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Modification of Polysaccharides from Callus Culture of *Silene vulgaris* (M.) G. Using Carbohydrases *in vitro*

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Abstract—Polysaccharides (pectin and intracellular and extracellular arabinogalactans) were isolated from campion callus culture cultivated on medium with varied concentrations of pectinase and β-galactosidase. A decrease in contents of arabinose residues in pectin and arabinogalactans and of galactose residues in arabinogalactans was associated with an increase in the activities of α-L-arabinofuranosidase and β-galactosidase upon addition of pectinase into the medium. Pectinase destroyed the high-molecular-weight (more than 300 kD) fraction of pectin and decreased the content of galacturonic acid residues. α-L-Arabinofuranosidase transformed arabinogalactan into galactan, and galactan was destroyed under the influence of galactosidase. The contents of arabinogalactan and/or galactan in the cells were decreased, and it was released into the culture medium. Pectin samples with low contents of arabinose and galactose in the side chains and galactan samples were obtained from the callus grown on the medium with β-galactosidase. Cultivation of the plant cells on medium containing carbohydrases resulted in modification of pectin and arabinogalactan of the cell walls.

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Plant polysaccharides possess a wide spectrum of physiological activities due to specific features of their structure [1-3]. Therefore, the search for effective raw materials and approaches for isolation and modification to obtain polysaccharides with prescribed properties and structure is a topical problem. Moreover, standardization of chemical composition and biological activities of polysaccharides isolated from native plants is also an urgent problem. These problems can be successfully solved using biotechnological methods for preparation and modification of polysaccharides, especially using plant cell cultures. In particular, polysaccharides with a modified structure can be obtained via regulation of their biosynthesis with glycosyl hydrolases. The cell extension, tissue differentiation, and production of oligosaccharides are accompanied by significant changes in the plant cell walls, which first of all are caused by the presence of enzymes in them. Enzymes degrading pectic substances are the first that affect the plant cell walls and modify them, and this releases other polysaccharides and wall-bound enzymes and promotes the further modification or degradation of the cell walls [4, 5]. At present genetic transformation of plants is under intensive studies in order to influence polysaccharide biosynthesis with glycosyl hydrolases and obtain polysaccharides with prescribed structure [6-8]. In particular, rhamnogalacturonan I (RGI) with 70 and 30% diminished contents of arabinose and galactose residues, respectively, was prepared as a result of expression of endo-α-1,5-arabinanase and endogalactanase in potato [6, 7]. Elaboration of approaches for directed modulation of enzymatic activity seems promising for preparation of polysaccharides with definite valuable properties and prescribed structure via physiological regulation of cell growth.

We have earlier shown that polysaccharides isolated from callus culture of campion *Silene vulgaris* (M.) G. (*Oberna behen* (L.) Ikonn) consist of arabinogalactan containing D-galacturonic acid residues and pectin, which was called silenan [9]. The silenan macromolecule consists of linear and ramified regions [10]. The linear region is represented by α-1,4-D-galacturonan and α-1,2-rhamno-α-1,4-D-galacturonan, which is also a

Abbreviations: AG1) arabinogalactan from callus; AG2) arabinogalactan from the agarized medium; RGI) rhamnogalacturonan I; SV) pectin silenan.

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backbone carbohydrate chain of the silenan ramified region represented by rhamnogalacturonan I. Side chains of the ramified region are built of residues of terminal and α-1,5-bound arabinofuranose and β-1,3-, β-1,4-, β-1,6-bound galactopyranose [11]. The carbohydrate chain of arabinogalactan consists of different parts of β-1,3-D-galactopyranan bound to one another by numerous residues of 3,6-di-O-substituted β-D-galactopyranose. The side chains contain residues of terminal and β-3-O-substituted β-D-galactopyranose, terminal α-arabinofuranose, and α-2-O-substituted rhamnopyranose. Residues of α-rhamnopyranose in the carbohydrate chain are substituted at the second position by residues of 4-O-substituted β-D-galactopyranosyluronic acid, and their presence determines the acidic character of arabinogalactan [12].

Qualitative and quantitative changes in polysaccharides have been studied during the growth cycle of the campion culture [13], and effects of hormones [9], carbohydrates [14, 15], calcium, phosphate, and nitrogen [16] on cell growth and production of polysaccharides have been shown.

We have earlier described the immunomodulating activity of polysaccharides from the campion intact plant and callus culture, in particular, increases in the uptake capacity and myeloperoxidase activity of human peripheral blood phagocytes and macrophages of rat abdominal cavity have been shown [17].

In the present work the effect of carbohydrases on biosynthesis and chemical characteristics of polysaccharides produced by campion callus culture was studied.

MATERIALS AND METHODS

Cultivation conditions of the callus culture. The campion callus culture was grown on a modified medium described by Murashige and Skoog [18]. The callus was cultivated on the medium with varied concentrations of pectinase (Pectofoetidin enzyme preparation, activity 2.2 U/mg; Privolzhskii Biochemical Factory, Russia) as follows: 10^{-5}, 10^{-2}, 1, 2, and 5 mg/ml. The callus was also grown on medium supplemented with β-galactosidase (EC 3.2.1.23; activity 11.8 U/mg; Sigma, USA) in the concentrations of 10^{-5}, 10^{-2}, 1, 2, and 5 mg/ml. In the control, the medium was without enzymes. The sterile enzymes were added after autoclaving the medium. The callus was subcultivated at the interval of 21 days at 26 ± 1°C in the dark.

Polysaccharides were isolated from the callus as described in [13], and polysaccharide fractions were obtained—a pectin silenan (SV) and arabinogalactan AG1.

Extracellular polysaccharides were isolated from the agarized culture medium. The medium was centrifuged (10,000*g*, 20 min), the supernatant was concentrated under vacuum on a rotary evaporator at 40-50°C, centrifuged (10,000*g*, 20 min), and the polysaccharides were precipitated with a double volume of 96% ethanol. The precipitate was separated by centrifugation, dissolved in distilled water, dialyzed against distilled water for 3 days, centrifuged, and the resulting supernatant was lyophilized. As a result, the polysaccharide fraction arabinogalactan AG2 was obtained from the agarized medium.

Contents of the polysaccharide fractions were expressed in percent of the dry biomass and as a quantity of polysaccharide produced per liter of the medium (g/liter). The experiments were performed in four repeats.

General analytical methods. Contents of glycuronic acids were determined in the polysaccharide fractions by reaction with 3,5-dimethylphenol in the presence of concentrated sulfuric acid [19], and the protein content was determined by the Lowry method [20]. The total content of carbohydrates was determined by reaction with phenol in the presence of sulfuric acid [21]. Spectrophotometry was performed with an Ultrospec 3000 instrument (Great Britain), GLC was performed on a Hewlett-Packard 4890A apparatus (USA) with a plasma-ionization detector and an HP 3395A integrator on an RTX-1 capillary column (0.25 mm × 30 m; Restek), with argon as a carrier gas. Liquid chromatography of polysaccharides was performed as described in [22]. A sample (2-3 mg) was dissolved in 1 ml of 0.15 M NaCl solution in bidistilled water and filtered. The sample was analyzed using a chromatographic system consisting of an SD-200 pump (Dynamax, USA), Shodex Asahipak GS-620HQ column (7.6 mm × 30 cm), Shodex GS-26 7B precolumn (7.6 mm × 5 cm), CTO-10AS thermostat, and RID G136A detector-refractometer (Shimadzu, Japan). The column was eluted with 0.15 M NaCl at 40°C and the flow rate of 0.5 ml/min. The column was calibrated using dextran sulfates with molecular weights of 36-50, 400-600, and 1400 kD (Sigma).

Full acidic hydrolysis. Polysaccharide fractions (2.0-2.5 mg) were hydrolyzed with 1 ml of 2 M trifluoroacetic acid (TFA) at 100°C for 3-4 h, and the hydrolyzates were evaporated under vacuum in the presence of methanol until the complete removal of TFA. Myoinositol (0.5 mg/ml) was used as an internal standard. Monosaccharides were identified by GLC as corresponding polyol acetates [23].

Molecular weight distribution of polysaccharides. The AG1 and SV fractions (30-40 mg) were dissolved in 30-50 ml of distilled water and successively separated by molecular weight in a Millipore ultrafiltration cell (USA) using polysulfone ultrafiltration membranes (Vladisart, Russia) with pores size corresponding to 300, 100, and 50 kD. The fractions were concentrated and lyophilized, and the following fragments were obtained: AG1-I, AG1-I-C (control) and SV-I, SV-I-C (control) with molecular weight of more than 300 kD; AG1-II, AG1-II-C (con-

trol) and SV-II, SV-II-C (control) with molecular weights of 100-300 kD; and AG1-III, AG1-III-C (control) and SV-III, SV-III-C (control) with molecular weights of 50-100 kD.

Analysis of carbohydrase activities. The raw biomass was homogenized in 0.05 M sodium acetate buffer (pH 5.0) at biomass/buffer ratio of 1 : 10 and centrifuged (10,000*g*, 20 min), and the supernatant was dialyzed against 0.05 M sodium acetate buffer (pH 5.0) for 3 days at 4°C and centrifuged. The intracellular enzyme activities were determined in the supernatant. The agarized culture medium was centrifuged (10,000*g*, 20 min), the supernatant was dialyzed against 0.05 M sodium acetate buffer (pH 5.0) for 3 days at 4°C, and centrifuged. The extracellular enzyme activities were determined in the supernatant.

The pectinase activity was determined by accumulation of reducing sugars upon incubation of the enzyme solution for 10 min at 50°C with 1% polygalacturonic acid (ICN, USA) in 0.05 M sodium acetate buffer (pH 4.6). The resulting reducing sugars were determined as described in [24]. D-Galacturonic acid was used for the calibration curve. The enzyme quantity which under the given conditions released from polygalacturonic acid 1 μmol D-galacturonic acid in 1 min was taken as the pectinase activity unit.

The activities of α-L-arabinofuranosidase and β-galactosidase were determined spectrophotometrically at 400 nm, with 4-nitrophenyl-α-L-arabinofuranoside and 2-nitrophenyl-β-D-galactopyranoside (Sigma) as substrates, respectively. *p*-Nitrophenol was used for the calibration curve. The enzyme quantity which cleaved 1 μmol substrate within 1 min at pH 4.2 and 30°C was taken as the unit of α-L-arabinofuranosidase and β-galactosidase activities.

The enzyme activities were expressed as the units per mg protein (U/mg protein). The experiments were performed in three to five repeats.

Statistical analysis. The statistical processing of data included calculation of the arithmetic mean and mean square deviation. The significance was evaluated using Student's *t*-test.

RESULTS AND DISCUSSION

Polysaccharides silenan SV and arabinogalactan AG1 were isolated from the campion callus culture grown on medium with different concentrations of pectinase. From the nutritional medium of the callus culture, the extracellular polysaccharide AG2 was prepared.

The yield of silenan (4.5-5.5%) and productivity (0.30-0.36 g/liter) per liter of the nutritional medium were similar in the absence of pectinase (control) and at its concentrations of 10^{-5} and 10^{-2} mg/ml (Table 1). On elevation of the enzyme concentration to 1-5 mg/ml the pectin yield was significantly increased (to 6.7-8.4%) with respect to the control, but the productivity per liter of the medium was five to eightfold lowered, which was associated with a decrease in the culture productivity per biomass. The pectin yield seemed to increase as a result of the pectinase action on the cell walls, which caused the more complete extraction of pectin from the cells.

The yield of arabinogalactan AG1 (5.2-5.4%) and productivity (0.34 and 0.36 g/liter) were similar in the absence of pectinase and at its concentration of 10^{-5} mg/ml; on elevation of the enzyme content in the medium from 10^{-2} to 5 mg/ml both the yield and productivity of the polysaccharide decreased to 1.9-3.6% and 0.02-0.24 g/liter, respectively (Table 1).

The content of extracellular arabinogalactan AG2 did not change and was 0.06-0.10 g/liter under both the pectinase absence in the nutritional medium and its concentration of 10^{-5} and 10^{-2} mg/ml (Table 1). High concentrations of the enzyme caused a considerable increase in the AG2 content in the nutritional medium (to 0.19-

Table 1. Effect of pectinase on polysaccharide contents in campion callus

Pectinase concentration, mg/ml	SV		AG1		AG2
	yield, %	g/liter	yield, %	g/liter	g/liter
0	5.5 ± 0.7	0.36 ± 0.04	5.2 ± 0.4	0.34 ± 0.03	0.08 ± 0.03
10^{-5}	4.5 ± 0.9	0.30 ± 0.06	5.4 ± 0.5	0.36 ± 0.03	0.06 ± 0.01
10^{-2}	5.2 ± 1.7	0.34 ± 0.09	3.6 ± 0.4*	0.24 ± 0.03*	0.10 ± 0.01
1	7.4 ± 0.4*	0.06 ± 0.01*	2.3 ± 0.7*	0.02 ± 0.005*	0.19 ± 0.04*
2	8.4 ± 1.9*	0.07 ± 0.02*	1.9 ± 0.3*	0.02 ± 0.003*	0.25 ± 0.05*
5	6.7 ± 0.7*	0.05 ± 0.01*	3.4 ± 0.7*	0.02 ± 0.003*	0.38 ± 0.07*

Note: Here and in Tables 2-4, in the control there was no enzyme.

* Here and in Tables 2-4, difference is significant at $p < 0.05$.

0.38 g/liter) that was likely to be associated with destruction of cell walls by the pectinase and release of the polysaccharide into the medium.

When the callus was cultivated in the absence of pectinase (control) and at its concentrations of 10^{-5} and 10^{-2} mg/ml, silenan contained many residues of D-galacturonic acid (66-69%), and neutral components were mainly residues of galactose, arabinose, and rhamnose. The arabinose/galactose ratio was 1 : (1.0-1.4). Pectinase at 1-5 mg/ml caused a decrease in the contents of arabinose and galacturonic acid in pectin by 44 and 18-37%, respectively (Fig. 1a). The arabinose/galactose ratio was 1 : (1.7-3.3). The increase in the arabinose/galactose ratio indicated the detachment of arabinose residues from the arabinogalactan side chains of rhamnogalacturonan. On varying the pectinase concentration in the medium, the content of rhamnose residues in silenan changed insignificantly (0.8-1.0%).

Two peaks with molecular weights of 400-500 and 300-400 kD were revealed by HPLC, which corresponded to the control sample of silenan obtained in the absence of pectinase in the medium. Silenan samples obtained in the presence of 1-5 mg/ml pectinase in the medium were characterized by a peak with molecular weight of 100-300 kD. The absence of fragments with molecular weight higher than 300 kD indicated the pectinase-caused destruction of the high-molecular-weight fraction and decrease in the molecular weight of SV.

In the absence of pectinase and also at its concentration of 10^{-5} mg/ml arabinose and galactose in the ratio of 1 : 6.6 were major neutral components of arabinogalactan AG1. Upon the addition of 10^{-2}-5 mg/ml pectinase, we obtained polysaccharide samples with the 49-86% decreased content of arabinose (Fig. 1b). The arabinose/galactose ratio was 1 : (11.9-24.7). On the addition of 1-5 mg/ml pectinase, the content of galactose residues in arabinogalactan was decreased by 40-69%. Glucose, xylose, and mannose residues were present in the AG1 samples in small quantities and more likely were components of attendant hemicelluloses. Galacturonic acid residues (3-6%) were also found in AG1. The protein content varied from 8 to 18%.

Thus, the addition of 10^{-2} mg/ml pectinase promoted a decrease in the content of arabinose residues in arabinogalactan and production of galactan. On the further increase in the enzyme concentration, the galactan was degraded.

The molecular weight distribution of AG1 and SV samples obtained from the callus grown in the presence of 10^{-2} mg/ml pectinase was studied. The molecular weight distribution and monosaccharide composition of AG1 in the experimental and control samples were different. The yield of the major fraction AG1-I with molecular weight higher than 300 kD in the experiment was 20% higher (67% yield) than in the control (56% yield). The yields of

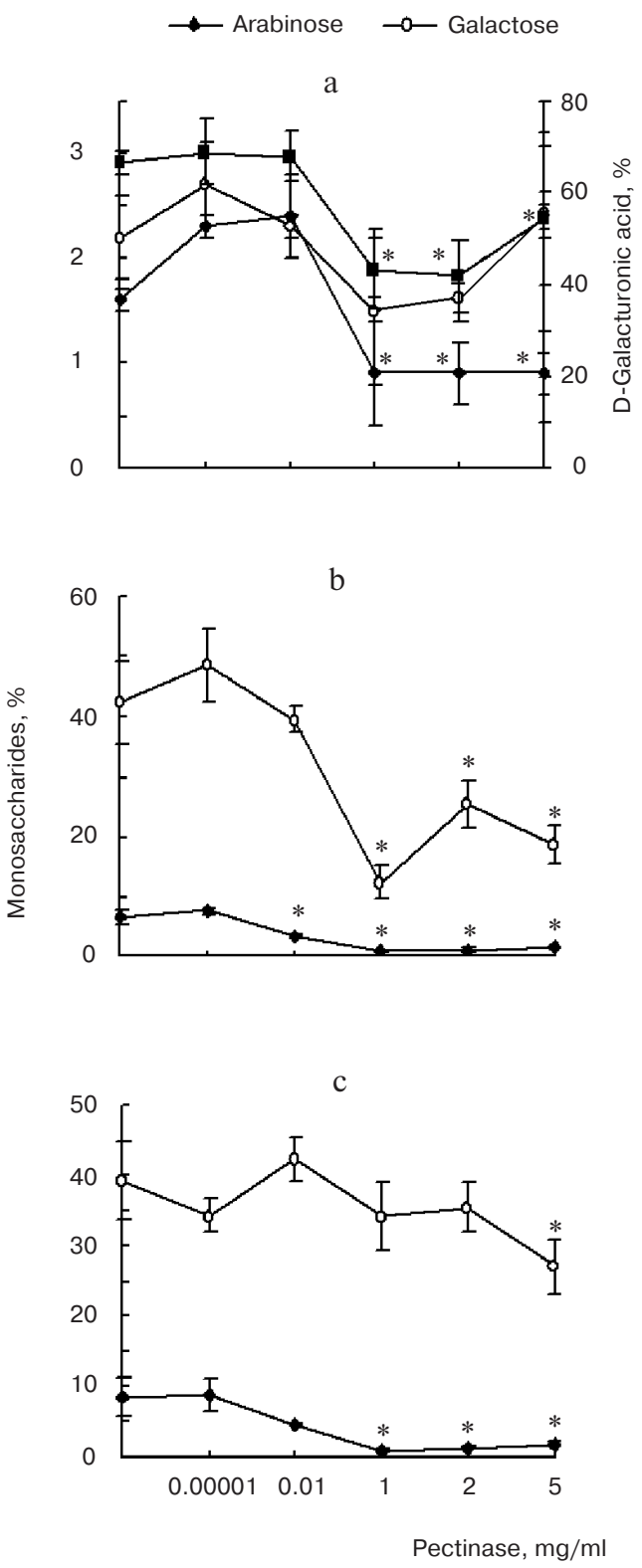

Fig. 1. Effect of pectinase on the monosaccharide composition of silenan (a), intracellular arabinogalactan AG1 (b), and extracellular arabinogalactan AG2 (c). * The differences are significant at $p < 0.05$. In the control there was no enzyme.

the minor fractions AG1-II (3%) with molecular weight 100-300 kD and AG1-III (1.3%) with molecular weight 50-100 kD were 78% lower. The increase in the relative content of the high molecular weight fraction in AG1 could be associated with an admixture of arabinogalactan detached from pectin as a result of activation of galactanase and/or arabinase degrading arabinogalactan side chains of pectin.

Galactose was a dominating monosaccharide in the experimental AG1 sample fractions with molecular weights 300, 100-300, and 50-100 kD, whereas in the fractions of the control sample of AG1-C galactose and arabinose were prevalent. In the AG1-III-C fraction with molecular weight of 50-100 kD the admixture of glucose and xylose was increased, and in the AG1-III fraction there was an increased glucose admixture. Thus, on callus cultivation in the presence of 10^{-2} mg/ml pectinase, galactan was synthesized, whereas in the absence of the enzyme arabinogalactan is synthesized.

The molecular weight distribution and monosaccharide composition of SV were similar in the experimental and control samples of pectin. The SV-I fraction with molecular weight higher than 300 kD was prevalent (53% yield). The fractions SV-II with molecular weight of 100-300 kD (0.4% yield) and SV-III with molecular weight of 50-100 kD (0.8% yield) were minor.

Contents of arabinose and galactose residues were significantly decreased in extracellular arabinogalactan AG2 at the pectinase concentration of 1-5 and 5 mg/ml, respectively (Fig. 1c). These findings indicated that high concentrations of pectinase were favorable for production and release of galactan into the culture medium. In AG2 samples, small amounts of glucose, xylose, and mannose residues were found, which seemed to be components of attendant hemicelluloses (xyloglucans). Galacturonic acid residues were also present in AG2 (13-16%).

The campion callus was cultivated through ten growth cycles on the medium supplemented with pectinase (10^{-5} and 10^{-2} mg/ml). Changes in the monosaccharide composition of AG1 represented by decreased content of arabinose residues were retained on the long-term cultivation in the presence of 10^{-2} mg/ml pectinase. Moreover, the content of arabinose residues was also significantly decreased in extracellular AG2. The pectinase concentration of 10^{-5} mg/ml did not influence the monosaccharide composition of AG1 and AG2. Low concentrations of pectinase had no effect on the composition of silenan SV on the long-term cultivation in the presence of the enzyme.

The effect of pectinase on activities of intracellular carbohydrases in the campion callus was determined. On cultivation in the pectinase-containing medium, the pectinase activity in the cells increased with the elevation of the enzyme concentration (Table 2). Pectinase at the concentration of 1-5 mg/ml increased the activity of α-L-arabinofuranosidase in the cells. The β-galactosidase activity was maximal at the pectinase concentration of 10^{-5} and 2 mg/ml.

The activity of extracellular carbohydrases was determined in the presence of pectinase in the nutritional medium. The pectinase activity was low in the absence of exogenous enzyme and at 10^{-2} mg/ml and was considerably higher at high concentration of the enzyme (1-5 mg/ml) (Table 2). The activity of extracellular α-L-arabinofuranosidase was maximal in the presence of high concentration of pectinase. Upon addition of pectinase into the medium, the activity of β-galactosidase increased with respect to the control.

It was earlier shown that the treatment of primary cell walls with exogenous enzymes, such as pectinases, could produce biologically active oligogalacturonides [26]. Oligogalacturonides stimulate transcription of certain genes that results in appearance of mRNAs the translation of which determines synthesis of particular enzymes. Oligogalacturonides can change the permeability of cytoplasmic membranes for potassium and calcium cations and induce phosphorylation of membrane proteins, which regulates the enzyme activities [5, 26].

Table 2. Effect of pectinase on activities of intracellular and extracellular carbohydrases (in U/mg protein)

Pectinase		Intracellular carbohydrases			Extracellular carbohydrases		
mg/ml	U/ml	pectinase	α-L-arabino-furanosidase	β-galacto-sidase	pectinase	α-L-arabino-furanosidase	β-galacto-sidase
0	0	0	129 ± 10	119 ± 31	2.0 ± 0.2	177 ± 15	8.3 ± 6.2
10^{-5}	0.03	0	130 ± 11	279 ± 75*	1.2 ± 0.6	389 ± 188	1315 ± 341*
10^{-2}	0.2	5.8 ± 1.1*	199 ±17	232 ± 84	0	205 ± 18	382 ± 151*
1	20.4	12.1 ± 1.8*	684 ± 60*	122 ± 31	575 ± 147*	3420 ± 294*	292 ± 39*
2	41.2	18.4 ± 7.4*	3232 ± 278*	247 ± 45*	554 ± 118*	3470 ± 298*	306 ± 45*
5	100.0	46.9 ± 18.0*	2831 ± 244*	153 ± 30	565 ± 76*	2856 ± 246*	313 ± 50*

Similar mechanisms are likely to be involved in the activation of the carbohydrases under study. Biologically active oligosaccharide fragments are produced from pectin and hemicellulose, and other enzymes, such as glucanases [26] and galactosidases, may be involved in their formation.

Changes in the contents of arabinose residues in SV and arabinose and galactose residues in AG1 and AG2 in the presence of pectinase in the nutritional medium suggest changes in the structure of side chains of rhamnogalacturonan and arabinogalactan. The decreased content of arabinose residues in polysaccharides SV, AG1, AG2, and of galactose residues in AG1 and AG2 is associated with an increase in the activities of α-L-arabinofuranosidase and β-galactosidase upon addition of pectinase into the medium (Table 2). The decrease in the content of galacturonic acid residues in silenan SV is caused by the increase in the activity of pectinase, which cleaves this pectic polysaccharide.

Thus, pectinase is responsible for cleavage of pectin and destruction of its high molecular weight fraction (above 300 kD), whereas the side chains of pectin, in particular, arabinose residues located on the outer parts of the ramified regions of the macromolecule, are destroyed under the influence of α-L-arabinofuranosidase. The transformation of arabinogalactan into galactan occurs with involvement of α-L-arabinofuranosidase, and then galactan is destroyed under the influence of galactosidase. Concurrently, the content of arabinogalactan and/or galactan is decreased in the cells, and it is released into the culture medium.

From the campion callus culture grown on medium with different concentrations of galactosidase, silenan SV and arabinogalactan AG1 were isolated. Extracellular arabinogalactan AG2 was obtained from the nutritional medium.

The relative content and productivity of silenan were increased twofold on the medium supplemented with 10^{-5} mg/ml galactosidase (Table 3). At the enzyme concentration of 1-5 mg/ml, the yield of silenan was higher (5.6-9.1%) than in the control (3.5%), whereas the productivity per liter of the medium was 4-6-fold lower owing to diminished production of the culture per biomass (Table 3). The increase in the pectin yield seems to be a result of the hydrolyzing effect of β-galactosidase on the cell walls and more complete extraction of pectin from the cells.

The yield and productivity of AG1 were close to control at the galactosidase concentrations of 10^{-5}, 10^{-2}, and 5 mg/ml, whereas the biosynthesis was significantly lower on addition of the 1 and 2 mg/ml of the enzyme into the medium (Table 3). The content of extracellular AG2 in the nutritional medium increased to 0.20-0.31 g/liter on the addition of 10^{-5} to 5 mg/ml galactosidase.

When the plant cells were cultivated on the medium with β-galactosidase, low concentrations of the enzyme did not influence the monosaccharide composition of pectic substances. The arabinose/galactose ratio in pectin was 1 : (1.4-1.8). In the presence of high concentrations of the enzyme, pectin samples contained decreased contents of arabinose (by 68-73%), galactose (by 30-46%), and galacturonic acid (by 19-29%) (Fig. 2a). The arabinose/galactose ratio was increased to 1 : (3.1-4.5).

Analysis by HPLC of SV samples obtained in the presence of 1-5 mg/ml of β-galactosidase revealed, in addition to the fragment with molecular weight of 300-500 kD, a fragment with molecular weight of 36-50 kD (with the peak area of 15-25%). This low molecular weight fragment seemed to be generated due to activation of extracellular pectinase (Table 4). The fragment detachment from silenan could also be associated with an increase in the activity of intracellular galactanase and/or arabinase, which are involved in the detachment of pectin side chains containing arabinogalactan, galactan, and arabinan.

Elevated concentrations of β-galactosidase (1-5 mg/ml) decreased in AG1 the content of galactose by

Table 3. Effect of β-galactosidase on the polysaccharide contents in the campion callus

Concentration of β-galactosidase, mg/ml	SV		AG1		AG2
	yield, %	g/liter	yield, %	g/liter	g/liter
0	3.5 ± 0.4	0.23 ± 0.01	6.0 ± 1.1	0.39 ± 0.06	0.14 ± 0.02
10^{-5}	6.5 ± 1.4*	0.41 ± 0.07*	4.3 ± 0.5	0.28 ± 0.03	0.20 ± 0.02*
10^{-2}	4.3 ± 0.4	0.25 ± 0.03	5.5 ± 0.4	0.33 ± 0.03	0.14 ± 0.05
1	5.6 ± 1.0*	0.04 ± 0.01*	2.8 ± 0.4*	0.02 ± 0.001*	0.20 ± 0.01*
2	9.0 ± 1.1*	0.06 ± 0.003*	1.9 ± 0.5*	0.01 ± 0.002*	0.25 ± 0.04*
5	9.1 ± 0.8*	0.05 ± 0.01*	6.1 ± 1.4	0.04 ± 0.01*	0.31 ± 0.04*

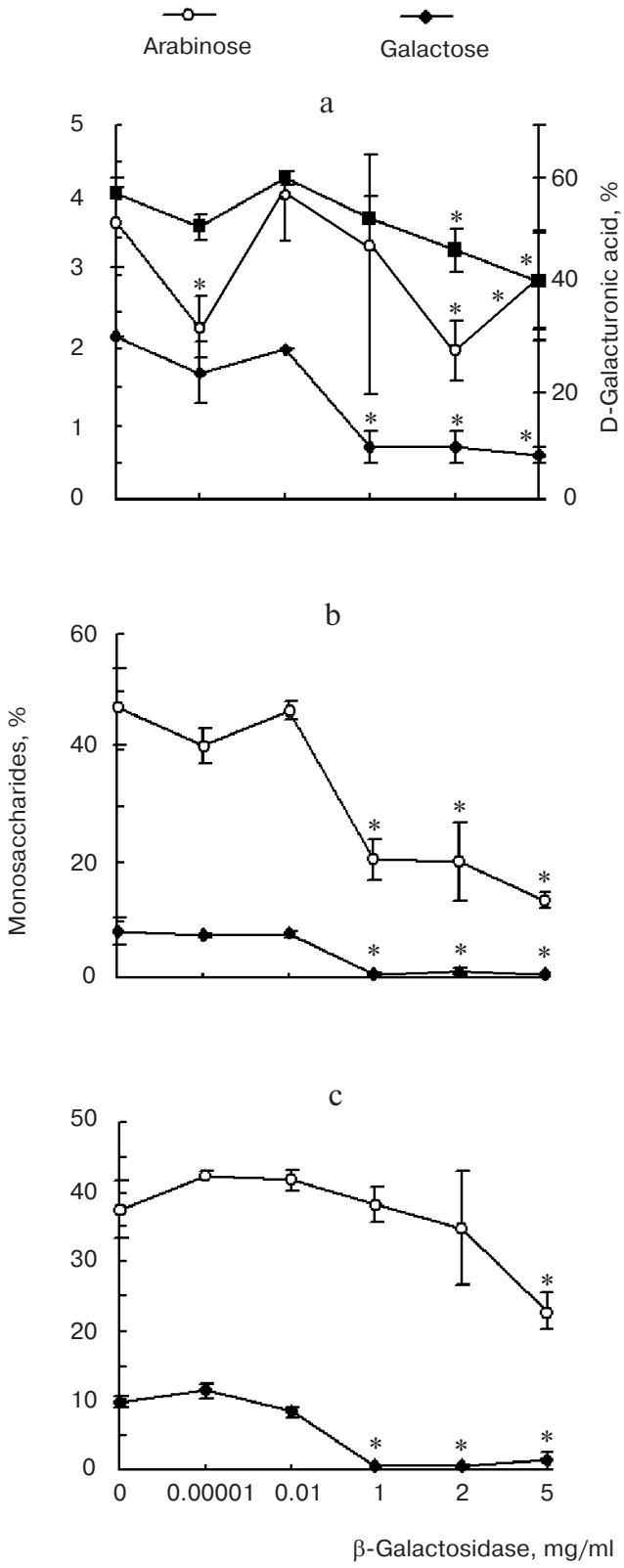

Fig. 2. Effect of β-galactosidase on the monosaccharide composition of silenan (a), intracellular arabinogalactan AG1 (b), and extracellular arabinogalactan AG2 (c). * Difference is significant at $p < 0.05$. No enzyme in the control.

56-71% and the content of arabinose by 89-91% (Fig. 2b). The arabinose/galactose ratio was 1 : (21.2-34.4). Thus, the addition of 1-5 mg/ml β-galactosidase promoted destruction of the galactan core of AG1 and side chains consisting of arabinose and galactose residues.

In the samples of extracellular arabinogalactan AG2, the content of arabinose residues was significantly (by 87-95%) decreased at the β-galactosidase concentration of 1-5 mg/ml and the content of galactose was decreased by 39% at the β-galactosidase concentration of 5 mg/ml (Fig. 2c). Thus, in the presence of high concentrations of the enzyme, galactan was produced and released into the medium.

There were little amounts of glucose, xylose, and mannose residues in the AG1 and AG2 samples. They seemed to be components of the attendant hemicelluloses. Moreover, residues of galacturonic acid (5-14%) and protein (5-21%) were detected in AG1.

Exogenous β-galactosidase (1-5 mg/ml) significantly increased the activities of intracellular α-L-arabinofuranosidase and β-galactosidase, but in the presence of 10^{-5} and 10^{-2} mg/ml β-galactosidase the activities of these intracellular enzymes did not differ from those in the control (Table 4). The pectinase activity in the callus was not greatly changed on the addition of β-galactosidase into the culture medium.

The specific activities of extracellular carbohydrases increased with the increase in the β-galactosidase concentration in the medium (Table 4). The activities of α-L-arabinofuranosidase and pectinases were maximal on addition into the medium of 1-5 mg/ml β-galactosidase.

Thus, cultivation of plant cells on medium with β-galactosidase resulted in modification of the structure of pectin and arabinogalactan of the cell walls. β-Galactosidase (EC 3.2.1.23) identified in higher plants was earlier shown to be involved in destruction of galactan side chains of cell wall pectins [27].

Changes in the monosaccharide composition of polysaccharides are associated with increases in the activities of α-L-arabinofuranosidase, β-galactosidase, and pectinase. The enzyme α-L-arabinofuranosidase detaches arabinose residues from the side chains of rhamnogalacturonan and arabinogalactan, and β-galactosidase is involved in detachment of galactose residues from the side chains of SV, AG1, and AG2, as well as in destruction of the galactan core of AG1 and AG2.

Thus, activities of the above-mentioned enzymes resulted in transformation of cell wall polysaccharides. The cultivation of campion callus on media with different concentrations of pectinase allowed us to obtain samples of pectin and arabinogalactan with diminished content of arabinose in the side chains. From the callus grown in the presence of β-galactosidase, samples of pectin were obtained with low contents of arabinose and galactose in the side chains, as well as samples of galactan.

Table 4. Effect of β-galactosidase on activities of intracellular and extracellular carbohydrases (in U/mg protein)

β-Galactosidase		Intracellular carbohydrases			Extracellular carbohydrases		
mg/ml	U/ml	pectinase	α-L-arabino-furanosidase	β-galacto-sidase	pectinase	α-L-arabino-furanosidase	β-galacto-sidase
0	0	1.5 ± 0.8	114 ± 10	137 ± 44	1.6 ± 0.5	40.8 ± 3.5	8.3 ± 6.2
10^{-5}	1.8	2.8 ± 0.4	130 ± 11	170 ± 72	0.8 ± 0.4*	389 ± 34*	105 ± 15*
10^{-2}	23.9	3.2 ± 2.2	199 ±17	101 ± 49	1.0 ± 0.5	205 ± 18*	459 ± 65*
1	2523	4.2 ± 0.9*	4240 ± 365*	45208 ± 8535*	3.4 ± 0.4*	2479 ± 213*	41320 ± 4242*
2	4544	2.6 ± 1.1	2196 ± 189*	56434 ± 5041*	3.7 ± 0.9*	1422 ± 122*	52518 ± 4361*
5	10501	2.5 ± 1.5	3991 ± 343*	45668 ± 8947*	3.2 ± 0.8*	646 ± 56*	35451 ± 11712*

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