^{1}H NMR Techniques in Studies of Transport of Paramagnetic Ions in Multicellular Systems

S. RATKOVIĆ[1] AND G. BAČIĆ[2]

1 *Department of Technology and Chemical Research, Maiz Research Institute "Zemun Polje", P.O.B. 89, 11080 Zemun-Beograd, Yugoslavia*

2 *Department of Physical Chemistry, Faculty of Science, University of Beograd, Beograd 11000, Yugoslavia*

Abstract. Two different pulse sequences used in ^{1}H NMR spectroscopy termed free induction decay amplitude recovery (FIDAR) and spin-echo recovery (SER) were applied to studies of transport of paramagnetic ions in multicellular systems. The molar relaxivity of several paramagnetic species (Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Mn^{2+}, $MnEDTA^{2-}$, dextran-magnetite) in water solutions was measured at 32 MHz resonance frequency. Ionic transport was studied using Mn^{2+} and $MnEDTA^{2-}$ as models for cations and anions, respectively, and plant root tissue as a model of a multicellular system.

Key words: Proton NMR — Paramagnetic ions — Molar relaxivity — Multicellular systems — Ionic transport

Introduction

Nuclear magnetic resonance (NMR) spectroscopy has been successfully applied in studies of transport of ions and molecules across artificial or biological membranes (Andrasko 1976; Brindle et al. 1979; Degani and Bar-on 1981; Gupta et al. 1984). NMR signal of the corresponding nucleus ($^7Li^+$, $^{23}Na^+$, $^{27}Mg^{2+}$, ^{31}P, $^{45}Ca^{2+}$ etc.) allows to directly monitor translocations of an ion from one side of the membrane to the other. Another approach is to use paramagnetic ions and look for changes in ^{1}H or ^{31}P NMR spectra of the intra or extracellular compartment. The presence of paramagnetic species may change the following properties: (1) chemical shift in high resolution NMR spectra, (2) spin-relaxation times, T_1 or T_2, of the solvent protons, and (3) bulk magnetic susceptibility.

Most NMR studies of transport processes have been performed on phospholipid vesicles and red cells. Andrasko (1976) studied penetration of Li^+ through membranes of human red blood cells by pulse gradient NMR, while

Degani and Elgavish (1978) used ^{7}Li and ^{23}Na NMR to follow ion transport across the membrane of phosphatidylcholine vesicles. The transport of Pr^{3+} across phospholipid vesicles was studied by observing its effect on chemical shifts of the choline group protons in NMR spectra at both sides of the membrane (Hunt 1975). Similarly, the disappearence of ^{1}H NMR signal of the choline group was used to monitor influx of paramagnetic Mn^{2+} ions into vesicles mediated by inophore X-537A (Degani 1978).

An interesting use of spin-echo NMR in membrane transport studies of small molecules have been described by Brindle et al. (1979) who applied this technique to study transport of alanine and lactate in human erythrocytes. The method is based on differences in bulk magnetic susceptibilities between the inside and the outside of the cells; the difference increases by the addition of paramagnetic DyDTPA to the external medium, e.g. the influx of alanine was reflected by an increase in line intensity (in the negative sense) of CH_3 protons in the high resolution ^{1}H NMR spectrum.

In the present paper two proton NMR techniques are described which are sensitive to the presence of paramagnetic centers, and can be used to monitor translocations of paramagnetic ions within complex multicellular systems. The methods have been termed Free Induction Decay Amplitude Recovery (FIDAR) and Spin-Echo Recovery (SER), and were developed on a pulsed low resolution NMR spectrometer.

^{1}H NMR techniques

I. *Free Induction Decay Amplitude Recovery (FIDAR)*

This method for monitoring translocation of paramagnetic centers from one compartment to another is based on a pulse sequence $(180°—\tau—90°—T)_n$ shown in Fig. 1*A* and is usually used for T_1 measurement in liquids (Farrar and Becker 1971). The function of the 180° pulse is to invert magnetization from $+Z$ to $-Z$; the second 90° pulse then monitors the FID amplitude after a selected time interval τ between the pulses. Magnetization M_τ relaxes with a characteristic time constant T_1 (spin-lattice relaxation time) and can be described by

$$M_\tau = M_0[1 - 2e^{-\tau/T_1}] \tag{1}$$

The interpulse interval may be set $\tau = \tau_0$ so that $M_{\tau_0} = 0$. This means that the proton FID signal from a compartment (cell or tissue) is zero (Fig. 1*A*). If a certain amount of a paramagnetic species is now added which can penetrate from the external medium into the compartment, the signal at τ_0 arises (M_{τ_0})

since proton relaxation time T_1 inside the compartment decreases, and according to Eq. (1) $M_\tau \rightarrow M_0$ (Fig. 1*B*). The curve $M_{\tau_0} = f(t)$ describes influx of the respective paramagnetic species into the compartment (Fig. 1*C*).

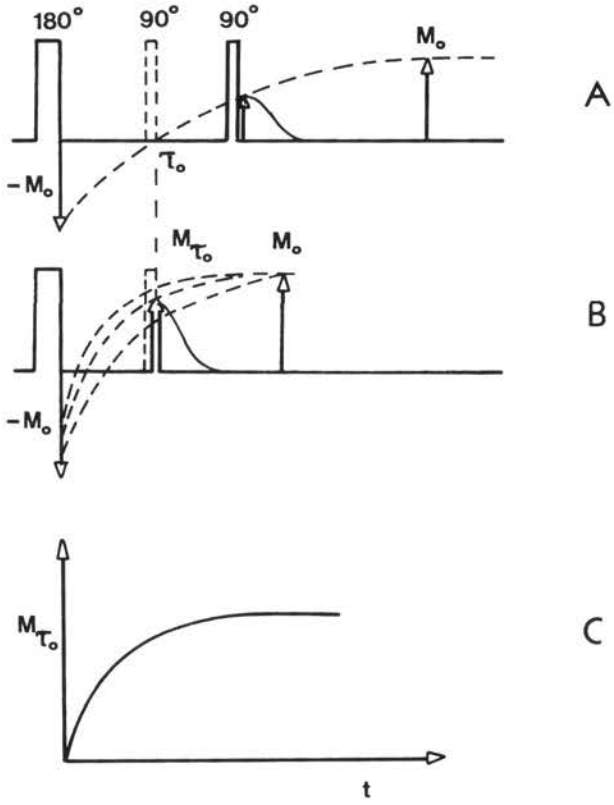

Fig. 1. Pulse protocol, the FIDAR technique. *A*: Standard 180°—τ—90° pulse sequence used to measure spin-lattice relaxation time T_1 in water solutions, single cells or in multicellular systems. The second 90° pulse can be set $\tau = \tau_0$ so that the FID signal intensity $M_{\tau_0} = 0$. *B*: After the addition of paramagnetic ions to a medium in contact with cells or tissue, ions start flowing in and proton T_1 of internal water begins to decrease producing the appearence of the FID signal, i.e. recovery of the FID amplitude. *C*: Recovery of M_{τ_0} over time actually gives the influx curve for a particular paramagnetic species.

II. *Spin-Echo Recovery (SER)*

This method, which has been described earlier (Bačić and Ratković 1984), uses a $(90°—\tau—180°—T)_n$ pulse sequence to produce spin-echo NMR signal of water protons from a certain compartment under the condition that the waiting

multicellular systems (or into single cells). The method enable fast, noninvasive and continual registration of the transport kinetics of paramagnetic ions.

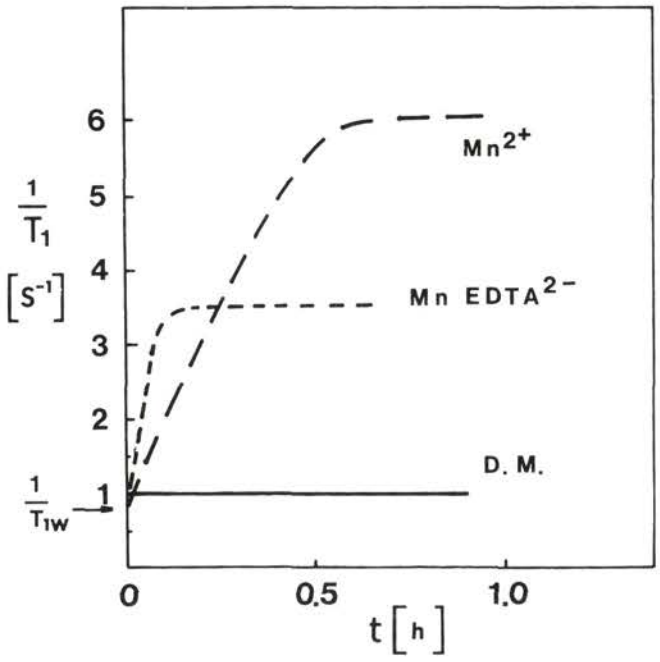

Fig. 2. Proton relaxation rate ($1/T_1$) of tissue water, or amplitude of the recovered FID signal versus soaking time, in accordance with the recorder tracing; primary root of *Zea mays* (hybrid ZP SC 46A), solutions: (1) $MnCl_2$, 50 mmol.l^{-1}, (2) MnEDTA, 50 mmol.l^{-1}, (3) dextran-magnetite, $\sim 1.5 \times 10^{-6}$ mol.l^{-1}.

References

Andrasko J. (1976): Measurement of membrane permeability to slowly penetrating molecules by a pulsed gradient NMR method. J. Magn. Reson. **21**, 479—484

Bačić G., Ratković S. (1984): Water exchange in plant tissue studied by proton NMR in the presence of paramagnetic centers. Biophys. J. **45**, 767—776

Bloembergen N., Purcell E. M., Pound R. V. (1948): Relaxation effects in nuclear magnetic resonance absorption. Phys. Rev. **73**, 679—712

Brindle K. M., Brown F. F., Campbell I. D., Grathwohl C., Kuchel P. W. (1979): Application of spin-echo nuclear magnetic resonance to whole-cell systems. Membrane transport. Biochem. J. **180**, 37—50

Degani H. (1978): NMR kinetic studies of ionophore X-537A- mediated transport of manganous ions across phospholipid bilayers. Biochim. Biophys. Acta **508**, 364—369

Degani H., Elgavish G. A. (1978): ^{23}Na and ^{7}Li NMR studies of ion transport across the membrane of phosphatidylcholine vesicles. FEBS Lett. **90**, 357—360

Degani H., Bar-on Z. (1981): Nuclear magnetic resonance kinetic studies of diffusion and mediated transport across membranes. Period. Biolog. **83**, 61—67

Farrar T. C., Becker E. D. (1971): Pulse and Fourier Transform NMR. Introduction to Theory and Methods. Academic Press, New York

Gupta R. K., Gupta P., Moore R. D. (1984): NMR studies of intracellular metal ions in intact cells and tissues. Annu. Rev. Biophys. Bioeng. **13**, 221—246

Haynes R. J. (1980): Ion exchange properties of roots and ionic interactions within the root apoplasm: their role in ion accumulation by plants. Bot. Rev. **46**, 75—99

Hunt G. R. A. (1975): Kinetics of ionophore-mediated transport of Pr^{3+} ions through phospholipid membranes using ^{1}H NMR spectroscopy.FEBS Lett. **58**, 194—196

Oakes J., Smith E. G. (1981): Structure of Mn-EDTA^{2-} complex in aqueous solution by relaxation nuclear magnetic resonance. J. Chem. Soc., Faraday Trans. 2 **77**, 299—308

Ohgushi M., Nagayama K., Wada A. (1978): Dextran-magnetite: A new relaxation reagent and its application to T_2 measurements in gel systems. J. Magn. Reson. **29**, 599—601

Final version accepted March 25, 1987