splitting for isochronous spins is

$$\Delta\nu = 1.5 \times D_{\mathrm{obs}}(3\cos^2\theta - 1), \tag{4}$$

where $D_{\mathrm{obs}} = SD_0$ is the dipolar coupling constant scaled by the order parameter $S$ that reflects molecular reorientation and $\theta$ is the preferred orientation of the internuclear vector, $\langle\vec{r}_{ij}\rangle$, with respect to $\vec{B}_0$ (anisochronous dipolar systems exhibit different line splittings, [1]). The transformation creates a linear combination of the initial product states for two of the new eigenfunctions. This transformation must be also applied to the operator $\mathbf{R}_\pi$ representing the $\pi$ pulse in the spin echo experiment. A common notation for the effects of a $\pi_x$ pulse on uncoupled spins is $|+\rangle \xrightarrow{\pi_x} \mathrm{i}\,|-\rangle$ and $|-\rangle \xrightarrow{\pi_x} \mathrm{i}\,|+\rangle$. Using the set of product states, this type of spin-flip operator can be represented by

$$\mathbf{R}_\pi = \begin{pmatrix} 0 & 0 & 0 & -1 \\ 0 & 0 & -1 & 0 \\ 0 & -1 & 0 & 0 \\ -1 & 0 & 0 & 0 \end{pmatrix} \tag{5}$$

and the rotation of the two-dimensional subspace yields

$$\mathbf{R}_\pi^{(r)} = \mathbf{U}^{-1}\mathbf{R}_\pi\mathbf{U} = \begin{pmatrix} 0 & 0 & 0 & -1 \\ 0 & \sin 2\alpha & -\cos 2\alpha & 0 \\ 0 & -\cos 2\alpha & -\sin 2\alpha & 0 \\ -1 & 0 & 0 & 0 \end{pmatrix}. \tag{6}$$

According to [6], the four transitions after the inversion pulse can be expressed by

$$|t\rangle\langle u| \xrightarrow{R_x} \sum_{r,s} R_{rs\,tu}\,|r\rangle\langle s| \tag{7}$$

using the transfer coefficients

$$R_{rs\,tu} = (R_\pi^{(r)})_{rt}(R_\pi^{(r)})^*_{su}. \tag{8}$$

A two-spin system exhibits four directly detectable transitions (listed in Table 1 with notations a, ..., d). Applying Eq. (7) to these transitions, we obtain for the transformation under the inversion pulse:

$$\begin{aligned}
|1\rangle\langle 2| &\xrightarrow{\pi_x} \cos 2\alpha|4\rangle\langle 3| - \sin 2\alpha|4\rangle\langle 2|,\\
|3\rangle\langle 4| &\xrightarrow{\pi_x} \cos 2\alpha|2\rangle\langle 1| + \sin 2\alpha|3\rangle\langle 1|,\\
|1\rangle\langle 3| &\xrightarrow{\pi_x} \cos 2\alpha|4\rangle\langle 2| + \sin 2\alpha|4\rangle\langle 3|,\\
|2\rangle\langle 4| &\xrightarrow{\pi_x} \cos 2\alpha|3\rangle\langle 1| - \sin 2\alpha|2\rangle\langle 1|.
\end{aligned} \tag{9}$$

The consequence of transformation **U** as manifested in Eq. (9) is a modified phase modulation, because of different frequencies that contribute to the evolution. The transitions of an AX system ($\alpha \approx 0°$) simply transform into the complementary doublet component (a ↔ b and c ↔ d). With increasing coupling strength, each resonance comprises a growing fraction of a transition of the other doublet Eq. (9). The consequences are illustrated in Fig. 1 and are explained as follows: Fig. 1A shows the case of an AX system. Each doublet consists of a fast and a slowly precessing ensemble, thus accumulating a phase difference during $TE/2$ determined by $J_{12}$. Application of the inversion pulse exchanges the frequencies (configuration at $t = TE/2 + \delta$). Hence, one vector is retarded and the other advanced relative to the uncoupled spins during echo formation at $t = TE$. The accumulated phase differences of the two resonances before and after the pulse are identical because the frequency difference of the two vectors depends on $J_{12}$. Therefore the phase evolves linearly with the product of time and coupling constant. This is also valid for further evolution prior to the second echo formation performing PRESS and the result is the well known relation for the phase modulation observed in a double spin-echo experiment, e.g., for the (scalar-coupled) lactate doublet:

$$\phi = 180° \times J_{12} \times TE. \tag{10}$$

The effect of an increasing perturbation parameter (as it occurs, e.g., for Cit) is shown in Fig. 1B. The evolution until $t = TE/2$ is the same. But the additional connectivity with the other doublet has different consequences for the two resonances. Consider doublet 1 with resonances a and b. Transition a is not only transferred into b, but is also connected to d. Both are of lower frequency and the accumulated phase of the downfield resonance relative to uncoupled spins is larger compared to the AX system. The upfield resonance of doublet 1 (b) is now connected to a and c. Hence, it is connected to faster and slower precessing ensembles and the phase accumulation is "decelerated."

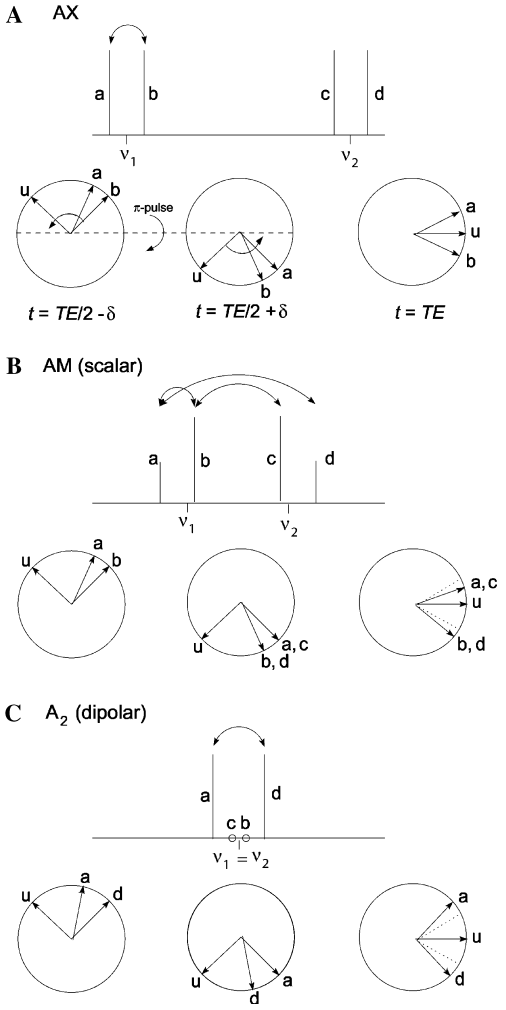


Fig. 1. Effects of an inversion pulse on two-spin systems of different coupling strength. The resonances are denoted according to Table 1, uncoupled spins are labelled with "u:" The first column of vector diagrams shows the situation just before irradiation of the pulse ($t = TE/2 - \delta$), the second the configuration immediately after the pulse ($t = TE/2 + \delta$), and the last column indicates the accumulated phases of the resonances during echo formation at $t = TE$. Connectivities following from Eq. (9) are indicated with curved arrows in the spectra. (A) The case of a weakly coupled AX system. The resonances of each doublet accumulate phases $+\phi$ and $-\phi$ relative to the uncoupled signals. (B) The changing connectivities—indicated by the letters at each vector—cause a retarded modulation of the inner resonances (b and c), while the phase of the outer resonances (b and d) still evolves rapidly. (C) The evolution of a dipolar $A_2$ system resembles that in (A).

Table 1
Effect of state mixing on intensities of the four single-quantum transitions of an AB system

| Transition | Intensity | |
|---|---|---|
| | Scalar AB | Dipolar $A_2$ |
| a: $\|2\rangle^{\dagger} \leftrightarrow \|4\rangle$ | Decreases | Doubles |
| b: $\|1\rangle \leftrightarrow \|3\rangle^{\ddagger}$ | Increases | Vanishes |
| c: $\|3\rangle^{\ddagger} \leftrightarrow \|4\rangle$ | Increases | Vanishes |
| d: $\|1\rangle \leftrightarrow \|2\rangle^{\dagger}$ | Decreases | Doubles |

Considering energy level $|1\rangle = |{+}{+}\rangle$ as the lowest and level $|4\rangle = |{-}{-}\rangle$ as the highest one, the letters a...d denote the transitions in the order of increasing frequency. The eigenstates that evolve to the singlet state of $A_2$ systems are marked with $^{\dagger}$ for scalar and $^{\ddagger}$ for dipolar coupling. In contrast to the case of strong scalar coupling, only two transitions remain in the case of a pseudo-strong dipolar interaction.

Likewise, the downfield resonance of doublet 2 (c) accumulates a smaller phase than the upfield one. Fig. 1B illustrates also the consequences of partial suppression of transitions between singlet and triplet states which will be totally forbidden for maximum mixing, i.e., $\alpha = -45°$. The singlet state arises from the former product state 2. Hence, transitions d and a have reduced, b and c increased intensity [1].

Using PRESS for localization, the second inversion pulse causes mutual contributions of all four resonances. In summary, the two intense inner resonances are connected to higher and lower frequencies, thus showing a retarded phase modulation; the small outer resonances are connected either to lower (a) or higher frequencies (d) and therefore undergo fast phase modulation. The specific circumstances for Cit at 1.5 T cause a fast evolution for the small resonances according to Eq. (10), while the main signals are always positive for $TE < 300$ ms as observed in experiments on model solutions and in vivo [2,3,7].

Finally, we consider the dipolar $A_2$ system ($\alpha = 45°$) illustrated in Fig. 1C. Transformation **U** yields symmetric eigenfunctions which can be combined to a triplet state with total spin $F = 1$ and a antisymmetric singlet state with total spin $F = 0$. The appropriate eigenbase is spanned by the $|F,m_F\rangle$ states. Because of the positive mixing angle, the triplet state of isochronous dipolar spins arises from the former product state $|-+\rangle$ and the resonances b and c vanish due to forbidden intercombination [1]. Eq. (9) implies that the remaining resonance of each doublet is now connected completely to the complementary one. A dipolar $A_2$ system exhibits two resonances with a frequency difference given by Eq. (4). Their frequencies are exchanged after the inversion pulse, thus the progress of the phase modulation is constant and resembles the behaviour of each resolved doublet of the AX system. The relation for the $A_2$ system is therefore in the case of a double-spin echo experiment

$$\pm\phi[^\circ] = \pm 180^\circ \Delta\nu(TE_1 + TE_2) + \beta. \qquad (11)$$

The additive parameter $\beta$ vanishes in the case of correct global phasing of the spectrum. Below, it is included in the fit of the experimental values in order to recognize any phase correction. Precise determination of $\Delta\nu$ with use of Eq. (11) can slightly differ from the visible peak-to-peak distance in the spectrum which was employed so far for the measurement of this parameter [8].

## 3. Experimental

The experimental setup is described in detail in the adjacent contribution of this issue [5]. Therefore, only the features important for the present study are depicted here.

A clinical whole-body MR scanner (operating at 63.63 MHz) with standard equipment for examinations of the lower human leg was employed. Spectra were acquired from ($2\,cm^3$) voxels in m. gastrocnemius from two male healthy volunteers. Before, informed consent according to procedures approved by the Institutional Review Board was obtained from the volunteers. The calf was oriented parallel to the $z$-axis of the magnet in order to minimize inclination of the muscle fibres with respect to the direction of the magnetic induction $\vec{B}_0$.

The residual dipolar coupling constant of the (P)Cr methylene protons was determined by means of a PRESS experiment. As an alternative to the measurement of the orientation-dependent line splitting Eq. (4) we employed the phase modulation of the doublet upon variation of $TE$ Eq. (11). Spectra were acquired with different $TE$ in the range of 36–146 ms and repetition time $TR = 2$ s, 1024 ms complex-data accumulation and 150 excitations each. Water signal suppression was achieved with a series of four 25.6-ms Gaussian-shaped chemical-shift selective (CHESS) rf pulses with different flip angles.

The phase of the (P)Cr doublet had to be adjusted manually by comparing the measured phase with that of the resonance fit. An error was estimated by varying this parameter to a certain degree.

Kreis et al. employed the (P)Cr methylene doublet to determine the orientation dependence of $D_{obs}$ by means of the apparent line positions. Conversely, the fibre orientation $\theta$ can be estimated from Eq. (4) by inserting the known value of the dipolar coupling constant $SD_0 = 4.92$ Hz [8] and the peak-to-peak distance of the doublet ($\Delta\nu_{pp}$) in the spectrum obtained with short echo time where phase modulation is insignificant (Fig. 2:

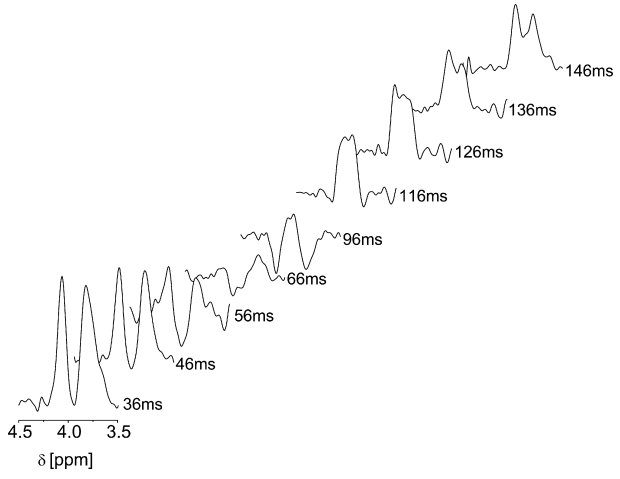


Fig. 2. Phase modulation of the dipolar-coupled methylene doublet of creatine/phosphocreatine ((P)Cr) centered at $\delta = 3.9$ ppm in localized water-suppressed in vivo 1.5-T $^1$H NMR spectra of m. gastrocnemius of a 26-year-old volunteer (study #1, Table 2) obtained with echo times $TE$ in the range of 36–146 ms. Experimental technique: PRESS with $TR = 2$ s, NEX = 150, voxel size = ($2\,cm^3$).

$TE$ = 36 ms); this method was proved to be reliable in other experiments [1].

Origin 6.1 (OriginLab, Northampton, MA, USA) was employed to display the data and to determine pace of phase modulation Eq. (11).

## 4. Results

The expected linear phase modulation of the (P)Cr methylene peak was clearly seen in vivo and a complete cycle of inversion and recovery of the doublet could be observed in examinations of two volunteers (Fig. 2). Severe perturbation of the signal owing to imperfect water-signal suppression (at $\delta = 4.7$ ppm) was not noticed. The evaluated phases (relative to the unmodulated signals) together with linear fits are shown in Fig. 3, the fit results are given in Table 2. In the second volunteer examination the measured line splitting ($\Delta\nu_{pp}$) for $TE = 36$ ms slightly exceeded the maximum splitting of $3 \times 4.92$ Hz; therefore $\theta = 0$ was assumed in this case.

These values of $\theta$ then allowed correction of the observed coupling strengths $\Delta\nu$ with the orientation-dependent term $1.5(3\cos^2\theta - 1)$ to obtain the coupling constant $D_{obs} = SD_0$. The two experiments yielded an average $D_{obs} = (6.3 \pm 0.1)$ Hz (Table 2), which is consistent with the value of 5.6 Hz obtained in zero-quantum (ZQ) evolution studies [4].

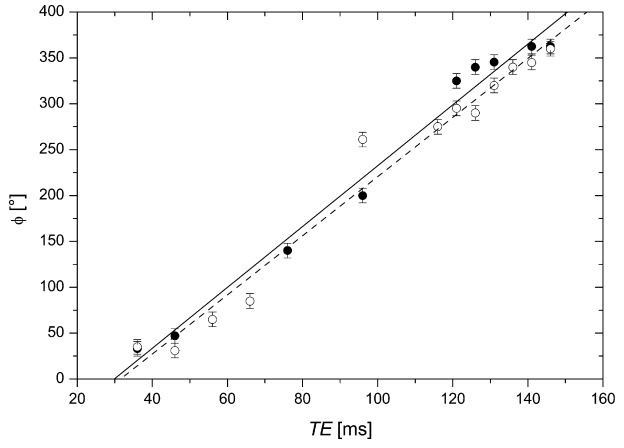


Fig. 3. Phase angle of the in vivo (P)Cr methylene doublet as a function of echo time $TE$ from examinations of two volunteers. Study #1 ($\phi(TE)$ from evaluation of the spectra in Fig. 2): (○) linear fit (- - -), Eq. (11) (correlation coefficient, $R = 0.987$); study #2: (●) linear fit (—) ($R = 0.992$). Fit results are given in Table 2.

Table 2
Spectral parameters including the observed dipolar coupling constant $D_{obs}$ of methylene protons of creatine and phosphocreatine ((P)Cr) from $^1$H NMR phase-modulation experiments in two volunteers

| Study # | $\Delta\nu$ (Hz) | $\beta$ (°) | $\Delta\nu_{pp}$ (Hz) | $\theta$ (°) | $D_{obs}$ (Hz) |
|---|---|---|---|---|---|
| 1[a] | 17.90 ± 0.34 | (101 ± 7) | 13.6 | 13.2 | 6.5 ± 0.2 |
| 2 | 18.42 ± 0.38 | (99 ± 8) | 15.2 | 0 | 6.1 ± 0.1 |
| Mean | | | | | 6.3 ± 0.1 |

*Note.* $-\Delta\nu$ and $\beta$ by fit of observed phase modulations using Eq. (11); fiber orientation $\theta$ from the observed frequency difference (peak-to-peak) $\Delta\nu_{pp}$; $D_{obs}$ from $\Delta\nu$ by correction with $1.5(3\cos^2\theta - 1)$.

[a] Corresponding series of spectra displayed in Fig. 2.

## 5. Discussion

The derived model of linear phase modulation $\phi(TE)$ for a dipolar-coupled $A_2$ system was clearly confirmed by the experiment and demonstrates a quite simple evolution despite the strong mixing of states. The correlation coefficient of the fit of Eq. (11) to the data was $R \approx 0.99$ for both datasets (Fig. 3). Since linear regression analysis uses only two parameters, a reliable evaluation of the coupling strength at a given orientation $\theta$ is possible and the method could be employed to expand the knowledge of residual dipolar couplings in living muscle tissue.

The phase modulation of the (P)Cr methylene doublet upon variation of $TE$ yields coupling strengths consistent with those derived from resolved line splittings and the zero-quantum evolution period (ca. 120 ms, corresponding to a splitting of 16.7 Hz) for an orientation of the calf muscle parallel to $\vec{B}_0$ [4]. However, the evaluation of the line splitting $\Delta\nu_{pp}$ yields a somewhat *smaller* value than the phase modulation method (e.g., 13.6 Hz vs. 17.9 Hz in study #1, Table 2 and Fig. 2). Actually, we cannot explain this unexpected difference. Considering only the line positions in spectra obtained with long $TE$ without taking phase evolution into account could lead to a systematic error since the accumulated phase distorts first the central dip of the doublet. This effect would *enlarge* the peak-to-peak distance. Monitoring of phase modulation is time-consuming, but may allow determination of the coupling strength with higher precision.

There are two obvious advantages of the phase-modulation method in comparison to the experiment where the lower leg is tilted in subsequent steps: the VOI does not change during the study thus providing constant shim and tissue conditions (only one shim adjustment is required) and, of course, it is more convenient for the volunteer to keep the calf always in straight posture.

At first sight, neglect of the angular dependence seems to be inappropriate since considering the spatial anisotropy permits discrimination of dipolar and scalar coupling and $\Delta\nu(\theta)$ might contain information about the relation between the laboratory frame and the geometry of the molecule, in particular, the preferred orientation of $\langle\vec{r}_{ij}\rangle$. However, an Euler transformation describing the transition from the molecular to the laboratory frame was found to be not necessary [8]. Since interactions with the anisotropic muscle structure are suspected to cause the restrictions in molecular reorientation, it is not surprising that the fibre structure reflects the only

preferred orientation which is detectable and which determines $\langle \vec{r}_{ij} \rangle$. Additionally, parallel orientation of the leg with respect to $\vec{B}_0$ yields the maximum observable coupling strength in good approximation since the $\cos^2$ function is quite insensitive to variations of $\theta$ in this regime. Therefore, the calculated coupling strength suffices to estimate the degree of restriction of molecular reorientation by means of the order parameter $S$. In an accompanying paper we discuss the concept of a common director in more detail for other dipolar-coupled systems [5].

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