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COMMUNICATION

Detection of Taurine in Biological Tissues by ^{33}S NMR Spectroscopy

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The potential of ^{33}S NMR spectroscopy for biochemical investigations on taurine (2-aminoethanesulfonic acid) is explored. It is demonstrated that ^{33}S NMR spectroscopy allows the selective and unequivocal identification of taurine in biological samples. ^{33}S NMR spectra of homogenated and intact tissues are reported for the first time, together with the spectrum of a living mollusc. Emphasis is placed on the importance of choosing appropriate signal processing methods to improve the quality of the ^{33}S NMR spectra of biological tissues.

Key Words: **^{33}S NMR spectroscopy; taurine; signal processing.**

The aim of this Communication is to demonstrate that ^{33}S NMR spectroscopy could be a reliable and easy-to-use technique for observing taurine (2-aminoethanesulfonic acid) in biological tissues and living organisms. Taurine is a naturally occurring β-aminoacid widely distributed in the biosphere (*1*). Despite the intensive studies, many mechanisms of the biochemical reactions involving taurine remain unknown or uncertain, probably because of the difficulty in detecting taurine in intact tissues. Indeed, routine procedures for the analysis of taurine are based upon destructive techniques, and the use of ^{1}H and ^{13}C NMR spectroscopy is severely hampered by the overlap between taurine ^{13}C and ^{1}H resonances and signals of other metabolites, even at high field strengths (*2, 3*).

^{33}S is a quadrupolar nucleus ($I = \frac{3}{2}$) with a low natural abundance (0.76%), a low magnetogyric ratio (2.0517×10^7 rad $T^{-1}\,s^{-1}$), and a receptivity to NMR detection that is 1.71×10^{-5} that of the proton. Since ^{33}S has a large quadrupole moment ($Q = -6.4 \times 10^{-28}\,m^2$), nuclear relaxation is dominated by the quadrupolar mechanism and, in consequence, ^{33}S NMR signals are very broad unless the electronic surrounding of sulfur is highly symmetric (*4*). Our previous experience on ^{33}S NMR spectroscopy of sulfones and sulfonates (*5*) suggested to us the possibility of detecting the ^{33}S NMR signal of taurine at natural abundance in biological samples.

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In this paper ^{33}S NMR spectra of biological tissues are reported for the first time. Homogenates and tissues of lamb and calf hearts, lamb brain, and two species of molluscs, *Mytilus edulis* and *Lithophaga lithophaga*, were examined, and the spectrum of *L. lithophaga* was obtained *in vivo*. In addition, it was demonstrated that signal processing could play an important role in improving the quality of ^{33}S NMR spectra with poor signal-to-noise ratio (S/N), thus expanding the potential of this technique in the detection of taurine in biological tissues.

The samples examined were selected based on both their high content of taurine and the relevance of the functions in which taurine is involved. In mammalian heart, taurine has protective effects against hypoxia and congestive failure. A high concentration of taurine is necessary to ensure the correct development of brain before birth. In molluscs taurine is one of the most important osmolytes (*1*).

^{33}S NMR spectra of taurine in aqueous solution. Due to the lack of literature data, it was necessary to determine the ^{33}S NMR parameters of taurine in aqueous solution. ^{33}S NMR spectra were recorded at 11.7 T on a Bruker AM 500 spectrometer operating at a frequency of 38.36 MHz for ^{33}S and equipped with a 10-mm broadband probe head. Typical acquisition parameters were: spectral width = 100 ppm, acquisition time = 0.25 s, 90° observing pulse. Chemical shifts were referred to the ^{33}S signal of 1 M Na_2SO_4 in H_2O in a coaxial cell. Taurine aqueous solutions in H_2O/D_2O (1 : 1) were used.

In the pH range 5−7.5 and in the concentration range 0.02–0.7 M, both ^{33}S chemical shift (-6.7 ± 0.1 ppm) and linewidth (LW = 11 ± 2 Hz) are constant at least within the limits of experimental error. On the basis of this LW value, the ^{33}S of taurine was expected to give a quite sharp peak also in the spectra of tissues, without any interference from other sulfur metabolites. Signals arising from other sulfur functional groups, commonly occurring in biological compounds, such as $-S^-$, $-SO_2^-$, −SH, −SR, –S–S–, are characterized by different chemical shift values and should be broad enough to disappear in the background noise (*4*). These experiments assessed the possibility of detecting the ^{33}S NMR signal of taurine at biological concentrations (10^{-3}–10^{-2} M) in reasonable times. At 11.7 T, two hours was

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sufficient to obtain the spectrum of a 2×10^{-2} M taurine solution with $S/N \approx 5$.

^{33}S NMR spectra in biological samples. ^{33}S NMR spectra were recorded at 11.7 T. The following recording parameters were used for all the samples examined: spectral width = 220−540 ppm, acquisition time = 0.020−0.125 s, 90° observing pulse. A preacquisition delay between 60 and 100 μs was used in order to reduce the rolling baseline. The number of accumulated transients ranged between 20,000 (*M. edulis*) and 1.5×10^6 (lamb brain).

In the chemical shift range −240 to +300 ppm the ^{33}S spectra of both homogenates and intact tissues exhibited a single signal that was assigned to the $-^{33}SO_3^-$ group of taurine on the basis of its chemical shift value (-6.8 ± 0.4 ppm). The attribution was confirmed by adding pure taurine to the samples.

In the spectral range examined, no ^{33}S NMR signals were detected from other sulfur-containing biological molecules, for instance, cystine, cysteine, methionine, and hypotaurine.

Taurine ^{33}S chemical shift did not significantly change in the examined samples and was in good agreement with the value measured in aqueous solution. Undoubtedly, the invariability of the chemical shift could facilitate the assignment of the taurine resonance in unknown samples. ^{33}S LW (40–80 Hz) was significantly larger than that in aqueous solution. Besides microscopic variations in the magnetic susceptibility of the samples, possible causes for the broadening of the signal may include interaction with metal ions (e.g., Ca^{+2}) or small molecules (e.g., other aminoacids), and exchange between free and bound taurine. To determine which mechanisms are operant, more experimental data are required.

The S/N values of the ^{33}S NMR spectra depend on the concentration of free taurine. The largest amount of free taurine was found in molluscs, the lowest in lamb brain, in agreement with the data reported in the literature (*1*).

Since *L. lithophaga* has a cylindrical shape, it was possible to acquire its ^{33}S NMR spectrum *in vivo*, using a standard 10-mm broadband probe head. A living mollusc within its shell was put into a 10-mm NMR sample tube filled with sea water that was previously saturated with oxygen. The ^{33}S NMR signal of taurine (Fig. 1) in live *L. lithophaga* was detected in about 8 min (40,000 scans) with S/N of about 3.

Data processing (6). Preliminary results are reported that concern the use in ^{33}S NMR spectroscopy of power spectrum representation and linear prediction (*7*) in the reconstruction of the FID.

In the processing of ^{33}S NMR spectra of biological samples with poor S/N, no significant improvement can be obtained using different apodization functions (exponential multiplication, Hanning, Hamming, etc.) (*6*) as evident by comparing Figs. 1a and 1b. A remarkable improvement in the visual detection of the taurine signal can be achieved by processing the data to display the power spectrum (Fig. 1c). It must be pointed out that

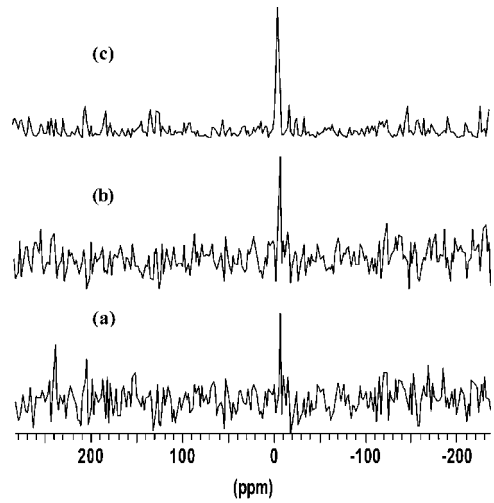

FIG. 1. *In vivo* ^{33}S NMR spectrum of an *L. lithophaga* at 11.7 T. Spectral width = 520 ppm, acquisition time = 0.0125 s, number of data points = 512, preacquisition delay = 90 μs, number of scans = 40,000. (a) FT of the FID, (b) FT of the FID multiplied by the Hamming function, (c) power spectrum of (b).

problems associated with power spectrum representation such as unreliability of relative intensities of different peaks and loss of phase information are not important if a single peak with constant linewidth is detected.

^{33}S NMR spectra are often affected by baseline distortions because the first data points of the FID are corrupted by transitory signals (*8*). This problem is not particularly evident at high magnetic fields (Fig. 1a) but becomes quite dramatic with the decreasing of the magnetic field strength and can severely deteriorate the quality of the spectrum beyond an acceptable limit in the case of low-concentration samples. In order to verify whether ^{33}S NMR spectroscopy can be used to detect taurine in biological samples also at magnetic fields lower than 11.7 T, the spectrum of a taurine 5×10^{-3} M solution was acquired at 7.04 T. The quality of the spectrum obtained is likely to be the minimum acceptable (Fig. 2a). The baseline distortions can be partly reduced by appropriate scaling of the first data points of the FID (Fig. 2b). This procedure could result in a loss of signal intensity for fast-relaxing nuclei, because the first data points of the FID contain most of the information about the signal intensity. It has been found that the problem can be circumvented by using linear prediction (*7*) in the reconstruction of the FID (Fig. 2c). Note the dramatic improvement in the quality of this spectrum in comparison with those in Figs. 2a and 2b.

Quantitative analysis: Some preliminary results. A lamb heart was finely homogenated and divided into two weighted parts. A known amount of Na_2SO_4 was added as an internal standard to each part (7.3×10^{-3} and 3.9×10^{-3} g SO_4^{2-}/g wet tissue). The ^{33}S NMR spectra were acquired at a repetition

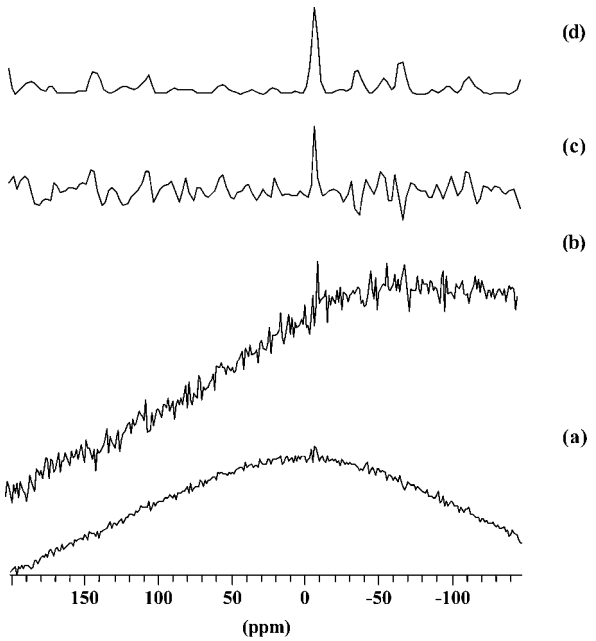

FIG. 2. ^{33}S NMR spectrum of a 5×10^{-3} M taurine solution, acquired at 7.04 T on a Bruker AM 300 spectrometer. Spectral width = 638 ppm, acquisition time = 0.035 s, number of data points = 1024, preacquisition delay = 90 μs, number of scans = 1.65×10^6, 75° observing pulse. (a) FT of the FID, (b) FT of the FID left-shifted by two data points and multiplied by an exponential function (10 Hz line broadening), (c) FT of the FID extrapolated by linear prediction (linear prediction was performed using the Win-NMR program by Bruker), (d) power spectrum of (c).

rate that allowed the complete nuclear relaxation. The number of scans was regulated so as to permit accurate measurements of the peak areas. The taurine content in each sample (53 and 58 μg/g wet weight) was easily determined by the ratio of the integrals of taurine and sulfate peaks and is consistent with the values reported in the literature for mammalian hearts. It follows that ^{33}S NMR spectroscopy might be a suitable tool also in quantitative determination of taurine. Obviously, the routine application of this technique requires the standardization of the analytical method but this is beyond the aim of the present work.

In conclusion, the use of the ^{33}S NMR spectroscopy in the detection of taurine could be very attractive for the following reasons: (a) spectra can be acquired directly for intact tissues or homogenates; (b) taurine can be observed selectively, which means that spectra are easy to interpret; and (c) due to short ^{33}S T_1 values (7–10 ms by saturation recovery), ^{33}S NMR spectra of taurine can be acquired using fast repetition rates, so that good S/N can be obtained in total experimental times equal or shorter than those needed for ^{13}C NMR spectra.

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