Biochemistry (Moscow), Vol. 66, No. 5, 2001, pp. 556-563. Translated from Biokhimiya, Vol. 66, No. 5, 2001, pp. 688-697.

Ion-Exchange Properties of Cell Walls Isolated from Lupine Roots

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Received October 23, 2000
Revision received December 18, 2000

Abstract—Acid–base properties of cell walls isolated from various root tissues of 7-day-old lupine seedlings and 14-day-old lupine plants grown in various media were studied. The ion-exchange capacity of root cell walls was estimated at various pH values (from 2 to 12) and constant ionic strength (10 mM). The parameters determining the qualitative and quantitative composition of cell wall ionogenic groups along the root length and in its radial direction were estimated using Gregor's model. This model fits the experimental data reasonably well. Four types of ionogenic groups were found in the cell walls: an amino group ($pK_a \sim 3$), two types of carboxylic groups ($pK_a \sim 5$ and 7.3, the first being the carboxylic group of galacturonic acid), and a phenolic group ($pK_a \sim 10$). The number of functional groups of each type was estimated, and the corresponding ionization constant values were calculated. It is shown that the chemical composition of the ionogenic groups was constant along the root length as well as in its radial direction and did not depend on either physiological state or root nutrition, while the number of different groups varied. The content of carboxylic groups of α-D-polygalacturonic acid in the root cell walls of 14-day-old plants was shown to depend on the distance from the root tip, being maximal in the zone of lateral roots. The number of these groups was 10- and 2-fold less in the central cylinder compared to that of cortex for 14-day-old plants and 7-day-old seedlings, respectively.

Key words: lupine roots, ion uptake, cell wall, potentiometric titration, ionogenic groups, p*K*

Ion-exchange processes in cell walls of roots have long been of interest for investigators. Structural and ionic characteristics of this compartment are important for the physiology of the plant because (a) they determine the ionic composition of the medium that bathes the cell membrane, (b) they control the extracellular transport of solutes, and (c) they affect the mechanical and osmotic phenomena involved in cell growth [1]. It has been shown that the plant cell wall behaves like a weakly basic cation exchanger with a low cross-linking degree of the polymeric chains, and properties of the cell wall are similar to those of ionites. The ability to absorb ions is related to the presence of carboxylic groups of α-D-polygalacturonic acid (PGA) in the apoplast. The carboxylic groups are characterized by their dissociation constant value, which determines the pH range where the ionogenic groups are ionized and can be involved in ion-exchange reactions with cations from the outer medium. There have been many attempts to determine the value of the dissociation constant for the carboxylic groups of PGA [2-7]. However, in spite of the similarity of the experimental approaches used, the pK_a values obtained differ significantly—from 3 [2-5] to 5 [6, 7]. These differences are related to the fact that the authors [2-5] did not consider other types of ionogenic groups in the apoplast polymer structure, and the titration range for PGA carboxylic groups was estimated approximately. Using a phenomenological approach to study the acid–base equilibria, the authors of the latter papers not only determined the pK_a value for PGA carboxylic groups ($pK_a \sim 5$ in 10 mM KCl), but also identified the quantitative and qualitative composition of ionogenic groups in the three-dimensional structure of the cell wall. Four types of ionogenic groups were found, including three cation-exchange groups (two carboxylic groups, one of which was a moiety of PGA, and a phenolic group), and the fourth being an anion-exchange group [6, 7]. Analysis of the structure of ionogenic groups in cell walls of various plants showed that their qualitative composition did not depend on plant species, while the numbers of different groups varied.

Water and ions in plant roots are known to be transported in two ways—by the apoplast and by the symplast. Under certain environmental conditions, the apoplast transport prevails, it being determined mainly by the cell wall properties [8]. Inside a root, water and ions move

Abbreviations: PGA) α-D-polygalacturonic acid.

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both in the radial direction and along its length. The structure of cells and tissues, including the structure and chemical composition of cell walls, changes in the same directions [9]. Thus, it is reasonable that the acid–base properties and, consequently, ion exchange capacity of cell walls also vary along the root length as well as in its radial direction. However, there are no experimental data either confirming or disproving this.

We studied the acid–base properties of cell walls isolated from various parts of lupine roots. The results allow us to characterize differences in ion exchange properties of the apoplast in various anatomical structures as well as depending on conditions of nutrition and the physiological state of the plants.

MATERIALS AND METHODS

Roots of *Lupinus albus* L. variety Nemchinovskii belyi were studied. Seeds were soaked for 3 h in tap water at room temperature and then germinated in a thermostat at 27°C in the dark. Experiments were performed with 7-day-old seedling roots (variant I) grown in tap water, and 14-day-old plants (variant II) grown from 7-day age in complete Knop's solution. In both cases, the conditions used were 20-22°C, illumination of 110 μM/sec per m^2, day length of 14 h, and aeration of 8 h per day. Thus, age of the plants and ionic strength of the solutions varied in the experiments.

The average root length was 8 ± 1.6 cm in variant I and 14 ± 2.1 cm in variant II. The roots were separated and cut into sections as follows: root tip, 0-1.5 cm; zone of lateral roots together with lateral roots, 1.5-6 cm in variant I and 1.5-12 cm in variant II; basal part, 6-8 cm and 12-14 cm in variants I and II, respectively. In variant I there were 4-7 of lateral roots of 1-5 mm length. In variant II there were 10-15 lateral roots of 1-10 mm length. Tissues of the cortex and the central cylinder were separated from the basal zone of 7-8 cm roots (variant I) and 13-14 cm roots (variant II). Microscopic analysis indicated that the cortex tissue contained epidermal cells and cortex cells; the central cylinder tissue also included endodermal cells.

The root tissues were fixed for 5 min at 100°C, dried at 55-60°C to constant weight, and stored in glass containers at room temperature until needed.

Cell walls were isolated as described previously [6, 7]. Fixed and dried material was placed into a glass column (200 ml) and washed under dynamic conditions with 10 mM KOH (~0.5 liter), distilled water (~2 liters), 10 mM HCl (~0.5 liter), and distilled water until no Cl^- was detected in the eluate, and then dried to constant weight at 50°C. Chloride ions were determined by titration with mercuric nitrate. Using the standardization method, cation exchange groups of cell walls were put into their H^+-form, this making it possible to study the ion-exchange properties of samples containing functional groups of different structure [6, 7, 10]. Microscopic analysis of the cell walls isolated from various root zones did not reveal any inner cell structures in all cases nor any disorder in cell position in the root tissues.

Potentiometric titration was performed using a number of samples [6, 7]. Dry cell wall samples (40 ± 0.1 mg) were placed into flasks with glass stoppers and 12.5 ml of KOH or HCl solutions of different concentrations were added. Constant ionic strength (10 mM) was maintained by the addition of KCl solution. Concentrations of the acid and the base were varied from 0 to 10 mM. After 24 h, the material was discarded and the pH of the resulting solution was measured using Jenway model 3320 pH meter (England). The concentration of the remaining acid and base was determined by titration of the solution with bromthymol blue. Ion-exchange capacity of a sample at fixed pH_i was calculated from the change in H^+ or OH^- concentration using the equation:

$$S_{cat(an)} = (c_o - c_{eq})V/g\,, \qquad (1)$$

where S is the cation-exchange capacity (S_{cat}), or anion-exchange capacity (S_{an}), μmole per g dry weight of cell walls; c_o and c_{eq} are the original and equilibrium concentrations of KOH or HCl in the solution, mM; V is the volume of the solution, ml; g is the sample weight, g.

Phosphorous assay in cell wall samples. A dried and pounded cell wall sample was weighed (20-40 mg) and placed into a quartz Kjeldahl flask; then 2 ml of 98% H_2SO_4 and 0.02 g of selenium catalyst were added. The mixture was heated in an electric oven for 3-4 h until the solution became colorless. Then the solution was cooled and placed into a volumetric flask (50 ml), and the volume was brought up to the mark with distilled water. An aliquot (2-10 ml, depending on the phosphorous content) was taken from the resulting solution, placed into a volumetric flask (50 ml), and neutralized with 40% NaOH solution using phenolphthalein as an indicator. Then 5 ml of reducing mixture and 5 ml of molybdenum solution were added. After 10 min of incubation, 10 ml of sodium acetate was added [11]. The volume was brought up to the mark with distilled water. The resulting solution was tested photometrically at 670 nm. A calibration curve was constructed using samples containing 10-100 μg of phosphorous prepared by dilution of a standard solution (0.25 mg phosphorous per ml).

RESULTS

Figure 1 presents experimental titration curves $pH = f(S_i)$ for cortex and central cylinder tissues of lupine roots of various age. All the variants investigated have similar dependences. The complex poly-sigmoidal character of the curves reveals a number of different functional groups in the carbohydrates of the cell walls. The ion-exchange

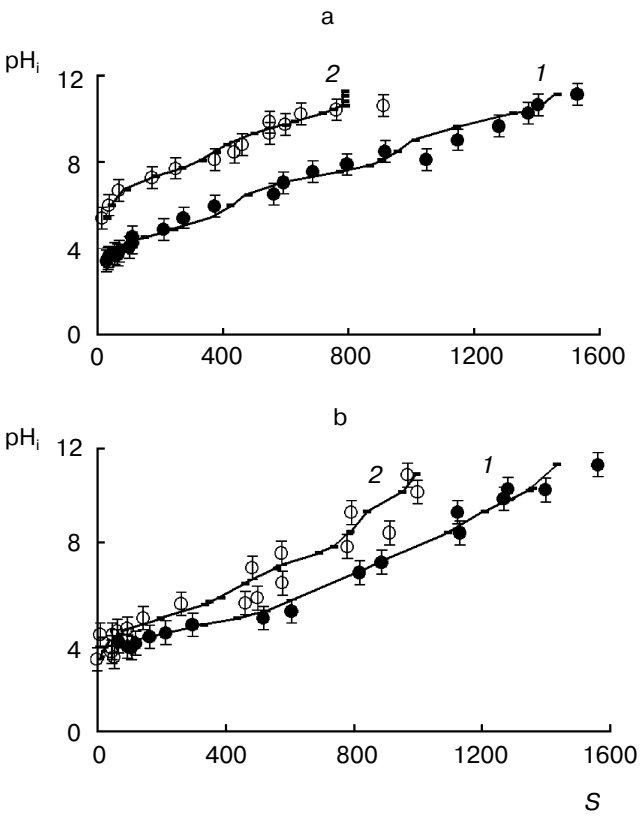

Fig. 1. Potentiometric titration of cell walls isolated from tissues of cortex (*1*) and central cylinder (*2*) of lupine roots. The ionic strength of the solution was 10 mM (*S*, ion-exchange capacity, μmole per g dry weight of the sample). Experimental data are shown by points (means ± SD). Curves plotted using Eq. (4) and the parameters given in Tables 2 and 3 are shown by solid lines. The cell walls were isolated from 7-day-old seedlings (a) and from 14-day-old plants (b).

capacity for KOH (S_{cat}) and HCl (S_{an}) are maximal at pH > 10.8 and pH < 2.8 (Table 1). These values characterize the total number of acidic and basic groups in the polymeric structure of the cell walls that can be involved in ion-exchange reactions at appropriate pH values of the nutrient solution [6, 7].

The experimental curves were divided into single sigmoidal sections using the derivative curves (dS_i/dpH_i) = $f(pH_i)$, which show minima of the functions corresponding to the beginning ($\alpha = 0$) and the end ($\alpha = 1$) of ionization for each functional group of the *j*th type [12]. Differentiation of the experimental curves made it possible to determine the number of ionogenic groups of each type (ΔS_j) (Tables 2 and 3) and the dissociation degree α_i^j for each pH_i value using the following equation [10]:

$$\alpha_i^j = (S_i - S_{j\,min})/\Delta S_j\,, \qquad (2)$$

where S_i is the ion-exchange capacity of the sample (μmole per g dry weight of cell walls) at fixed pH_i value; $\Delta S_j = \Delta S_{j\,max} - \Delta S_{j\,min}$, where $\Delta S_{j\,max}$ and $\Delta S_{j\,min}$ are the maximal and minimal values of the ion-exchange capacity determined by the positions of the extremes of the derivative curves and corresponding to the beginning and the end of ionization of the *j*th type group (μmole per g dry weight).

To calculate the dissociation constant values, the Henderson–Hasselbach equation as modified by Gregor was used [13]:

$$pH = pK'_a + n \cdot \log[\alpha/(1-\alpha)]\,, \qquad (3)$$

where pK'_a is the apparent ionization constant of ionogenic groups of a polymer, α the dissociation degree, and *n* a constant that depends on the polymer structure and the nature of the counterion. Being somewhat empirical, Eq. (3) nevertheless is successfully used for description of acid–base equilibrium processes in polyfunctional synthetic [10, 14] and natural ion exchangers [6, 7]. Using Eq. (3) and the values of α_i^j calculated for each pH_i (Eq. (2)), pK_{aj} and n_j values for each ionization step were calculated using regression analysis (Tables 2 and 3). These parameters were used to calculate the values $S_i = f(pH_i)$ (Fig. 1) for each experimental pH_i value according to Eq. (4) [10]:

$$S_{i\,cal} = S_{cat} - \sum_{\substack{j=1\\ i=1}}^{k,m} \Delta S_j/\{1+\exp[10[(pK_{aj}-pH_i)/n_j]]\}\,, \qquad (4)$$

where S_{cat} is the maximal ion-exchange capacity of cell walls (μmole per g dry weight); ΔS_j is the number of iono-

Table 1. Maximal cation-exchange (S_{cat}) and anion-exchange (S_{an}) capacities (μmole per g dry weight of cell walls) of cell walls isolated from different parts of roots of lupine seedlings and plants

Root part	S_{cat}	S_{an}
14-day-old plants		
Zone of lateral roots	1600 ± 70	50 ± 15
Basal root zone	1450 ± 50	55 ± 10
Cortex	1450 ± 50	50 ± 12
Central cylinder	1000 ± 70	35 ± 15
7-day-old seedlings		
Cortex	1500 ± 60	50 ± 20
Central cylinder	840 ± 40	30 ± 10

Table 2. Parameters of Gregor's equation (3) calculated for root cell walls from 14-day-old lupine plants

Part of root	j	$pK_{a\,j}$	n_j	r_j	ΔS_j, μmole per g dry weight of cell walls
Root tip	2	4.9	—	—	220
	3	7.3	—	—	500
Zone of lateral roots	1	3.15	1.05	0.955	50
	2	4.73	1.10	0.986	900
	3	7.29	1.01	0.944	400
	4	9.56	1.14	0.988	300
Basal root zone	1	3.20	0.95	0.988	55
	2	4.74	1.10	0.998	750
	3	7.31	0.80	0.959	350
	4	10.2	1.11	0.966	350
Central cylinder	1	3.12	1.15	0.940	35
	2	4.91	1.05	0.969	450
	3	7.22	0.93	0.943	350
	4	9.73	0.81	0.943	200
Cortex	1	3.20	1.05	0.990	50
	2	4.62	1.21	0.998	750
	3	7.37	1.11	0.999	350
	4	9.71	1.29	0.982	350

Note: In Tables 2 and 3: j) type of group; pK_{aj}) ionization constant for groups of the *j*th type; n_j) constant of Eq. (3) for groups of the *j*th type; r_j) correlation factor; ΔS_j) number of groups of the *j*th type; 1) amino groups; 2) carboxylic groups of PGA; 3) other carboxylic groups; 4) phenolic groups.

Table 3. Parameters of Gregor's equation (3) calculated for root cell walls from 7-day-old lupine seedlings

Part of root	j	$pK_{a\,j}$	n_j	r_j	ΔS_j, μmole per g dry weight of cell walls
Central cylinder	1	3.21	0.95	0.940	30
	2	5.0	1.01	—	30
	3	7.43	1.03	0.993	360
	4	9.79	1.00	0.965	450
Cortex	1	3.5	0.95	0.993	50
	2	4.81	1.02	0.995	450
	3	7.33	0.8	0.983	500
	4	9.90	1.04	0.988	550
Zone of lateral roots	2	4.9	—	—	350
	3	7.3	—	—	400
Basal root zone	2	4.9	—	—	400
	3	7.3	—	—	500

Table 4. Adequacy of calculated and experimental potentiometric curves for lupine root cell walls

Part of root	*a*	*b*	r^2
14-day-old plants			
Zone of lateral roots	27.2	0.99	0.96
Basal root zone	−8.21	1.0	0.99
Cortex	−7.97	0.99	0.99
Central cylinder	8.92	0.95	0.96
7-day-old seedlings			
Cortex	9.53	0.95	0.99
Central cylinder	−1.39	0.99	0.97

Note: *a* and *b*) factors of the regression equation $S_{cal} = bS_{exp} + a$; S_{cal}) ion-exchange capacity of cell walls calculated using Eq. (4) and the parameters given in Tables 2 and 3; S_{exp}) ion-exchange capacity values obtained in the experiment at pH_i; *r*) correlation factor.

genic groups of the *j*th type (μmole per g dry weight); $S_{i\,cal}$ is the calculated ion-exchange capacity of cell walls at a certain pH_i (μmole per g dry weight); pK_{aj} is the apparent ionization constant for ionogenic groups of the *j*th type; n_j is the constant of Eq. (3) for ionogenic groups of the *j*th type; *k* is the number of points of the experimental potentiometric curve; *m* is the number of types of ionogenic groups.

The adequacy of the approach used for the description of acid–base equilibrium was estimated by regression analysis [12], calculating the parameters of the equation:

$$S_{cal} = b \cdot S_{exp} + a\ .$$

The values of the correlation factors, as well as parameters *a* and *b* (Table 4) indicated that the model fit the experimental data reasonably well.

For cell walls of root tip tissues of 14-day-old plants and for both the basal zone and the zone of lateral roots of 7-day-old seedlings, the number of carboxylic groups was calculated using separate sections of the potentiometric curve, not the whole curve (Tables 2 and 3). This was due to the small quantities of these root tissues. In these cases, the following facts were taken into consideration. The results of the present study (Tables 2 and 3) as well as previous results [6, 7] demonstrated that pK_a values for the groups of a certain type did not depend on tissue, age of plants, or nutrition conditions (Tables 2 and 3). According to these data, 3-5 points of the potentiometric curve $pH_i = f(S_i)$ were obtained for cell walls of root parts at pH_i values in the range 4.5-5.5 and 7-8. Assuming that pK_a values for the carboxylic groups are 4.9 and 7.3, ion-exchange capacity values were estimated at pH 4.9 and 7.3 using 3-5 points within the indicated intervals [12]. These values were used to calculate the number of the groups given in Tables 2 and 3.

DISCUSSION

The total number of anion-exchange groups (S_{an}) in root cell walls of lupine seedlings (calculated per g dry weight) remains constant both along the root length and in the radial direction. The total amount of cation-exchange groups (S_{cat}) varies depending on the type of tissue (Table 1). In all samples investigated, S_{cat} values exceed significantly (30-50-fold) S_{an} values (Fig. 2). These results support the idea that root cell wall is a natural ion exchanger exhibiting mainly cation-exchange properties [1]. The changes in S_{cat} along the root length do not exceed 10% (basal root zone, zone of lateral roots), while they reach 50% in the radial direction (Table 1).

According to data in the literature, cell walls include four types of functional groups that can be ionized within the pH range 2-12 [6, 7, 15]. Considering the pK_a values (Table 2 and 3), the data on chemical composition of the cell wall [15], and pK_a values of different types of groups in low molecular weight compounds [16], the section of the potentiometric curve within the pH range 8-11 can be attributed to the titration of phenolic groups of the cell wall matrix because $pK_a = 9.98$ for phenol. Low molecular weight amides are weak bases with pK_a of 1-3.5. In our experiments, proton uptake (not proton release) was detected at pH values in the range 2-3.7. This suggests ionization of a basic group in this range. Thus, the groups with pK_{a1} of 3 observed in cell wall components are supposed to be amide groups.

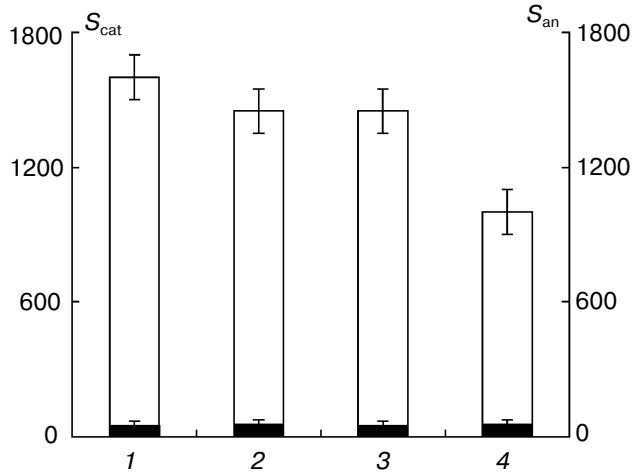

Fig. 2. Content of cation-exchange (S_{cat}, open rectangles) and anion-exchange (S_{an}, filled rectangles) groups in cell walls of various root tissues of 14-day-old lupine plants (μmole per g dry weight of cell walls): *1*) zone of lateral roots; *2*) basal root zone; *3*) cortex; *4*) central cylinder. The data are given as mean ± SD.

Table 5. Phosphorus content in various root tissues of 14-day-old lupine plants

Part of root	Phosphorus content			
	intact roots		cell walls	
	μmole/g*	%	μmole/g*	%
Zone of lateral roots	93	0.29 ± 0.03	38	0.12 ± 0.02
Basal zone	134	0.42 ± 0.05	12	0.036 ± 0.03
Cortex	132	0.41 ± 0.08	0	0
Central cylinder	153	0.47 ± 0.01	10	0.032 ± 0.03

Note: Phosphorus content was determined by Lowry's method, burning the dry material in 98% H_2SO_4 in the presence of selenium catalyst with subsequent spectrophotometric assay of the phosphate ion [11]. The means of three experimental values are given.

* Calculated per g dry weight of intact roots and cell walls, respectively.

For galacturonic acid, the pK_a was shown to be 3.42 [3]. However, the pK_a values for low molecular weight and polymer acids differ greatly. The transition from a low molecular weight compound to three-dimensional polymer structure results in pK_a shift as follows: acrylic acid, 4.26 [16], polyacrylic acid, 4.8 [17], and its three-dimensional analog, 5-7.5 [17] depending on the type and concentration of the linking agent. Thus, it can be assumed that the functional groups with pK_a ~ 5 observed in the three-dimensional structure of the apoplast are the groups of galacturonic acid. The ionization constant value for these groups determined previously by potentiometric titration (pK_a 3.2-3.4 [2-5]) differ significantly from that obtained in the present work. This discrepancy may be due to the fact that the authors of the earlier work did not consider other types of ionogenic groups in their calculations. Consequently, the pH range for the graduated dissociation of PGA groups was estimated incorrectly.

The ionogenic groups with pK_a ~ 7.3 may belong to either acid (phosphorous or carboxyl containing groups) [17], or base (imidazolic or α-amino groups) [16]. A compound containing acid groups as well as basic ones is considered an ampholyte or zwitterion [16]. Such compounds exhibit only anion-exchange properties in strongly acid solutions (maximal anion uptake) and only cation-exchange properties in strongly basic solutions (maximal cation uptake) [16]. Based on this material and the data obtained in this work, it can be concluded that cell wall components contain 50 and 1000-1600 μmoles (depending on root zone) of anion and cation exchange groups per g dry weight of cell walls, respectively (Table 1). We also demonstrated that the number of ionogenic groups with pK_a ~ 7.3 (S_3) was 350-500 μmoles per g dry weight of cell walls depending on the type of tissue (Tables 2 and 3).

Consequently, for this ionization step, 300-450 μmoles of groups per g dry weight of cell walls (the difference between S_1 and S_3) are certain to be cation-exchange groups. It is also known that the mentioned groups can be either carboxylic or phosphoric acid derivatives [17]. However, analysis of cell wall preparations by Lowry's method revealed low phosphorous content (no more than 40 μmoles per g dry weight of cell walls, Table 5). Consequently, the groups with pK_a ~ 7.3 constituting 350-450 μmoles per g dry weight of cell walls are mainly carboxylic groups.

Thus, the data of the present investigation indicate that there are four types of ionogenic groups in the polymer structure of cell walls isolated from different parts of lupine roots: amino groups with pK_a ~ 3, carboxylic groups of PGA with pK_a ~ 5, carboxylic groups of a second type with pK_a ~ 7.3, and phenolic groups with pK_a ~ 10 (Tables 2 and 3). These values are in good agreement with our earlier data on the potentiometric titration of root cell walls of various plants at 10 mM ionic strength [7].

The qualitative composition of functional groups in cell walls is constant for different parts of roots, as seen from the similar values of the ionization constants pK_{aj} (Tables 2 and 3), but the content of each group varies significantly (ΔS_j, Tables 2 and 3). The data demonstrate that the number of PGA groups varies along the root length as well as in the radial direction (Figs. 3 and 4). The value ΔS_2 increases sharply towards the zone of lateral roots and decreases towards the basal root part. This parameter is 2-5-fold lower in the root tip than in the other root zones. The content of PGA groups is 10- and 2-fold less in the central cylinder than in the cortex for 7-day-old seedlings and 14-day-old plants, respectively (Figs. 3 and 4). These results can be considered in terms of functional peculiarities of various root tissues. The main function of cell walls of epidermis as well as cortex is uptake and concentrating of cations from the envion-

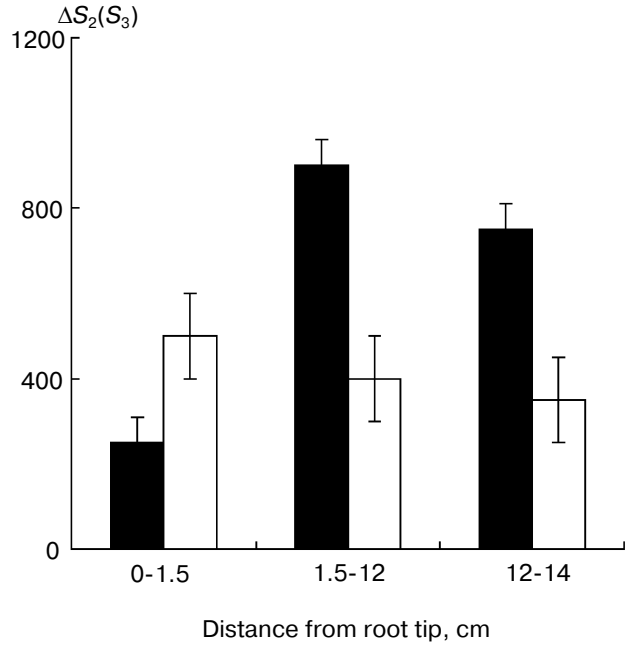

Fig. 3. Content of carboxylic groups (ΔS_j) in root cell walls of 14-day-old plants—dependence on the distance from the root tip. ΔS_2 and ΔS_3) total number of PGA carboxylic groups (filled rectangles) and carboxylic groups of the second type (open rectangles), respectively (μmole per g dry weight). Data are given as mean ± SD.

mental medium, while cell walls of the central cylinder transport the cations to the aerial part of the plant. It is accepted that apoplast vessels providing for transport of cations and water along xylem are involved in bulk transpiration stream transfer. The results of our investigation suggest that the stream composition may be changing while moving up due to the interaction between the ionogenic groups of the vessel cell walls and cations of the solution. These changes could take place in the cases of increase or decrease in pH or concentration of ions in the xylem sap, resulting in decrease or increase in concentration of cations in the solution moving up to the aerial part of the plant.

The number of PGA carboxylic groups in the cell walls of cortex depends on the age of plants and the nutrition conditions: 14-day-old plants grown in complete Knop's solution contain 2-fold more carboxylic groups of PGA than 7-day-old seedlings grown in tap water. The ratio of carboxylic groups with ionization constants ~5 and ~7.3 in the central cylinder and in the cortex depends on the age. In the cortex tissues of 7-day-old seedlings the ratio (S_2/S_3, Tables 2 and 3) is 1 : 1; in the case of 14-day-old plants the ratio is 2 : 1.

The ionogenic groups in the root apoplast structure are of weak acids, their ionization depending on two factors: pH and pK_a. The latter value is known to be a constant for an acid or a base. Consequently, ionization degree at a fixed pH value depends only on the nature of the acid (base), independently of their neutralization degree [16]. To calculate the dissociation degree α for ionogenic groups of cell walls at fixed pH of the medium (Fig. 5), the following equation was used:

$$\alpha = \{1/[1 + 1/10^{[(pH-pK_a)/n]}]\}. \quad (5)$$

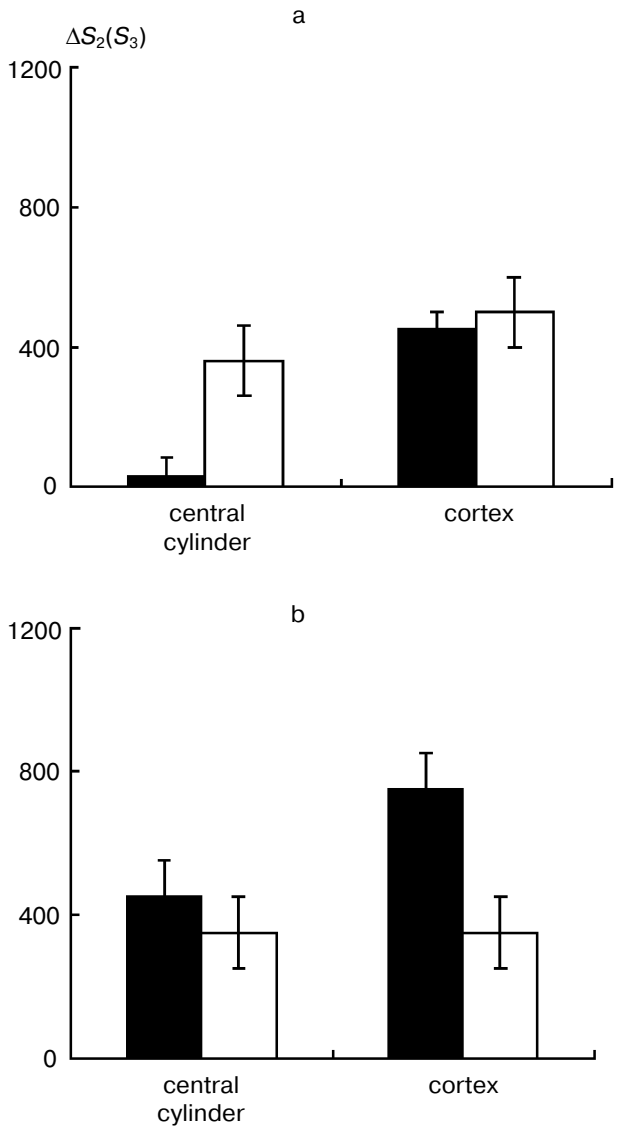

Fig. 4. Changes in the content of carboxylic groups in the radial direction of lupine root. ΔS_2 and ΔS_3) content of carboxylic groups of α-D-polygalacturonic acid (filled rectangles) and carboxylic groups of the second type (open rectangles), respectively (μmole per g of dry weight). Data are shown as mean ± SD. a, b) Root cell walls of 7-day-old seedlings and 14-day-old plants, respectively.

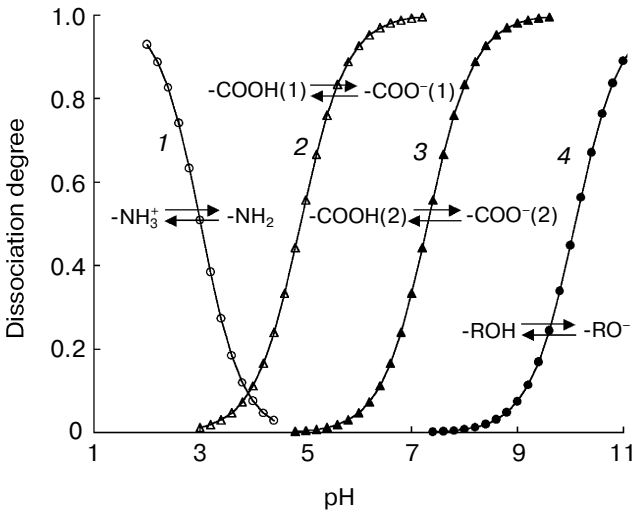

Fig. 5. The pH dependence of the dissociation degree for cell wall ionogenic groups: *1*) amino groups; *2*) α-D-polygalacturonic acid carboxylic groups; *3*) carboxylic groups different from *2*; *4*) phenolic groups. Data are calculated from Eq. (5) using mean values of pK_{aj} and n_j: $pK_{a1} \sim 3.2$, $pK_{a2} \sim 5.0$, $pK_{a3} \sim 7.3$, $pK_{a4} \sim 10.3$, $n_j = 1$ for all cases.

For example, at pH 6.0, 90% of the carboxylic groups with $pK_{a2} \sim 5$ are ionized, while the carboxylic groups with $pK_{a3} \sim 7.3$ are unable to exchange cations; at pH 8.0, both groups are ionized, by 100 and 75%, respectively. It is worth noting that amino and phenolic groups are always closed (non-dissociated) under physiological conditions (pH 4-8) and consequently cannot be involved in ion-exchange reactions with environmental ions (Fig. 5). Considering that pK_a and n values differ slightly for various types of tissues, the dependences describe the behavior of ionogenic groups in cell walls in all lupine root tissues at various pH and constant ionic strength (10 mM).

The data of the present work indicate that the original composition of the external solution can be altered significantly in the apoplast due to the physicochemical properties of the cell walls. Ion-exchange reactions between ionized groups of the cell wall and cations of the solution result in a local decrease in pH and change in the solution composition. According to the results obtained, proton concentration in the extracellular solution passed through the cell wall and contacting the plasmalemma must be higher than in the external solution; cation concentration in this compartment must be lower than that of the external solution, and anion concentration must be not lower than that of the external solution. The consumption of anions by a cell is known to increase when pH decreases [15]. This is a reason to conclude that the cell wall acidifies the external solution to provide the conditions for anion transport into the cell.

Thus, the results demonstrate that ion-exchange capacity of lupine root apoplast determined by physicochemical properties of the cell walls is not a constant value. It changes during the development of root absorptive function, and its value depends on pH and composition of the nutrient solution. The differences in the values of these parameters in various tissues correlate with the changes in chemical structure of cell wall matrix and reflect its role in the processes of ion uptake in the root and ion transfer to aerial organs.

This work was supported by the Russian Foundation for Basic Research (grant No. 98-04-48867 and 01-04-48717) and by the Program "Universities of Russia" (St. Petersburg).

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