ISSN 0012-4966, Doklady Biological Sciences, 2006, Vol. 411, pp. 488–490. © Pleiades Publishing, Inc., 2006.
Original Russian Text © T.A. Sibgatullin, F.J. Vergeldt, A.V. Anisimov, H. Van As, 2006, published in Doklady Akademii Nauk, 2006, Vol. 411, No. 3, pp. 427–429.

GENERAL BIOLOGY

Translational Dynamics of Water in the Cytoplasm of Parenchymal Cells of *Malus domestica* Fruit: A Pulsed NMR Approach

T. A. Sibgatullin[a, b], F. J. Vergeldt[a], A. V. Anisimov[b], and H. Van As[a]

Presented by Academician I.A. Tarchevsky May 6, 2006

Received May 19, 2006

DOI: 10.1134/S0012496606060184

It is generally believed that cytoplasmic streamings (CSs) are characteristic of all eukaryotes, including plants [1]. CSs stir the cytoplasm, free the membrane surface of transmembrane transport products, and deliver substances, including signal molecules, to the sites where they are used. CSs are driven by the activity of the actomyosin complex using the energy of ATP [2]. The CS velocity varies from several micrometers per second in terrestrial plants to about one hundred micrometers per second in aquatic ones, such as *Characea* [3, 4]. The parameters of CSs and cell metabolism are interrelated, so that actively metabolizing cells and cells with slack metabolism, e.g., parenchymal storage cells, should be expected to differ in CS intensity. For most living objects, however, the existence of CSs is postulated rather than substantiated by experimental data. The point is that commonly available techniques of CS measurement are based on optical methods of observation of the movement of stained organelles (usually, chloroplasts [5]) serving as natural markers of cytoplasm flow. However, the velocity of organelle movement may not correspond to the true CS intensity, because it is determined by their own propellers; in addition, the organelles are large, and their movement is retarded by cytoplasmic elements, mainly, the cytoskeleton. The movement of smaller, optically indiscernible objects (vesicles and macromolecules) does not encounter substantial obstacles and may occur at a velocity different from that of organelle movement. It is also noteworthy that optical methods are inapplicable to most of nontransparent tissues. The diffusion NMR method [6, 7], which permits tracing directly the translational displacement of water molecules, offers new possibilities of recording CSs. For example, NMR experiments on CS activation by temperature or Gd^{3+} ions showed that the water diffusion coefficient in the cytoplasm of the cells of the *Elodea canadensis* stem was higher than that of pure water [7]. Taking into account the notion that cytoplasmic water molecules are involved in CS, it is logical to explain the abnormally high diffusion coefficients by the superposition of displacement caused by the CS and diffusional water displacement. However, no attempts have been made at estimating the CS velocity by differentiation between the contributions of the displacement caused by diffusion and by CS. The goals of our study were to demonstrate experimentally the CSs were formed in the parenchyma of the storage tissue of the *Malus domestica* fruit and to evaluate the CS velocity by means of pulsed field gradient echo–spin NMR. The experimental procedure was based on recording the integrated displacement of cytoplasmic water followed by the correction for the contribution of the diffusional movement of water molecules.

We used a 30-mm^3 sample of a ripe apple of the *M. domestica* cultivar Granny Smith. Parenchymal cells insignificantly vary in shape and size. Microscopic examination showed that the linear size of the cells was 150–200 μm (on average, 175 μm).

Compartments of the parenchymal cells of *M. domestica* fruits differ in the time of T_2 spin–spin relaxation of water [8]. The water of the vacuole occupying the largest part of the cell's volume, has the longest T_2 relaxation time. The water of the apoplast and intercellular space related with protons of the cell wall via rapid exchange is characterized by the shortest relaxation time. The cytoplasm is a layer between the vacuole and the plasma membrane. The cytoplasmic water has an intermediate T_2 relaxation time. Combined analysis of the relaxation and diffusion behavior of water molecules makes it possible to determine the differences between the compartments of apple parenchymal cells with respect to the self-diffusion coefficient

[a] *Wageningen University, Dreienlaan 3, Wageningen, 6703 HA Netherlands*

[b] *Institute of Biochemistry and Biophysics, Kazan Scientific Center, Russian Academy of Sciences, Kazan, 420111 Tatarstan, Russia*

(SDC) of water [9], which cannot be made by the classic method of diffusion measurement because of similarity between SDC values.

In this study, we attempted to determine the pattern of the translational mobility of water molecules. For this purpose, we analyzed the dependence of the observed SDC of water in the vacuole and the cytoplasm on the diffusion time (t_d) in the range from 0.012 to 4.0 s.

For simultaneous measurement of the diffusion and relaxation characteristics, we used an STE–PFG–CPMG pulse train [10]. It consists of a stimulated magnetic pulsed field gradient echo train and the Carr–Parcell–Meibum–Gill pulse train. The stimulated echo signal was subdivided into three components corresponding to different cell compartments according to the results of analysis of the echo signal relaxation decay. Then, the SDC was determined for each component on the basis of the initial slope of the corresponding signal attenuation plot.

The figure shows the experimental dependences of the observed SDC of water in the vacuole (D_{vac}) and cytoplasm (D_{cyt}) of the apple parenchymal cell on the observation time. We found that D_{cyt} increased with increasing t_d. At short diffusion times, $D_{vac} > D_{cyt}$ and the observed increase in D_{cyt} was largely determined by diffusion exchange with the vacuole; at $t_d > 0.5$ s, the effect of restrictions (cell membranes) became noticeable, and D_{vac} decreased so that $D_{vac} < D_{cyt}$. The contributions of the signals of the apoplast and intercellular water was considerably decreased because of the rapid T_1 relaxation during the observation and did not affect other components. Therefore, subsequent growth of D_{cyt} with increasing t_d cannot be explained by diffusion exchange.

If there is a rotational water streaming with the mean linear velocity V in the cytoplasm, then the mean square displacement of water molecules ($\Delta\overline{X}^2$) in the cytoplasm is the sum of squares of the displacements accounted for by the diffusion and the streaming:

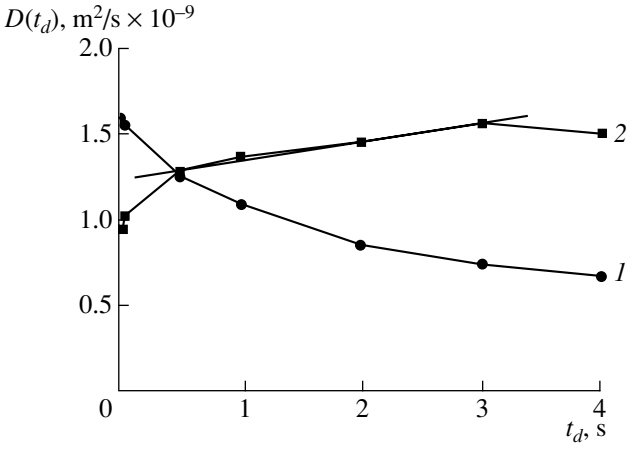
$$\Delta\overline{X}^2(t_d) = 2D_0t_d + (Vt_d)^2, \quad (1)$$

where D_0 is the limit of short diffusion times.

On average, there is no distinct direction of cytoplasm movement in the entire sample. The cytoskeleton structure makes the CS stochastic; therefore, it may be regarded as "quasi-diffusion" in terms of NMR. Therefore, taking into account Einstein's equation and Eq. (1), the observed coefficient of water diffusion in the cytoplasm is determined by the equation

$$D_{app}(t_d) = D_0 + \frac{V^2}{2}t_d. \quad (2)$$

This equation is true under the conditions of slow exchange with the vacuole characteristic of the apple parenchyma. It is applicable in a certain range of diffusion times: on the one hand, t_d should be large enough for the inequality $D_{cyt} > D_{vac}$ to be true; on the other hand, t_d should be small enough for the total mean square displacement of water molecules in the cytoplasm (determined by both diffusion and CS) not to exceed the size of the cell.



The dependence of the observed water diffusion coefficient in the (*1*) vacuole and (*2*) cytoplasm on the observation time. The straight line shows the dependence $D(t_d)$ for a rotational movement with a velocity $V = 15$ μm/s.

We analyzed various trajectories of water molecule movement in a closed impermeable sphere (an analogue of the vacuole) and a closed impermeable thin spherical layer (an analogue of the cytoplasm). The results showed that the mean square displacement of the molecules could not exceed the limit value $\max(\Delta\overline{X}^2) = (2/5)R^2$ for the sphere and $\max(\Delta\overline{X}^2) = (2/3)\ R^2$ for the thin spherical layer, where R is the radius of the sphere (the cell). Thus, it should be expected that, because of geometrical differences, the effect of restrictions on the translational mobility in the vacuole will be observed at shorter times than in the cytoplasm. Indeed, the linear increase in the observed $D_{cyt}(t_d$ predicted by Eq. (2) against the background of a decreasing $D_{vac}(t_d$ was observed in the time range 0.5–3.0 s. We used the slope of the $D_{cyt}(t_d$ dependence curve to determine the velocity of the rotational water movement in the cell cytoplasm (V), which was found to be 15 μm/s.

The obtained estimate of the velocity falls within the range of CS velocities determined by observation of the movement of chloroplasts in cells of *E. canadensis* leaves with the use of optical methods [5, 7] and the movement of plant myosins along actin microfilaments [11]. This allows us to conclude that the functioning of the actomyosin complex leads to the involvement of the most of the cytosol water into the CS, along with organelles. Thus, CS, being a process common for all plant cells, makes a considerable contribution into the acceleration of the redistribution and transport of

water-soluble substances via the symplast pathway. Signal molecules participating in intra- and intercellular information exchange are also likely to be transported with the use of this mechanism. The results of this study show that NMR is a promising tool for studying CSs in plants.

Thus, we can make the following conclusions.

(1) Translational mobility of water molecules classified as CS whose mechanism is not related to diffusion has been found and evaluated in the cytoplasm of apple parenchymal cells with the use of NMR.

(2) The CS has been found to involve most of the water contained in the cytosol.

REFERENCES

1. Pickard, W.F., *Plant Cell Environ.*, 2003, vol. 26, pp. 1–15.
2. Kamiya, N., *Annu Rev. Plant Physiol.*, 1981, vol. 32, pp. 205–235.
3. Yamamoto, K., Hamada, S., and Kashiyama, T., *Cell. Mol. Life Sci.*, 1999, vol. 56, pp. 227–232.
4. Shimmen, T. and Yokota, E., *Curr. Opin. Cell Biol.,* 2004, vol. 16, pp. 68–72.
5. Vorob'ev, V.N. and Anisimov, A.V., *Biofizika*, 1995, vol. 40, pp. 551–555.
6. Anisimov, A.V., Vorob'ev, V.N., and Zholkevich, V.N., *Dokl. Biochem. Biophys.*, 2003, vol. 392, no. 6, pp. 292–293 [*Dokl. Akad. Nauk*, 2003, vol. 392, no. 6, pp. 823–824].
7. Vorob'ev, V.N., Anisimov, A.V., and Dautova, N.R., *Protoplasma*, 2004, vol. 224, pp. 195–199.
8. Snaar, J.E.M. and Van As, H., *Biophys. J.,* 1992, vol. 63, pp. 1654–1658.
9. van Dusschoten, D., de Jager, P.A., and Van As, H., *J. Magn. Reson. Ser. A*, 1995, vol. 116, pp. 22–28.
10. van Dusschoten, D., Moonen, C.T.W., de Jager, P.A., and Van As, H., *Magn. Reson. Med.*, 1996, vol. 36, pp. 907–913.
11. Tominaga, M., Kojima, H., Yokota, E., et al., *EMBO J.*, 2003, vol. 22, pp. 1263–1272.