Spin-Spin Relaxation Time Manifestations of Tissue Degradation*

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The changes of spin-spin ($T_2$) relaxation time in rat liver and muscle biopsies after tissue excision were investigated by low resolution NMR spectrometry. Relaxation times $T_2$ were determined during the first four hours after the tissue excision at 9-minute time intervals, keeping the tissue at constant temperature. Series of measurements were performed at three different temperatures (7, 20, 37 °C). Changes in pH were measured under the same experimental conditions.

Due to degradation processes after tissue excision, molecular and structural changes take place in the cells and are manifested in variations of $T_2$ value. An empirical relation is suggested to describe the observed correlation between $T_2$ and pH, as well as temperature in the excised liver and muscle tissue.

INTRODUCTION

The NMR relaxation times of protons in different biological tissues have been studied extensively. The importance of such studies has been increased with the development of NMR tomography, since relaxation times could be compared with those measured in vivo. However, the variety of relaxation mechanisms and their influence on the $T_1$ (spin-lattice) and $T_2$ (spin-spin) relaxation time are still not fully understood.

It was proposed by several groups that the changes in pH and temperature have an influence on $T_2$ in biological tissues. 2,3

The goal of the present study was to investigate this dependence on $T_2$ in rat liver and muscle tissue on the degradation process.

EXPERIMENTAL

In all experiments, male sprague – dawley rats of 200–300 mg body weight were used. The animals were sacrificed by cervical dislocation, the median lobe of the liver or skeletal muscle (M. extensor digitorum longus) were excised immediately, slightly blotted and cut into smaller pieces.

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* Dedicated to Prof. Dušan Hadži on the occasion of his 70th birthday.
The proton $T_2$ values were measured by a 20 MHz low resolution pulse spectrometer (pc 120 minispec, Bruker, FRG). The pH was measured by a pH-meter (Seybold G104, Austria), using a regular pH-electrode (o.d. = 3 mm). Temperature of the sample was controlled by a thermostat (Haake F3).

The $T_2$ data were determined by applying the Carr-Purcell-Meiboom-Gill (CPMG) method with 1690 180° RF-pulses. The shortest possible pulse separation of 200 $\mu$s was used. For technical reasons, only every 10-th echo was sampled. The repetition time was 3.0 s, allowing a complete relaxation of the tissue protons.

For each animal, $T_2$ and pH were determined immediately after the sacrifice and excision of the tissue (~5 minutes) and afterwards at 9-minute intervals over a period of 4 hours. Thus, the degradation effects were followed by 26 $T_2$ as well as 26 pH measurements per animal.

The $T_2$ and pH values were measured at three different temperatures (7, 22 and 37 °C). A group of 5 animals was used for experiments at each temperature.

RESULTS

Figures 1 and 2 show a good correlation between the changes in $T_2$ and pH in rat liver tissue with the time elapsed after the excision of the tissue. In Figures 3 and 4 a similar time dependence for $T_2$ and pH in muscle tissue with increased degradation time is shown.

Based on this observation, we propose a linear relationship in order to describe the dependence of $T_2$ on pH and temperature of rat liver and muscle tissue:

$$T_2(T,\Delta pH) = T_2(T_o,0) + \left( \frac{\partial T_2}{\partial T} \right)_{\text{pH}=0} \cdot (T - T_o) + \left( \frac{\partial T_2}{\partial \Delta pH} \right)_{T_o} \cdot \Delta pH$$

(1.1)

where:

$T$ — temperature of the excised tissue

$T_o$ — in vivo temperature of the tissue (37 °C)

$\Delta pH$ — difference between in vivo and measured pH values

$T_2(T_o,0)$ — constant; representing the initial value of $T_2$ and 37 °C and in vivo pH

![Figure 1. The in vivo proton $T_2$ relaxation time alternation of excised liver tissue as a function of the time after organ excision (sacrifice). $T_2$ (mean±SD) time courses are drawn for different tissue temperatures (Δ − 37 °C, □ − 22 °C, △ − 7 °C).](image-url)
Figure 2. Alterations of pH of excised liver tissue as a function of the time after organ excision (sacrifice). $T_2$ (mean±SD) time courses are drawn for different tissue temperatures ($\Delta - 37^\circ C$, $\Box - 22^\circ C$, $\O - 7^\circ C$).

Figure 3. The in vitro $T_2$ relaxation time alteration of excised muscle tissue as a function of the time after tissue excision (sacrifice). $T_2$ (mean±SD) time courses are drawn for different tissue temperatures ($\Delta - 37^\circ C$, $\Box - 22^\circ C$, $\O - 7^\circ C$).

Figure 4. The pH alteration of excised muscle tissue as a function of the time elapsed after tissue excision (sacrifice). $T_2$ (mean±SD) time courses are drawn for different tissue temperatures ($\Delta - 37^\circ C$, $\Box - 22^\circ C$, $\O - 7^\circ C$).
TABLE I

Constants and correlation coefficients obtained by fitting the experimental data to linear relationship (1.1) for excised liver and muscle tissue

<table>
<thead>
<tr>
<th></th>
<th>$T_2(T_0,0)(\text{ms})$</th>
<th>pH$_{\text{in vivo}}$</th>
<th>$C_T(\text{ms}/^\circ\text{C})$</th>
<th>$C_{pH}(\text{ms})$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>24±3.8</td>
<td>7.25</td>
<td>0.19±0.002</td>
<td>5.25±2.2</td>
<td>0.877</td>
</tr>
<tr>
<td>Muscle (skeletal)</td>
<td>21.6±2.6</td>
<td>7.03</td>
<td>0.46±0.05</td>
<td>28±3.3</td>
<td>0.928</td>
</tr>
</tbody>
</table>

These constants, determined by 390 data points for each type of tissue, are given in Table I. The correlation coefficients $R^2$ (Table I) indicate the quality of our fit for liver and muscle tissue to expression (1.1).

DISCUSSION

Recently, an empirical model correlating $T_2$, temperature and pH in mouse liver was presented. In order to separate various effects influencing $T_2$ in different types of tissue, we decided to apply a linear relation to fit the data. By this approach, the interdependence between $T_2$, temperature and pH changes in rat liver and muscle tissue is described.

The changes in hydrogen ion concentration are correlated with $T_2$ variations of normal liver and muscle tissue after biopsy at constant tissue temperature. However, pH dependence is much stronger in muscle (28 ms/pH unit) than in liver (about 5 ms/pH unit) tissue. Also, the temperature dependence of $T_2$ of the skeletal muscle (0.46 ms/$^\circ\text{C}$) is about twice as large as that of liver (0.19 ms/$^\circ\text{C}$) tissue.

We may conclude that pH and temperature are the most important parameters that influence the changes of $T_2$ in normal soft tissue during early post mortem processes.

REFERENCES


SAŽETAK

Očitovanje raspada tkiva relaksacijskim vremenom spin-spin

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Promjene relaksacijskog vremena spin-spin ($T_2$) pri biopsijama jetre i mišića štakora pro-
učavane su NMR spektroskopijom niskog različivanja.

Relaksacijska vremena $T_2$ određivana su pri stalnoj temperaturi tijekom prva četiri sata nakon izrezivanja u devetminutnim intervalima. Izveden je niz mjerenja pri tri različite tempera-
ture (7, 20 i 37 $^\circ\text{C}$). Mjere su promjene pH pod istim eksperimentalnim uvjetima.

Uslijed raspadnih procesa nakon izrezivanja tkiva dolazi do molekulskih strukturalnih prom-
jena, koje se očituju u promjenama vrijednosti $T_2$. Predlaže se empirijska relacija, koja opisuje zapaženu korelaciju između $T_2$ i pH, kao i temperature izrezanog jetrenog i mišićnog tkiva.