Curious Consequences of Strong Coupling in NMR Experiments Involving Selective Pulses

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This study is concerned with the effects of applying selective pulses to systems with strong second-order scalar couplings in isotropic phase, where different transitions (rs) are associated with different transition matrix elements $F_{ss'}$. Two unusual features can be distinguished: the nutation angle ("flip angle") depends on the matrix element of the irradiated transition (rs), and, in contrast to the behavior of an isolated spin-$\frac{1}{2}$ system, the norm of the three single-transition operators $[I_{1}^{(rs)}, I_{2}^{(rs)}, I_{3}^{(rs)}]$ associated with the fictitious spin-$\frac{1}{2}$ space of the irradiated transition (rs) is generally not conserved. It is necessary to consider the single-transition operators $[I_{x}^{(rs)}, I_{y}^{(rs)}, I_{z}^{(rs)}]$ and $[I_{x}^{(rp)}, I_{y}^{(rp)}, I_{z}^{(rp)}]$ associated with all connected transitions (rp) and (sq) that share a common energy level r or s with the irradiated transition (rs). If the pulse applied to the (rs) transition is sufficiently selective, the transverse components $I_{x}^{(rs)}$, $I_{y}^{(rs)}$, $I_{z}^{(rs)}$, and $I_{x}^{(rp)}$ can be neglected, since their expectation values remain equal to zero after application of a selective pulse to the (rs) transition, but the longitudinal components $I_{x}^{(rp)}$ and $I_{x}^{(rs)}$ acquire nonvanishing expectation values. When the selective pulse affects several transitions simultaneously, the response varies from one transition to another, depending on the matrix elements and the connectivities. These effects manifest themselves in unusual amplitudes and phases of signals excited by selective pulses, in particular in selective two-dimensional correlation spectra.

INTRODUCTION

Second-order effects due to strong scalar coupling in isotropic phase have received comparatively little attention in recent years. This may be ascribed to at least three reasons: (i) the increasingly widespread use of very high magnetic fields, (ii) the formal analysis of strongly coupled systems is often cumbersome because of the failure of simple product-operator formalisms, and (iii) in practical applications of two-dimensional spectroscopy, it is often assumed that strongly coupled pairs of spins merely lead to cross peaks that are close to the diagonal, and it is often believed that such features need not be taken into account. In actual fact, none of these factors constitute a sufficient reason for neglecting second-order effects: (i) even at very high static fields (750 MHz and beyond), nearly degenerate spin pairs (AB systems) are common in proteins and nucleic acids, particularly for diastereotopic protons, (ii) although the density operator representing a strongly coupled system cannot be easily expanded in product operators, single-transition operators are quite suitable, and tools for numerical simulations such as the GAMMA program (I) are readily available, and (iii) strong coupling can have dramatic effects on multiplets that are far from the diagonal, even in simple cases such as ABX systems.

Second-order effects have recently been encountered in an unexpected context. Several research groups have advocated the use of selective two-dimensional correlation ("soft-COSY") methods (2–7) to record individual cross-peak multiplets. So far, it has generally been assumed that such multiplets would have similar patterns as multiplets observed in nonselective correlation spectra obtained with conventional COSY (8) with a mixing pulse with a small nutation angle $0 < \beta \ll 90^\circ$ (9, 10). It was believed that selective methods would simply yield better digital resolution, while allowing one to record the relevant information in a shorter minimum time, insofar as sensitivity requirements do not compel one to record a large number of transients. However, these assumptions are not warranted in strongly coupled systems, where multiplets recorded with soft-COSY and related methods may suffer severe anomalies in phase and amplitude. In this paper, we shall explain the origin of these anomalies.
FIG. 1. Conventional 400 MHz proton NMR spectrum of 4-ethenyl-3-hydroxy-5-iodomethyl-3-methyl-dihydro-2(3H) furanone in CDCl₃ at 295 K, excited by a single nonselective 90° pulse of 11 μs duration with an RF amplitude of 22 kHz. Expansions are shown for the Hₐ−H₈ region and for the multiplets of spins H₇ and H₈. The latter multiplet has a width of 16.2 Hz; the other expansions are plotted on the same scale. The spectrum was recorded with a Bruker ARX 400 spectrometer.

EXPERIMENTAL EVIDENCE

We have chosen a sample of 4-ethenyl-3-hydroxy-5-iodomethyl-3-methyl-dihydro-2(3H) furanone (I) to illustrate the anomalies in phase and amplitude due to second-order effects. Figure 1 shows the conventional one-dimensional ¹H NMR spectrum excited by a “hard” 90° pulse, with expanded multiplets belonging to the HₐH₈H₇H₈ subsystem.

Figure 2 shows a multiplet enlarged from a normal DQF-COSY and Fig. 3 shows the corresponding soft-COSY multiplet. Both are due to coherence transfer between the strongly coupled HₐH₈ subsystem and spin H₇. The multiplet structure in Fig. 2 is composed of a superposition of squares, each spanned by two positive and two negative peaks, separated by the active coupling constants. Note that some of the amplitudes in Fig. 2 do not fulfill ideal C₂ symmetry with respect to the center of the multiplet, presumably in part because of second-order effects (11) and in part because the mixing pulses in the DQF-COSY sequence were not properly calibrated to β = 90°. No phase anomalies can be seen in the rows and columns extracted from the DQF-COSY spectrum in Fig. 2. By contrast, the corresponding soft-COSY multiplet in Fig. 3 suffers from severe anomalies of the phases and amplitudes of the individual peaks. At first sight, one might be tempted to incriminate imperfections of the truncated 270° Gaussian G¹ pulses used for recording the soft-COSY multiplet. Indeed, the self-refocusing properties of these pulses are imperfect, and they may lead to substantial phase dispersion across their excitation bandwidth. However, the G¹ pulses used for Fig. 3 had durations of 20 ms, which is sufficient to excite widths of ±20 Hz with a phase dispersion of less than ±20°. It can easily be verified that the same pulses lead to well-behaved, pure absorptive multiplets if the “frequency window” is shifted to coincide with a weakly coupled mult-
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If we consider the motion of a semiclassical magnetization vector \( M_{rs} \) associated with the fictitious spin-1/2 space of the irradiated transition \((rs)\), with three components \( M_{rs}^{x} \), \( M_{rs}^{y} \), and \( M_{rs}^{z} \) that are proportional to the expectation values of the three single-transition operators \( I_{rs}^{x} \), \( I_{rs}^{y} \), and \( I_{rs}^{z} \) \((13, 14)\), we may consider the nutation angle ("flip angle") \( \beta_{rs} \) experienced by the vector \( M_{rs} \) during a pulse of duration \( \tau \), i.e., the angle subtended between the vectors \( M_{rs}^{(\tau)}(\tau_-) \) and \( M_{rs}^{(\tau)}(\tau_+) \) before and after the pulse. If all transitions in the system are simultaneously affected by a nonselective pulse of amplitude \( \gamma B_{1} \) and duration \( \tau_{p} \), the nutation angle is the same for all transitions \((rs)\) in the system:

\[
\beta_{rs} = -\gamma B_{1} \tau_{p} \quad \text{for all} \ (rs).
\]

However, if a selective pulse with a time-dependent RF

We consider the effect of a selective pulse such as a 270° Gaussian truncated at 2.5% \((12)\). This pulse will be applied to a strongly coupled spin system, where the different transitions \((rs)\) are associated with different transition matrix elements \( F_{rs} \). In conventional one-dimensional spectra, the lines have unequal intensities ("roof effect" in AB systems). If we consider the motion of a semiclassical magnetization vector \( M_{rs}^{(\tau)} \) associated with the fictitious spin-1/2 space of the irradiated transition \((rs)\), with three components \( M_{rs}^{x(\tau)} \), \( M_{rs}^{y(\tau)} \), and \( M_{rs}^{z(\tau)} \) that are proportional to the expectation values of the three single-transition operators \( I_{rs}^{x(\tau)} \), \( I_{rs}^{y(\tau)} \), and \( I_{rs}^{z(\tau)} \) \((13, 14)\), we may consider the nutation angle ("flip angle") \( \beta_{rs}^{(\tau)} \) experienced by the vector \( M_{rs}^{(\tau)} \) during a pulse of duration \( \tau \), i.e., the angle subtended between the vectors \( M_{rs}^{(\tau)}(\tau_-) \) and \( M_{rs}^{(\tau)}(\tau_+) \) before and after the pulse. If all transitions in the system are simultaneously affected by a nonselective pulse of amplitude \( \gamma B_{1} \) and duration \( \tau_{p} \), the nutation angle is the same for all transitions \((rs)\) in the system:

\[
\beta_{rs}^{(\tau)} = -\gamma B_{1} \tau_{p} \quad \text{for all} \ (rs).
\]

However, if a selective pulse with a time-dependent RF
amplitude of duration $\tau_p$ is applied, the nutation angle depends on the matrix element:

$$\beta^{(rs)} = -\gamma [F^{+}_\nu] \int_0^\tau B_1(\tau)d\tau. \quad [2]$$

Provided that the pulse is truly selective, i.e., provided that the RF amplitude is too weak to affect any of the other transitions, one expects the norm of the three single-transition operators associated with the fictitious spin-$\frac{1}{2}$ space of the irradiated transition to be conserved,

$$n^{(rs)} = \{ \langle I_z^{(rs)} \rangle^2 + \langle I_y^{(rs)} \rangle^2 + \langle I_x^{(rs)} \rangle^2 \}^{1/2}. \quad [3]$$

in analogy to the behavior of an isolated spin-$\frac{1}{2}$ system. Even for a truly selective irradiation, however, it is important to remember that there is an effect on the longitudinal components $I_z^{(rp)}$ and $I_z^{(sq)}$ associated with connected transitions ($rp$) and ($sq$) that share common energy levels $r$ and $s$ with the irradiated transition ($rs$).

The situation is quite different if the shaped pulse is not truly selective, i.e., if it is strong enough to affect other connected transitions in the system. In this case, one must also consider the transverse components $I_y^{(rp)}$, $I_y^{(sq)}$, and $I_y^{(st)}$ that are associated with connected transitions ($rp$) and ($sq$).

**SIMULATIONS OF TRAJECTORIES**

In order to visualize the effects of selective pulses on an AB spin system, we simulated the trajectories of the single-transition operators of the four fictitious spin-$\frac{1}{2}$ spaces of the allowed transitions with the help of the GAMMA program. Figure 4a shows the simulated one-dimensional spectrum of an AB system for $\Delta \Omega/2\pi = 20$ Hz and $J = 10$ Hz. It is instructive to compare the behavior of a strongly coupled AB system with a weakly coupled AMX system that possesses four transitions with the same resonance frequencies. The labeling of the A transitions of the AMX system used for this comparison is shown in Fig. 4b.

Figure 5 shows the evolution in the four single-transition spaces of the AB system during a very selective $G^1$ Gaussian 270° pulse with a duration $\tau_p = 500$ ms and a peak amplitude $\gamma B_1^{\max}/2\pi = 3.25$ Hz, truncated at 2.5%, and applied to the ‘‘inner’’ transition (13) along the $y$ axis of the rotating frame. The RF amplitude was calibrated on an isolated spin-$\frac{1}{2}$ system to yield a ‘‘nominal’’ 270° nutation angle. The unusual length of the pulse (which would hardly be practical because of relaxation) was chosen for the sake of clarity to ensure that only a single line of the AB system was affected. The upper part of the Fig. 5 shows so-called ‘‘grapefruit’’ diagrams, where the axes are spanned by the Cartesian components of the single-transition operators, e.g., $I_{z}^{(12)}$, $I_{y}^{(12)}$, and $I_{x}^{(12)}$ in the first diagram to the left. The time-dependence of the norm $n^{(rs)}$ and the expectation values of the single-transition operators $I_z^{(rs)}$, $I_y^{(rs)}$, and $I_x^{(rs)}$ are drawn in the lower part. Figure 5 shows the trajectories when one of the strong inner transitions of the AB system is irradiated, while Fig. 6 shows trajectories when the selective pulse is applied to a weak outer transition of the AB system.

Note that the thick curves that represent the trajectories of the tips of the vectors do not necessarily remain on the surface of the globes. In Fig. 5, the transitions (12) and (34) that share a common energy level with the irradiated transition show a significant violation of the conservation of the norms $n^{(12)}$ and $n^{(34)}$. Even the norm of the single-transition operator $I_z^{(13)}$ is not perfectly conserved as one would expect for an isolated spin-$\frac{1}{2}$ system. Most importantly, there is a ‘‘overshoot’’ of the magnetization vector of the irradiated transition in Fig. 5, so that the effective nutation angle...
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FIG. 5. Trajectories of the four vectors with components \( (I^{(rs)})_x \), \( (I^{(rs)})_y \), and \( (I^{(rs)})_z \), associated with the four single-quantum transitions of the AB system of Fig. 4a. The wavy arrow indicates the transition that is irradiated, which is one of the strong “inner” transitions in this case. A very selective \( G^1 \) Gaussian pulse with a duration \( \tau_p = 500 \) ms and a peak RF amplitude \( \gamma B_1^\max = 3.25 \) Hz is applied to the (13) transition along the \( +y \) axis. The nominal nutation angle (i.e., the angle which would be observed if the pulse were applied on-resonance to an isolated spin-1/2 system) is 270°, but the effective nutation angle of \( I^{(13)} \) approaches 342°. The graphs at the bottom show the time dependence of the norms \( (I^{(rs)})_x \), \( (I^{(rs)})_y \), and \( (I^{(rs)})_z \) (heavy continuous lines) and of the individual components \( (I^{(rs)})_x \), \( (I^{(rs)})_y \), and \( (I^{(rs)})_z \) (dash-dotted, dashed, and thin continuous lines, respectively) in the course of the \( G^1 \) Gaussian pulse. Note that the norm of the (24) transition, which is parallel (and therefore not connected) to the irradiated (13) transition, is barely affected. The norm of the regressively connected (12) transition drops almost to zero in the middle of the \( G^1 \) pulse, and the norm of the progressively connected (34) transition nearly doubles in the middle of the pulse. Toward the end of the pulses, both norms are nearly restored to their equilibrium values.

is much larger than 270°. The resulting \( x \) magnetization achieves only 60% of its maximum magnitude. This can easily be explained by considering the matrix element \( F^{(24)} \) of the relevant transition. The matrix representation of the operator in the eigenbase of the strongly coupled two-spin system is

\[
F^+ = \begin{bmatrix}
0 & v & u & 0 \\
0 & 0 & 0 & v \\
0 & 0 & 0 & u \\
0 & 0 & 0 & 0
\end{bmatrix}
\]  

with \( u = \cos \theta + \sin \theta \) and \( v = \cos \theta - \sin \theta \), where \( \theta = \frac{1}{2} \arctan[2\pi J_{AB}/(\Omega_A - \Omega_B)] \). With a difference of chemical shifts \( (\Omega_A - \Omega_B)/2\pi = 20 \) Hz and a coupling constant \( J_{AB} = 10 \) Hz, one obtains \( \theta = 13.28^\circ \) and hence \( u = 1.2 \) and \( v = 0.74 \).

Figure 6 shows the behavior when a weak transition (24) is irradiated with a Gaussian pulse, again with a nominal flip angle of 270°. Because the matrix element \( [F^+]_{(24)} \) is smaller than unity, there is an “undershoot” of the trajectory of the single-transition operator \( I^{(24)} \). At the end of this trajectory, only 40% of the maximum \( x \) magnetization is generated and the \( z \) magnetization does not vanish as for an isolated spin-1/2 system. The trajectory ends near the “south pole” of the frame, so that, instead of exciting transverse magnetization, an almost complete inversion is unwittingly achieved.

The upper half of Fig. 7 shows a more realistic situation
where a 30 ms $G^1$ pulse with a peak RF amplitude of 55 Hz was applied along the +y axis to the center of the spectrum of the AB system. In this case, the $G^1$ pulse is strong enough to affect all four transitions simultaneously. Note that the trajectories of $I_{x}^{(12)}$ and $I_{x}^{(24)}$ of the two outer transitions (12) and (24) are identical, while those of their $I_{y}^{(12)}$ components are antisymmetrical. Similar symmetries hold for the $I_{x}^{(34)}$, $I_{y}^{(34)}$, and $I_{z}^{(34)}$ components of the inner (34) and (13) transitions. Clearly, the norms are not conserved: at the end of the pulse, the norms $n^{(12)}$ and $n^{(24)}$ of the weak outer transitions (12) and (24) are larger than unity and the norms $n^{(13)}$ and $n^{(34)}$ of the strong inner transitions (13) and (34) are smaller than unity. The arithmetic mean of the norms of the four transitions (i.e., the total angular momentum) is, of course, conserved. The norms of the two pairs of parallel transitions (12), (34) and (13), (24) show a complementary behavior.

For comparison, the lower half of Fig. 7 shows the trajectories of the magnetization vectors that belong to the A multiplet of the AMX system of Fig. 4b in the course of the same selective $G^1$ pulse that was used in the simulations of the upper half of Fig. 7. The RF carrier was set in the center of the A region (i.e., at 0 Hz in our convention). In this weakly coupled case, all norms remain equal to unity during the entire duration of the selective pulse. At the end of the pulse, the magnetizations of the four transitions have undergone the same flip angles as if the pulses were applied to isolated spin-$\frac{1}{2}$ systems with different offsets.

In a typical selective two-dimensional experiment such as soft-COSY, the transverse magnetization that has been excited by the initial selective pulse at the beginning of the evolution period is subjected to one or several other selective pulses. In a strongly coupled system, each transition has a different response, depending on its inherent matrix element and its connectivity to other transitions. As an example, the upper half of Fig. 8 shows the behavior of the four single-quantum transitions of the AB spin system of Fig. 4a in the course of a second $G^1$ pulse of 30 ms duration, identical to the $G^1$ pulse used in Fig. 7, which is applied at the center of the four-line spectrum along

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**FIG. 6.** Graphs similar to those of Fig. 5, except that one of the weak "outer" transitions is irradiated in this case (see wavy arrow). The nominal nutation angle is again 270°, as in Fig. 5, but the effective nutation angle of $I^{(24)}$ is only about 198°. Note that the norm of the (13) transition, which is parallel to the irradiated (24) transition, is barely affected, while the norm of the regressively connected (34) transition drops almost to zero during the $G^1$ pulse, and the norm of the progressively connected (12) transition nearly doubles toward the end of the pulse.
FIG. 7. (Top) Time dependence of the norms $n^{(rs)}$ (dotted lines) and of the individual components $<I^{(rs)}_x>$, $<I^{(rs)}_y>$, and $<I^{(rs)}_z>$ (continuous, dashed, and dashed-dotted lines, respectively) for a selective $G^1$ pulse with a nominal 270° flip angle ($\tau_p = 30\ ms$, $gB_1^{\text{max}}/2\pi = 55\ Hz$), truncated at 2.5%, applied in the center of the AB spectrum of Fig. 4a with an RF phase along $+y$ axis of the rotating frame. (Bottom) Trajectories obtained when the $G^1$ pulse is applied along the $+y$ axis to the center of the weakly coupled A multiplet of Fig. 4b. In the weakly coupled AMX system, all norms are conserved in the course of the $G^1$ pulse, whereas in the AB system the norm for the inner transitions $I^{(34)}$ and $I^{(13)}$ is reduced, while the norms for the outer transitions $I^{(12)}$ and $I^{(24)}$ are increased in the course of the pulse.

The $+y$ axis of the rotating frame, immediately after the first $G^1$ pulse. For comparison, the lower half of Fig. 8 illustrates the responses of the four lines of spin A in the weakly coupled AMX system of Fig. 4b. Once again, the norms are not conserved in the AB system, while they are invariant in the AMX case. In the latter case, the $G^1$ pulse applied along the $+y$ axis leaves the $y$ components almost unaffected (although not exactly, because the effective RF fields are slightly tilted). As expected for the AMX case, the $x$ components are effectively converted into $z$ components; i.e., one observes a net rotation $I^{(rs)}_x \rightarrow I^{(rs)}_z$ as under a nonselective 270° pulse. In the AB case, it is surprising that the transverse components $I^{(12)}_x$ and $I^{(24)}_x$ of the weak outer transitions of the AB system are in effect transformed into $I^{(12)}_z$ and $I^{(24)}_z$ by the second $G^1$ pulse, much as in a well-behaved weakly coupled system. On the other hand, the transverse components $I^{(13)}_x$ and $I^{(34)}_x$ of the strong inner transitions of the AB spectrum are not rotated back to the $z$ axis, but remain essentially along the $x$ axis. Thus Fig. 8 demonstrates that selective pulses have different effects on different transitions of strongly coupled systems, even if the pulses affect all transitions of the multiplet simultaneously. This observation is directly relevant for a soft-COSY experiment, where the second pulse affects the four transitions of an AB system differently. As a result, the multiplets in soft-COSY spectra suffer from severe anomalies in phases and amplitudes.

CONCLUSIONS

It has been shown why the phases and amplitudes of cross-peak multiplets in strongly coupled systems that are recorded by selective correlation methods can be quite different from those observed in nonselective correlation spectra. The anomalies are due to transition-dependent nutation angles and to the lack of conservation of the norms of the magnetization vectors associated with individual transitions. In strongly coupled spin systems, selective pulses have differ-
FIG. 8. Graphs similar to those of Fig. 7, but calculated during the second selective \( G^1 \) pulse of a soft-COSY sequence, applied immediately after the first selective \( G^1 \) pulse, both with the same durations and amplitudes \( \tau_p = 30 \) ms, \( \gamma B_{1\text{rms}}/2\pi = 55 \) Hz, truncated at 2.5%, and phases along the \(+y\) axis. (Top) AB system; (bottom) weakly coupled A multiplet.

ent effects on transverse magnetization components belonging to different transitions.

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