SQUID-NQR of nitrogen-14 in amino acids and small peptides

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We present a technique for the study of nuclear quadrupole resonance of ^{14}N interacting with low electric field gradients as found, for example, in amino acids. A superconducting quantum interference device (SQUID) is used to detect directly small NMR signals via cross relaxation of the ^{14}N polarization to adjacent proton spins, eliminating the need for field cycling. When one nitrogen quadrupolar transition matches the proton Zeeman splitting, the remaining two quadrupolar transitions can be observed by sweeping a saturating rf field through resonance. In addition, signal enhancement by simultaneous excitation of two nitrogen resonances helps to identify connected transitions.

1. Introduction

^{14}N nuclear quadrupole resonance (NQR) and nuclear magnetic resonance (NMR) spectroscopy have the potential to provide valuable structural information concerning the nitrogen environment in, for example, polymers and biopolymers. However, detection of the spectra poses significant problems due to the low nitrogen quadrupole frequencies (about 1 MHz) combined with the quenching of the magnetic moment of nuclei with spin $I=1$ in low magnetic fields [1,2].

A variety of field cycling techniques that detect the nitrogen signals indirectely through their effect on adjacent protons have been developed to overcome those difficulties [3–7]. These methods involve the polarization of proton spins in high magnetic fields, followed by cycling to lower fields and subsequent rf irradiation of the nitrogen spins. Matching and cross polarization between the proton and nitrogen spin systems occurs either in the laboratory frame, the rotating frame or by the solid effect [4–6]. After returning to the original high magnetic field the proton magnetization is measured. By repeating this procedure with a series of different rf-irradiation frequencies the nitrogen absorption spectrum is assembled point by point in the frequency domain. Recent developments have brought some sensitivity improvements in this approach [7].

In this Letter, we describe the detection of the proton mediated nitrogen signals in *low magnetic field*. The necessary sensitivity is provided by means of a dc superconducting quantum interference device (SQUID). The elimination of field cycling for each irradiation frequency simplifies the measurements substantially, since it allows us to observe a wide frequency range in a single scan. We demonstrate various properties of this technique with results of ^{14}N experiments on amino acids and small peptides.

2. Theoretical background

2.1. ^{14}N NQR

The spin $I=1$ ^{14}N nucleus is subject to a quadrupole interaction, $H_Q=\frac{1}{4}e^2qQ[3I_z^2-I^2+\eta(I_x^2-I_y^2)]$, between its quadrupole moment eQ and a local electric field gradient, eq, with asymmetry parameter, η. The electric field gradients are a sensitive reflection of the identity and arrangement of the surrounding nuclei [8]. The quadrupole coupling con-

stants of ^{14}N in amino acids and terminal nitrogen atoms of peptides are typically $e^2qQ/h \approx 1.1$–1.3 MHz, with asymmetry parameter in the range $\eta = 0.1-0.5$, and the coupling constants for the nitrogen nuclei in peptide bonds are around $e^2qQ/h \approx 3$ MHz, with $\eta \approx 0.4$–0.8 [9,10]. In zero magnetic field the quadrupolar eigenstates $|x\rangle \equiv (|1\rangle + |-1\rangle)/\sqrt{2}$, $|y\rangle \equiv (|1\rangle - |-1\rangle)/\sqrt{2}$, and $|z\rangle \equiv |0\rangle$ (where $|1\rangle$ and $|-1\rangle$ denote the magnetic quantum numbers), give rise to three transitions at frequencies

$$\nu_+ = \tfrac{1}{4}C_Q(3+\eta),$$

$$\nu_- = \tfrac{1}{4}C_Q(3-\eta),$$

$$\nu_0 = \nu_+ - \nu_- = \tfrac{1}{2}C_Q\eta, \qquad (1)$$

where $C_Q = e^2qQ/h$ is the quadrupole coupling constant. In a magnetic field these transition energies shift by an amount that can be calculated analytically [11] or estimated by perturbation expressions [12]. In the magnetic fields we choose (between 80 and 160 G) these shifts are less than five kHz for the maxima of the powder spectra. Knowledge of two transition frequencies is sufficient to determine the quadrupolar parameters, if the transitions can be assigned to ν_+, ν_- or ν_0. In section 2.2, we demonstrate how the magnitude and sign of the signals, together with estimates of the quadrupole coupling constant and the asymmetry parameter, help to identify the transitions.

2.2. Level matching between nitrogen and proton transitions

The expectation values of the magnetic moments for non-degenerate quadrupolar eigenstates are zero for all nuclei with integer spin [1,2]. Even with the small magnetic moment of the eigenstates induced by the Zeeman perturbation in low magnetic field, the nitrogen signals remain difficult to observe directly. To overcome this problem both field cycling techniques and the present method involve the observation of the nitrogen transitions via their influence on adjacent proton spins through the dipole–dipole interaction. In contrast to field cycling techniques, our experiments begin with thermal equilibrium between the proton system, the nitrogen subsystems and the lattice (temperature T_L) at low magnetic fields. To obtain efficient coupling between the spin systems, the proton Larmor frequency, ν_L^P, or double the frequency, $2\nu_L^P$, must match one of the nitrogen transitions. Assume, for example, that the magnetic field is such that the proton Zeeman splitting, ν_L^P, resonates with the ν_- transition of the nitrogen spins (fig. 1). In this case the populations $P(|y\rangle)$ and $P(|z\rangle)$ of the nitrogen states and $P(|1/2\rangle)$ and $P(|-1/2\rangle)$ of the proton states fulfill the conditions

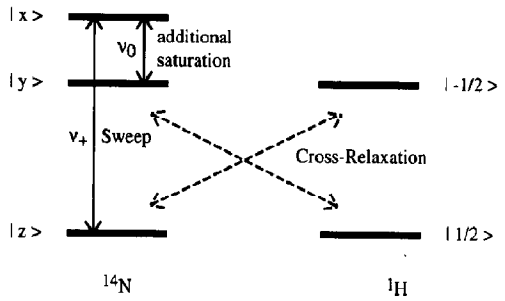

Fig. 1. Energy level scheme illustrating the matching between proton and nitrogen states (ν_-) and the processes during signal detection (ν_+) for single irradiation. Additional saturation of the third transition (ν_0) enhances the signal.

$$\frac{P(|y\rangle)}{P(|z\rangle)} = \frac{P(|-1/2\rangle)}{P(|1/2\rangle)} = \exp(-h\nu_-/kT_L). \qquad (2)$$

Saturation of one of the two remaining nitrogen transitions (ν_+ in fig. 1) changes the populations of one of the eigenstates thermally coupled to the protons (in this example $P(|y\rangle)$). The proton–nitrogen subsystem is no longer in equilibrium since the temperature of the nitrogen subsystem has been modified. Depending on whether the irradiation affects the energetically higher or lower sublevel coupled to the protons, the temperature of the subsystem can be reduced or increased. Through cross relaxation, the nitrogen spin sublevels and the proton spins will now relax toward a new common spin temperature. The included transitions between the proton spin states during this process change the total magnetization that can be detected.

The magnitude and the sign of the magnetization change can be calculated for different pairs of matched and observed transitions assuming complete saturation and fast cross-relaxation times (table 1) (following procedures described in refs. [5,6]). The signal at the ν_+ resonance is largest. Only

Table 1
Sign and sizes of the signal calculated for complete saturation and fast cross relaxation [a]

Observed transition	Matched transition	Relative signal $\times C_Q/4kT(4+4.5R)$
ν_+	ν_-	$3+\eta$
ν_+	ν_0	$3+\eta$
ν_-	ν_+	$3-\eta$
ν_-	ν_0	$-3-\eta$
ν_0	ν_+	2η
ν_0	ν_-	-2η

[a] $C_Q=e^2qQ/h$. Level-crossing with ν_L^P: R = number of protons/number of nitrogen nuclei. Level-crossing with $2\nu_L^P$: R = number of protons/2 × number of nitrogen nuclei.

in this case does the sign of the signal not depend on which of the remaining transitions is used for level crossing. The smallest signal is predicted for the ν_0 transition, which is indeed the most difficult to observe in our experiments.

Further enhancement of the signal amplitude can be achieved by additional saturation of the third transition (ν_0 in fig. 1). Given complete saturation and fast cross-relaxation times the populations of all three nitrogen states and the proton states equalize thus utilizing the full proton signal to detect the nitrogen resonances. The increased signal intensity due to the double irradiation makes possible the detection of previously unobservable nitrogen resonances. Additionally, saturation of a second transition helps to identify resonances arising from the same nitrogen site.

3. Experiments

The samples DL-serine, glycyl-glycine and L-alanine were purchased from Sigma Chemical Company and used without further purification. 0.2 cm^3 (roughly 100 mg) of the samples were sealed under vacuum in 5 mm glass tubes.

The basic design of the SQUID spectrometer is described in an earlier paper [13]. The dc-SQUID detects the changes in magnetic flux proportionally to ΔI_z along the direction of the static magnetic field. Both the SQUID and the samples are kept at 4.2 K. A static magnetic field (up to a few 100 G) is applied, and the rf-irradiation frequency is swept over a range of several megahertz. The frequency range is typically divided into sections of roughly 250 kHz bandwidth with scans lasting 100–250 s. When rf irradiation saturates a nitrogen resonance, cross relaxation can induce proton transitions, if one nitrogen and a proton resonance are matched. The proton transitions change the total magnetization of the sample, which yields a signal given by an increase or decrease in the flux detected by the SQUID. The spectra of sweeps from high to low frequency and reverse sweeps from low to high frequency are combined to exclude distortions from saturation, relaxation and instrumental artifacts. Having found one nitrogen transition frequency, the magnetic field is changed so as to match that resonance with the proton Larmor frequency in order to search for the other two connected transitions.

4. Results and discussion

Coupling between the protons and the nitrogen nuclei is efficient when ν_L^P or $2\nu_L^P$ is matched to one of the nitrogen transition frequencies. When the frequencies coincide, the nitrogen resonance cannot be observed, because the stronger proton transition at the same frequency is saturated directly, and not indirectly through the nitrogen polarization. However, in this case the signal intensity of the other two nitrogen transitions is maximized. On the other hand, if the proton and the nitrogen transition frequencies differ slightly, the first nitrogen may also be detected, most efficiently by sweeping through its resonance frequency before saturating the ν_L^P or $2\nu_L^P$ proton resonance. Despite less efficient cross relaxation in this case the other two nitrogen transitions can be observed as well, though they are less intense than under exact matching conditions.

The dependence of the cross efficiency on the matching condition, which manifests itself as the field dependence of the signal, is demonstrated with DL-serine (fig. 2). The changes in the magnetic field can be followed by observing the broad proton signal at twice the Larmor frequency move across the spectral window. The two nitrogen signals at $\nu_- = 882 \pm 3$ kHz and $\nu_+ = 961 \pm 3$ kHz grow and decay as they pass the matching condition. The spectra yield values $e^2qQ/h = 1227 \pm 2$ kHz, $\eta = 0.128 \pm 0.005$ (literature

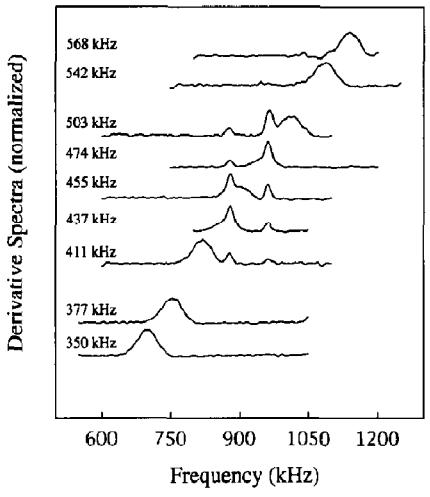

Fig. 2. Derivative spectra of DL-serine for different magnetic field strengths. The broad proton line appears at double the Larmor frequency. The nitrogen transitions ν_- (882 kHz) and ν_+ (961 kHz) become visible near the level crossing.

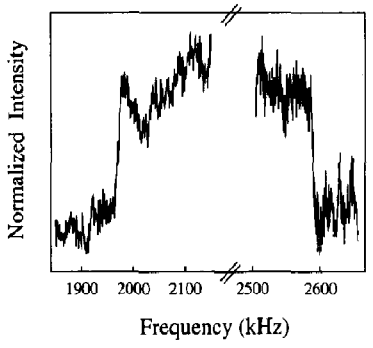

Fig. 3. Spectra of glycyl-glycine taken by matching the proton Larmor frequency with the ν_0 frequency (618 kHz) of the amino acid nitrogen and sweeping over the ν_- (1972 kHz) and ν_+ (2590 kHz) transitions.

[5] for $T=77$ K: $e^2qQ/h=1217\pm1$ kHz, $\eta=0.118\pm0.003$).

As mentioned in section 2.2, the signs of the signal may vary depending on which nitrogen state is coupled to the protons. The positive and negative signals are most easily observed by coupling the ν_0 transition to the proton resonance and observing the ν_+ or ν_- transitions. Fig. 3 shows this signal inversion for glycyl-glycine. The transitions at $\nu_-=1972\pm3$ kHz and $\nu_+=2590\pm3$ kHz yield $e^2qQ/h=3041\pm3$ kHz with $\eta=0.407\pm0.005$ (literature [5] at $T=77$ K: $e^2qQ/h=3030\pm10$ kHz, $\eta=0.41\pm0.02$) and originate from the peptide-NH group.

As mentioned above, additional irradiation of the third transition (which is not directly involved in the cross relaxation or observation) enhances the detected signal, as demonstrated with L-alanine (fig. 4). The upper traces for each of the ν_0, ν_- and ν_+ spectra show the signal while sweeping only through the observed transition. The lower traces show the same experiment with additional irradiation of the third transition (fig. 1). The most drastic change in the magnitude of the signal is found for the ν_0 line (160 ± 3 kHz), which is too weak to be seen under single irradiation. Additional excitation of the ν_+ resonance (988 ± 3 kHz) clearly brings out the ν_0 signal. Even if the additional frequency is not known exactly, irradiation of a broad frequency range, that includes the third transition, before the actual experiment improves the signal. This method takes advantage of the long relaxation times typically found in amino acids at 4.2 K. As shown in fig. 4, sweeping over the weak ν_0 line before the observation scan enhances the intensity of the ν_+ resonance. The quad-

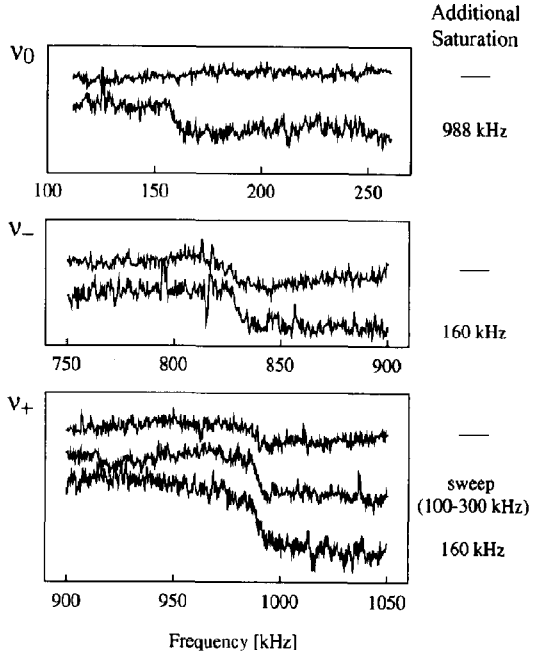

Fig. 4. Comparison of the three nitrogen transitions of L-alanine ($\nu_0=160$ kHz, $\nu_-=828$ kHz, $\nu_+=988$ kHz) under single and double irradiation.

rupolar parameters obtained from the three frequencies of $e^2qQ/h = 1208 \pm 3$ kHz, $\eta = 0.267 \pm 0.005$ agree well with previous results at 77 K giving $e^2qQ/h = 1205 \pm 1$ kHz, $\eta = 0.261 \pm 0.003$ [5].

5. Conclusion

The use of a dc-SQUID spectrometer has made it possible to detect ^{14}N NQR at low magnetic fields. The high sensitivity of the SQUID eliminates the necessity for field cycling and thus enables the observation of a broad spectra range in one scan. Cross relaxation between nitrogen and proton spin systems allow us to overcome the detection problem caused by the low effective magnetic moments of the pure quadrupolar eigenstates. Furthermore, simultaneous irradiation at two frequencies helps to enhance the nitrogen signal and to identify transitions originating from the same nitrogen site. We have demonstrated this new technique and some of its properties with ^{14}N NQR spectra of amino acids and small peptides.

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