Magnetic Resonance Imaging, Vol. 16, Nos. 5/6, pp. 565–568, 1998

Printed in the USA.
0730-725X/98 $19.00 + .00

ELSEVIER

PII S0730-725X(98)00053-8

● *Contributed Paper*

WATER DIFFUSION IN BIOLOGICAL POROUS SYSTEMS: A NMR APPROACH

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The spin-echo nuclear magnetic resonance (NMR) technique together with paramagnetic ion doping are used to study structural parameters of plant samples, such as restricted dimensions, and cell interconnection both through membranes and by cell contact by studying simultaneous restricted diffusion and intercellular water transfer via various pathways. Also, peculiarities of water diffusion on the surface of cell-wall cellulose are studied over a wide range of water content. © 1998 Elsevier Science Inc.

Keywords: **Nuclear magnetic resonance; Relaxation; Diffusion; Water transfer; Plant tissue.**

INTRODUCTION

Biological cells and tissues as a complex class of porous systems are certainly an interesting object for research[1] and for testing the methods of investigation of porous systems. The aim of the present paper was the investigation of structural and dynamic parameters of plant samples, characteristic porous systems, over a wide range of water content using the spin-echo nuclear magnetic resonance (NMR) technique with pulsed magnetic field gradient.

MATERIALS AND METHODS

The scheme of investigations included two types of experiments. 1) The first was the study of the peculiarities of water diffusion on cotton fiber with water content in the range 20–100%. The initial water content of cotton fiber samples was obtained by vacuum infiltration in water with subsequent centrifugation. 2) The second was the study of structural parameters of the excised roots of 7-day-old maize plants (var. Sterling) carried out by water diffusion measurements.

The samples were studied using spin-echo NMR with the pulsed magnetic field gradient method.[2] Self-diffusion coefficients of sample water were measured using a homemade spin-echo instrument at the resonance frequencies 19.2 MHz for cotton fiber and 60 MHz for maize roots. The relative echo amplitude (R) dependence on amplitude (g), and duration (δ) of magnetic field gradient pulses, and the interval between pulses (t_d), i.e., diffusion time, were recorded. The average and effective diffusion coefficients were calculated using the relation:

$$R = \exp[-\gamma^2\delta^2 g^2 (t_d - \delta/3)\, D_{ef(av)}], \qquad (1)$$

where γ is the gyromagnetic ratio, D_{ef} was calculated from the tangent to the "tail" of the curve $\ln R(g^2)$, and D_{av} was calculated from the tangent to the echo envelope $\ln R(g^2)$ as $g \to 0$ for various t_d.

The doping of the sample by paramagnetic ions[1] was used in a number of experiments. Manganese ions were doped into the extracellular space by exposing the roots of intact maize plants to $MnCl_2$ solution (0.025 M) for 10–60 min.

RESULTS AND DISCUSSION

Figure 1 shows the dependence of D_{ef} on water content for the samples of immature (3) and mature (1, 2) cotton fiber in oriented and entangled arrangements. The peculiarity of the dependence is its extreme behaviour at 75% water content, where the diffusion coefficient exceeds that for bulk water, and the D_{ef} anisotropy depends

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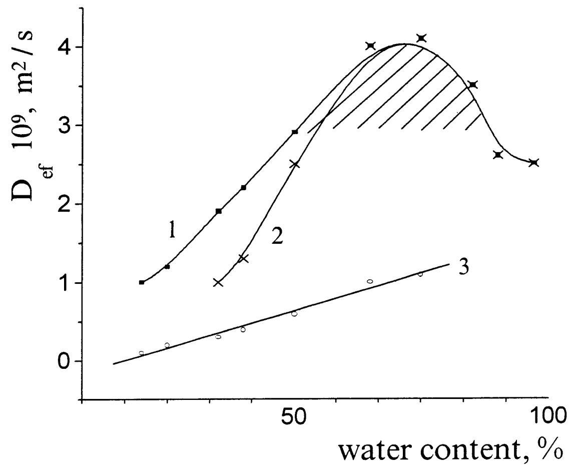

Fig. 1. The dependence of diffusion coefficient on water content for mature (1 and 2) and immature (3) cotton fiber. (1 and 2) Sample arrangement, providing maximal and minimal diffusion echo attenuations, respectively. The dashed region shows the data variation in the experiments.

on fiber orientation with respect to the direction of diffusion registration. The diffusion decrease at a water content higher than 75% is due to capillary-condensed water on the sorption surface, with diffusion similar to that in bulk water. It should be noted that the enhanced D_{ef} value was observed by other authors, and it is connected with sorbed molecule diffusion inside sorbent pores in a vapour or gas phase.[3,4] This mechanism may occur in our experiment, but the absence of the effect in the experiment with immature cotton fiber of low cellulose content testifies in favour of specific properties of the sorbent (cellulose) surface.

The surface diffusion together with the effect of restricted diffusion explain the observed anisotropical phenomena. The diffusion of sorbed molecules along the sorbing surface is known to be determined by the energy relief of the surface. In cotton fiber this relief is given by highly arranged cellulose microfibrils, and this arrangement may determine diffusion anisotropy. The large D_{ef} values are observed only at small t_d. This definitely points to an effect of restricted diffusion.

The dependence of magnetization diffusion attenuation on the pulse amplitude over a range of three orders of magnitude for maize roots shows a complicated nonexponential behaviour for all diffusion times (Fig. 2). The extrapolation of the "tails" of decays as exponentials and subsequent subtraction of these components from the summary envelopes for each diffusion time produce a fast attenuating exponential decay independent of diffusion time in the range 5–1500 ms with diffusion coefficient $D_{ef} = 1.9 \cdot 10^{-9}$ m^2/s and representing a proportion of 40%. The subtraction of this component from the summary envelopes for different diffusion times resulted in a set of slowly decaying nonexponential components with the dependence of D_{av} on diffusion time shown (Fig. 3).

The doping of paramagnetic ions into the intercellular space shortens the extracellular water relaxation time, and at $t_d \geq 20$ ms completely excludes from the total attenuation curve the fast decaying component connected with extracellular water in the porous cell wall system. The remaining slowly decaying magnetization compo-

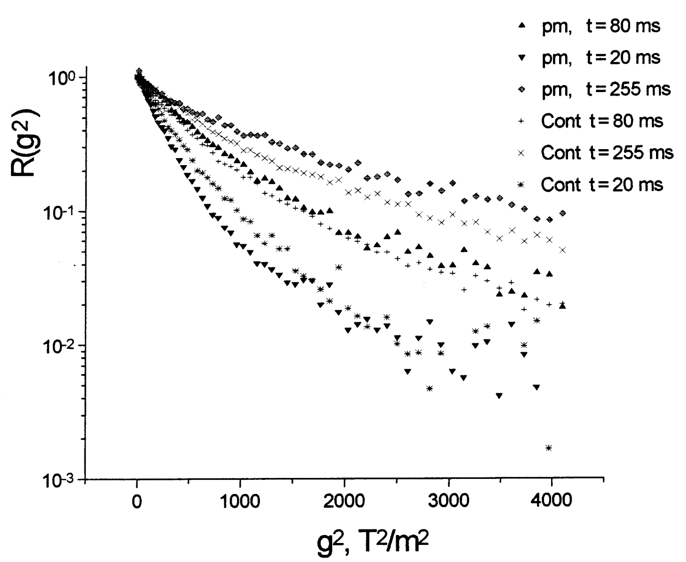

Fig. 2. Diffusion attenuation for maize root samples at various t_d values at baseline and after paramagnetic ion doping.

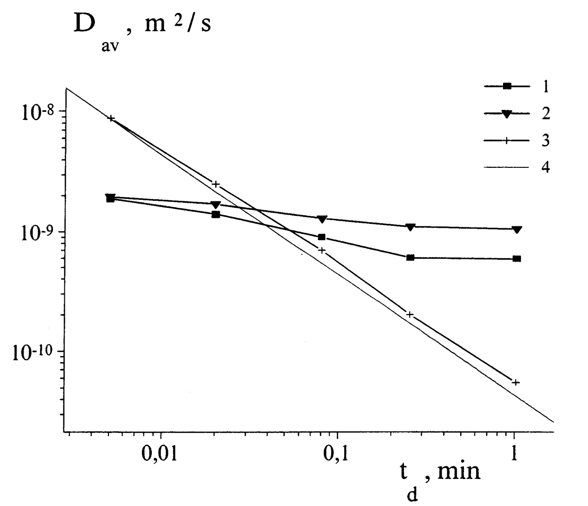

Fig. 3. The dependence of the average diffusion coefficient (ADC) on diffusion time. (1) ADC after subtraction of fast decaying component; (2) original ADC; (3) ADC after taking into account the contributions of free and hindered diffusion; (4) theoretical curve $D_{av} \propto t_d$.

nent is obviously attributable to intracellular water diffusing from cell to cell via intercellular contacts, i.e., plasmodesmata.

The interesting parameters of the porous system are pore size and their connectivity. This information can be found in the dependence, $D(t_d)$. The S-shaped dependence of the average diffusion coefficient on diffusion time (Fig. 4) is known to be expected for porous systems with interpore connectivity. The curve can be divided into three regions: 1) the region of free diffusion; 2) the region of restricted diffusion where the averaging of the

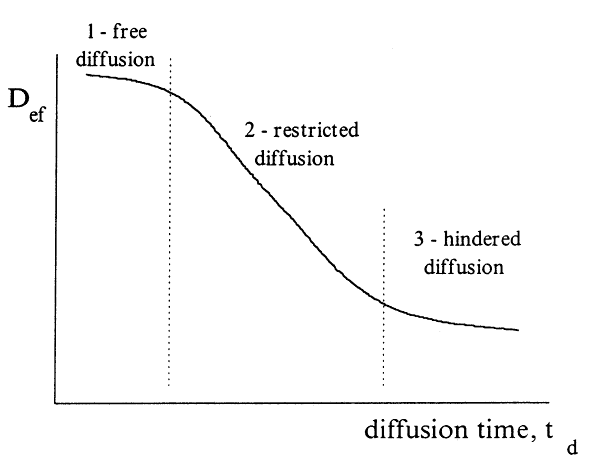

Fig. 4. Idealized dependence of D_{ef} on diffusion time t_d for the system of water containing compartments with permeable walls.

local properties over a large enough volume does not occur yet; and 3) the region of hindered diffusion where the effect of the total averaging of intrapore diffusion is achieved due to coherence and/or restricting permeability of pore walls.

Nonexponential dependencies $R(t_d)$ (Fig. 2) and corresponding dependence $D(t_d)$ (Fig. 3) related to intracellular water obviously contain information about cell size. The key problem is to separate from the $D(t_d)$ dependence the constituent that is connected with the restriction effect only. The solution of this problem is tested experimentally on model samples and results in the renormalisation of $D(t_d)$ given elsewhere:[5] 1) for the short t_d range, the exclusion of the contribution of freely diffusing molecules in $D(t_d)$ is obtained using the relation:

$$D_s(t_d) = D_o D(t_d)/(D_o - D(t_d)), \qquad (2a)$$

and 2) for the large t_d range, the exclusion of the contribution of interporous permeability in $D(t_d)$ is obtained using the relation:

$$D_1(t_d) = [D(t_d) - D^p][D_o/(D_o - D^p)], \qquad (2b)$$

where D_o is the self-diffusion coefficient at $t_d \rightarrow 0$, $D(t_d)$ is the measured self-diffusion coefficient, and D^p is the diffusion coefficient measured at large t_d and independent of t_d. After application of these relations to the experimental curve 1 (Fig. 3) the $D(t_d)$ dependence can be obtained, which is characteristic of the entire restricted diffusion behaviour. It is described as a linear dependence of D on t_d^{-1} (Fig. 3, curve 4). The characteristic restricting size is estimated to be 15 microns using the Einstein-Smolukhovsky relation $\langle a^2 \rangle = 6 t_d D_o$, where D_o is assumed to be equal to the bulk water self-diffusion coefficient 2.5×10^{-9} m^2/s, for the obtained D_{av} and is identified as the cell dimension in the transverse direction. It is important to note that the definition of the restricting size, even if it is not identified with particular morphological structures, might be a useful characteristic parameter for biological sample heterogeneity. The D_{av} value for the region of hindered diffusion obtained in experiments with paramagnetic doping results from water transfer via plasmodesmata, because the transfer via intercellular space becomes invisible in the presence of paramagnetic ions. Then the effective permeability of plasmodesmata can be estimated using the relationship obtained from the analysis of the resistance to water current through a series of semipermeable membranes (thin walls) separated by the distance a:[6]

$$(D^p)^{-1} = D_o^{-1} + (p_{ef}\, a)^{-1}, \qquad (3)$$

where p_{ef} is the effective permeability coefficient. For the transverse cell size $a = 15$ μm, the value of $D_{av} = D^p = 0.55 \times 10^{-9}$ m^2/s at the maximal diffusion time $t_d = 1$ s, and $D_o = 1.1 \times 10^{-9}$ m^2/s at $t_d = 5$ s, the effective permeability coefficient is estimated to be 5×10^{-5} m/s. It is comparable to plasmalemma permeability (10^{-5} m/s).[1]

The independence of the fast decaying component of $R(g^2)$ of diffusion time beginning at the minimal experimental diffusion time, $t_d = 5$ ms, allows us to assert that under these conditions the regimen of hindered diffusion is fulfilled for water encompassed in cell-wall pores. Using the Einstein–Smolukhovsky relation, the maximal pore size, which is experimentally resolved at $t_d = 5$ ms, is estimated to be 8.7 μm. In its turn, the lower limit of the effective cell wall pore permeability coefficient can be estimated using the Eq. (3) as 10^{-6} m/s for $D^p = 1.9 \times 10^{-9}$ m^2/s and $D_o = 2.5 \times 10^{-9}$ m^2/s.

CONCLUSION

The method of analysis of NMR measurements of porous systems is adapted for plant tissues. Determinations of the parameters of cell interconnection, the characteristic restricting cell dimension, and registration of diffusion coefficient for a wide range of water contents are demonstrated.

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