BIOLOGIJA. 2007. Vol. 18. No. 1. P. 60–63
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Organic cation diffusion in root cell walls of different plants

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A comparative study of the uptake of cation type dye (methylene blue) of the isolated root cell walls and of the roots of whole transpirating seedlings was performed. Seven day seedlings of the *Triticum aestivum* L., *Zea mays* L., *Cucumis sativus* L., *Lupinus albus* L. and *Vigna radiata* L. grown at low concentration of all nutrients (~0.2 mM) in the medium were used. We determined the amount of ionogenic groups per 1 g of dry and fresh weight of root cell walls, their swelling capacity, ion exchange capacity on methylene blue (M^{cw}) depending on adsorption time and diffusion coefficients of organic cation into the polymeric matrix of cell walls (D^{cw}). It was shown that both M^{cw} depended on šĶ (or dissociation degree of the carboxyl groups), changed in accordance with the amount of the carboxyl groups per 1 g of cell walls dry weight and decreased as follows: cucumber > mungbean > lupin > wheat > corn. The results indicate that D^{cw} also depended on plant species and decreased in the order: lupin > mungbean > maize > cucumber > wheat. We conclude that the mechanism of methylene blue absorption by plant root cell walls is stimulated by ion exchange reactions between the methylene blue cation and carboxyl groups of cell walls, but the rate of absorption is mainly defined by methylene blue cation diffusion into the cell wall polymeric matrix.

Key words: coefficients of diffusion, cell walls, roots, dye uptake

INTRODUCTION

In plant roots, water and ions are transported via the apoplast and the symplast. These two pathways are more or less equivalent for the transport of mineral nutrient ions and water. The predominance of one of them is mainly determined by environmental conditions and the type of transported compound. Under certain conditions, the apoplast pathway of water and ion movement predominates [1], and thus it is mainly determined by the properties of cell walls.

The properties of cell walls as cation exchangers can be characterized by such physicochemical parameters as ion-exchange capacity, ionization constants for active groups, swelling the coefficient of polymer matrix and the coefficient of ion diffusion in the matrix [2, 3]. The first two parameters characterizing cell walls properties and their effects on mineral ion uptake have been studied in several laboratories [3, 4]. Little is known about ion diffusion in cell walls [3].

Many authors believe that diffusion is the determining stage during ion movement in the root; however, no experimental data on the rate of ion diffusion in the polymeric matrix of plant cell walls and the effect of this property on ion transport are available. In the present study we have quantitatively evaluated ion diffusion in the cell walls and its contribution to the absorbing function of the root.

MATERIALS AND METHODS

Grain and seeds were soaked in tape water for 3 h at room temperature and germinated at 27.5 °C in the dark. Experiments were carried out at 20–22 °C using 7-day-old seedlings of *Triticum aestivum* L., *Zea mays* L., *Cucumis sativus* L., *Lupinus albus L.* and *Vigna radiata L.*grown in tape water at a concentration of K^+, Na^+, NO_3^-, Cl^-, PO_4^{3-} of ~0.2 mM. Illumination was 110 μM/s per m^2 for 14 h per day and the solutions were aerated during 8 h per day.

Cell walls were isolated and standardized as previously described [2]. Cell walls swollen in water were blotted with filter paper, weighed and placed into 0.1 mM solution of methylene blue (0.032 mg/ml). The experiments were carried out under constant shaking (60 oscillations/min). Aliquots of the mixture (1ml) were taken after 2, 4, 6, 10, 15, 20, 30, 40, 50, 60, 120 and 180 min of incubation and diluted for spectrophotometric determination of methylene blue concentration at 650 nm using a Uniplan analyzer (Russia).

The amount of methylene blue accumulated by plant preparation was calculated using the following formula:

$$M_i^t = \frac{C_o V_o - C_i^t V_i^t - \sum_{i=2}^{n} \left(C_{i-1}^t V_{al} \right)}{319g},$$

where M_i^t is the amount of methylene blue accumulated during time interval t (μmol per 1 g root wet weight); C_0 and C_i^t are dye concentration (μg/ml) at zero time (t = 0) and after incubation (t_i); V_0 and V_i^t represent the initial volume (ml) and the volume at the moment of the aliquot collection, respectively; n is the number of aliquots; i is the aliquot number; V_{al} is the aliquot volume (ml); C_{i-1}^t is methylene blue concentration (μg/ml); g is root weight (in grams); 319 is the relative molecular mass of methylene blue.

To determine the maximal uptake of methylene blue, cell walls were blotted with filter paper, weighed and placed into Erlenmeyer flasks (~250 ml) with 150 ml 0.1 mM methylene blue solution. After two days, the plant material was discarded and solution dye concentration was determined as described above. Maximal uptake was calculated using the following formula:

$$M_{max} = \frac{(C_{in} - C_f)V}{319g},$$

where M_{max} is the maximal ion-exchange capacity (in μmol per 1 g wet or dry weight) of cell walls with respect to methylene blue under certain conditions (pH, dye concentration, root/solution ratio, temperature), C_{in} and C_f represent the initial (C_{in}) and the final (C_f) concentrations of methylene blue (μg/ml), V is the volume of the incubation mixture (150 ml), g is the weight of roots used for isolation of cell walls (in grams), 319 is the relative mass of methylene blue.

Potentiometric titration of isolated cell walls was carried out using the method of separate weights. The amount of ionogenic groups per 1 g of dry and fresh weight of root cell walls, their swelling capacity and ionization constants of cation exchange groups were determined using potentiometric titration curves as described earlier [4].

RESULTS

In all variants of the measurements, there was exponential time-dependent dye uptake by both cell walls (M^{cw}). The values of M^{cw} were in the following order: cucumber > mungbean > lupin > wheat > corn (Fig. 1).

Since ionization of methylene blue results in the formation of a colored cation, it is reasonable to suggest that its uptake occurs via exchange reaction between this cation and carboxyl groups of the cell walls. Data on pH dependence of methylene blue uptake seem to support this suggestion. In all cases, change in pH value was accompanied by a corresponding change of the amount of methylene blue in cell walls (Fig. 2). Such

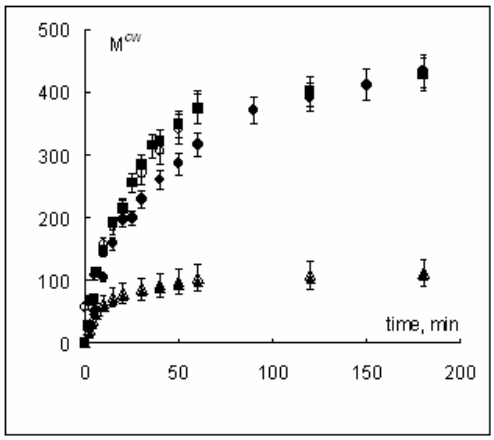

Fig. 1. Time course of methylene blue uptake (M^{cw}) by cell walls isolated from roots of cucumber (closed squares), mungbean (closed circles), lupin (open circles), wheat (closed triangles) and maize (open triangles) plants

In all experiments, the volume of solution and dye concentration was 150 ml and 0.1 mM, respectively. Results (μmol per 1 g root cell wall dry weight) represent mean ± SD of 3–5 experiments.

pH-dependent behavior of adsorption capacity suggests that methylene blue binding to cell walls is an ion-exchange process and, consequently, the adsorption value should be linked to the number of ionogenic groups in cell walls.

It has been established that the polymer structure of root cell walls of all plants studied contains four types of ionogenic groups: amino groups with pK_a~3, carboxyl groups of α-D-polygalacturonic acid with pK_a~5, the carboxyl groups of hydroxylcinnamic acids with pK_a~7.3, and the phenolic groups with pK_a~10. In all cases the amount of bound dye was 1.5–3-fold higher than the amount of carboxyl groups of a-D-polygalacturonic acid. This suggests involvement of both types of

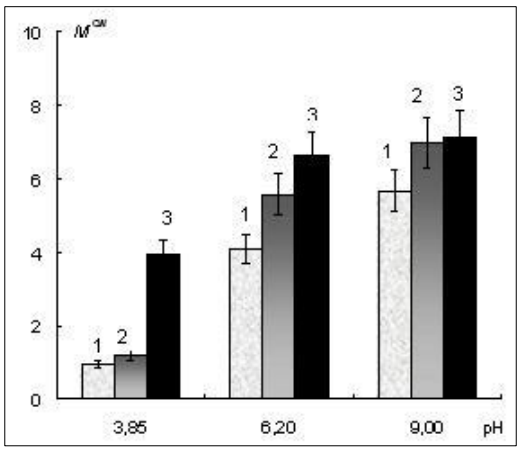

Fig. 2. Effect of pH on methylene blue uptake (M^{cw}) by cell walls isolated from cucumber (*1*), mungbean (*2*), and cucumber roots (*3*) during 3 h of exposure to 150 ml of standard 0.1 mM dye solution. Results (μmol per 1 g root wet weight) represent mean ± SD

carboxylic groups into methylene blue cation binding. The ion-exchange mechanism of methylene blue binding is also confirmed by the dependence of maximal ion-exchange capacity (methylene blue) of cell walls (M_{max}) on the total amount of carboxyl groups (S) which may be potentially involved in the exchange reactions (Fig. 3). Results of statistical treatment indicate that under our experimental conditions M_{max} and S are related parameters. All these considerations provide a convincing evidence that the mechanism underlying methylene blue binding involves ion-exchange reactions in the cell wall and thus the experimental kinetic curves can be analyzed by known equations describing ion-exchange kinetics [5].

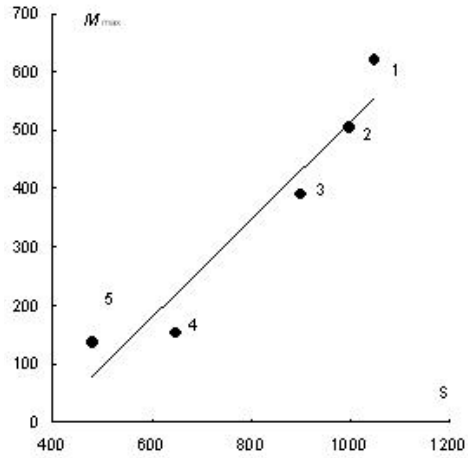

Fig. 3. Dependence of maximal uptake of methylene blue cation (M_{max}) by cell walls under given experimental conditions (dye concentration, pH, ionic strength, roots/solution ratio) on total number of carboxylic groups in cell walls

In all cases the volume of 0.1 mM methylene blue solution was 150 ml. The results are expressed as μmol per 1 g cell wall dry weight. Points represent experimental results and solid line shows trend (M_{max} = 0.85S + 330.3; r^{corr} = 0.959).

DISCUSSION

The coefficient of methylene blue diffusion in root cell walls (D^{cw}) depends on plant species (Table 1). Since parameter D^{cw} has not been quantitatively evaluated in plant roots, the D^{cw} obtained in the present study was compared with that for synthetic ion-exchange materials. The diffusion coefficient for methylene blue cation in sulfur-containing cationites varied from $3 \cdot 10^{-10}$ to $4 \cdot 10^{-12}$ cm^2/sec depending on the amount of cross-linking reagent (2–16%) [6]. According to our data, plant cell wall is more permeable for methylene blue than for synthetic ionites because the diffusion coefficients in cell walls are 2–4 orders of magnitude higher. This is obviously determined by a characteristic structure of the cell wall polymeric matrix, which is characterized by a lower cross-linkage compared with the synthetic ion-exchange materials. The values of diffusion coefficients obtained in the present study, together with results on root cell wall swelling in water (Table), provide additional arguments supporting our conclusion that cell walls represent a weak cross-linked ion exchanger or they are characterized by a low degree of cross-links between linear chains of polymers (~1–2%). The coefficient of cell wall swelling in water (K^{cw}) and the coefficient of methylene blue diffusion in cell walls (D^{cw}) are parameters characterizing the same property of a matrix, cross-linkage or permeability of its polymeric structure. This suggests a correlation between K^{cw} and D^{cw}. Our calculation actually revealed the existence of a statistically significant relationship between these parameters (Fig. 4). This also supports the correct choice of the model for calculation of diffusion coefficients, because K^{cw} and D^{cw} values were obtained in independent experiments.

One of the physiological functions of cell walls as the ion-exchange material consists in accumulation of ions from the environment. Using data of the present study, it is possible to evaluate quantitatively the methylene blue cation concentrating ability of cell wall phase at a low ionic strength of the external medium. The concentrating coefficient $k_{conc} = C_{cw}/C_0$ (where C_{cw} and C_0 represent volume concentrations of methylene blue in cell walls and in initial solution (0.1 mM)) is 745, 265, 200, 170 and 90 in cucumber, mungbean, wheat, lupin and maize, respectively. These results show that cation concentration in cell walls is 100–700-fold higher than in solution. Although the methylene blue cation is significantly bigger than mineral nutrition cations, these results clearly demonstrate that cell wall accumulates cations during the first stage of cation consumption by cells.

In the physiology of mineral nutrition, it is generally accepted that by studying the kinetics of ion accumulation it is possible to discriminate its transport in cell walls from transport through the cell membrane [7, 8]. Results of our study suggest that diffusion, the slowest stage of ion exchange, is very important for ion accumulation by plant roots, and the exponential type of the

Table. **Mean values of the coefficient of cell wall swelling in water (K^{cw}); of the coefficients of methylene blue diffusion into cell walls (D^{cw}); the number of carboxyl groups in cell wall structure (S) and the maximal uptake of methylene blue by the cell walls (M_{max}). K^{cw} is expressed in gram H_2O per 1 gram of cell wall dry weight. D^{cw} is expressed in cm^2 per second. S and M_{max} are expressed in micromole per 1 gram of cell wall dry weight and in micromole of methylene blue per 1 gram of cell wall dry weight respectively**

Plant	K^{cw}	$D^{cw} \cdot 10^8$	S	M_{max}
Cucumber	7.3	3.1	1050	619
Mungbean	18	13.1	1000	504
Lupin	22	14.1	900	389
Wheat	6.6	1.3	650	152
Maize	14	8.4	480	135

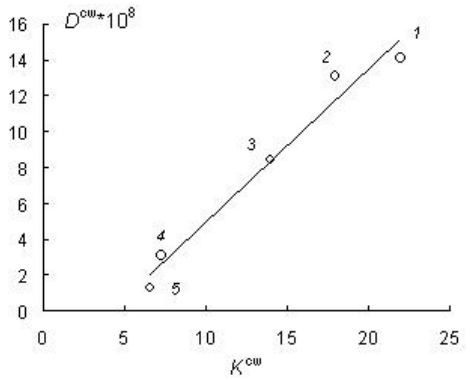

Fig. 4. Dependence of coefficients of methylene blue diffusion (D^{cw} cm^2 per 1 second) in root cell walls of lupin (*1*), mungbean (*2*), maize (*3*), cucumber (*4*) and wheat (*5*) on coefficient of cell wall swelling in water (K^{cw}, grams H_2O per 1 g dry weight of cell walls)

The coefficients D^{cw} and K^{cw} characterized the same property of the polymer matrix, the rigidity of its polymeric structure (or degree of its cross-linkage, or permeability). Calculations revealed a statistically significant relationship which confirms the correctness of the model for the calculation of diffusion coefficients because K^{cw} and D^{cw} were obtained in independent experiments. Points indicate experimental results and solid line shows trend ($D^{cw} = 0.85K^{cw} - 3.54$, $r^{corr} = 0.99$).

kinetic curve is not the criterion for discriminating the ways of ion transportation (via symplast or apoplast). Sometimes cation movement into cell walls is not a rapid process as was suggested earlier [7]. According to the results of the present report, during 3 h of exposure cell walls are saturated with methylene blue by 60–90% (depending on plant species).

In conclusion, the mechanism of methylene blue absorption by plant root cell walls is stimulated by ion exchange reactions between the methylene blue cation and carboxyl groups of cell walls, but the rate of the adsorption is mainly defined by the diffusion of methylene blue cation into the cell wall polymeric matrix. Diffusion, the slowest stage of ion exchange, is very important for ion accumulation by plant roots, and the exponential type of the kinetic curve is not the criterion for discriminating the ways of ions transportation (via symplast or apoplast).

ACKNOWLEDGEMENT

We are grateful to the Russian Foundation of Fundamental Research (Project number 04-04-49379).

Received 19 November 2006
Accepted 22 December 2006

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