ISSN 0006-2979, Biochemistry (Moscow), 2006, Vol. 71, No. 8, pp. 893-899. © Pleiades Publishing, Inc., 2006.
Original Russian Text © N. R. Meychik, N. P. Matveyeva, Yu. I. Nikolaeva, A. V. Chaikova, I. P. Yermakov, 2006, published in Biokhimiya, 2006, Vol. 71, No. 8, pp. 1103-1111.
Originally published in Biochemistry (Moscow) On-Line Papers in Press, as Manuscript BM06-104, July 9, 2006.

Features of Ionogenic Group Composition in Polymeric Matrix of Lily Pollen Wall

N. R. Meychik*, N. P. Matveyeva, Yu. I. Nikolaeva, A. V. Chaikova, and I. P. Yermakov

Department of Plant Physiology, Faculty of Biology, Lomonosov Moscow State University, 119899 Moscow, Russia; fax: (495) 939-4309; E-mail: meychik@mail.ru

Received April 12, 2006
Revision received April 24, 2006

Abstract—The composition of ionogenic groups and ion-exchange capacity were studied in the polymeric matrix of cell walls isolated from the pollen grain and tissues of vegetative organs (leaves and stems) of *Lilium longiflorum* Thunb. The ion-exchange capacity was evaluated at different pH values and ionic strength of 100 mM. In the two-layered pollen wall and the somatic cell walls four types of ionogenic groups were found: amino groups, two carboxyl groups (represented by residues of uronic and hydroxycinnamic acids), and phenolic OH-groups. The groups of all four types are present in the intine, whereas the exine contains one type of anion-exchange and two types of cation-exchange groups. The contents of each type group and their ionization constants were determined. The qualitative and quantitative compositions of structural polymers of the pollen intine and somatic cell walls are significantly different. It is suggested that hydroxycinnamic acids should be involved in cross-linking of polysaccharide chains in both the intine and somatic cell primary walls, and such cross-links play a crucial role in the structural organization and integrity of the pollen grain wall.

DOI: 10.1134/S0006297906080116

Key words: pollen grain, polymeric matrix, exine, intine, ionogenic groups

During the development of the male gametophyte of angiosperms, a complicated specialized wall is formed which, together with the external hydrophobic lipid–protein layer (tryphine), plays a crucial role in the defense of the pollen grain, its transfer to the stigma surface and interaction with it, and also in triggering the germination and formation of the pollen tube [1-3]. In the majority of angiosperm species, the pollen wall includes the external and internal layer, or exine and intine, respectively. The main component of the exine is a polymer sporopollenin consisting of fatty acid derivatives and phenols. Sporopollenin provides for the resistance of exine to physical and chemical exposures, which is, in particular, manifested by conservation of the pollen wall sculpture in geological deposits during thousands of years [4-6]. In some cases, sporopollenin is joined into a group of protective polymers, together with lignin, cutin, and suberin [5, 7]. The intine contains structural polymers specific for the primary wall of plant somatic cells, such as a microfibrillar cellulose component, matrix of pectins and hemicelluloses, and also structural and mobile proteins [1]. The intine has a unique ability to sharply increase its surface during hydration that precedes the pollen grain germination. Under *in vitro* conditions, the pollen grain volume for a few seconds increases 2-3-fold. But what features of the intine structural polymers are responsible for a rapid change of the rigid wall to a loose and elastic state is poorly investigated, but *a priori*, ionogenic groups of the intine are likely to be of importance during such processes. Both layers of the pollen wall can be considered as a three-dimensional polymeric matrix with ionogenic groups in the structure. As it has been shown for the isolated walls of somatic cells [8, 9], the type, distribution, and concentration of such groups significantly determine the matrix behavior during the hydration, as well as the diffusion in the matrix of physiologically active molecules and ions.

So far, the state and location of pectins in the intine have been mainly studied by immunochemical approaches. However, the findings of different authors are contradictory. Thus, Aouali et al. [10] found in the lily intine both esterified and acidic pectins, but according to Hasegava et al. [11], acidic pectins appeared only after the pollen grain germination, while esterified pectins were present both before and after germination. In work [12], methyl-esterified pectins were absent in the germi-

* To whom correspondence should be addressed.

nated pollen grain and the intine contained a very small amount of acidic pectins. But the question about the contents in the intine of free carboxyl groups of uronic acids is important because they can interact with calcium ions and thus strengthen the rigidity of the matrix polymeric structure. So the determination of their contents in the intine is essential for comprehension of mechanisms which control the functioning of the extracellular compartment of the pollen grain during germination. It should be noted that pectin esterification is also believed to play the central role in the regulation of elasticity and permeability of the polymeric matrix of the walls of somatic cells [13, 14] and pollen tube [15]. Other mechanisms responsible for regulation of properties of the somatic cell wall matrix are also under discussion, in particular, the features associated with the presence of different phenolic derivatives providing for the cross-linking of the polymers [16, 17]. There are no available literature data concerning these compounds in the intine.

Thus, qualitative data on the structural polymers of the pollen wall are contradictory and quantitative data are insufficient; therefore, new approaches must be developed to study this issue. A physicochemical description of features of the pollen wall polymeric matrix as a natural ion exchanger can be used as such an approach. The present work is a comparative study of the ionogenic group composition in the pollen wall (intine and exine) of lily and in the cell walls of vegetative organs of the same plant.

MATERIALS AND METHODS

Cut-off *Lilium longiflorum* Thunb. cv Worldwhite plants were studied. Dry pollen grains were collected from open anthers and stored at –20°C. The cell walls of vegetative organs were studied in the intermediate parts of the leaves of the four upper circles and stem fragments on the same level.

Cell walls were isolated from the unfrozen vegetative tissues, as described in [8, 18]. The severed leaves or stems were weighed, placed into a glass ion-exchange column (V = 250 ml), and washed successively in 1% alkali and acid solutions and in distilled water until the disappearance of Cl^- in the washing waters, and then dried to constant weight in the presence of $CaCl_2$ at 55-60°C.

To isolate and purify the pollen two-layered walls, which included the exine and intine, the pollen was washed free from the tryphine in some portions of diethyl ether and fixed in the mixture of equal volumes of absolute ethanol and acetone for 2 h at 6°C. After the fixation, the pollen was precipitated by centrifugation (600*g*, 1 min) and washed in distilled water. Then the material was treated successively with 2% SDS, 1% NaOH, and 1% HCl and filtered across a nylon filter with 11-μm pores (Millipore, USA) using a vacuum pump. Each treatment was performed for 30 min and was alternated with washing in distilled water. After the purification, a part of the material was left to prepare the exine, and the rest was dried and used for the subsequent potentiometric analysis.

To prepare exine, isolated and purified two-layered walls were incubated for 24 h at 25°C in 25 mM MES-NaOH buffer (pH 4.5) supplemented with 1% cellulase (from *Trichoderma viride*; ICN, USA) and 1% pectinase (from *Aspergillus niger*; ICN). Then the preparation was washed free of the enzymes in distilled water, incubated for 24 h in 2% SDS solution, washed in water, and dried to constant weight at 55-60°C.

To evaluate the purification degree and integrity of the isolated cell walls, the preparations were examined with a light microscope. The purification of the cell walls was considered complete in the case of absence in the preparation of nuclei and their fragments (the largest fragments of the protoplast). The nuclei were stained with DAPI (4′,6-diamidino-2-phenylindole) using the conventional technique [19]. Axioplan 2 imaging MOT microscope (Zeiss, Germany) was used supplemented with the AxioVision 4.2 software (Zeiss) and an AxioCam HRc digital camera (Zeiss).

The qualitative and quantitative compositions of the ion-exchange groups were determined by potentiometric titration using separate weights [8]. Dry weights of the preparations (40 ± 0.1 and 10 ± 0.1 mg for the vegetative organ cell walls and pollen wall, respectively) were placed, respectively, in 50- and 10-ml ground conic flasks with ground stoppers and filled with 12.5 or 3 ml, respectively, of KOH or HCl solutions of varied concentration but with the same ionic strength of 100 mM adjusted with the appropriate solutions of NaCl. The concentration of the alkali and acid was varied in the range from 0 to 10 mM. After 48 h, the preparations were separated from the solution, which was used to determine pH with a Model 3320 pH Meter (Jenway, England) and the concentration of the remaining acid or alkali by titration with Bromothymol Blue as the indicator. By changes in the H^+ or OH^- concentrations the sorption capacity of the cell wall was calculated at the fixed pH_i value using the formula:

$$S_i = \frac{(C^{in} - C^{eq})V}{g}, \qquad (1)$$

where S_i (μmol/g dry weight of cell walls) is the cation-exchange capacity of the samples at the corresponding value of pH_i; C^{in} and C^{eq} (mM) are the initial and equilibrium concentrations of KOH or HCl in the solution; V (ml) is the solution volume; g (g) is the sample weight.

The titration curves were calculated as described in [8, 20]. The contents of each type ion-exchange group (ΔS^j, j is the group type) were determined from experimental curves of the pH-dependence of the sorption capacity of the pollen wall or somatic cell walls. The con-

tents of free amino groups in the samples were determined by non-aqueous titration in acetic acid [21].

Ionization constants of ionogenic groups were calculated using the Henderson–Hasselbach equation as modified by Gregor [22]:

$$pH = pK_a + n\log_{10}(\alpha/1 - \alpha), \quad (2)$$

where pK_a is an apparent ionization constant of the polymer ionogenic group, α is the dissociation degree, n is a constant depending on the polymeric matrix structure and the counter-ion type. On calculating the corresponding value of $\log_{10}(\alpha_i/1 - \alpha_i)$ for every pH_i value and using regression analysis, the pK_a^j and n^j values were obtained for each step of the ionization.

Using the resulting values of the parameters (ΔS^j, pK_a^j, n^j), the calculated curves of the $S_i = f(pH_i)$ dependence were determined for all points of the experimental pH_i values using the summarized equation [23]:

$$S_i^{calc} = S_t^{cat} - \sum_{j,i=1}^{k,m} \Delta S^j \left[1 + 10^{(pK^j_a - pH_i)/n^j}\right]^{-1}, \quad (3)$$

where S_t^{cat} is the maximal cation-exchange capacity of the samples; ΔS^j is the amount of the j-type ionogenic groups; S_i^{calc} is the calculated ion-exchange capacity of the pollen wall or somatic cell walls at the corresponding value of pH_i. S_t^{cat}, ΔS^j, and S_i^{calc} are expressed in μmol per g dry weight of the walls; pK_a^j is the apparent ionization constant of the j-type ionogenic groups; n^j is the constant of Eq. (2) for the j-type ionogenic groups; k is the number of points on the potentiometric curve; m is the number of ionogenic group types.

The adequacy of the approach used for description of the acid-base equilibrium was evaluated by regression analysis determining parameters of the equation:

$$S_i^{calc} = B \cdot S_i^{exp} + A, \quad (4)$$

where S_i^{exp} and S_i^{calc} (μmol per g dry weight of cell walls) are the ion-exchange capacity, experimental and calculated from the Eq. (3) at the corresponding value of pH_i; A and B are regression parameters. The calculations have shown complete adequacy of the model chosen to the experimental data, which is supported by the correlation coefficients (r^{corr}) of the dependences $S_i^{calc} = f(S_i^{exp})$ and the A and B coefficients of Eq. (4). In all variants $r^{corr} \to 1$, the A value is not more than the experimental error, and $B \to 1$.

The composition of the ionogenic groups in the structural polymers of the intine were calculated from the ratio of the intine and exine mass fractions (M_{int} and M_{ex}, respectively) in the two-layered pollen wall using the formulas:

$$M_{int} = (1 - N_w/N_{ex}), \quad (5)$$

$$M_{ex} = N_w/N_{ex}, \quad (6)$$

where M_{int} and M_{ex} are mass fractions of the intine and exine in two-layered pollen wall, respectively; N_w and N_{ex} are numbers of two-layered pollen walls and exines per mg dry weight, respectively, determined with a Fuchs–Rosenthal counting chamber. The N_w and N_{ex} values were found to be $(8.8 \pm 0.3)\cdot 10^3$ and $(25.0 \pm 1.9)\cdot 10^3$, respectively. Using these values and formulas (5) and (6), the ratio M_{int}/M_{ex} was found to be 0.65 : 0.35.

The contents of the j-type ionogenic groups in the intine (ΔS_{int}^j) were calculated by the formula:

$$\Delta S_{int}^j = (\Delta S_w^j - 0.35\Delta S_{ex}^j)/0.65, \quad (7)$$

where ΔS_w^j and ΔS_{ex}^j (μmol per g dry weight of the sample) are contents of the j-type ionogenic groups in two-layered pollen wall and exine, respectively.

RESULTS

In the cell wall preparations obtained by successive extraction of the protoplast components and the wall soluble components, the architecture of vegetative organs, leaves and stem (Fig. 1, a-c), and also the structure of the pollen wall (Fig. 2, a and b) were retained. But these preparations contained no nuclei and their fragments, which indicated a rather high degree of purification from the protoplast components. The exine prepared by enzymatic hydrolysis was free of intine fragments (Fig. 2c).

In the experimental titration curves of the pollen wall and cell walls isolated from the vegetative organ tissues (Fig. 3), the region of positive S_i values corresponds to release of protons in accordance with the reaction:

$$\sim COOH + Na^+ \to \sim COONa + H^+,$$

where ~ indicates the polymeric chain, and the region of negative S_i values corresponds to uptake protons according to the reaction:

$$\sim NH_2 + H^+ \to \sim [NH_3]^+.$$

At pH > 11 and pH < 2, values of the cation-exchange (S_t^{cat}) and anion-exchange capacities (S_t^{an}) reach the maxima, and the S_t^{cat} and S_t^{an} values characterize the total contents of acidic and basic groups in the polymeric structure which are capable of involvement in the exchange reactions at the appropriate values of the environmental pH.

The polymeric matrix of the pollen wall and of the lily leaf and stem cell walls contained four types of ionogenic groups capable of being involved in the exchange reactions with environmental ions under appropriate conditions: three types were cation-exchange groups, and

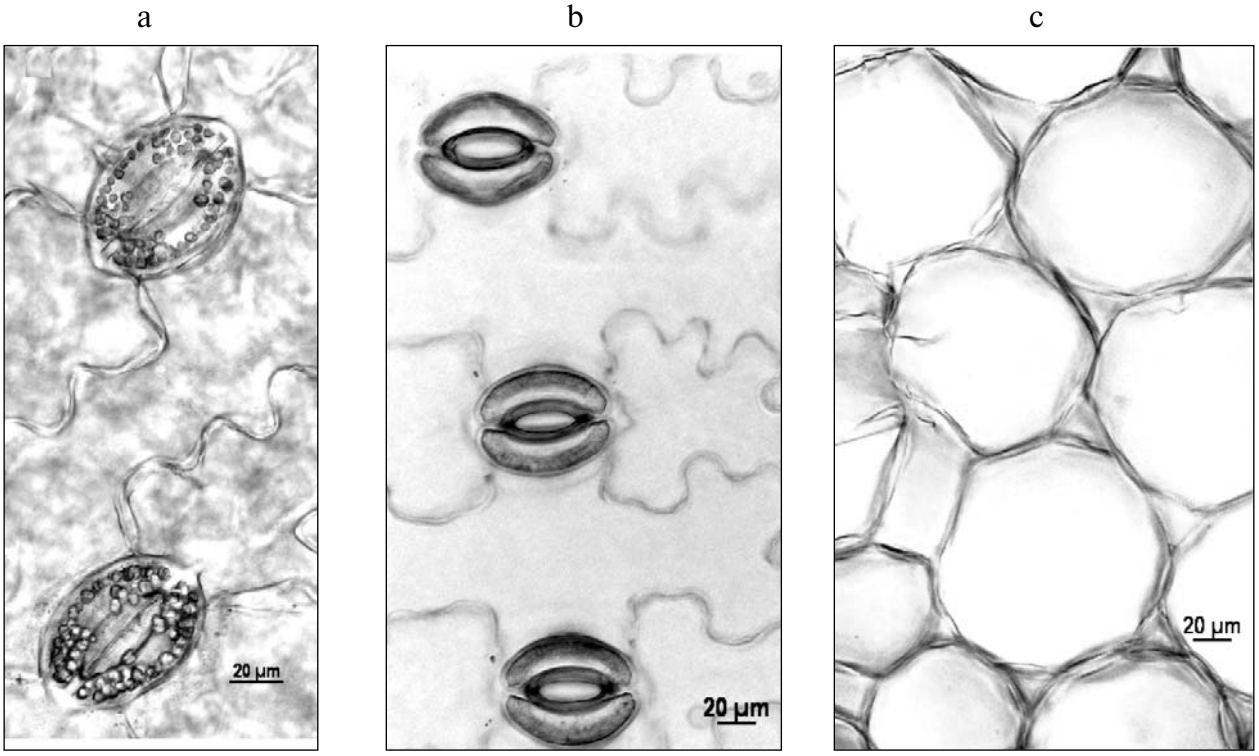

Fig. 1. Preparations of the intact leaf (a) and cell walls isolated from the lily vegetative organs (b, c). One can see chloroplasts in the guard cells of the intact leaf stomata (a). In the preparations of the cell walls isolated from the leaf (b) and stem (c) the organ's architecture is preserved and organelles are absent.

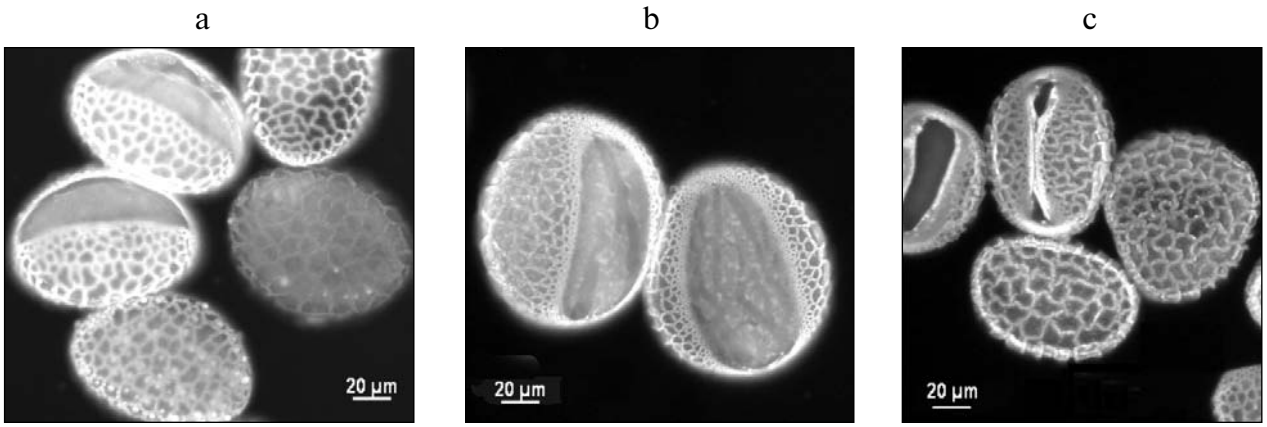

Fig. 2. Pollen grains (a), isolated two-layered pollen walls (b), and exines (c) of lily. The autofluorescence (excitation at λ_{max} = 365 nm). The intact pollen grains (a) are encircled with a two-layered pollen wall (the intine and reticular exine) and covered with the tryphine layer seen as lipid drops. The structural organization is retained in the preparations of two-layered pollen wall (b) and exine (c). There are no intine fragments in the isolated exines (c).

the fourth type exchanges anions (Tables 1 and 2). The intine contained all the four types, whereas in the exine one anion-exchange group and two-cation exchange groups were present (Table 1).

The calculated values of pK_a (Table 2) were compared with the ionization constants of ionogenic groups in the cell walls of other plant species [8, 18, 20], and the groups with pK_a values of 3.5-4.3 were suggested to be carboxyl groups of uronic acids, the groups with pK_a of 7.1-8.3 seemed to be carboxyl groups of hydroxycinnamic acids, and the groups with pK_a of ~10 were likely to be phenolic OH-groups.

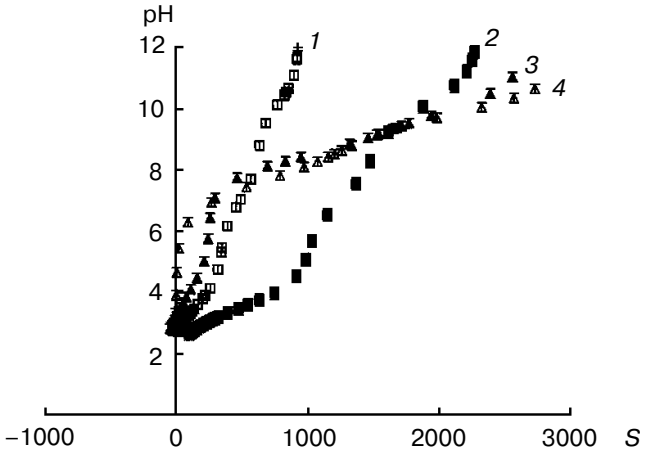

Fig. 3. Potentiometric titration curves of the polymeric matrix of the cell walls isolated from the lily pollen grains and vegetative organs: *1*) stem; *2*) leaf; *3*) two-layered pollen wall; *4*) exine. The ionic strength of the solution during titration was 100 mM. *S* (μmol per g dry weight of the cell walls) is the ion-exchange capacity of the cell wall polymeric matrix. The bars show ± standard error.

The content of free amino groups and carboxyl groups of hydroxycinnamic acids in the intine is many-fold higher than that in the leaf and stem cell walls (Table 1). The contents of carboxyl groups of uronic acids were virtually the same in the intine and stem cell walls, and they were nearly threefold lower than those in the leaf cell walls (Table 1). It should be noted that the intine was different from the somatic cell walls in the qualitative composition of both hydroxycinnamic and uronic acids (Table 2).

The exine was similar to the intine in the contents of free amino groups and carboxyl groups of hydroxycinnamic acids, but the contents of phenolic OH-groups in the exine were considerably higher than in the intine and somatic cell wall: the OH-group contents in the exine are 1.7-, 2-, and 5-fold higher than those in the intine, leaf, and stem, respectively. The exine was characterized by the absence of carboxyl groups of uronic acids (Table 1). The qualitative composition of hydroxycinnamic acids of the exine was different from that of the intine and somatic cells, which was indicated by different ionization constants of carboxylic groups of these acids (Table 2).

The qualitative composition of ionogenic groups in the structural polymers of the cell walls isolated from the leaf and stem was the same (Table 2). However, these two vegetative organs were significantly different in the ratio of all ionogenic groups in the walls. In the leaf cell walls the contents of uronic and hydroxycinnamic acid carboxyl groups were 3- and 1.7-fold higher, respectively, than in the stem cell walls, the contents of phenolic OH-groups was 2.7-fold higher, but the contents of amino groups was 1.4-fold lower (Table 1).

Thus, clear distinctions were shown between the exine and intine, on one hand, and the cell walls of the leaf and stem, on the other hand. The two layers of the pollen wall were characterized by significantly higher contents of amino groups and carboxyl groups of hydroxycinnamic acids.

DISCUSSION

The total contents of cation-exchange groups (S_t^{cat}) were shown to be approximately the same in the two layers of the pollen wall and the leaf cell walls (purified from the protoplast but retaining integrity), but 2.5-3-fold higher than in the stem cell walls. However, in all cases the S_t^{cat} value was 5-18 times higher than S_t^{an} (Table 1).

Table 1. Quantitative composition of ionogenic groups in pollen wall and somatic cell walls of lily

Ionogenic group	Two-layered pollen wall	Intine*	Exine	Stem	Leaf
Amino groups (S_t^{an})	469 ± 35	465 ± 125	476 ± 25	175 ± 14	129 ± 12
Carboxyl groups of uronic acids	250 ± 30	385 ± 85	not detectable	335 ± 20	1000 ± 50
Carboxyl groups of hydroxycinnamic acids	1300 ± 95	1246 ± 274	1400 ± 100	300 ± 28	500 ± 45
Phenolic OH-groups	1100 ± 65	884 ± 168	1500 ± 120	300 ± 25	800 ± 50
S_t^{cat}	2650 ± 120	2515 ± 427	2900 ± 180	935 ± 93	2300 ± 120

Note: S_t^{cat} (S_t^{an}) is the total cation-exchange (anion-exchange) capacity of the cell wall polymeric matrix. All values in the table are expressed in μmol per g dry weight of the preparation.

* Values for intine are calculated on the basis of the experimentally determined exine and intine fractions in two-layered pollen wall (see "Materials and Methods").

Table 2. Ionization constants (pK_a) of cation-exchange groups in the polymeric matrix of the pollen wall and the somatic cell walls of lily

Ionogenic group	pK_a				
	two-layered pollen wall	intine*	exine	stem	leaf
Carboxyl groups of uronic acids	4.25	~4.2	not detectable	3.64	3.55
Carboxyl groups of hydroxycinnamic acids	8.29	> 7.6	7.56	7.10	7.21
Phenolic OH-groups	10.07	~10.0	10.13	10.21	10.11

* The pK_a is evaluated approximately on the basis of the corresponding data for two-layered pollen wall and exine.

This means that the structural polymers of the pollen wall, as well as those of the leaf and stem cell walls, are natural cation exchangers.

The data on the quantitative composition (Table 1) and ionization constants (Table 2) of ionogenic groups in the pollen wall and cell walls of the leaf and stem tissues allowed us to reveal specific features of the structural polymers of the intine, exine, and leaf and stem cell walls.

Specific features are especially pronounced in the exine: it has no uronic acids, but the contents of phenolic OH-groups and carboxyl groups of hydroxycinnamic acids are the highest. These findings are consistent with the literature data and confirm the idea that phenolic compounds are important structural components of sporopollenin. The presence of phenolic compounds in the exine polymers has been shown by many authors using various approaches. Thus, by ^{1}H-NMR analysis, sporopollenin of *Typha angustifolia* was found to have four different three- and four-substituted phenolic compounds [6]. By pyrolysis chromatography combined with mass spectrometry, *p*-coumaric acid was shown to be the quantitatively prevalent phenolic compound of sporopollenin from *Pinus mugo* [24]; *p*-coumaric and ferulic acids were also found in sporopollenin from *Helleborus foetidus*, *Pinus silvestris*, *Betula pendula*, and *Vicia faba* [25]. In the purified exine of *Cucurbita maxima*, *p*-coumaric acid was revealed immunocytochemically, mainly in the region of the germination pore, in the inner layer of the exine, and also in micro- and macrospinules [26].

The high contents of free amino groups found in the walls (Table 1) suggest the presence of noticeable amounts of structural proteins, and this is in agreement with biochemical data. In the purified wall of the *Arabidopsis* leaf the protein fraction was 14%, similarly to the cellulose fraction [27]. Values of the same order for the protein fraction (10-20% of the cell wall weight) were obtained for cell walls of other higher plants [28, 29]. Note, that amino acids which are prevalent in the structural proteins of somatic cells include positively charged lysine and histidine, as well as tyrosine which has the phenolic OH-group [30]. These amino acids are also found in structural proteins of the maize exine, the fraction of which was 0.3% of the pollen grain total protein [31]. And many structural proteins of the exine are glycosylated [31, 32].

The same types of ionogenic groups are revealed in the intine and cell walls of the lily stem and leaf (Tables 1 and 2). The finding of free carboxyl groups of uronic acids and amino groups in the intine (Tables 1 and 2) are in agreement with the cytochemical data and the resulting idea that the intine is a highly organized complex of polysaccharides and structural proteins, which is similar to the primary wall of other cells of plants [1].

The contents of free carboxyl groups of uronic acids in the intine are low relatively to the leaf walls (Table 1), but they are comparable with the contents of these groups in the cell walls of the lily stem (Table 1) and somatic cells of other plant species [8, 20]. The data on the high contents of carboxylic groups in the leaf walls (Table 1) are in consistence with the data of other authors. Thus, the pectin fraction in the *Arabidopsis* leaf is 43% of the cell wall weight [27]. We have shown that pectins of the intine are specified by uronic acids different of those of the lily somatic cells (Table 2).

Phenolic OH-groups and carboxyl groups of hydroxycinnamic acids were first found in the intine (Table 1). The contents of the hydroxycinnamic acids are significantly higher than in the somatic cell walls, and these acids are also different in their qualitative composition (Table 2). Note that the question of phenolic compounds in the intine was not considered earlier in the available literature. Nevertheless, studies on somatic cells revealed an important structural function of hydroxycinnamic acids, such as ferulic, sinapic, *p*-coumaric, and caffeinic acids [17]. The residues of these acids bound to the side chains of structural polysaccharides can be involved in oxidative dimerization reactions and thus cross-link polymeric molecules to one another [33].

Hydroxycinnamic acids are suggested to form cross-links between polysaccharide molecules in the intine, as they do in the primary walls of somatic cells. The contribution of hydroxycinnamic acids to the matrix rigidity in the intine has to be significantly higher in the dehydrated pollen grain than in the somatic cell walls (Table 1). During hydration at the initial stages of the pollen grain germination, these cross-links (probably, ester bonds) can be hydrolyzed, and this can result in a rapid loosening of the intine polymeric matrix.

Thus, the physicochemical approach to studies on properties of the male gametophyte wall of angiosperms has allowed us to reveal specific features of the polymeric matrix composition of the intine, which can play a determining role for its structural organization and functioning. The presence in the intine of carboxyl groups of uronic and hydroxycinnamic acids that are involved in ion-exchange reactions suggest that the intine can act as a buffer capacity, which can locally regulate ion-exchange flows between the gametophyte vegetative cell and the surface cells of the stigma.

The work was supported by the Russian Foundation for Basic Research (project Nos. 05-04-49370 and 04-04-49379).

REFERENCES

1. Heslop-Harrison, J. (1987) *Int. Rev. Cytol.*, **107**, 1-78.
2. Knox, R. B. (1984) *The Pollen Grain*, in *Embryology of Angiosperms* (Johri, B. M., ed.) Springer-Verlag, Berlin, pp. 197-272.
3. Edlund, A. F., Swanson, R., and Preuss, D. (2004) *Plant Cell*, **16**, S84-S97.
4. Osthoff, K. S., and Wiermann, R. (1987) *J. Plant Physiol.*, **131**, 5-15.
5. Scott, R. J. (1994) *Pollen Exine: the Sporopollenin Enigma and the Physics of Pattern*, in *Molecular and Cellular Aspects of Plant Reproduction* (Scott, R. J., and Stead, M. A., eds.) Cambridge University Press, Cambridge, pp. 49-81.
6. Ahlers, F., Thom, I., Lambert, J., Kuckuk, R., and Wiermann, R. (1999) *Phytochemistry*, **50**, 1095-1098.
7. Scott, R. J., Spielman, M., and Dickinson, H. G. (2004) *Plant Cell*, **16**, S46-S60.
8. Meychik, N. R., and Yermakov, I. P. (2001) *Plant and Soil*, **234**, 181-193.
9. Meychik, N. R., Yermakov, I. P., and Prokoptseva, O. S. (2003) *Biochemistry (Moscow)*, **68**, 760-771.
10. Aouali, N., Laporte, P., and Clement, C. (2001) *Planta*, **213**, 71-79.
11. Hasegawa, Y., Nakamura, S., Uheda, E., and Nakamura, N. (2000) *Grana*, **39**, 46-55.
12. Parre, E., and Geitmann, A. (2005) *Plant Physiol.*, **137**, 274-286.
13. Willats, W. G. T., McCartney, L., Mackie, W., and Knox, J. P. (2001) *Plant Mol. Biol.*, **47**, 9-27.
14. Willats, W. G. T., Orfila, C., Limberg, G., Buchholt, H. C., Alebeek, G.-J. W. M., Voragen, A. G. J., Marcus, S. E., Christensen, T. M. I. E., Mikkelsen, J. D., Murray, B. S., and Knox, J. P. (2001) *J. Biol. Chem.*, **276**, 19404-19413.
15. Parre, E., and Geitmann, A. (2005) *Planta*, **220**, 582-592.
16. Faulds, C. B., and Williamson, G. (1999) *J. Sci. Food Agric.*, **79**, 393-395.
17. Fry, S. C. (2004) *New Phytologist*, **161**, 641-675.
18. Meychik, N. R., Yermakov, I. P., and Savvateeva, M. V. (1999) *Fiziol. Rast.*, **46**, 742-747.
19. Johnson-Brousseau, S. A., and McCormick, S. (2004) *Plant J.*, **39**, 761-775.
20. Meychik, N. R., Nikolaeva, J. I., and Yermakov, I. P. (2005) *Plant and Soil*, **277**, 163-174.
21. Cheronis, N. D., and Ma, T. S. (1973) *Micro- and Semimicromethods of Organic Functional Analysis* [in Russian], Khimiya, Moscow.
22. Gregor, H. P., Luttinger, L. D., and Loeble, E. M. (1954) *J. Amer. Chem. Soc.*, **76**, 5879-5880.
23. Leikin, Yu. A., Meychik, N. R., and Solov'ev, V. K. (1978) *Zh. Fiz. Khim.*, **52**, 1420-1424.
24. Wehling, K., Niester, Ch., Boon, J. J., Willemse, M. T. M., and Wiermann, R. (1989) *Planta*, **179**, 376-380.
25. Rozema, J., Broekman, R. A., Blokker, P., Meijkamp, B. B., Bakker, N., Staaij, J., Beem, A., Ariese, F., and Kars, S. M. (2001) *J. Photochem. Photobiol.*, **62**, 108-117.
26. Niester-Nyveld, C., Haubrich, A., Kampendonk, Y., Gubatz, S., Tenberge, K. B., Rittscher, M., Wilmesmeier, S., and Wiermann, R. (1997) *Protoplasma*, **197**, 148-159.
27. Zablackis, E., Huang, J., Mullerz, B., Darvill, A. C., and Albersheim, P. (1995) *Plant Physiol.*, **107**, 1129-1138.
28. Reiter, W.-D. (1998) *Tr. Plant. Sci.*, **3**, 27-32.
29. Sharova, E. I. (2004) *Cell Wall of Plants* [in Russian], St. Petersburg University Publishers, St. Petersburg.
30. Showalter, A. M. (1993) *Plant Cell*, **5**, 9-23.
31. Chay, C. H., Buehler, E. G., Thorn, J. M., Whelan, T. M., and Bedinger, P. A. (1992) *Plant Physiol.*, **100**, 756-761.
32. Abreu, I., and Oliveira, M. (2004) *Protoplasma*, **224**, 123-128.
33. Ralph, J., Quideau, S., Grabber, J. H., and Hatfield, R. D. (1994) *J. Chem. Soc. Perkin. Trans.*, **1**, 3485-4398.