The Effect of Osmotics on Plant Cells: A Study Using ^{1}H NMR Relaxation and Self-Diffusion of Water

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Abstract. The osmotic changes in root cells of *Zea mays L.* under the effect of mannitol (concentration range 1–15%; range of osmotic pressures: −0.13 to −2.01 MPa) were studied by measuring time and concentration dependence of water self-diffusion constant (D_{eff}) and proton spin-lattice relaxation time (T_1) using proton NMR relaxation spectroscopy. In addition, *in vivo* uptake of Mn^{2+} by roots after their incubation in mannitol solutions were studied.

At low ($\leq$ 5%) and medium (5–10%) concentrations of the osmoticum dehydration takes place proportional to the concentration, whereas membrane destruction occurs at higher concentrations (10–15%). It seems that there is a distribution of cells within root tissue regarding their sensitivity to osmotic stress.

Key words: Osmotic pressure — Mannitol — Proton NMR relaxation — Self-diffusion — Membrane rupture — Mn^{2+} — *Zea mays*

Introduction

Cells of various plant species exhibit different sensitivity to osmotic stress. Differences may also occur within a species or even between different organs of the same plant (Meidner 1983; Morgan 1984). For instance, epidermal cells of onion can survive water stress of −12.9 MPa during 20 hours (Palta et al. 1977). On the other hand, growth of maize stem, silk and leaves was completely inhibited at water potentials of −0.50, −0.75 and −1.0 MPa, while root growth continued to −1.4 MPa (Westgate and Boyer 1985) **. In previous studies by Palta et al.

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** According to Slavík (1974) water potential (ψ_w) of a plant cell is defined as the sum of three contributions: $\psi_w = \psi_s + \psi_p + \psi_m$, where ψ_s is osmotic (solute) potential

(1977) cell survival was controlled by protoplasmic streaming or by plasmolysis in a hypertonic mannitol solution.

In this work we used proton NMR relaxation method to test the action of an osmoticum (mannitol) in a concentration range of 1–15%, on the intactness of the cell membrane. Proton spin-lattice relaxation time, T_1, of cellular water was measured in roots of *Zea mays L*, to follow dehydration of cells upon increasing external mannitol concentration. Measurements of restricted diffusion of cellular water (self-diffusion constant, D_{eff}) in the same range of osmotic concentrations could be interpreted in terms of increasing populations of ruptured cells with the increasing concentration. Additional evidence for an increased permeability of membranes at higher osmotic pressures was obtained from ion transport studies on intact plants using Mn^{2+} as a paramagnetic ionic probe.

Materials and Methods

Plants

The experiments were done with 8–9 days old maize plants (*Zea mays L.*, var ZP SC 46A). The previously washed seeds were placed between sheets of wet filter paper and grown in dark at 25 °C. Three to four days old plants were then transferred into aerated half-strength Knopp solution, and grown for 5 days under controlled environment until the roots reached a length of 15–20 cm.

Spin-lattice relaxation time (T_1) measurements

The spin-lattice relaxation time, T_1, was measured with a proton spin echo NMR (model IJS-2-71 modified relaxometer and a home made spin-echo instrument) using either 90°-*t*-90° or 180°-*t*-90° pulse sequence at a resonance frequency of 32 MHz (Farrar and Becker 1971).

In a first series of experiments the roots of intact plants were kept for 1 hour in mannitol solution of a specific concentration, then blotted, cut into segments, and placed into an NMR sample tube to measure T_1. In another series of experiments the roots of intact plants were placed after pretreatment with mannitol (1 h) in a specially constructed NMR unit with continual perfusion of nutrient solution containing paramagnetic label $MnCl_2$ (Ratković and Vučinić 1990).

Self-diffusion (D_{eff}) measurements

Self-diffusion constant (D) of root tissue water was measured using the method of pulsed magnetic field gradient spin echo (PGSE) (Steiskal and Tanner 1965; Anisimov 1982). The diffusion constant was calculated from the ratio of the relative amplitudes of the spin echo in presence $A(2\tau)$ and absence of the pulsed gradient, $A(0)$, and the intensity of the pulsed magnetic field gradient (g):

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or osmotic pressure, ψ_p is hydrostatic or pressure potential, and ψ_m is matric potential (surface interactions in the cytoplasm and the cell wall). Therefore, the osmotic potential ψ_s describes decrease of the total water potential due to substances dissolved in water (e.g. mannitol). There are also alternative terminologies (Slavík 1974).

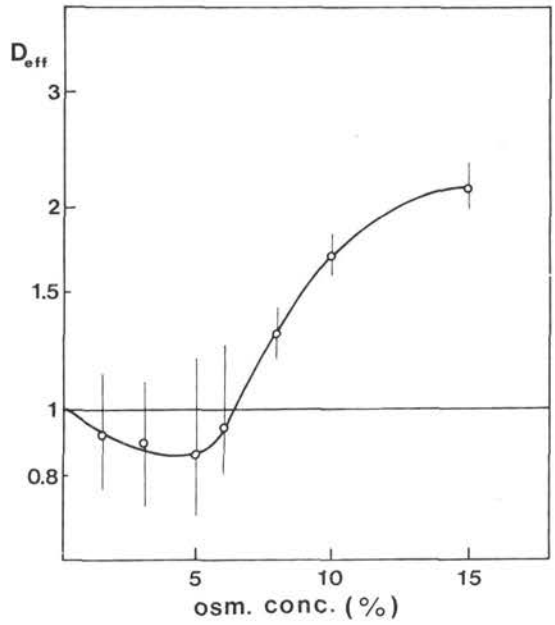

Figure 1. Effective water diffusion coefficient, D_{eff}, in roots of *Zea mays L.*, obtained by dividing the diffusion coefficient of the root after incubation (1 h) in mannitol solutions of different concentrations, by the diffusion coefficient for control roots (no treatment with mannitol). The solid curve is a guide to the eye rather than statistical average. $\Delta = 300$ ms.

$$\ln[A(2\tau)/A(0)] = -\gamma^2\delta^2 g^2 \left(\Delta - \frac{1}{3}\delta\right) D$$

where γ is the gyromagnetic ratio for protons, δ is the width of the gradient pulse and Δ is the time delay between the consecutive gradient pulses.

The vector of the magnetic field gradient was oriented perpendicularly to the longitudinal root axis, i.e the radial diffusion of water in root was measured. The value D_{eff} shown in Figs. 1 and 2 was obtained by dividing the diffusion constant of the tissue treated with mannitol with that of the control (untreated) samples.

Results and Discussion

The osmotic dehydration of maize roots was studied by measuring time and concentration dependences of D_{eff} and T_1 of the root tissue water.

As shown in Fig. 1, only slight changes of D_{eff} were observed at osmotic concentrations below 5%, with a tendency to decrease; between 5 and 15%, the increase reached almost the threefold of the initial values. At low concentrations of

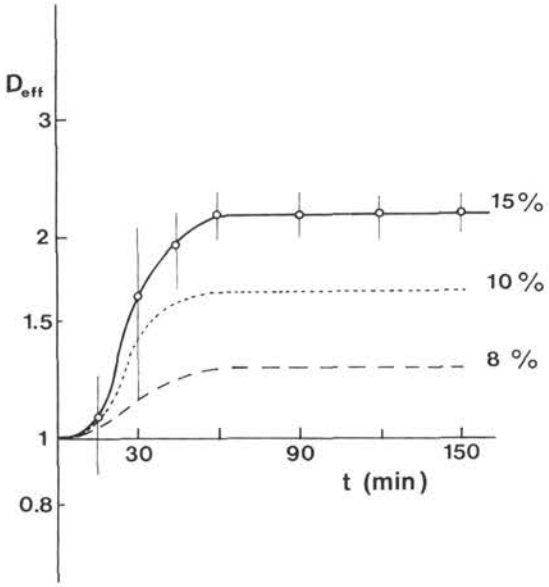

Figure 2. Time dependence of D_{eff} during the incubation of roots in concentrated mannitol solutions.

the osmoticum, water diffusion could be related to adaptive reactions of cells during dehydration involving the system of intercellular contacts (plasmodesma). On the other hand, the permeability of membranes to water under the same conditions increases as suggested by T_1 values (Fig. 3).

At medium and high concentrations of the osmoticum (5–15%), D_{eff} increases with time (Fig. 2) and reaches after 40–60 min a plateau, the level of which depends on the osmotic concentration. The fact that D_{eff} of the tissue water increases with the increasing concentration of the osmoticum in the external solution probably is a consequence of a breakthrough of the cellular membranes and cell destruction, since in such cells there is no more restricted diffusion (Cooper at al. 1974). The different levels of the plateau, as well as relaxation data shown below indicate that there is a distribution of cells within plant roots with respect to their sensitivity to osmotic stress.

Proton relaxation of water in roots is multiexponential. The slow decaying component of relaxation makes up 80–90% of the total NMR signal, and it corresponds to the major part of the intracellular and extracellular water. The characteristic time constant T_1 is sensitive to changes in water exchange rate across the cellular membrane (plasmalemma). The dependence between T_1 and concentration

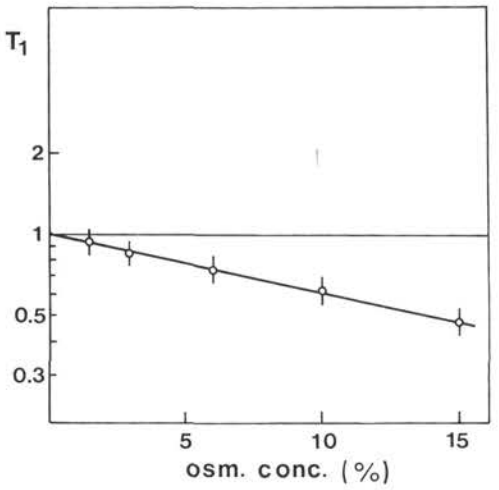

Figure 3. Proton spin-lattice relaxation time (T_{1exp}/T_{1con}) of tissue water in roots of *Zea mays L.* after incubation (1 h) in mannitol solutions of increasing concentrations (1.5–15%).

of mannitol solutions is shown in Fig. 3. The decrease in T_1 upon increasing the concentration of the osmoticum can be related to redistribution of protons between the different fractions during cell dehydration, to an increase in water permeability of the membranes, and partly to the destruction of cellular membranes at high osmotic pressures.

As in experiments with diffusion, low (1–5%) and medium, and high (5–15%) concentrations of osmoticum can be considered. The relaxation measurements indicate the operation of complex processes within the first 40–60 min of root treatment with mannitol, due to adaptive reactions of roots on dehydration and possibly to a restricted rate of propagation of the osmoticum within the root extracellular space. At relatively high concentrations of mannitol solutions (5–15%) there is a fast (10–15 min) decrease in the intensity of the corresponding slow-decaying component and an increase in the intensity of the fast-decaying component.

In a further step we studied T_1 of the uptake kinetics of paramagnetic Mn^{2+} ions following an osmotic treatment. Fig. 4 shows changes in $(1/T_1)_n$ in roots of *Zea mays L.* perfused with 100 μmol/l $MnCl_2$ solutions following 1h treatment with 1%, 5%, 10% and 15% mannitol solutions, respectively. Each point on the curves was obtained by dividing the corresponding $1/T_1$ value with the control value (in the absence of the osmoticum). In this way the abscissa corresponds to control roots.

The T_1 curves in Fig. 4 show that the cells recover from dehydration in man-

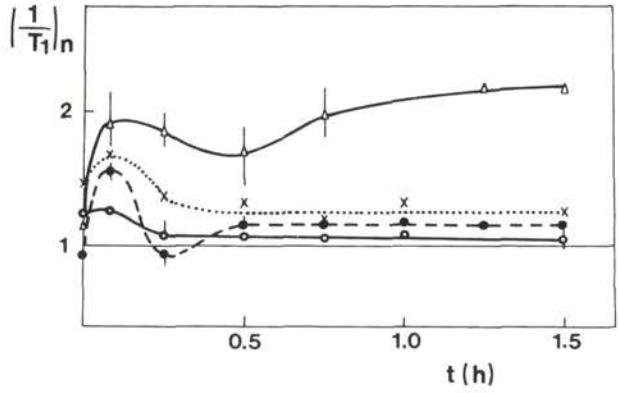

Figure 4. Proton relaxation rates $(1/T_1)_n$ in roots of intact plants of *Zea mays L.* continually washed with 100 μmol/l $MnCl_2$ solution. The normalized values $(1/T_1)_n$ were obtained by dividing $1/T_1$ values on the $MnCl_2$ uptake curves in the presence and absence of the osmoticum. All roots were incubated for 1 h in 1% (o), 5% (•), 10% (×) or 15% (△) mannitol solutions.

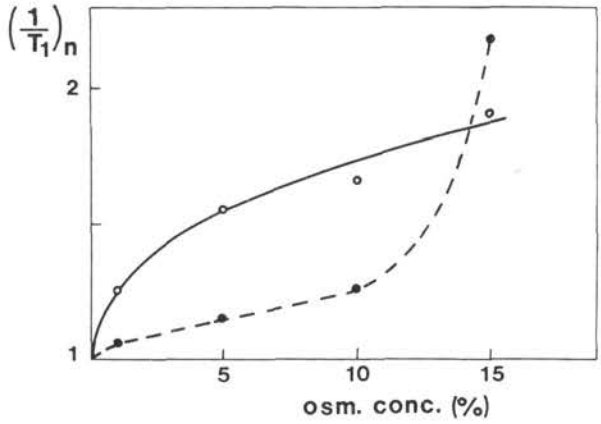

Figure 5. Normalized relaxation rates $(1/T_1)_n$ in root tissue plotted against mannitol concentration at two washing times with 100 μmol/l $MnCl_2$: t=5 min (o) and t=90 min (•).

nitol solutions during the first 0.5 h circulation of 100 μmol/l $MnCl_2$ around the roots. During that period $1/T_1$ first increases over the control value due to influx of Mn^{2+} ions, but afterwards it is slowed down by rehydration of the cells and dilution of the paramagnetic centers.

Replotting the data after 5 min and 90 min washing with $MnCl_2$ as a function

of concentration of the osmoticum, a positive correlation between $(1/T_1)_n$ and mannitol concentration is obtained (Fig. 5). The 90 min curve shows two regions of mannitol concentrations: at concentrations below 10% the relaxation rate steadily increases, whereas between 10–15%, $(1/T_1)$ abruptly increases. This jump in the relaxation rate at concentrations above 10% (0.55 mol/l) can also be assigned to rupture of cell membranes producing more direct contact of Mn^{2+} ions in the extracellular space with the vacuolar water pool. It is worth to note at this point that the osmotic value of the epidermal cell sap of onion cells, as determined by incipient plasmolysis was found to be 0.50 osmol (Palta et al. 1977), i.e. close to our value on the 90 min curve (Fig. 5). In conclusion, it can be stressed that at low concentrations of the osmoticum there is no obvious membrane rupture and cell destruction. This region is characterized by oscillatory dynamic processes and adaptation to osmotic perturbations during 40–60 min, the adaptation is even faster if the solution circulates around the roots. These processes concern cell membranes as well as intercellular contacts. Water exchange across the membranes or across the intercellular contacts may be asynchronous; this aspect is worth of being further investigated (Anisimov et al. 1983 a;b).

At a high osmotic concentration the membranes undergo destruction, but a number of cells still remain unchanged keeping their characteristics. The amounts of unchanged cells depend on the concentration of the osmoticum suggesting that there is a distribution of cells in the roots of *Zea mays L.* with different sensitivity to osmotic stress.

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