

IMPROVED CHARACTERIZATION OF HEALTHY AND MALIGNANT TISSUE BY NMR LINE-SHAPE RELAXATION CORRELATIONS

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ABSTRACT We performed a relaxation-line-shape correlation NMR experiment on muscle, liver, kidney, and spleen tissues of healthy mice and of mouse tumor tissue. In each tissue studied, five spin groups were resolved and characterized by their relaxation parameters. We report a previously uncharacterized semi-solid spin group and discuss briefly the value of this method for the identification of malignant tissues.

INTRODUCTION

During the past two decades nuclear magnetic resonance (NMR) has been used extensively for the investigation of the properties of biological systems on a molecular level (1–24). Many studies (25–37) of relaxation times in healthy and malignant tissues were prompted by the observation (25) that the spin-lattice relaxation time (T_1) and spin-spin relaxation time (T_2) in a tumor generally were longer than in a healthy tissue. In these studies, the NMR relaxation measurements have been taken at only one time window on the free induction decay (FID). The resolution of the heterogeneous spin distribution was improved if the relaxation measurements were taken at two time windows (23, 24). A further improvement of the resolution of spin distributions in these complex systems was achieved by a two-dimensional (2D) NMR technique (38, 39). This new approach is also called line-shape relaxation correlation NMR, or 2D-correlation NMR.

In the 2D-correlation spectroscopy the time evolution of the transverse decay, along the t -axis, and of the longitudinal recovery, along the τ -axis (38) are recorded and correlated. Typically 20 values of t and about 25 values of τ are used as compared with 1 value of t and about 25 values of τ in a standard NMR relaxation measurement. At each FID window, t , the magnetization recovery function,

$M_x(t) - M_x(t, \tau)$, is plotted as a function of τ and graphically decomposed into components. The $\tau = 0$ intercepts and T_1 values of the components at each t are obtained. A plot of the $\tau = 0$ intercepts vs. t yields the main part of the FID of each magnetization component. Finally these FIDs are fitted to a Gaussian or an exponential. Projections to $t = 0$ of the FID, which are the equilibrium magnetizations, are obtained. If T_1 of magnetization components differ by more than a factor of ~ 5 , the characterization of the spin groups is unambiguous. If the T_1 difference is smaller, additional corroboration, such as the $T_{1\rho}$ -line-shape correlation, is needed.

In this report, we present the results of the standard FID and T_2 experiments as well as of the 2D-correlation NMR experiments in the mouse muscle, liver, kidney, spleen, and tumor tissues. With the 2D correlation approach, we also resolve a previously uncharacterized semi-solid spin group. In addition, proper measurements of the nuclear spin-relaxation times yield an increased malignancy index without increasing the time of the measurement.

EXPERIMENTAL METHODS

Normal tissue samples were removed from 8–10-wk old BALB/c mice. The tumor studied was the EMT6 fibrosarcoma (obtained from R.F. Kallman, Stanford, CA) grown in the dorsal subcutaneous tissue of BALB/c mice. The tissue samples were blotted free of blood and cut into $\sim 0.1\text{-cm}^3$ pieces. These were placed in $\sim 25\text{-mm}$ glass tubes with 5-mm diameter and flame sealed.

NMR measurements were performed at 10°C with a Bruker SXP pulse spectrometer (Bruker Instruments, Inc., Billerica, MA) at 38 MHz. Since the receiver dead time was $\sim 8\text{ }\mu\text{s}$, the FIDs were recorded starting 10 μs

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after the 90° pulse. The T_2 was measured using the Carr-Purcell-Gill-Meiboom (CPMG) pulse sequence with 100- μ s spacing between the successive 180° pulses. The T_1 was measured with the inversion recovery sequence 180°- τ -90° using ~ 25 values of τ .

The signal averaging and the data analysis were done with a Hewlett-Packard model 9845. A desk-top computer (Hewlett-Packard Co., Palo Alto, CA), interfaced with the spectrometer via a Biomation 805 transient waveform recorder (Biomation, Cupertino, CA). 10 and 100 FIDs were typically averaged in the T_1 and T_2 experiments, respectively.

RESULTS

The results of the proton FID experiment combined with a proton spin-echo envelope experiment on the five tissues are summarized in Table I. In each tissue the decay curve was graphically decomposed into five components, (Table I). These components are labeled solid (S), semi-solid (SS), semi-liquid (SL) and liquid (L_2 and L_1) according to their T_2 of ~ 20 μ s, ~ 300 μ s, ~ 5 ms, ~ 50 ms, and ~ 200 ms, respectively. Each S-component was fitted to a Gaussian curve. The T_2 of the Gaussian was taken to be the time in

which the magnetization decays to one-half of its initial value.

All values shown represent the average of measurements on five different samples of a particular type of tissue. The accuracy of a typical measurement is $\pm 3\%$. However, because of biological sample differences, the standard deviations shown are much larger.

The FID and T_2 results in muscle agree with the published data (12, 23, 24). The SS-component, which was deduced indirectly a few years ago from measurements in fully deuterated muscle (24), is resolved in the present experiment in the natural muscle.

All the recovery curves of the proton magnetization were graphically decomposed into two components. Fig. 1 shows the T_1 values obtained from such decomposition, at 20 time windows along the FID in muscle, plotted as a function of time, t . The long and the short T_1 (represented by circles and crosses, respectively) are independent of the window position. Each of the T_1 values, given in Table I as the 2D

TABLE I
SPIN RELAXATION RESULTS IN MOUSE MUSCLE, LIVER, KIDNEY, SPLEEN, AND TUMOR

Sample	Spin group character	FID and CPMG T_2		2D Time evolution T_1 (FID)		
		Magnetization fraction	T_2	T_1	Magnetization fraction	T_2
		(%)	(ms)	(ms)	(%)	(ms)
Muscle	L_1	6 ± 2	190 ± 50			
	L_2	80 ± 2	46 ± 4	610 ± 60	85 ± 4	$\sim 1.5^*$
	SL	3 ± 1	6 ± 1			
	SS	4 ± 1	0.29 ± 0.11	90 ± 10	7 ± 3	0.42 ± 0.16
	S	7 ± 1	$(18 \pm 1) \times 10^{-3}$	610 ± 60	8 ± 3	$(15 \pm 4) \times 10^{-3}$
Liver	L_1	4 ± 1	190 ± 50			
	L_2	78 ± 2	39 ± 6	380 ± 30	89 ± 4	$\sim 1.5^*$
	SL	6 ± 2	6 ± 2			
	SS	5 ± 2	0.27 ± 0.11	80 ± 10	5 ± 1	0.22 ± 0.09
	S	7 ± 1	$(16 \pm 3) \times 10^{-3}$	380 ± 30	6 ± 3	$(15 \pm 4) \times 10^{-3}$
Kidney	L_1	5 ± 3	190 ± 30			
	L_2	79 ± 3	50 ± 8	490 ± 50	91 ± 1	$\sim 1.5^*$
	SL	5 ± 2	8 ± 1			
	SS	5 ± 1	0.36 ± 0.11	85 ± 10	5 ± 2	0.22 ± 0.10
	S	6 ± 1	$(21 \pm 1) \times 10^{-3}$	490 ± 50	4 ± 1	$(17 \pm 4) \times 10^{-3}$
Spleen	L_1	9 ± 4	210 ± 20			
	L_2	76 ± 4	60 ± 4	530 ± 20	90 ± 2	$\sim 1.5^*$
	SL	5 ± 1	6 ± 2			
	SS	5 ± 1	0.28 ± 0.03	85 ± 15	5 ± 1	0.17 ± 0.03
	S	5 ± 1	$(19 \pm 1) \times 10^{-3}$	530 ± 20	5 ± 2	$(14 \pm 3) \times 10^{-3}$
Tumor	L_1	15 ± 2	170 ± 50			
	L_2	75 ± 4	67 ± 15	760 ± 50	94 ± 1	$\sim 1.5^*$
	SL	3 ± 1	4 ± 1			
	SS	4 ± 1	0.30 ± 0.11	100 ± 10	3 ± 1	0.24 ± 0.07
	S	3 ± 1	$(19 \pm 6) \times 10^{-3}$	760 ± 50	3 ± 1	$(16 \pm 2) \times 10^{-3}$

All values shown represent the mean \pm SD obtained from 10–100 measurements on five different samples of each kind.

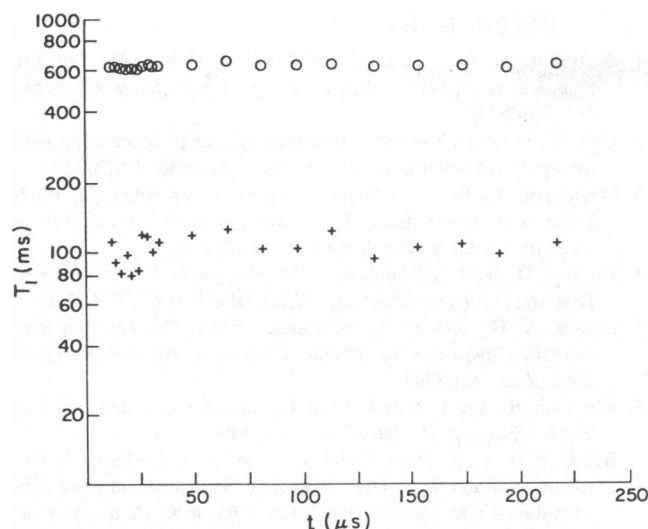


FIGURE 1 T_1 plotted as a function of window position in muscle tissue.

time evolution T_1 (FID), is the average of 100 values obtained from the analysis of the recovery curves at 20 windows in 5 different muscle samples. The T_1 values of the ($L_1 + L_2 + SL + S$) magnetization components (Table I) compare well with published results (27). The T_1 of the SS-magnetization component has not been detected accurately until now.

The $\tau = 0$ intercepts obtained from the graphical decompositions are plotted as a function of t in Fig. 2. The FID of the magnetization component with long T_1 (represented by circles, Fig. 2) was decomposed graphically into two components (Table I). The main component was fitted to an exponential with a time constant T_2^* of ~ 1.5 ms. This spin-spin relaxation time is due to the field inhomogeneity broadening and is given in Table I as $\sim 1.5^*$ ms. The short component was fitted to a Gaussian (S-component with $T_2 = [15 \pm 4] \mu s$ in Table I). The FID of the magnetization component with short T_1 (represented by crosses in Fig. 2) is characterized by a single exponential with a T_2 of $400 \mu s$ (Table I). This component is labeled SS. The scatter in the data points in Figs. 1 and 2 could not be decreased significantly by an iteration procedure (39). The equilibrium magnetization fractions obtained from the projections to $t = 0$ of the two FID in Fig. 2, are shown in Table I in the column labeled 2D time evolution T_1 (FID).

The results of the same experiments and analysis in liver, kidney, spleen, and tumor tissues are summarized in Table I.

DISCUSSION

The relaxation times and the relative magnitudes of the associated magnetizations are very similar in all tissues studied (Table I). In each tissue, with the FID and T_2 experiment, five magnetization components are resolved. In the muscle tissue, a preliminary assignment of the magnetization components to molecular protons has been

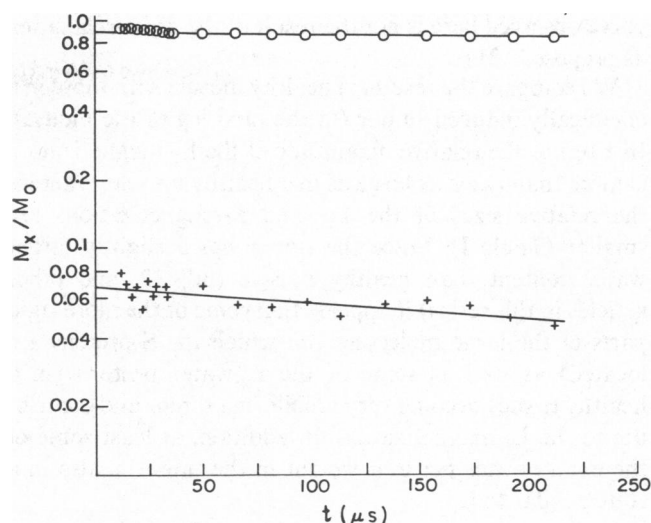


FIGURE 2 FID in muscle tissue obtained by the 2D NMR technique. The FID of the spin groups with long and short T_1 are represented by circles and crosses, respectively.

made. In the multiwindow study (23, 24) of natural and deuterated mouse muscle it was shown that in muscle the L_1 - and L_2 -protons are on the most mobile parts of large molecules and on molecules of the "free" water, respectively. The SL-protons are protons on water molecules with restricted mobility (bound water). The S-spins are on rigid parts of large molecules.

In the muscle tissue in which all water H and exchangeable large-molecule H were exchanged with deuterons (deuterated muscle) the SS-component was estimated (24) to have a magnitude of $(3.4 \pm 0.7)\%$ relative to the total magnetization of the natural muscle. In the experiment reported here the SS-component in the natural muscle amounts to $(4 \pm 1)\%$ and $(7 \pm 3)\%$ of the total equilibrium magnetization of this tissue, as determined from FID- T_2 and 2D-time evolution T_1 (FID) experiments, respectively (Table I). Within the experimental error these three estimates of its relative magnitude do not differ. Although it is reasonable to assign the SS-component magnetization to protons on large molecules (24), it cannot be ruled out that a part of the SS-component ($\sim 1\%$ of the total magnetization of the natural muscle) is due to bound-water protons.

It is interesting that in the muscle tissue the SS-proton group has $T_1 = 90 \pm 10$ ms, whereas all the remaining protons have $T_1 = 610 \pm 60$ ms (Table I). The implication of this large difference in T_1 is that the SS-protons are isolated from the other spins. In a recent study (21) of cross-relaxation effects in collagen and muscle it was shown that in the muscle the recovery curve for the magnetization of large molecules could be described by two apparent relaxation times of 17 and 625 ms. The fact that the shorter of these is about five times smaller than the T_1 of 90 ms resolved above (Table I) suggests that the apparent non-exponentiality in the magnetization recovery

curve observed here is not the result of the cross-relaxation as proposed (21).

We compare the results in healthy muscle with those in a chemically induced tumor (in the hind leg of the mouse). In a tumor the relative magnitude of the L_1 -magnetization is more than twice as large as in a healthy muscle, whereas the relative sizes of the L_2 - and S-magnetizations are smaller (Table I). Since the tumor has a slightly larger water content than healthy muscle (30, 32, and other articles in this series) it appears that some of the more rigid parts of the large molecules (on which the S-protons are located) as well as some of the L_2 -water protons (in a healthy tissue) become very mobile in a tumor and contribute to the L_1 -magnetization. In addition, at least some of the excess water per unit weight in the tumor is also in a state similar to L_1 .

In a tumor, the mean value of $T_2 = (67 \pm 15)$ ms of the water (L_2) magnetization is ~50% larger than in a healthy muscle tissue (Table I) in agreement with the earlier observations (25, 26). The well-documented fact that the tumors have longer T_1 than healthy tissues is also evident in the present results; i.e., T_1 of a tumor is 760 vs. 610 ms for the muscle tissue (Table I).

For a magnetization component, i , a so-called malignancy index (35, and other articles in this series) is defined as

$$\Delta(i) = \frac{(T_{1,i})_T}{(T_{1,i})_H} + \frac{(T_{2,i})_T}{(T_{2,i})_H}$$

where $(T_{1,i})_T$, $(T_{2,i})_T$, and $(T_{1,i})_H$, $(T_{2,i})_H$, are the relaxation times of the i th magnetization component in malignant and healthy tissue, respectively. For the muscle tumor $\Delta(L_1) = 2.1 \pm 0.3$, $\Delta(L_2) = 2.7 \pm 0.3$, $\Delta(SL) = 1.9 \pm 0.3$, $\Delta(SS) = 2.2 \pm 0.5$, and $\Delta(S) = 2.3 \pm 0.4$. Only the $\Delta(L_2)$ deviates significantly from $\Delta = 2$ (for healthy tissue).

In earlier NMR studies of healthy and malignant tissues an average T_1 and T_2 of a number of components was used in obtaining a Δ -value. As a result, the Δ -value was nearer to 2. It is proposed that the resolution can be improved by using the $\Delta(L_2)$ instead.

In summary, a semi-solid magnetization has been resolved and its relaxation times determined for each tissue. This magnetization is shown to be isolated from the other protons in the tissue. We also determined the so-called malignancy index for each of the five components into which the total tissue magnetization has been resolved. It was found that the index of the proton magnetization L_2 , which is associated with the free-water protons is considerably larger than the index of the total magnetization (consisting of all the L-, SL-, SS-, and S-components combined).

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