NMR Studies of Radial Exchange and Distribution of Water in Maize Roots: The Relevance of Modelling of Exchange Kinetics

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ABSTRACT

Radial transport of water in primary maize roots has been studied by following the kinetics of the H2O-D2O exchange using proton magnetic resonance. The method is based on the use of an impermeant relaxation agent, dextran-magnetite, which made possible separation of the surrounding proton signal from the root water proton signal. Two pulsed NMR techniques for continuously following the exchange are described. The exchange curve was fitted to several theoretical models in order to evaluate the dominant radial water pathway in roots. It was found that the choice of the appropriate model could not be made solely on the basis of goodness of fit and thus the previously calculated value of membrane water permeability, which is not dependent on the water transport pathways, was used as an additional testing criterion. It is concluded that the major radial water flux in root occurs through the cells, and not through the intercellular space, as previously assumed. The relatively low calculated apparent diffusion coefficient (D' = 3 x 10^-6 cm^2 s^-1) of water is mostly determined by water permeation through a series of membranes. A minor fraction of water in roots (10-15%), exchanged at a slower rate than the rate at which bulk diffusion would allow and was assumed to correspond to the water having restricted mobility due to the influence of macromolecules. The NMR measurements of relaxation of water in roots also suggest different water fractions and it is concluded, from both experimental approaches, that most of the cytoplasmic water has significantly different mobility from the bulk water.

Key words—Maize root, water exchange, proton NMR.

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INTRODUCTION
Much attention has been paid to the radial movement of water through plant roots and both experimental and theoretical aspects are reviewed by many authors (Newman, 1976; Bowling, 1976; Pitman, 1982; Zimmermann and Steudle, 1978; Weatherley, 1982). However, there is still no general agreement about the main pathway for radial flow. There are three potential parallel pathways: (a) the apoplast (free space/endodermis), (b) the symplast and (c)
the vacuolar system (transcellular pathway). There is no doubt that water can utilize all of these pathways but the relative amounts of water transported through each remain uncertain i.e. which pathway offers the lowest resistance to water movement? Most authors accept apoplastic water movement i.e. the traditional barrier is the endodermis where the Casparian band forces water flow through the cell membrane (Anderson, 1976; Bowling, 1976; Weatherley, 1982), although contrasting views can be found (Newman, 1976; Steudle and Jeschke, 1983).

The exchange of labelled water is widely used in attempts to establish the identity of the rate-limiting step for radial exchange (Woolley, 1965; Jarvis and House, 1967; Ginsburg and Ginzburg, 1970) and again there are two disputed views of the exchange process. In the first, water from the extracellular space is easily exchanged with labelled water and, therefore, cells exchange water with the extracellular space independently of each other. This is the so-called 'isolated cell' model, which favours the apoplastic transport. In the second, labelled water moves from cell to cell throughout the tissue in a manner that resembles bulk diffusion and this model favours two other possible pathways.

The lack of an unequivocal answer about the mechanism of water exchange arise from two major problems in exchange studies:

(1) Diffusion of labelled water through unstirred water layers could control water exchange and cause erroneous values for membrane permeability to be obtained (Dainty, 1963; House, 1974). Although the problem of the unstirred water layer at the root surface may not be serious (Jarvis and House, 1967), it is advantageous to eliminate its possible influence. Therefore, we suggest here the use of an NMR technique for following H₂O-D₂O exchange that successfully deals with this problem. In this technique an impermeable relaxing agent, dextran-magnetite (Ohgushi, Nagayama, and Wada, 1978), is used and the technique is based on a principle similar to the NMR technique introduced by Conlon and Outhred (1972) for the determination of cell permeability.

(2) There is no reliable criterion for choosing the correct model for water transport solely on the basis of exchange data since all previous experiments have shown nearly the same time course of exchange, but the values obtained for the diffusional permeability of root cell membranes (P_d) are in the range 10⁻³-10⁻⁶ cm s⁻¹. These discrepancies certainly cannot be attributed to species differences. In this paper we test several models of water exchange and show that the above range of values can be easily obtained with a single set of experimental data by fitting the results to different models. Therefore, an additional testing criterion is necessary and since P_d can be calculated from all models, the previously determined value of P_d (Bačić and Ratković, 1984) is used to test the correctness of each model because this value does not depend on the water transport pathways through the root.

Although exchange data inherently contain information about the state of water in the tissue, few attempts have been made to exploit this, especially in plant tissues. Therefore, we have tried to combine exchange measurements with proton NMR relaxation measurements with the aim of classifying the properties of different water fractions.

The primary root of maize is an obvious choice for these experiments because more data concerning water transport are available for maize roots than for any other plant tissue.

**MATERIALS AND METHODS**

*Plant material*

Maize seedlings (Zea mays L., hybrid ZP SC 46A) were as described previously (Bačić, Božović, and Ratković, 1978). Four-day-old seedlings were used in all experiments. For exchange studies, 4.0 cm long
segments (including root tips) from primary roots were used. The root segments were weighed prior to exchange measurements and the average root radius, $R$, was calculated assuming cylindrical geometry. Since the exchange kinetics may depend on the root radius, only roots with $R = 0.055 \pm 0.005$ cm were used. After completion of the exchange, the roots were blotted and weighed again. It was found that the increase in root weight was due only to the replacement of light water with heavy water, i.e. the $D_2O$ influx was counter-balanced by an equal $H_2O$ efflux.

**NMR measurements**

A pulsed coherent NMR spectrometer, IJS-2-71, operating at 32 MHz was used in all exchange experiments. Two or three root segments were placed in an NMR tube and the amplitude of the proton free induction decay (FID) signal was measured 250 $\mu$s after the 90° pulse. A large volume of 1.0 mg g$^{-1}$ dextran–magnetite (Meito-Sangyo Co., Inc, Tokyo) solution in $D_2O$ (Norskhydro-electrics, 99.8%) was then poured over the roots without allowing their cut ends to come into contact with solution, and radial exchange was followed using one of the procedures described below. As the dead time of this procedure was $\approx 0.4$ s, the exchange process was followed practically from the beginning since $H_2O$–$D_2O$ exchange in maize root occurs over a much longer time scale (Table 1). This is important because the early stage of exchange is essential for the accurate fit of the data to the models. The concentration of dextran–magnetite ($\approx 10^{-3}$ mol m$^{-3}$) was insufficient to produce any significant water flow via an osmotic effect and so only diffusional water exchange was measured. This concentration was, however, sufficient to reduce the water proton spin-lattice relaxation time ($T_1$) to a few milliseconds (Bacic and Ratkovic, 1984). When a root is placed in dextran–magnetite in $D_2O$, the proton signal from the solution is initially zero. As the exchange starts, water molecules are transported to the surrounding solution and, because of their interaction with the relaxation centres, give a fast decaying proton signal which can be easily separated from the root water proton signal ($T_1 = 1-3$ s). The water molecule protons are rapidly relaxed by dextran–magnetite as soon as they reach the root surface, but the relaxation of the water within the root is not influenced by dextran–magnetite because the large particles of dextran–magnetite do not penetrate (Bacic and Ratkovic, 1984). Thus, the exchange can be followed almost continuously by monitoring either the increase in the proton signal from the surrounding solution or the decrease in the signal from the root. These changes can be measured using two pulse sequences (Fig. 1):

(a) When the (180°-τ-90°-TR)$_h$ pulse sequence is applied to a root sample with $TR = 10$ s $> 5 T_1$ ($TR$—waiting time, $\tau$—pulse spacing) and $\tau_0 = T_1 \ln 2$ there will be no proton signal from the root. The typical $\tau_0$ was 0.8–0.9 s. At $t = 0$, there are no water protons in the external solution and so the total proton signal is zero. When exchange begins ($\tau > 0$), water protons in the external solution are relaxed completely in time $\tau_0$ by the dextran–magnetite and so the proton signal from the external solution is directly proportional to the amount of light water actually exchanged.

(b) The standard (90°-τ-180°-TR)$_h$ spin–echo sequence may also be used. Water protons that appear in the external solution are rapidly relaxed ($T_2 < 1$ ms) and if the pulse spacing is long enough (e.g. $\tau = 8$ ms), the echo signal from the external solution is zero. At the same time, the echo signal from the root water protons is still high and this signal decreases during the exchange due to the efflux of light water from the root.

If these techniques are to be used, the relaxation time of the root water must not change significantly during the exchange. Slightly higher values of $T_1$ were found during the exchange, but $T_2$ did not differ significantly from the value in untreated root after exchange was terminated, showing that the above condition was fulfilled. The non-exchangeable water fraction ($P_{ne}$) was calculated from the ratio of the FID amplitude in $D_2O$ treated and untreated roots. The same experiments were also performed with 4.0 cm long segments of isolated cortex.

For accurate determination of the non-exchangeable water fraction it is necessary to prove that the retention of light water in the root is not a consequence of equilibration with the light water that appears in the external solution during exchange, i.e. it is necessary to provide conditions conducive to the complete exchange of all exchangeable water. Therefore, the ratio of external $D_2O$ volume to root volume was varied in the range 10:1 to 50:1, but no change in the fraction of the non-exchangeable water was found. In addition, no further exchange was found when previously deuterated roots were transferred into fresh $D_2O$. For these reasons, it was assumed in the analysis of the exchange kinetics that the outer compartment had an infinite volume relative to the root, so that the concentration of the $D_2O$ in the external solution remained practically unchanged during the exchange.
The exchange curve is presented in Fig. 2 to show the efflux of light water from the root \((1 - M_t/M_e)\) against time, where \(M_t\) is the amplitude of the NMR signal at time \(t\), and \(M_e\) is the equilibrium value after termination of exchange. Curve fitting was performed on a computer and the sum of squares of errors \((\Sigma d^2)\) was used as a measure of the goodness of fit.

The measurements of the relaxation times of water in different parts of the root were made on a Bruker SXP-100 spectrometer at 90 MHz using the Carr–Purcell–Meiboom–Gill (CPMG) sequence for \(T_2\) and the inversion–recovery sequence for \(T_1\) (Farrar and Becker, 1971).

All experiments were performed at 22 ± 1°C. The average radius of the cortical cells \(r\) was determined with a light microscope on hand sections cut about 10 cm from the root tip.

RESULTS

Exchange measurements

Before analysing the kinetics of H_2O-D_2O exchange, it is important to realize that a non-exchangeable water fraction \(P_{ne}\) was found in maize roots. According to NMR measurements, \(P_{ne} = 0.13 ± 0.02\) \((n = 9)\) in intact roots and \(0.08 ± 0.01\) \((n = 6)\) in isolated cortex. These values were obtained from the proton NMR signals measured 250 \(\mu\)s after the 90° pulse and, therefore, the relatively static macromolecular protons \((T_2 = 10-20\ \mu\)s) could not have participated in the signal. However, certain biomolecules (lipids and proteins) can also have very mobile protons \((T_2 ≈ 150\ \mu\)s), but in roots the dry mass is only around 7% and the possible contribution of mobile macromolecular protons should be less than 2% of the total NMR signal. As non-exchangeable water has also been found using the near-IR technique (Bačić and Ratković, 1982), it seems that there is sufficient experimental evidence to demonstrate its existence in maize roots. This water fraction cannot be readily detected in THO-type experiments.
The exchange kinetics of the exchangeable water fraction ($P_e$) were analysed using the following theoretical models:

**1. Two compartments in series**

This is the most frequently used model and can be expressed as:

$$1 - M_t/M_\infty = V_1 \exp(-k_1 t) + V_2 \exp(-k_2 t)$$

where $V_1$ and $V_2$ are the apparent volumes of compartments 1 and 2, and $k_1$ and $k_2$ are rate constants (for the complete description see e.g. Rescigno and Beck, 1972). Using these values, the real relative volumes ($V_1$ and $V_2$) can be calculated and the exchangeable water fractions expressed as: $P_{1e} = V_1 P_e$ and $P_{2e} = V_2 P_e$, where $P_{1e} + P_{2e} + P_{ne} = 1$.

The computed best fit curve based on equation (1) is compared with the experimental points obtained on intact roots (using both NMR techniques) in Fig. 2. An almost identical pattern was obtained for isolated cortex (results not shown). Both tissues showed good agreement between the experimental points and the computed curve, except at small values of $t$ (see insert in Fig. 2). Results obtained using this model are presented in Table 1 together with those obtained previously using a near IR technique (Bačić and Ratković, 1982).

The most straightforward and frequently used explanation for two compartmental exchange in tissues is that the first compartment represents extracellular space, while the
Table 1. Two compartments in series model

Exchange half-times ($t_{1/2}$) in seconds and relative volumes ($P_t$) of the two exchanging compartments (1 and 2). The third column gives the results when exchange was monitored using near infrared spectroscopy (Bačić and Ratković, 1982).

<table>
<thead>
<tr>
<th></th>
<th>Root ($n = 9$)</th>
<th>Cortex ($n = 6$)</th>
<th>Root ($n = 6$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t_{1/2}$</td>
<td>61 ± 6</td>
<td>43 ± 5</td>
<td>136 ± 6</td>
</tr>
<tr>
<td>$P_{1e}$</td>
<td>0.72 ± 0.04</td>
<td>0.75 ± 0.04</td>
<td>0.64 ± 0.09</td>
</tr>
<tr>
<td>$P_{2e}$</td>
<td>0.15 ± 0.01</td>
<td>0.17 ± 0.01</td>
<td>0.12 ± 0.02</td>
</tr>
</tbody>
</table>

$a$ ($t_{1/2}$) = ln 2/$k_i$; see equation 1.

$n$—Number of samples.

second represents the part of the tissue enclosed by membranes. Although it could be assumed that the exchange of water from the extracellular space (cell walls and intercellular space) is rapid, the first compartment certainly cannot represent this space because it is well established that the extracellular space amounts to some 5-10% of the root volume (Tanton and Crowdy, 1972; Läuchli, 1976; Bačić and Ratković, 1984) while according to this model it should be around 70%. Therefore, it is not possible to apply the two compartment model in this way and alternative explanations for the exchange curve must be considered:

(a) Deviation of the exchange curve from linearity in a semi-logarithmic plot is often taken as proof that exchange is not rate-controlled by water permeation through the surface of the cell or tissue (Woolley, 1965; Jarvis and House, 1967; Ling, Ochsenfeld, and Karreman, 1967). It seems unlikely that this simple criterion can be applied even to single cells like algae (Srejic, personal communication), and there are many explanations for deviations from linearity in tissues. For example, permeation of water through the outer cell layer (epidermis or hypodermis) can still be rate-limiting for water exchange if:

(i) The two compartments in series are the cortex and stele because the hypodermis and endodermis may form two continuous concentric barriers for radial water movement. The relative volumes of these two regions correspond to the volumes of the two exchanging compartments (Table 1). It seems, however, that this interpretation can be easily ruled out because two compartments of similar dimensions were also found in isolated cortex.

(ii) There is a subcellular, slowly exchanging water compartment (e.g. organelles, 'bound' water, etc.). On this assumption, the diffusional permeability of hypodermal cells ($P_{d,h}$) can be calculated from the relation $P_{d,h} = k_t R/2$. The value for intact roots ($P_{d,h} = 3.4 ± 0.3 \times 10^{-4}$ cm s$^{-1}$) is in good agreement with that ($P_{d,h} = 2.6 ± 10^{-4}$ cm s$^{-1}$) for suberized sleeves of epidermal-hypodermal cells from onion roots (Clarkson, Robards, Sanderson, and Peterson, 1978). According to IR measurements, $P_{d,h} = 1.4 ± 0.1 \times 10^{-4}$ cm s$^{-1}$, but this lower value could be easily explained by the influence of an unstirred water layer at the root surface on IR measurements. However, there is no obvious explanation for the difference in calculated $P_{d,h}$ between intact roots and isolated cortex, where $P_{d,h} = 4.4 ± 0.5 \times 10^{-4}$ cm s$^{-1}$. Furthermore, in maize roots suberin lamellae are present in hypodermal cells around 10 cm from the tip, and so the above model seems inappropriate for the material used here.
For these reasons, it is unlikely that the hypodermis and endodermis formed significant barriers for diffusional water exchange.

(b) The small positive deviations of the experimental points from the theoretical curve at the beginning of the exchange period (Fig. 2) suggest that a three compartment model may be more appropriate, with the first phase representing exchange from the extracellular space. The compartment designated 1 in Table 1 would then represent the main intracellular water, but the second compartment has no obvious anatomical counterpart. This interpretation is essentially an 'isolated cell' model, since the cortical cells should all start to exchange with the labelled water practically simultaneously due to the fast exchange of the extracellular water. Therefore, the diffusional permeability of cortical cells can be calculated from \( k_t \) (Table 1).\(^1\) Using \( r = 20 \) \(\mu\)m, \( P_d \) values of \( 1.1 \pm 0.1 \times 10^{-5} \) cm s\(^{-1}\) and \( 1.6 \pm 0.2 \times 10^{-5} \) cm s\(^{-1}\) are obtained for intact roots and isolated cortex. With the IR technique, \( P_d = 5.1 \pm 0.2 \times 10^{-6} \) cm s\(^{-1}\), which is in good agreement with \( P_A = 4.5 \times 10^{-6} \) cm s\(^{-1}\) obtained from THO-H\(_2\)O experiments using the same model (Jarvis and House, 1967). The discrepancies between NMR and other measurements cannot be explained by an unstirred layer at the root surface because this model predicts that diffusion of water in the extracellular space and, therefore, in the unstirred layer, is fast compared with membrane permeation. Moreover, there is no explanation for the differences in \( P_d \) between isolated cortex and intact roots.

(2) Diffusion in the extracellular space with simultaneous permeation through the cell membrane

This model is much less frequently used (Elford, 1970a, b) although it provides a more realistic view of the isolated cells model. Namely, it can be shown that the diffusional exchange of extracellular water is not particularly fast when compared with the time required for complete exchange. The time taken for water molecules to diffuse from the root surface to the endoderm can be calculated from \( t = l^2/(2D_e) \), where \( l \) is the diffusion pathlength and \( D_e \) is the diffusion coefficient of water in the apoplasm. Assuming that \( D_e \) is not significantly different from the self-diffusion coefficient in pure water (\( D_w \)) and that \( l \) is equal to the radius of the cortex, it appears that \( t \) is around 30 s (the actual pathlength is much longer due to tortuosity, producing an even larger value for \( t \)). Since this value is too large for it to be safely assumed that exchange of labelled water starts simultaneously in all cortical cells, the application of this model appears justified.

The equations describing the water exchange may be written as follows (Elford, 1970a, b):

\[
E \frac{\partial C_0}{\partial t} = D_e/\lambda^2 \frac{1/R}{\partial \partial R (R \partial C_0/\partial R)} - (1 - E) \frac{\partial C_1}{\partial t} \tag{2a}
\]

where \( C_0 \) and \( C_1 \) are the concentrations of labelled water in the extracellular and intracellular space, \( E \) is the fractional volume of the extracellular space, and \( \lambda \) is the ratio of the effective diffusional pathlength to the geometrical pathlength in the apoplasm. Permeation of water through the cortical cell membrane can be described by:

\[
\frac{\partial C_1}{\partial t} = K(C_0 - C_1) \quad K = A/V P_d \tag{2b}
\]

where \( A \) and \( V \) are the cell area and volume. The above equation is valid only if exchange between cells and the extracellular space is controlled by membrane permeation and not by water diffusion inside cells. Solutions of equations 2a and 2b are given by Elford (1970a) and Keynes (1954), but it is important to note that a unique solution is unlikely if all four

\(^1\) The value of \( k_t \) will differ from that in Table 1 if the third compartment is included, but the change will not exceed experimental uncertainty due to the small volume of this compartment.
parameters \((D_e, K, E, \lambda)\) are varied simultaneously (Elford, 1970a). Therefore, \(D_e\) was fixed at \(2.5 \times 10^{-5}\ \text{cm}^2\ \text{s}^{-1}\) (the value for pure water), a value of \(0.07\) was taken for \(E\) (Bačić and Ratković, 1984) and \(K\) and \(\lambda\) were varied to obtain agreement with the experimental results. The theoretical best fit curve is shown in Fig. 2. Using the calculated value for \(K\) of \(8 \times 10^{-3}\ \text{s}^{-1}\) and the known cell radius, the diffusional permeability of cortical cells is \(P_d = 8 \times 10^{-6}\ \text{cm s}^{-1}\).

(3) **Cylinder diffusion**

Maize root segments can be approximated to a cylinder and the water exchange treated as being analogous to radial diffusion in a homogeneous cylinder. This process can be described by the equation:

\[
\frac{\partial C}{\partial t} = \frac{1}{R} \frac{\partial}{\partial R} \left( D'R \frac{\partial C}{\partial R} \right)
\]

where \(D'\) is the apparent diffusion coefficient of labelled water within the root. When there is no effective change in the external concentration of labelled water:

\[
1 - M_t/M_\infty = \sum_j 4/\alpha_j^2 \exp(-D'\alpha_j^2 t/R^2)
\]

(Crank, 1956), where \(\alpha\) is the j-th root of the Bessel function \(J_0(\alpha) = 0\). In equation 4, \(D'\) is the only parameter which is varied and the best fit was obtained for \(D' = 3.0 \times 10^{-6}\ \text{cm}^2\ \text{s}^{-1}\). Agreement with experimental data is less good than in the previous models (Fig. 2) and there is a pronounced deviation at large values of \(t\). Similar deviations have been observed in THO-H\(_2\)O exchange experiments (Jarvis and House, 1967, 1969). Equation 4 cannot be applied to isolated cortex because it is not a ‘homogeneous’ cylinder. Therefore, the model of diffusion in a ‘hollow’ cylinder (Crank, 1956) was substituted and a value of \(D' = 2.65 \times 10^{-6}\ \text{cm}^2\ \text{s}^{-1}\) obtained, which is very close to the value for intact roots. The theoretical curve for the isolated cortex (not shown) deviates from the experimental points in the same manner as for intact roots.

The calculated values of \(D'\) are within the range of \(1.2-5.0 \times 10^{-6}\ \text{cm}^2\ \text{s}^{-1}\) found previously for different roots (Woolley, 1965; Jarvis and House, 1969; Ginsburg and Ginzburg, 1970). These values are considerably smaller than the self-diffusion coefficient of water, but are also smaller than the \(D'\) value determined by an NMR pulsed gradient spin-echo (PGSE) technique, with which \(D' = 1.76 \times 10^{-5}\ \text{cm}^2\ \text{s}^{-1}\) (Abetsedarskaya, Miftakhudfinova, and Fedotov, 1965). This discrepancy between NMR and isotope exchange data can be explained as follows. The apparent diffusion coefficient of water in heterogeneous systems depends on the time allowed for diffusion, and, if measured by the NMR–PGSE method, will depend on the spacing of the gradient pulses \((D' = f(t_d))\). With short times \((t_d \to 0)\), water molecules will not experience the influence of barriers (membranes) and the measured value of \(D'\) depends only on the mobility of water inside the cells \((D' \to D_0)\). The above cited value of \(D'\) refers to this condition. As diffusion time is increased, the influence of membranes becomes more pronounced \((D' decreases)\) and at very long times \(D'\) approaches a constant value, \(D_\infty\). The \(D'\) values obtained in isotope exchange experiments correspond to this condition. For the case of equally spaced parallel permeable barriers, Tanner (1978, 1979) derived the following expression:

\[
1/D_\infty = 1/D_0 + 1/dP_d
\]

where \(d\) is the barrier spacing (cell diameter). The early model developed by Philip (1958) and used subsequently for similar calculations is the limiting solution of equation 5. Using \(D_0 = 1.76 \times 10^{-5}\ \text{cm}^2\ \text{s}^{-1}\) (Abetsedarskaya et al., 1965) and \(D_\infty = 3 \times 10^{-6}\ \text{cm}^2\ \text{s}^{-1}\) (this work) we calculated that \(P_d = 2 \times 10^{-3}\ \text{cm s}^{-1}\), which is 2-3 orders of magnitude higher than
the values obtained with previous models. However, this value is close to that obtained using the NMR technique (Bačić and Ratković, 1984).

It is also possible to explain the observed difference between the NMR and IR techniques using the cylinder diffusion model. The diffusion of water in the root cylinder can be expressed as:

\[ t_{1/2} = \frac{0.0621R + \delta^2}{D} \]  

where \( \delta \) is the thickness of the unstirred layer at the root surface. Since \( \lambda \) does not depend on the method by which the signal of light water is detected in the bathing solution, \( \delta \) can be calculated by comparing NMR and IR data. The value obtained of \( \delta = 300 \, \mu m \) is too high to represent only the unstirred layer (\( \delta = 90 \, \mu m \) in the HTO-\( H_2O \) experiments of Jarvis and House (1967)) and probably represents the distance between the root surface and the optical beam where light water is registered. Therefore, the difference in water exchange half-times is a consequence of an additional diffusional pathlength in IR experiments.

Relaxation measurements

The results are summarized in Table 2. The general features of water relaxation in different parts of the root are:

(a) The relaxation time \( T_1 \) was a single exponential in all samples, except perhaps in the root tips where an indication of a fast decaying component (population around 10%) was found. For \( T_2 \) relaxation, a good fit to the data was obtained with two exponentially decaying components \( T_{2a} \) and \( T_{2b} \). The exact values of both relaxation times and the relative populations of protons (\( P_a \) and \( P_b \)) were found to depend on the pulse spacing used in the CPMG sequence, and results are given for \( \tau = 0.2 \, ms \).

(b) The relaxation times of water in all samples were directly proportional to their water content, a feature regularly observed in many biological systems. The ratio of measured populations of the two \( T_2 \) components (\( P_a/P_b \)) increases with the decrease of the sample water content.

DISCUSSION

Water pathways in roots

Analysis of the \( H_2O-D_2O \) exchange kinetics in maize roots showed that: (1) the 'isolated cells' model provided \( P_d \) values of \( 10^{-5}-10^{-6} \, cm \, s^{-1} \), while the 'cell-to-cell' model gave a value of \( 2 \times 10^{-3} \, cm \, s^{-1} \); (2) none of the models fit the experimental data perfectly. Therefore,

<table>
<thead>
<tr>
<th>Sample</th>
<th>( T_1[s] )</th>
<th>( T_2[s] )</th>
<th>Water content [g ( H_2O ) per g of tissue dry weight]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>Intact root</td>
<td>1.35</td>
<td>0.120</td>
<td>0.410 (0.68)*</td>
</tr>
<tr>
<td>Cortex</td>
<td>1.40</td>
<td>0.110</td>
<td>0.415 (0.70)</td>
</tr>
<tr>
<td>Stele</td>
<td>1.16</td>
<td>0.085</td>
<td>0.275 (0.65)</td>
</tr>
<tr>
<td>Root tip</td>
<td>0.82</td>
<td>0.065</td>
<td>0.196 (0.58)</td>
</tr>
</tbody>
</table>

a and b denote fast and slow \( T_2 \) component.

* Numbers in brackets are relative populations of the slow relaxing component in all protons.

* First 0.5 cm.
the use of goodness of the fit as the only criterion for choosing the correct model seems highly inappropriate.

The main reason for disagreements about the water pathways in roots is the uncertainty over the permeability of cell membranes. $P_d$ should, therefore, be determined by methods that are independent of the pathways taken by water and free from the problems caused by the unstirred layer. Two types of experiments can provide such data. The NMR method involves doping of the extracellular space with paramagnetic agents and has provided a $P_d$ value of $6.3 \times 10^{-3} \text{ cm s}^{-1}$ for maize root cells (Bačić and Ratković, 1984). Alternatively, application of the pressure-probe technique (Steudle, Tyerman, and Wendler, 1983) to plant roots has given a value $1.2 \times 10^{-6} \text{ cm s}^{-1} \text{ bar}^{-1}$ for the hydraulic conductivity ($L_p$) of cortical cell membranes in both barley and wheat (Jones, Tomos, Leigh, and Wyn Jones, 1983; Steudle and Jeschke, 1983). $P_d$ is related to $L_p$ by the formula $P_d = L_p RT/V_w$ (Dainty, 1963), where $RT/V_w \approx 1.3 \times 10^{3} \text{ bar}$ under standard conditions. This gives a value for $P_d$ of $1.7 \times 10^{-3} \text{ cm s}^{-1}$. This formula is valid if no substantial water transport occurred through the membrane pores, but even if it did, this would hardly change the order of magnitude of the calculated $P_d$ (Finkelstein, 1984).

Both techniques suggest that $P_d > 10^{-3} \text{ cm s}^{-1}$, which is in the upper part of the range given in the literature (Newman, 1976). Apart from the root cells, these techniques have also been applied to a variety of algal and higher plant cells and in all cases $P_d$ values were in the range $10^{-4}$-$10^{-2} \text{ cm s}^{-1}$ (Stout, Cotts, and Stepokus, 1977; Stout, Stepokus, Bustard, and Cotts, 1978; Stout, Stepokus, and Cotts, 1978; Ratković and Bačić, 1980; Steudle et al., 1983). Furthermore, the typical $P_d$ of various lipid bilayers is around $10^{-4}$-$10^{-3} \text{ cm s}^{-1}$ and from structural considerations it is difficult to imagine that the permeability of root cell membranes can be 2-3 orders of magnitude lower than that of a pure lipid membrane. Therefore, it seems that the values of $P_d = 10^{-5}$-$10^{-6} \text{ cm s}^{-1}$ reported earlier are serious underestimates.

For these reasons, it is clear that the first two models (see Results) based essentially on apoplastic water movement in the root cortex must be rejected. The only model that gives a reasonable value for $P_d$ is the cylinder diffusion model. Furthermore, this is the only model that is compatible with other experimental evidence, such as differences in the time-course of water exchange between the root and the cortex, and between NMR and IR data. The value of $P_d = 2 \times 10^{-3} \text{ cm s}^{-1}$ obtained is slightly underestimated because the curve fitting was performed using all experimental points, although it is clear that the long curvilinear ‘tail’ (Fig. 2) cannot be adequately described by equation 4. Therefore, it can be concluded that a significant proportion of radial water movement occurs through the cells and that the apoplasm is not, as is usually assumed, a lower resistance pathway than the cell-to-cell pathway. In fact, the concept of three distinct pathways is probably not entirely correct for diffusional water exchange. According to the theory of Molz and Ikenberry (1974) and Molz (1976), when water exchange between cells and the extracellular space is fast compared to the time required for radial equilibration of water in the root, there will be a local equilibrium between all pathways and water exchange will be adequately described by a single diffusional equation (equation 4). Since the half-time of water exchange across the plasmalemma is only a few hundred milliseconds (Bačić and Ratković, 1984), it is clear that local equilibrium is attained in maize roots and that the amounts of water transported through the cell-to-cell and apoplastic pathways are directly proportional to their cross-sectional areas perpendicular to the radial direction. Therefore, some 80-90% of the water flux should occur through the cells, a conclusion in agreement with the finding that the water flux is reduced by some 80% when the root is treated with metabolic inhibitors (Ginsburg and Ginzburg, 1970; Pitman and Welfare, 1978).
Although the model of radial diffusion in the root cylinder represents most realistically the equilibration of roots with deuterated water, the relatively large deviations of the experimental efflux curve from the model for large values of time ($t > 5$ min) need to be explained. These deviations are positive, indicating that a certain fraction of water in root is exchanged more slowly than bulk diffusion would allow; it appears, from extrapolation of the tail to the $1 - M_I/M_0$ axis, that this fraction amounts to some 10-15% of the root volume. Similar results have previously been obtained with maize (Jarvis and House, 1967) and barley roots (Shone, private communication). Since the same curvilinear 'tail' was obtained in both cortex (this work) and stele (Jarvis and House, 1969), this phenomenon probably originates at the cellular level rather than at the level of root structure. The exchange of deuterium with hydrogen atoms in organic molecules cannot be the explanation because the volume of this slowly exchanging component is larger than the calculated mass of dry tissue. Therefore, we assume that this water fraction corresponds to the water having restricted mobility, due to the influence of macromolecules, relative to the main water fraction. This conclusion is substantiated by the fact that the rate constants for the slowly exchangeable water are the same for intact root and isolated cortex (Table 1), showing that the rate of exchange is determined by the state of this water and not by the time required for diffusion through the tissue i.e. by the tissue radius. Water fractions are defined above according to their accessibility for diffusion exchange and have no obvious anatomical counterparts. Thus, instead of defining water compartments in terms of their physical boundaries, these are defined in terms of their different free energies for diffusion exchange.

The NMR measurements seem to further support the existence of different water fractions in the root. The single exponential $T_1$ process and the non-exponential $T_2$ process found here (Table 2) has also been observed in a variety of plant cells and tissues (Juranic, Macura, Srejic, Djordjevic, and Vucelic, 1980; Rajashekar, Gusta, and Burke, 1979; Samuilov, Nikiforova, and Nikiforov, 1979; Stout et al., 1978; Ratkovic and Bacic, 1980) and the reported proton populations are quite similar to those observed here. This again suggests that we are dealing with cellular phenomena and implies that components a and b may represent cytoplasmic and vacuolar water, respectively. Relaxation behaviour can then be explained in terms of the effect of water exchange between different water environments. Namely, the average relaxation of water near macromolecular surfaces (cytoplasm, membranes) is fast compared to the relaxation of 'bulk' water (vacuolar water). Assuming that the permeability of the tonoplast is not significantly different from that of the plasmalemma, the time taken by water molecules to diffuse through the average cell radius is $\tau_d = 150-200$ ms (Bacic and Ratkovic, 1984). This seems to be fast enough to average the cell water spin-lattice relaxation time to a single $T_1$, which is often called the 'fast exchange limit'. Since $T_2$ is typically much shorter than $T_1$ in biological systems, the internal mixing is slower than intrinsic spin-spin relaxation, $T_{2a} < \tau_d$ (Table 2), and two components are observed. Correlation between water content and the $P_b/P_a$ ratio supports this conclusion because differences in water content between various root parts are mainly a consequence of differences in cytoplasmic to vacuolar volume.

However, the above assignment of relaxation component may not be entirely correct because the ratio of the populations $P_a$ and $P_b$ is slightly too high to represent the actual ratio of cytoplasm to vacuole volume. Moreover, in maize root cells with highly permeable membranes and diffusion times comparable to the intrinsic water relaxation time, it is not necessary to have a physical boundary (membrane) between populations to observe non-exponential behaviour. The influence of the tonoplast and relaxation sinks within the cytoplasm could extend well inside the vacuole. In addition, the plasmalemma and cell walls could alter water mobility within the extracellular space. Thus, all water molecules influenced...
by these interfaces would represent ‘the fast relaxing water fraction’. The analysis of the $T_2$ relaxation decay as two discrete components may be, therefore, in error and a continuous distribution of correlation times is likely to exist (Brownstein and Tarr, 1977).

It is difficult to distinguish between the above relaxation mechanisms in heterogeneous systems like maize root (see review by Belton and Ratcliffe, 1985). However, since $H_2O-D_2O$ exchange and NMR relaxation experiments both reveal the existence of different water fractions in the maize root cells, it is reasonable to assume that the majority of the fast relaxing water fraction corresponds to the slowly exchanging water in the $H_2O-D_2O$ experiment and is located in the cytoplasm; that is, that the mobility of most of the cytoplasmic water differs from that of the bulk water.

The existence of non-exchangeable water is again unlikely to depend on some specific structure of plant roots because $P_{ae}$ was found in both intact roots and isolated cortex and stele. Therefore, it may be concluded that this water is subcellular in origin and is either bound to macromolecules or ‘encapsulated’ inside subcellular structures. At this stage, the latter seems more probable because the relaxation times of the non-exchangeable water are not significantly different from the average root water relaxation times. More detailed analysis of the nature of this water will be presented in a subsequent publication (Bačić, Srejić, and Ratković, in preparation).

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