

Nitrogen-14 SQUID NQR of L-Ala-L-His and of Serine

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In honor of Professor Dr. Werner Müller-Warmuth on his 65th birthday

^{14}N nuclear quadrupole resonance spectroscopy, detected with a Superconducting Quantum Interference Device, makes possible the study of nitrogen environments in amino acids and small peptides. The present experiments characterize: 1) the effects of intermolecular interactions upon the amino nitrogen of serine upon cocrystallization of the stereoisomers in a range of concentrations; 2) the changes of the amino and imidazole nitrogen environments brought about by combining L-Alanine and L-Histidine into the dipeptide L-Ala-L-His.

Key words: SQUID, NQR, Amino acids, Nitrogen-14.

1. Introduction

^{14}N nuclear quadrupole resonance (NQR) and nuclear magnetic resonance (NMR) spectroscopy can provide valuable structural information about the nitrogen environment in biochemically, pharmaceutically and technologically important solids. The high natural abundance of the nitrogen-14 nuclei makes the study of the isotope desirable. However, the detection of the signal is difficult because of the quenching of the magnetic moment of nuclei with spin $I=1$ in low magnetic fields [1–3] and the low nitrogen quadrupolar frequencies [4–6]. For example, the quadrupole transition frequencies of ^{14}N in amino acids and the terminal nitrogen atoms of peptides typically lie below 1300 kHz [7, 8]. Field cycling techniques overcome these problems by detecting the nitrogen signals through their effect on adjacent protons that are polarized and detected in high magnetic fields [3–6]. Various field cycling techniques have been compared and a new method has been developed that involves resonant proton-nitrogen coupling and excitation of the two non-resonant ^{14}N NQR transitions [9]. In contrast to field cycling methods, in our experiments the proton mediated nitrogen signals are detected *directly in low magnetic fields* using the high sensitivity of a dc superconducting quantum interference device (SQUID). The elimination of the field cycling step (which probes the response of the sample at

each irradiation frequency separately) simplifies the measurements substantially, making it possible to observe a wide frequency range in a single scan; indeed, the spectral resolution is comparable to that of the newer field-cycling techniques. A further advantage of the direct detection with a SQUID is the possibility of studying samples with short relaxation times.

In a previous letter we demonstrated preliminary results using the SQUID technique for ^{14}N experiments [10]. In the present work we have applied the technique to study the structural changes of the nitrogen environment induced by cocrystallizing optically active isomers and by combining amino acids into small peptides.

2. Theoretical Background

Nitrogen-14 nuclei with spin $I=1$ are subject to the quadrupole interaction described by the quadrupole Hamiltonian $H_Q = \frac{e^2 q Q}{4} [3I_z^2 - I^2 + \eta(I_x^2 - I_y^2)]$. The nitrogen nuclear quadrupole moment eQ interacts with the electric field gradient, eq , with asymmetry parameter, η , created by the local environment of the nitrogen nuclei [11]; the quadrupole coupling constant $C_Q = e^2 q Q/h$ and the anisotropy parameter contain structural information about the local nitrogen surroundings. 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 $|1\rangle$, $|0\rangle$, and $|-1\rangle$ denote the magnetic quantum numbers), give rise to three

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transitions at the frequencies

$$\begin{aligned} \nu_+ &= C_Q(3 + \eta)/4, \\ \nu_- &= C_Q(3 - \eta)/4, \\ \nu_0 &= \nu_+ - \nu_- = C_Q\eta/2. \end{aligned} \quad (1)$$

Knowledge of two transition frequencies is sufficient to determine the quadrupolar parameters, if the transitions can be assigned to ν_+ , ν_- or ν_0 . The transition energies change slightly in the presence of a magnetic field [12]. Analytic calculations of the magnetic field strengths applied here (between 8 and 16 mT) show that the maxima of the powder spectra shift by less than five kHz.

The direct detection of the nitrogen transitions, however, is difficult since the expectation values of the magnetic moments for non-degenerate quadrupolar eigenstates, whose change upon excitation the SQUID detects, are zero in the absence of a magnetic field. The application of the low magnetic fields used here does not induce a sufficiently large moment to allow direct detection of the signal. As we have shown before, SQUID detection of the nitrogen transitions directly at low magnetic fields is possible via cross-relaxation to adjacent proton spins coupled by the dipole-dipole interaction. One or two nitrogen transitions are excited with a radio frequency field, while matching the third nitrogen resonance to a proton transition frequency. The dependence on good matching between the proton Larmor frequency ν_L^H or one of its multiples $n\nu_L^H$ ($n = 2, 3$) to a slightly Zeeman perturbed nitrogen transition frequency ν_0 , ν_- , or ν_+ is used to assign transitions coupled to one ^{14}N site. Simultaneous or consecutive excitation of two transitions, mainly used to enhance the signal, is also used advantageously to correlate resonances arising from the same nitrogen site. Additionally the magnitude, sign, and width of the signals, selective saturation, and estimates of the quadrupole coupling constant and the asymmetry parameter can support the assignment of the transition frequencies to different nitrogen sites.

3. Experiments

All samples were purchased from Sigma Chemical Company and used without further purification. 0.2 cm³ (roughly 100 mg) of the samples were sealed under vacuum in 5 mm glass tubes.

The homebuilt continuous wave spectrometer is described in [13]. The probehead containing the

dc-SQUID and the samples is kept at 4.2 K. The dc-SQUID detects the changes in magnetic flux proportional to ΔI_z along the direction of the static magnetic field. The static magnetic field (up to a few 10 mT) is applied by inducing current into a lead coil during the cooling process. The magnetic field strength can be changed by raising the temperature of the superconducting field trap above the critical temperature of lead (7 K) and inducing a different current. The rf-irradiation field is swept orthogonal to the static field over a frequency range of several megahertz. Typically the frequency range is divided into scans of 250 kHz bandwidth lasting 100–250 seconds.

As stated previously, the excitation of nitrogen transitions induces proton transitions via cross polarization. The SQUID detects the resulting increase or decrease of the total magnetization as a positive or negative signal. Having found one nitrogen transition frequency, the magnetic field is changed to match that resonance with the proton Larmor frequency to detect the other two transitions from the same nitrogen site. 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. Because the relaxation times are typically (but not exclusively) longer than the sweep times, the spectra typically display smooth steps and the spectra obtained from sweeping in both directions are subtracted in order to exclude these distortions. To identify the resonance frequencies more exactly, derivatives of the spectra are taken after applying a gaussian linebroadening (between 1–3 kHz width). Flux quantum jumps in the SQUID, identified by offsets or spikes within one random digitization point, are removed numerically.

4. Results and Discussion

4.1. Optically Active Isomers

The sensitivity of the nitrogen NQR frequency to small changes in the nitrogen environment can be demonstrated by comparing the spectra of the optically active isomers L-serine and D-serine ($\text{CH}_2(\text{OH}) \cdot \text{CH}(\text{NH}_3^+) \cdot \text{CO}_2^-$) to the spectra of the racemic mixture DL-serine. The NH_3^+ groups of D-serine and L-serine have identical resonance frequencies $\nu_+ = 979 \pm 4$ kHz and $\nu_- = 859 \pm 4$ kHz (Fig. 1), as expected. Because the crystal structures are related by

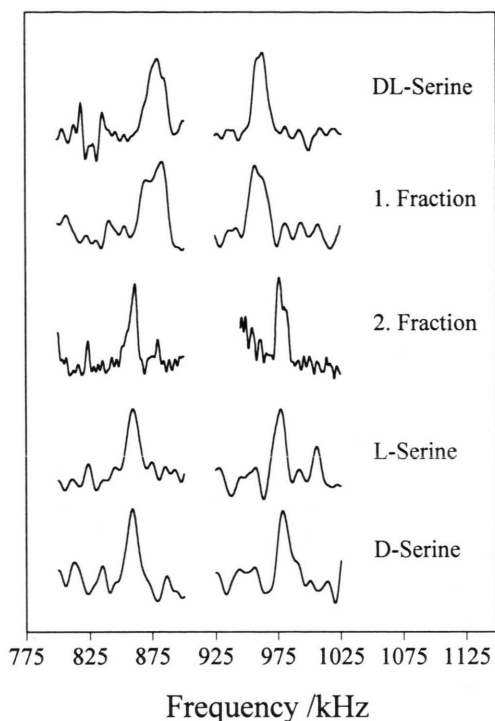


Fig. 1. Nitrogen-14 NQR spectra (normalized derivatives of SQUID detected continuous wave sweeps) of D- and L-serine in different degrees of optical purity. The crystals from a solution of 75% L-serine and 25% D-serine were collected in two fractions. The spectra were acquired by matching the nitrogen transitions ν_- and ν_+ with the proton resonance frequencies $2\nu_L^H$, and observing the remaining ν_+ and ν_- resonances, respectively.

reflection, the nitrogen quadrupolar parameters are identical $e^2qQ/h = 1223 \pm 4$ kHz, $\eta = 0.194 \pm 0.01$ (literature [5] for $T = 77$ K: $e^2qQ/h = 1215 \pm 1$ kHz, $\eta = 0.184 \pm 0.003$). On the other hand, the racemic mixture of DL-serine crystallizes in a different structure that accommodates both isomers. This leads to intermolecular nitrogen environments that differ from those of the optically pure crystals. However, the D- and L-molecules within this the racemic crystal (P_{21} /a symmetry) are related by reflection on a mirror plane [14], so they experience identical electric field gradients and have the same quadrupole parameters. The quadrupole transition frequencies are shifted by 23 kHz ($\nu_- = 882 \pm 3$ kHz) and -18 kHz ($\nu_+ = 961 \pm 3$ kHz) away from those of the optically active isomers. The derived quadrupole parameters $e^2qQ/h = 1227 \pm 2$ kHz, $\eta = 0.128 \pm 0.005$ change only slightly in the magnitude of the quadrupole constant, but significantly in the anisotropy parameter. The an-

isotropy parameter is a consequence of the changes in the hydrogen bonds in which the nitrogen atoms participate, caused mainly by the intermolecular crystal packing around the ammonium group. The small difference between the current data for the quadrupole parameters and the literature values [5] for $T = 77$ K ($e^2qQ/h = 1217 \pm 1$ kHz, $\eta = 0.118 \pm 0.003$) probably result from the different temperatures at which the data were obtained.

A mixture of 25% D-serine and 75% L-serine was dissolved and reprecipitated from aqueous solution. The high frequency resolution of the resonances from the pure and racemic compounds can be used to determine whether the 25:75 precipitate contains both isomers in random positions, or whether it crystallizes into conglomerates. The conglomerates can be composed of either the chiral crystals D- and L-serine or of the racemic crystal and the excess L-serine. Crystals containing the serine isomorphs in random positions would cause a broad distribution of resonances, while conglomerates of the pure materials should give resonances at the frequencies of the pure chiral or racemic materials.

The precipitate was collected in two fractions, the first one containing the crystals that formed early, the second one consisting of the remainder. The first precipitate shows resonances close to those of DL-serine (878 ± 8 kHz and 961 ± 5 kHz). The resonance frequencies of the second crystallization fraction resemble mostly those of the optically pure isomer (857 kHz and 979 kHz). These results show that most of the racemic crystallization has taken place first, leaving the excess L-serine to crystallize last. The preference of the crystallization into the racemic mixture indicates a greater stability of the DL crystal form, in agreement with earlier results [15].

4.2. L-Alanine and L-Histidine Forming L-Ala-L-His

L-Alanine: The results of combining two different amino acids into a dipeptide is illustrated by the formation of L-Ala-L-His from the amino acids L-alanine and L-histidine (Figure 2). All three transitions of L-alanine $\nu_+ = 988 \pm 3$ kHz, $\nu_- = 828 \pm 4$ kHz and $\nu_0 = 160 \pm 3$ kHz can be detected. The signal for the ν_0 resonance (160 ± 3 kHz), which is too weak to be detected under single irradiation techniques, can be enhanced sufficiently by exciting the ν_+ resonance (988 ± 3 kHz) [10]. The experimentally determined

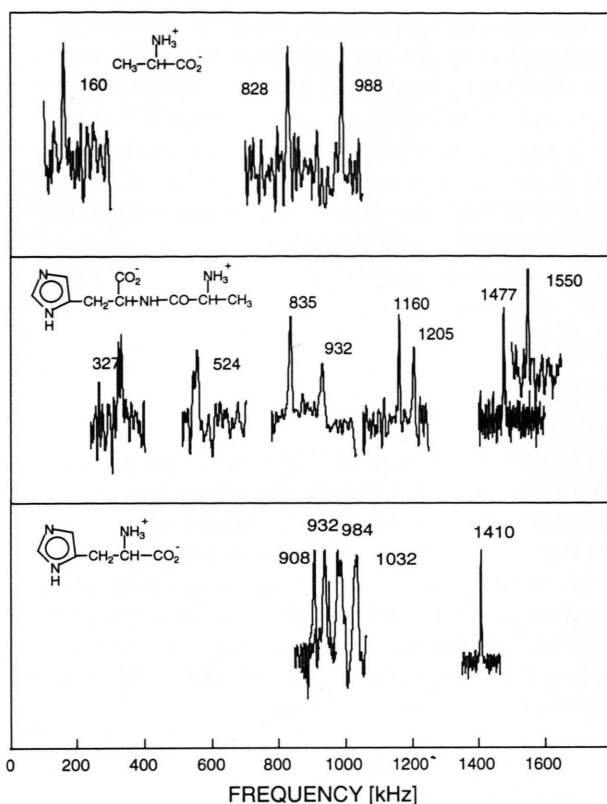


Fig. 2. Nitrogen-14 spectra (normalized derivatives of sweeps) for L-alanine (top), L-Ala-L-His (center) and L-histidine (bottom).

quadrupolar parameters $e^2qQ/h = 1208 \pm 3$ 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].

L-Histidine: The L-histidine spectra show five resonance frequencies at 908 ± 2 kHz, 939 ± 2 kHz, 984 ± 3 kHz, 1032 ± 3 kHz, and 1410 ± 3 kHz. The dependences of the signal intensities on the magnetic field strength allow us to identify connected transitions: The resonances at 908 ± 2 kHz and 984 ± 3 kHz result from one terminal nitrogen site while the resonances at 939 ± 2 kHz and 1032 ± 3 kHz originate from a second terminal nitrogen atom giving quadrupolar parameters $e^2qQ/h = 1258 \pm 2$ kHz and $\eta = 0.115 \pm 0.005$ and $e^2qQ/h = 1312 \pm 2$ kHz, $\eta = 0.140 \pm 0.005$, respectively. Since the existence of two different sites with $e^2qQ/h = 1251 \pm 3$ kHz and $\eta = 0.113 \pm 0.006$ and $e^2qQ/h = 1305 \pm 3$ kHz, $\eta = 0.143 \pm 0.006$ (at $T = 77$ K) was reported earlier and traced back to a mix-

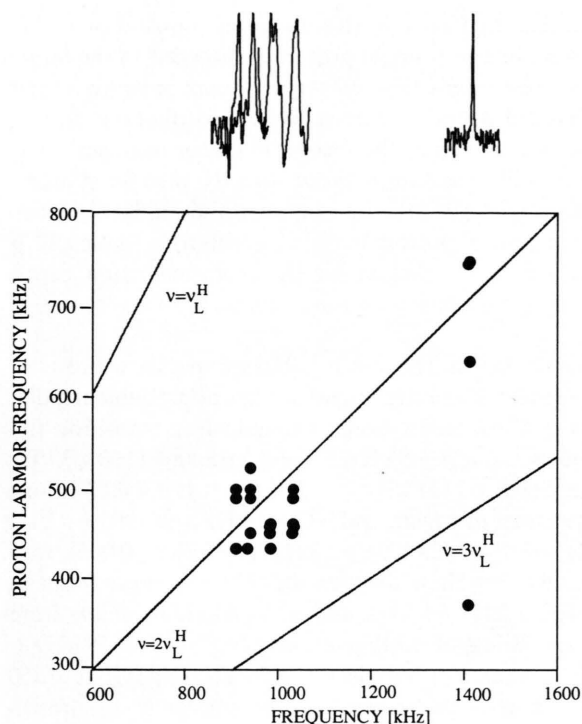


Fig. 3. Dependence of the nitrogen resonances of L-histidine on the matching with the proton Larmor frequency ν_L^H .

ture of orthorhombic and monoclinic L-histidine [8], respectively, no further effort was invested to separate those compounds. The transition frequencies connected with the site causing the resonance at 1410 ± 2 kHz have not been identified. However, using the matching condition of one nitrogen resonance with the proton Larmor frequency ν_L^H or one of its multiples, the lower transition frequencies can be estimated to be in the range between 610–670 kHz and 720–780 kHz. This explains why the peak at 1410 kHz becomes visible at fields with proton Larmor frequencies $\nu_L^H = 636$ kHz, 746 kHz, and with $2\nu_L^H = 740$ kHz (i.e. $\nu_L^H = 370$ kHz), respectively, but cannot be observed at a field with proton Larmor frequency $\nu_L^H = 702$ kHz (Figure 3). Earlier results for higher temperatures at $T = 77$ K, $e^2qQ/h = 1437$ kHz ± 1 kHz, $\eta = 0.915 \pm 0.003$ fall within the range of the estimated quadrupole coupling constant $e^2qQ/h = 1452$ kHz ± 30 kHz, $\eta = 0.88 \pm 0.07$. The magnitude of the quadrupole coupling constant and the anisotropy parameter suggest that this nitrogen resonance is caused by an imidazole nitrogen in the histidine ring. The fact that the resonance is sharper than those of the terminal nitrogen

atoms is consistent with a smaller dipole-dipole coupling due to a single proton as opposed to the larger dipolar interaction of the terminal nitrogen atoms bonded to three protons. We do not find any further resonances from the second imidazol nitrogen atom, probably the one without directly bonded protons. The hydrogen bonding between the imidazole nitrogen and a proton from a neighboring molecule is probably insufficient for the cross-relaxation necessary for detection in our technique.

L-Ala-L-His. The L-Ala-L-His spectra show eight resonances. Field dependent studies and double irradiation allow us to assign the following transition frequencies: 327 ± 5 kHz, 835 ± 4 kHz and 1160 ± 3 kHz ($e^2qQ/h = 1324$ kHz ± 3 kHz, $\eta = 0.49 \pm 0.005$) to one terminal nitrogen; and 932 ± 4 kHz and 1205 ± 4 kHz ($e^2qQ/h = 1423$ kHz ± 3 kHz, $\eta = 0.384 \pm 0.005$), to a second terminal nitrogen site. The resonance frequencies at 1477 ± 2 kHz, and 524 ± 4 kHz originate from one imidazol nitrogen with $e^2qQ/h = 1619$ kHz ± 2 kHz and $\eta = 0.646 \pm 0.005$. The signal at 1550 ± 2 kHz shows sharp features, similar to the transition in the imidazole ring both in L-Ala-L-His and L-histidine indicating that it probably also results from an imidazol resonance. The field dependence of the resonance seems to indicate a slightly higher quadrupole coupling constant. To our knowledge no literature values are available for this compound. In summary, we find evidence for two different terminal nitrogen sites and two imidazol nitrogens.

In the L-Ala-L-His peptide, the terminal nitrogens are members of the L-alanine fraction of the molecule. The quadrupole parameters of these terminal nitrogens are larger both in magnitude and asymmetry parameter than those of pure L-alanine indicating a

more distorted environment. This arises probably mainly from a change of the hydrogen bonds to the neighboring molecules. Similarly, the environment of the imidazol nitrogens is more asymmetric than that in the pure L-histidine crystal structure. The asymmetry parameter here reflects mostly the imidazole ring structure. The lower value of the asymmetry parameter indicates a greater deviation from planar symmetry, which means that the ring also is deformed by the crystal packing of the peptide.

5. Conclusion

The direct detection of ^{14}N NQR at low magnetic fields has become possible by using a dc-SQUID spectrometer. We have used this technique to study the changes of the nitrogen-14 environments of amino acids resulting from the mixture of optically active isomers in the L- and D-serine and DL-serine. The results show that the applied technique is sensitive to the optical purity of the said compounds. The comparison of the spectra of L-alanine and L-histidine combined to form L-Ala-L-His has demonstrated the influence of the intermolecular surroundings even on nitrogen sites in the intact imidazol ring.

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