transition. Weaker bands separated by 844 cm^{-1} (and 831 cm^{-1}) with deuterium counterparts near 720 cm^{-1} correspond, however, to the out-of-plane C–H bending mode at 874 cm^{-1} (deuterium counterpart 752 cm^{-1}). This b_{3g} mode is vibronically allowed with *z*-polarized radiation.

The much weaker band at 461 nm in solid argon can be assigned to the short-axis-polarized, dipole-allowed $^2B_{3g} \leftarrow {}^2A_u$ transition based upon $^2B_{3g}$ idenification[10] of the fourth PES band. The dipole change along the long axis is much greater than along the short axis as shown by the relative intensities of the 675- and 461 -nm N$^+$ absorptions. The 381-nm absorption, corresponding to an 11.4-eV PES peak,[10] is probably due to an allowed $\sigma \leftarrow \pi$ transition.

Comparison of vibrational intervals in the third photoelectron band and the structured matrix absorption is complicated by the 250-cm^{-1} PES bandwidth. The first three vibronic peaks are separated by 500 ± 20 cm^{-1}, in agreement with the matrix spectrum; the fourth vibronic peak is broadened and probably contains some 1420-cm^{-1} and (3 × 500)-cm^{-1} vibrational spacing.

For large rigid molecular cations like N$^+$ with relatively low electron affinities (8.15 eV) compared to the ionization energy of argon (15.8 eV), the argon matrix–cation interaction is minimal, as shown by comparison of the electronic band origin with the PES. The observed vibrational intervals show little change from fundamentals for the neutral molecule; this contrasts the case of smaller parent molecular ions like $CH_2F_2^+$ where the hole has a greater effect on the vibrational potential function of the molecule.[18]

Acknowledgment. N.S.F. support is gratefully acknowledged.

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High-Resolution NMR Spectra of Quadrupole Nuclei in Solution Using Multiple Quantum Coherence

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Received June 8, 1981

The utilization of multiple quantum coherence (MQC) in NMR experiments probing molecular structure and dynamics is becoming increasingly common. The principal attraction has been the simplification of both spectra and spin relaxation mechanisms that results from the reduced number of MQ transitions between energy levels. To date, MQC experiments have been performed on quadrupole nuclei in ordered systems,[1,2] dipolar coupled spin-$^1/_2$ nuclei in ordered systems,[3-6] and scalar coupled spin-$^1/_2$ nuclei in solution.[7-10] These experiments have in many cases yielded structural and dynamic information inaccessible via one quantum spectra alone.

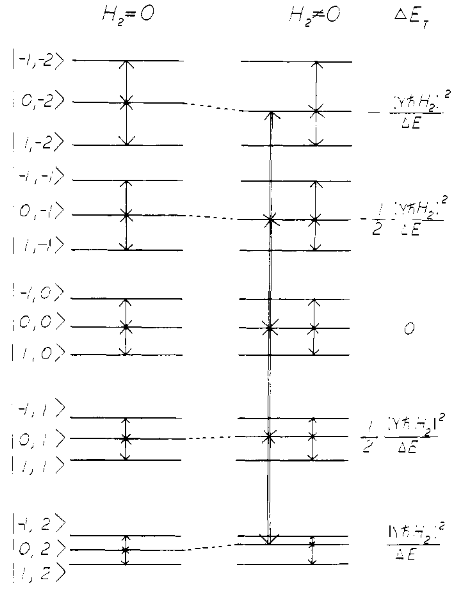

Figure 1. $^{14}NH_4^+$ energy level diagram showing the effect of spin tickling. ^1H spin tickling is applied at the central line of the ^1H triplet as shown by the double arrows. Single arrows show the 1Q transitions. Spin states are indicated by *Z*-magnetization quantum numbers for ^{14}N states and total ^1H states. The energy changes ΔE_T due to spin tickling, listed to the right of the figure, were calculated from second-order perturbation theory using $H' = \gamma\hbar H_2 I_x$, where γ is the gyromagnetic ratio, H_2 is the tickling field, and I_x is the *x* component of the spin operator.

A relatively unexplored area of MQC applications is that of quadrupole nuclei in solution. Quadrupole nuclei are subject to the same chemical shieldings and scalar couplings which are valuable structural parameters for spin-$^1/_2$ nuclei. These parameters are, however, obscured by the broad lines and resultant poor resolution usually found in spectra of quadrupole nuclei. MQC offers a possibility for improvement of resolution in that both chemical shifts and splittings due to spin–spin coupling scale as the number of quanta involved, while line widths due to quadrupole broadening can remain independent of or actually decrease with number of quanta. Development of techniques for observation of MQC of quadrupole nuclei in solution is, therefore, worthy of exploration.

Unfortunately, direct application of commonly used pulse sequences does not result in observable MQC for most quadrupole nuclei in solution. This arises because of the degeneracy of one quantum (1Q) transitions and necessitates explicit modification of experimental procedures. We present here one option which proves successful in observing MQC for spin-1 nuclei which show some residual spin–spin coupling. This option employs removal of degeneracy by spin tickling. We will illustrate use of this method on ^{14}N spectra of NH_4Cl in aqueous solution.

MQC spectra are commonly observed by using the pulse sequence 90°-τ-90°-t_1-90°-t_2 in which 90° represents rf pulses of sufficient length to rotate magnetization by 90° in the rotating frame and τ, t_1, and t_2 represent time delays.[6,7,10] The effect of this sequence is best seen by employing density matrix methods. For the $^{14}NH_4^+$ case, each ^{14}N line associated with a particular ^1H spin state can be described by using a 3 × 3 matrix. One quantum coherence, represented by spin density matrix elements σ_{12} and σ_{23}, is excited by the first 90° pulse. Under favorable conditions, the 1Q elements evolve during τ such that their phases differ at the time of the second 90° pulse. The second pulse then mixes these density matrix elements to produce a finite MQC that evolves during t_1 according to MQ spectral parameters. The final 90° pulse mixes MQC back into 1Q density matrix elements which can be observed via their relationship to transverse magnetization during the time period t_2. The time period t_1 is incrementally increased over a period appropriate for decay of the MQC and the data transformed in the two dimensions, t_1 and t_2, to produce normal 1Q spectra in the F_2 dimension and MQC spectra in the F_1 dimension.

The reason this sequence is ineffective for a spin-1 nucleus in solution can best be illustrated following a treatment given by

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Bodenhausen et al.[1] This treatment shows that after the second 90° pulse, the 2Q element $\sigma_{13}(\tau^+) = i \cos \Delta\omega\tau \sin \omega_Q\tau$, where $\Delta\omega$ is the offset of the carrier frequency from exact resonance.[11] $2\omega_Q$ represents the residual quadrupole splitting in ordered media or more generally the frequency difference between the two 1Q transitions. For degenerate 1Q transitions, $\omega_Q = 0$ and $\sigma_{13}(\tau^+) = 0$, clearly giving no MQC.

Excitation and observation of MCQ can be restored by making 1Q transitions nondegenerate. Spin tickling provides a means of removing the degeneracy in situations where spin coupling to a second nucleus is partially resolved. The ^{14}N spectrum of NH_4^+ is a symmetric pentet in which each line can be associated with a particular proton spin state. Relevant 1Q frequencies ω_{12} and ω_{23} under normal and spin-tickled conditions can be deduced from an energy level diagram such as that in Figure 1 where the effect of a weak spin-tickling field, H_2, at the proton frequency of the central line is calculated by using second-order perturbation theory. In practice, it is necessary to produce perturbations which are large compared to line widths. While it appears that this requires H_2 fields of a magnitude which may not be fully described by perturbation theory, qualitative predictions should remain valid. Clearly, $\omega_{12} \neq \omega_{23}$ for most of the lines of the $^{14}NH_4$ multiplet when the rf field is applied. The central line is a clear exception, and intensity of this line should not show pronounced effects of multiple quantum coherence even under spin-tickling conditions. For the lines other than the central line, a choice of $(\omega_{12} - \omega_{23})\tau = n\pi$ for $n = 1, 3, 5...$ should lead to maximum excitation of MQC.[1] For just two transitions, this condition can be optimized as shown by Bodenhausen et al.[1] However, for the NH_4^+ system it will not be possible to optimize all lines simultaneously, and some additional distortion of multiplet intensities will result.

The predicted effects of spin tickling were tested on a 6 M aqueous solution of reagent grade NH_4Cl. The sample was acidified with concentrated HCl (pH < 1.0) and temperature held to 25 °C to slow proton exchange. Spectra were run at 14.4 MHz on a Bruker CXP200 spectrometer using a probe with a 10-mm horizontal solenoid coil for observation and an orthogonal 22-mm Helmholtz pair for ^{1}H irradiation. 90° pulse times were typically 20 μs.

For MQC experiments, data were accumulated in quadrature by using the 90°-τ-90°-t_1-90°-t_2 pulse sequence with spin tickling during τ and acquisition (t_2). Proton decoupling was in some cases applied during the evolution period t_1. As suggested by Bodenhausen et al.[1] phases of rf pulses were cycled by 90° for excitation and 180° for observation in order to suppress 1Q data. This was not entirely successful, presumably because of imperfections in pulses. Data were transformed after trapezoidal multiplication by using standard Bruker software. Only magnitude spectra are displayed.

A single quantum ^{14}N spectrum of NH_4Cl normally shows a pentet with 52 ± 4 Hz, ^{1}H–^{14}N spin–spin coupling. The spin-tickled spectrum shows splitting of the normally degenerate lines as expected. Although the presence of spin tickling complicates the spectrum greatly, it is important to realize that tickling is only necessary for excitation and observation of MQC. Tickling is not employed during the MQ evolution period t_1, and spectra viewed from the F_1 dimension should not show these complications. In fact, decoupling can be employed during t_1 to reduce the spectrum to a single line.

Figure 2 shows sections of 2-D plots for decoupled experiments employing a τ value of 0.1 s and conducted with (Figure 2b) and without (Figure 2a) spin tickling during τ and acquisition. In the absence of spin tickling only a single 1Q line is observed at an offset of 30 Hz from the carrier. When tickling is employed two lines appear, one at the normal 1Q position and a second line at 60 Hz, a position appropriate for the 2Q line. Clearly, the application of spin tickling leads to observation of multiple quantum coherence.

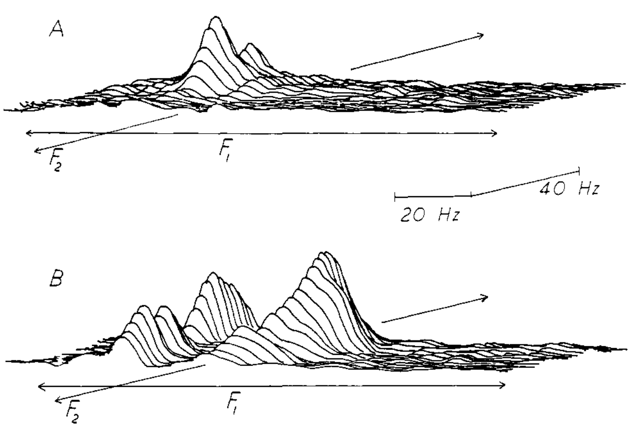

Figure 2. Sections of ^{14}N 2-D spectra of NH_4Cl obtained using a 90°-τ-90°-t_1-90°-t_2 pulse sequence (a) without and (b) with ^{1}H spin tickling during τ and t_2. Data were accumulated as 64 1 K spectra, each consisting of 16 pulses with a 1-s recycle delay. ^{1}H decoupling was employed during t_1. An optimum value of τ = 0.1 s was determined empirically. t_1 was incremented by 3.9 ms in successive spectra.

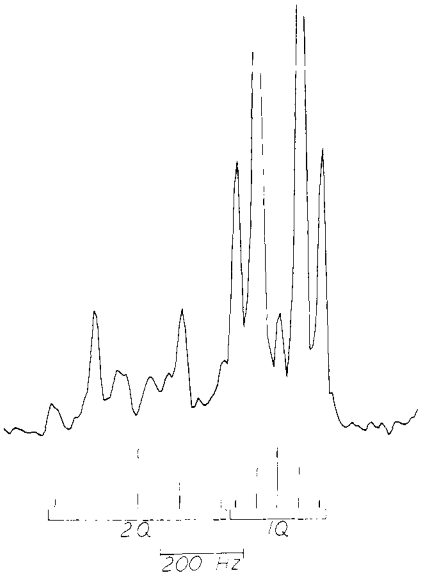

Figure 3. ^{1}H-coupled ^{14}N MQC spectrum of NH_4Cl. The same pulse sequence as in Figure 2 was used but without decoupling during t_1. 128 spectra each containing 128 points were collected with t_1 incremented by 0.49 ms to increase the F_1 spectral width. The spectrum was obtained by summing the 56-column central segment of the 2-D data set.

Figure 3 shows a sum of cross sections in the F_1 dimension for a 2-D spectrum accumulated with spin tickling during τ and acquisition but without the use of ^{1}H decoupling during t_1. A residual set of lines in the single quantum region is visible from 250 to 450 Hz. A set of four lines is also visible in the double quantum region. With the exception of a missing central line, these would form a pentet with splitting of 100 Hz, a value equal to twice the coupling constant observed in a normal 1Q spectrum. The missing central line is in fact predicted based on the lack of spin-tickling perturbation of these transition frequencies.

Resolution of multiplet structure in the 2Q regime is slightly better than in the 1Q regime. This is largely due to the scaling of splittings due to spin–spin coupling. A factor of 2 improvement is not achieved because line widths also increase. NH_4Cl is a poor choice to illustrate improvement in resolution because quadrupole broadening is small, and the finite field homogeneity contributions which dominate the line width scale up as the number of quanta. Nevertheless, the experiments presented here demonstrate that is is possible to obtain MQC for quadrupole nuclei in solution. The method employed is limited to cases showing residual coupling but recognition of underlying reasons for failure of normal observation techniques should lead to yet other procedures for acquiring data.

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[11] We have corrected a minor sign error in eq 6 of ref 1 for the rotation matrix in applying it to our analysis.

Acknowledgment. This work was supported by grants from the National Institute of General Medical Sciences (GM 19035) and the Yale University NSF Regional Instrumentaion Center (NSF CDP 7916210).

A Ferrous, High-Spin Heme *a* Model for Cytochrome *a*$_3$ in the Dioxygen Reducing Site of Cytochrome Oxidase

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Received July 20, 1981

Cytochrome oxidase catalyzes the four electron reduction of dioxygen to water in its role as the terminal oxidase in mitochondrial electron transport.[1] The catalysis is remarkably efficient: the enzyme operates at an overvoltage which is only 300-400 mV above the reversible potential of the water/dioxygen couple.[2] The structural and mechanistic basis for this efficiency appears to lie in the binuclear nature of the dioxygen reducing site. A copper atom, denoted Cu_{a_3}, and a heme *a* bound iron atom, cytochrome *a*$_3$, comprise this site and in the oxidized enzyme are strongly exchange coupled;[3] in the reduced enzyme they apparently act in concert to reduce dioxygen to a bound peroxy intermediate which is subsequently reduced to water.[4] Some evidence exists which indicates that the dioxygen reducing site is apolar[5] but that it communicates with bulk solution by a hydrophilic channel.[6] Sporadic attempts to prepare heme *a* model compounds for deoxycytochrome a_3^{2+} have been reported;[7] however, these have been limited to aqueous solution and have been only partially successful. For the experiments reported here we developed techniques for producing both high- and low-spin ferrous heme *a* in aprotic solvents. A comparison of the optical and resonance Raman properties of these models, and of the same species in water, with key spectroscopic properties of the enzyme shows that the iron of deoxycytochrome *a*$_3$ is high spin and that the dioxygen reducing site is hydrophobic.[8]

Reduction of heme *a* in aprotic solvents was carried out in an inert atmosphere by titrating solutions of the oxidized chromophore in the solvent of interest with a near-stoichiometric amount of sodium dithionite/[2.2.2]-cryptand solution, a procedure derived from that used by Mincey and Traylor.[9] Anaerobicity was achieved either by purging the solutions with oxygen-free argon gas or by using successive freeze-pump-thaw cycles. The extent of heme *a* reduction was monitored optically during the titration; excess dithionite was found to alter the spectrum of heme *a*, possibly by inducing adduct formation at the formyl group on the porphyrin periphery. Other reducing systems (e.g., hydrazine hydrate, hydrogen/Pd, $NaBH_4$) attack this group and were not examined as potential reductants in any detail. The technique of overlaying the organic solvent/heme *a* solution with aqueous dithionite was also found unsuitable owing both to the amphiphilic nature of heme *a* and to the slight solubility of H_2O in the solvents of interest. For five-coordinate high-spin models, 2-methylimidazole was employed as the sole axial ligand.[10] Low-spin models had bis(*N*-methylimidazole) ligation in the axial sites. Raman spectroscopy with Soret laser excitation was carried out as described previously.[5a]

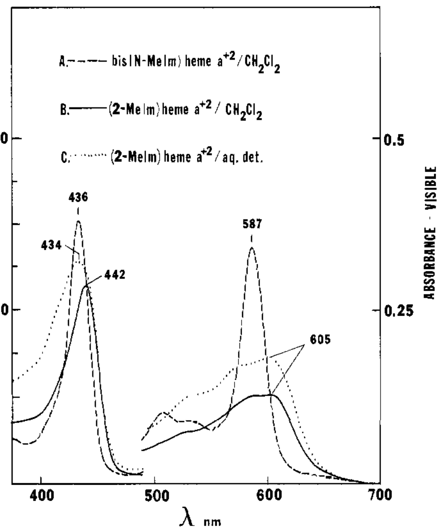

Figure 1. Optical spectra of reduced heme *a* model compounds (cell path length is 0.5 cm). (A) Low-spin reduced heme *a*; ~27 μM in heme, 0.3 M in *N*-methylimidazole; aprotic solvent is CH_2Cl_2; (B) high-spin reduced heme *a*; approximately 22 μM in heme, 2.5 mM in 2-methylimidazole; aprotic solvent is CH_2Cl_2; (C) high-spin reduced heme *a*; approximately 33 μM in heme, 1.2 mM in 2-methylimidazole; aqueous solvent is 0.07 M cetyltrimethylammonium bromide and 0.1 M potassium phosphate buffered to pH 7.4.

Figure 1 presents optical spectra of low-spin heme a^{2+} in CH_2Cl_2 and of high-spin a^{2+} in both CH_2Cl_2 and aqueous detergent solution. The high-spin model in aprotic solution has optical properties clearly resembling the 443-nm Soret maximum and the weak 600-nm visible absorption determined for cytochrome a_3^{2+}.[11] When the ferrous high-spin species is prepared in water, there is a shift to 434 nm in the Soret region with little change in the visible spectrum. Low-spin heme *a* shows a significant increase in visible absorption, and the Soret maximum occurs at 436 nm. Interestingly, there is very little solvent dependency in the absorption properties of the low-spin species.[12] Evidence presented elsewhere indicates that low-spin bis(imidazole) heme *a* is a reasonable first-order model for the other heme *a* chromophore in cytochrome oxidase, cytochrome *a*.[7b,13]

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