Eur Biophys J (2008) 37:975–978
DOI 10.1007/s00249-008-0316-x

ORIGINAL PAPER

Water diffusion in cytoplasmic streaming in Elodea internodal cells under the effect of antimitotic agents

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Received: 5 December 2007 / Accepted: 4 February 2008 / Published online: 27 March 2008

Abstract The translational displacement of the cytoplasmic water in Elodea stem cells resulting from protein motor activity was measured using the NMR method. A 24-h treatment with vincristine results in a reduction of the translational displacement of the cytoplasmic water. With a constant cytoplasmic streaming velocity, the dynamics of the translational displacement of the cytoplasmic water under the effect of taxol are characterized by a continuous increase at a concentration of 0.05 mM, and reaching a plateau at a concentration of 0.5 mM.

Keywords Elodea densa · Nuclear magnetic resonance · Cytoskeletal tubulin component · Translational diffusion

Introduction

The cytoskeleton is known to play a crucial role in the time and space arrangement of the cellular function of plants (Tiwary et al. 1984). The most important indication of this function is the intracellular transport. Transport along cytoskeletal structures occurs using the energy of specialized mechanical–chemical ATPases called motor proteins (Minin and Kulik 2004). In plant cells, myosin interacting with actin moves along the cytoskeletal actin filaments. The average velocity of this movement is approximately 10 μm/s, and the maximum velocity is approximately 100 μm/s (Yokota et al. 1995; Morimatsu et al. 2000; Kimura et al. 2003). Vesicle and organoid transport in biocolloids with the low Reynolds number to which the cytoplasm belongs should not significantly affect the diffusion (Pickard 2003). At the same time, the spaces in subcellular compartments where reactions occur are supplied with reaction agents using diffusion (Luby-Phelps 2000). Obviously, the intracellular transport including the transport of signaling molecules to the nucleus region is provided by the cytoplasmic streaming and their penetration depends on diffusion.

There is a number of mechanisms of regulation of the cytoplasmic streaming velocity, which result from the interaction of cytoskeletal components (Kobayashi et al. 1988; Takesue and Shibaoka 1998; Hasezawa et al. 1998; Tominaga et al. 1997); therefore, it is reasonable to suppose the availability of the mechanism of diffusion regulation.

The vital determination of diffusion coefficients of molecules inside the cytoplasm is far from being a trivial problem, which requires some special technique. One of such noninvasive methods of the diffusion study is the method of spin-echo nuclear magnetic resonance (NMR) with pulsed magnetic field gradient.

Selective measurements of the translational diffusion of the cytoplasmic water using the spin-echo NMR method (Anisimov et al. 2003; Vorob'ev et al. 2004; Anisimov et al. 2004) with modified hydrodynamic characteristics allow us to come close to understanding the self-regulation mechanisms of the diffusional constituent of intracellular transport. In this study, an attempt to study the dependence of the translational displacement of cytoplasmic water on microtubular cytoskeletal integrity modified by taxol and vincristine has been made.

Material and methods

The anatomy of Elodea cells allows us to determine the parameters of cytoplasmic streaming using both the

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microscopic method of synchronous tracing (Vorob'yev and Anisimov 1995) and the NMR spin-echo method with selective registration of diffusional transfer along the cytoplasm alone (Anisimov et al. 2003).

Sample preparation

After removing the leaves, a stem of *Elodea densa* was cut into segments up to 1 cm long, and each segment was infiltrated with solutions of vincristine and taxol at a pressure of 10 Pa for 60 min. Upon completion of the experiment, the samples were placed into the original solutions, where they were kept under normal conditions. Viability of cells was determined by the presence of the cytoplasmic streaming.

Solution preparation

The water solution of vincristine was prepared from the drug "Vincristine–lans" (Veropharm, Russia), which consisted of the lyophilized powder of vincristine sulfate. The water solution of taxol was prepared from the drug Taxol (paclitaxel) (Bristol-Myers Squibb, Italy). Each milliliter contained 6 mg paclitaxel, 527 mg Cremaphor EL (polyoxyethylated castor oil) and 49.7% (v/v) dehydrated alcohol, USP. The study of the solvent effect was carried out using water solution of Cremaphor EL (Fluka BioChemica, Germany) and alcohol at corresponding proportions.

Visual assessment of the cytoplasmic streaming

The cytoplasmic streaming velocity was determined using the synchronous tracing method (Vorob'yev and Anisimov 1995) in intact cells of the longitudinal dissection of an internode. The average cell size was 500 μm. The measurements were carried out at room temperature. The values of the cytoplasmic streaming velocity were measured prior to infiltration, immediately after infiltration, and 24 h later prior to the last NMR measurement.

NMR method

Measurements were carried out on a home-made NMR spin-echo relaxation-diffusion-meter at a frequency of 16 MHz using the pulsed magnetic field gradient (Steiskal and Tanner 1965). The pulse sequence of stimulated echo (Tanner 1970) was used as a basic one.

The obtained diffusional echo decay can be described by:

$$R = \exp\left\{-\gamma^2\delta^2 g^2[t_d - (1/3)\delta]D\right\} \quad (1)$$

where γ is the gyromagnetic ratio, D is the self-diffusion coefficient, δ and g are the duration and amplitude of pulses of magnetic field gradients, respectively, t_d is the interval between pulses (diffusion time) and R is the relative echo amplitude, $R = A(g)/A(0)$. The pulsed magnetic field gradient was created along the stem axis. Diffusional decays were obtained with g as a variable (32 steps with a maximum of 4.2 T/m) at fixed δ ($\delta = 250$ μs) and diffusion time ($t_d = 15$ ms). The control of the diffusion-meter and treatment of diffusional decays was performed using a computer.

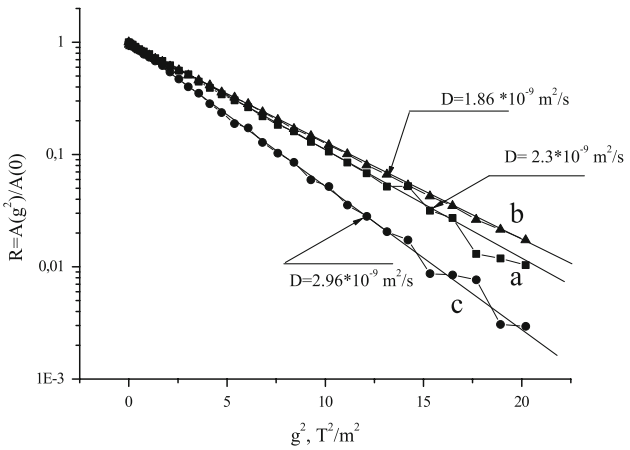

Fig. 1 Diffusional decays and diffusion coefficients of water at 25°C: **a** bulk water, **b** sample water without application of the inversing 180° radio-frequency pulse, **c** cytoplasmic water after application of the inversing 180° radio-frequency pulse

To eliminate the vacuole water signal, the sequence for diffusion measurement was switched on after applying the inversing 180° radio-frequency pulse at the moment when the vacuole water magnetization crossed zero (the zero point) (Vorob'ev et al. 2004; Anisimov et al. 2004). The self-diffusion coefficient of water in the studied samples, when the inversing 180° radio-frequency pulse was not applied (Fig. 1b), was lesser than the self-diffusion coefficient of bulk water (Fig. 1a). The application of the inversing 180° radio-frequency pulse did not affect the self-diffusion coefficient of water in a sample tube, but when applied to the studied sample to measure the cytoplasmic water diffusion coefficient (D^*) (Fig. 1c), it showed a difference caused by the actomyosin motor operation. The temperature (24–25°C) during measurements was achieved and maintained using a liquid scheme of thermal stabilization. The temperature in the NMR relaxation-diffusion-meter probe was maintained with an accuracy of better than 1°C.

Data handling

The measured value of the factor R varied according to a signal/noise ratio within the range of up to 10%, but after averaging over several measurements, it was approximated

by an exponential so that the accuracy of the determined diffusion coefficient was better than 5%. Diffusion coefficients were taken as an average of independent measurements of three assays for each treatment.

Results and discussion

The cytoplasmic streaming in plant cells accomplishes at least two functions: (1) it is the driving force of the intracellular and symplast transport; (2) it provides the cytoplasm stirring (Pickard 2003). The efficiency of stirring depends on the cytoplasm hydrodynamic parameters, which are related to the diffusion of water and dissolved compounds. In our experiments, insignificant changes in the cytoplasmic streaming velocity under the effect of vincristine and taxol [in the control samples it was equal to 17 ± 2 μm/s (SD, $n = 9$) and in treated samples it was equal to 18 ± 1.5 μm/s (SD, $n = 6$)] indicated that the actomyosin motor was still functioning. At the same time, changes in the cytoplasm water diffusion differed (Figs. 2, 3).

After infiltration of the control sample in distilled water, the self-diffusion coefficient of cytoplasmic water modified by cytoplasmic streaming (D^*) increased by 5% (Fig. 2a). The effect of vincristine on D^* was visible from the first hour of exposure for both concentrations of 1 mM (Fig. 2b) and 0.5 mM (Fig. 2c). The difference between the diffusion coefficients at the beginning of the measurements D^*_{control} and at the end of the measurements $D^*_{24\text{hours}}$ $\left(\Delta D^* = D^*_{\text{control}} - D^*_{24\text{hours}}\right)$ showed the effect of vincristine. The value of ΔD^* equaled 0.63 ± 0.1 for the concentration 1 mM and 0.31 ± 0.12 for the concentration 0.5 mM.

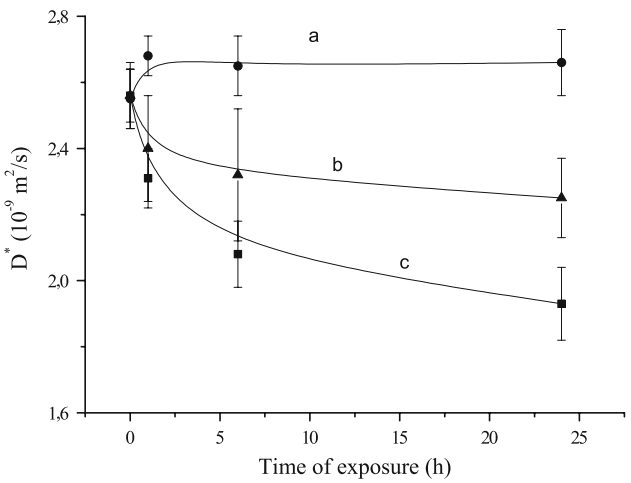

Fig. 2 Coefficients of translational diffusion of the cytoplasmic water (D^*): **a** control sample, **b** sample treated with 0.5 mM vincristine, **c** sample treated with 1 mM vincristine

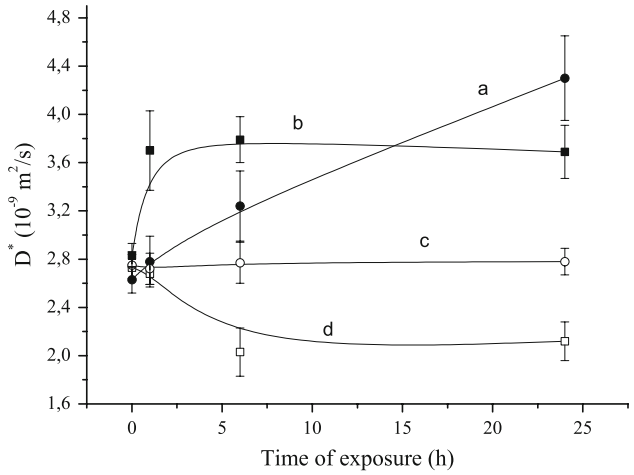

Fig. 3 Coefficients of translational diffusion of the cytoplasmic water (D^*): **a** sample treated with 0.05 mM taxol, **b** sample treated with 0.5 mM taxol, **c** sample treated with Cremophor at concentration corresponding to 0.05 mM taxol, **d** sample treated with Cremophor at concentration corresponding to 0.5 mM taxol

Vincristine, as well as the whole class of vincaalcaloids, suppresses polymerization of microtubules binding to tubulin at both "+" and "−" ends. Moreover, it inhibits the hydrolysis of GTP bound to β-tubulin (Gupta and Bhattacharyya 2003). As a result, the concentration of nonmicrotubular tubulin in the cytoplasm increases, slightly altering the hydrodynamic characteristics of the cytoplasm. This results in a minor decrease in D^*, since the amount of water free for hydratation is limited in liquids with low Reynolds numbers. The dynamics of D^* under the effect of taxol was characterized by a gradual increase at the concentration of 0.05 mM (Fig. 3a) and reached a plateau at the concentration of 0.5 mM (Fig. 3b).

Taxol stimulates the assembling of microtubules. It shifts the equilibrium between tubulin dimers and polymers towards polymers and causes the reduction of tubulin concentration in the cytoplasm (Schiff et al. 1979; Schiff and Horwitz 1980). It is probable that more liquid cytoplasm is stirred more efficiently by the cytoplasmic streaming. This should explain the increase in D^* up to $(4.3 \pm 0.35) \times 10^{-9}$ m^2/s under the effect of 0.05 mM taxol during the 24-h treatment. The higher concentration resulted in the increase in D^* during the first hours of exposure [$D^* = (3.7 \pm 0.33) \times 10^{-9}$ m^2/c] and only slightly changed for the rest 24 h. The study of the effect of the solvent, which according to Ibrahim et al. (2002) has highly toxic properties, showed that, at the Cremophor concentration corresponding to 0.5 mM solution of taxol, D^* decreased by the sixth hour of exposure (Fig. 3d). The lower Cremophor concentration corresponding to 0.05 mM taxol solution showed no effect on D^*.

The study shows the significance of the correlation of processes of polymerization and depolymerization of the cytoskeletal tubulin component in the translational diffusion of the cytoplasm water. It is reasonable to suppose that the regulation of the diffusional constituent of the cytoplasmic transport is implemented by the cytoskeletal tubulin component.

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