CROATICA CHEMICA ACTA CCACAA **65** (1) 211–214 (1992)

ISSN 0011-1643
UDC 543.42
CCA–2054

Original Scientific Paper

Spin-Spin Relaxation Time Manifestations of Tissue Degradation*

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Received July 17, 1991

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.[1] 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.

* 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 μs 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:

$$T_2(T,\Delta\text{pH}) = T_2(T_o,0) + \left(\frac{\partial T_2}{\partial T}\right)_{\Delta\text{pH}=0} \cdot (T - T_o) + \left(\frac{\partial T_2}{\partial \Delta\text{pH}}\right)_{T_o} \cdot \Delta\text{pH} \qquad (1.1)$$

where:

T — temperature of the excised tissue
T_o — *in vivo* temperature of the tissue (37 °C)
ΔpH — 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

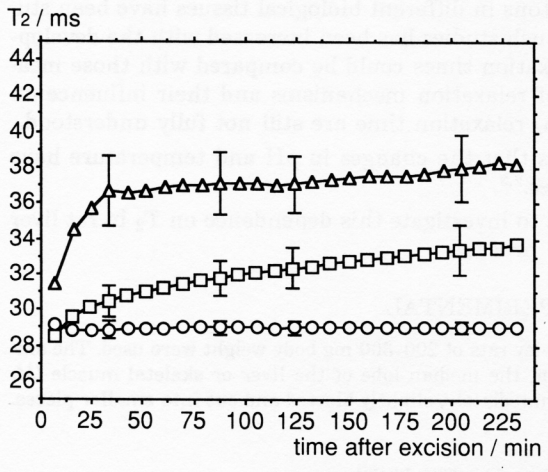

Figure 1. The *in vivo* proton T_2 relaxation time alternation of excised liver tissue as a function of the time after organ excision (sacrifice). T_2 (mean±SD) time courses are drawn for different tissue temperatures (Δ – 37 °C, □ – 22 °C, O – 7 °C).

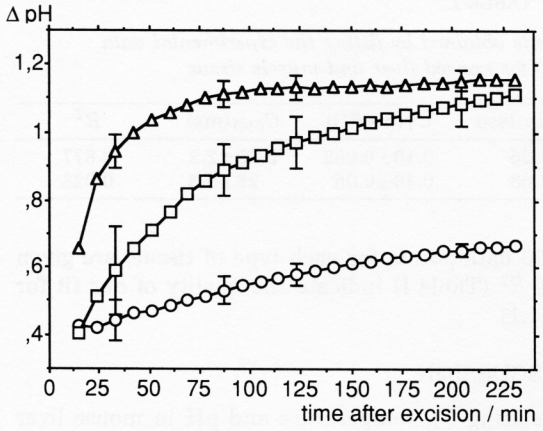

Figure 2. Alterations of pH of excised liver tissue as a function of the time after organ excision (sacrifice). T_2 (mean±SD) time courses are drawn for different tissue temperatures (Δ – 37 °C, □ – 22 °C, O – 7 °C).

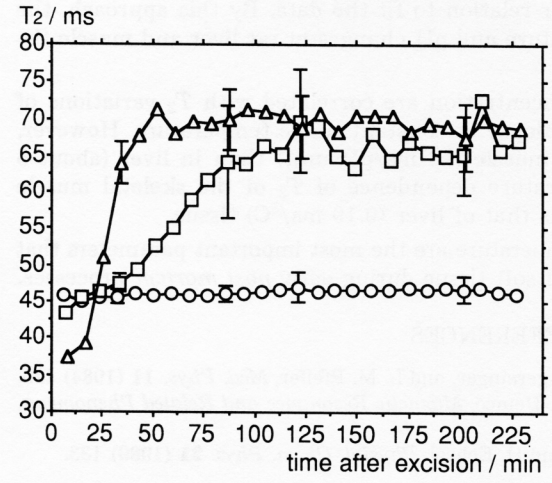

Figure 3. The *in vitro* T_2 relaxation time alteration of excised muscle tissue as a function of the time after tissue excision (sacrifice). T_2 (mean±SD) time courses are drawn for different tissue temperatures (Δ – 37 °C, □ – 22 °C, O – 7 °C).

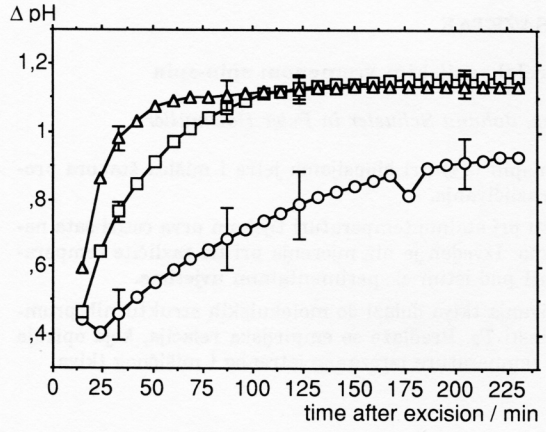

Figure 4. The pH alteration of excised muscle tissue as a function of the time elapsed after tissue excision (sacrifice). T_2 (mean±SD) time courses are drawn for different tissue temperatures (Δ – 37 °C, □ – 22 °C, O – 7 °C).

TABLE I

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

	$T_2(T_o,0)$(ms)	pH*in vivo*	C_T(ms/°C)	$C_{\Delta pH}$(ms)	R^2
Liver	24±3.8	7.25	0.19±0.002	5.25±2.2	0.877
Muscle (skeletal)	21.6±2.6	7.03	0.46±0.05	28±3.3	0.928

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.[3] 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/°C) is about twice as large as that of liver (0.19 ms/°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.

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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 proučavane 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 temperature (7, 20 i 37 °C. Mjerene su promjene pH pod istim eksperimentalnim uvjetima.

Uslijed raspadnih procesa nakon izrezivanja tkiva dolazi do molekulskih strukturnih promjena, 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 mišičnog tkiva.