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RESEARCH PAPERS

Agrobacterial *rol* Genes Modify Thermodynamic and Structural Properties of Starch in Microtubers of Transgenic Potato

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Abstract—Wild-type (WT) plants of potato (*Solanum tuberosum* L.) and their transgenic forms carrying agrobacterial genes *rolB* or *rolC* under the control of *B33* class I patatin promoter were cultured in vitro on MS medium with 2% sucrose in a controlled-climate chamber at 16-h illumination and 22°C. These plants were used as a source of single-node stem cuttings, which were cultured in darkness on the same medium supplemented with 8% sucrose. The tubers formed on them were used for determination of the structure of native starch using the methods of differential scanning microcalorimetry (DSC), X-ray scattering, and scanning electron microscopy. It was found that, in starch from the tubers of *rolB*-plants, the temperature of crystalline lamella melting was lower and their thickness was less than in WT potato. In tubers of *rolC* plants, starch differed from starch in WT plants by a higher melting temperature, considerably reduced melting enthalpy, and a greater thickness of crystalline lamellae. Deconvolution of DSC thermogram makes it possible to interpret the melting of starch from the tubers of *rolC* plants as the melting of two independent crystalline structures with melting temperatures of 65.0 and 69.8°C. Electron microscopic examination confirmed the earlier obtained data indicating that, in the tubers of *rolC* plants, starch granules are smaller and in the tubers of *rolB* plants larger than in WT plants. Possible ways of influence of *rol* transgenes on structural properties of starch in amyloplasts of potato tubers are discussed.

Key words: Solanum tuberosum, rolB, rolC, transgenes, tubers, starch, lamellae, melting temperature.

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INTRODUCTION

Tuber formation in potato is regulated by numerous external and internal factors with phytohormones being of special importance [1]. In order to investigate the hormonal control over tuber formation, some researchers used transformed potato plants expressing *rolB* and *rolC* genes of *Agrobacterium rhizogenes* [2]. Biochemical mechanism of the operation of *rol*-transgenes remains debatable; however, it was shown that their role in higher plants resembles to the effect of phytohormones: auxins in case *rolB* and cytokinins in case *rolC* [3]. It is assumed that hormone-like effect of *rol* genes may be related to their influence on the metabolism of auxins and cytokinins or to the changes in plant cell sensitivity to these phytohormones [3]. It was also shown that the intensity of the effect of *rol* genes on morphogenesis of potato plants greatly depends on the used promoter governing organospecificity of gene expression [4].

It order to investigate the effect of genes *rolB* and *rolC* on tuber formation, we used *B33* class I patatin promoter, which ensured preferred expression of *B33::rolC* and *B33::rolB* in growing potato tubers [5]. These experiments showed that transgenes induced numerous morphological changes accompanied by changes in the activity of some enzymes of carbohydrate metabolism, the content of soluble sugars [6], and the number and size of starch granules [7]. The obtained data suggested that transgenes *rolB* and *rolC* might also modify qualitative characteristics of storage starch, which by volume and biological role is the

Abbreviations: DSC—differential scanning microcalorimetry; ΔH_{mlt}—melting enthalpy; ΔH^{VH}—van't Hoff enthalpy; L_{crl}—thickness of crystalline lamella; *rolB*-plants—potato plants expressing *B33::rolB* transgene; *rolC*-plants—potato plants expressing *B33::rolC* transgene; T_{mlt}—temperature of crystalline lamellae melting; WT—wild-type potato plants.

main metabolite in tubers. Presently, starch is widely used in different industries, with composition and structural features of this carbohydrate being of great practical importance [8]. The biosynthesis of storage starch is a complicated process; therefore, the formation of its structure is obscure and debatable [9]. Transformed plants are an efficient up-to-date instrument of investigating the regulation of starch structure formation [10].

Influence of the genes *rolB* and *rolC* on the qualitative characteristics of storage starch in tuber amyloplasts is so far unexplored. It this relation, the aim of this work was to study thermodynamic and structural features of starch produced in the tubers of potato plants expressing transgenes *B33::rolB* and *B33::rolC*.

It is known that storage starch in potato is deposited in tuber amyloplasts as water-insoluble granules that consist of alternate crystalline (mainly amylopectin) and amorphous (mainly amylose) layers (lamellae). There are two main types of crystalline assembly of amylopectin double helixes in lamellae: type A (monoclinic) and type B (hexagonal). As a rule, potato starch is crystallized according to type B but, under certain experimental conditions, the type of starch crystalline structure changes [11]. The biological role of such changes is not evident.

Structural characteristics of starch granules are described by numerous parameters. In this work, we determined the type of crystalline assembly of the investigated starch types, analyzed the thermograms of their melting describing heat stability and the degree of order of lamellar structure, and determined the thickness of crystalline lamellae and dimensions of starch granules.

MATERIALS AND METHODS

Plant material. Investigations were conducted with tubers of wild-type potato (*Solanum tuberosum* L., cv. Désirée) and transgenic lines of this cultivar expressing *rolB* and *rolC* genes of *Agrobacterium rhizogenes* under the control of the *B33* class I patatin promoter. The plants were transformed, and expression of the transferred genes was analyzed at the Institute of Molecular Plant Physiology (Max-Planck Institute für Molekulare Pflanzenphysiologie, Germany) [5]. The experiments were conducted with independently transformed lines (*B33::rolC* and *B33::rolB*) notable for typical morphological features described in our previous papers [12, 13].

Plants were propagated by in vitro cloning on MS agar medium containing 60 mg/l mesoinositol, 0.4 mg/l thiamine, 0.5 mg/l pyridoxine, and 2% sucrose and cultured in a controlled-climate chamber at Timiryazev Institute of Plant Physiology, Russian Academy of Sciences, at 22°C and 16-h illumination with luminescent lamps producing white light. Low content of sucrose was unfavorable for tuber formation but promoted vegetative growth of plants. These plants were a starting material for subsequent experiments.

In the experiments, we used single-node stem cuttings with one leaf taken from the middle part of initial plants. The cuttings were transplanted to the agar medium of the same composition supplemented with 8% sucrose conducive to tuber formation. From the 6th day after planting, the cuttings were cultured in darkness at 20°C, which promoted the formation of microtubers. Starch was analyzed in 4-week-old tubers.

Starch content. Starch was determined in freeze-dried microtubers after prior extraction of soluble sugars with 80% boiling methanol. Residues were washed with water, lyophilized, repeatedly weighed, and used for hydrolysis of starch as described by Sergeeva et al. [14]. The content of starch was calculated from the level of glucose produced as a result of hydrolysis. Glucose was assayed by HPLC combined with pulse amperometric detection using a Dionex system (Dionex, United States) equipped with Carbo Pac PA1 columns (4 × 250 mm and 4 × 500 mm). Elution was conducted with 85 mM NaOH at room temperature and the flow rate of 1 ml/min.

Differential scanning microcalorimetry. Starch was extracted from fresh microtubers at room temperature according to [15] with repeated washing with deionized water. Thermodynamic parameters of melting of 0.15% water dispersions of investigated starch samples [15] were determined by high-sensitivity differential scanning microcalorimetry (DSC), using a DASM-4 microcalorimeter (Russia) with the sample volume of 0.5 cm^3 in a closed cell. Measurements were conducted in the temperature range from 20 to 100°C (figures show the results in the range from 40 to 85°C) at a constant pressure of 2.5 bar and heating rate of 2°C/min. The scale of excess heat capacity for each experiment was calibrated using the effect of Joule–Lenz. Under the chosen experimental conditions, there was no need to take into consideration a thermal lag and duration of the sample treatment in the calorimetric cell [16]. As a reference, deionized water was used.

Average values of thermodynamic parameters were calculated on the basis of three measurements and standardized per mole of anhydroglucose (162 g/mol).

Melting temperature corresponded to the peak on the DSC-thermogram. Repeated scanning showed that the structures melted irreversibly. Melting enthalpy was determined as the area under the peak above the extrapolation curves.

In order to evaluate cooperativity and the thickness of crystalline lamella in starch granules, we used a single-stage model of melting [17]. Values of van't-Hoff enthalpy (ΔH^{VH}) were calculated as described earlier [18] using the following equation:

$$\Delta H^{VH} = 2\,R^{1/2}\,T_{mlt}(C_p - 0.5\Delta C_p^{exp})^{1/2},$$

Table 1. Growth characteristics of tubers and content of starch therein in WT and transgenic (*rolB* and *rolC*) plants of potato after 4 weeks of culturing in continuous darkness

Plant material	Tuber fr wt, mg	Length/width ratio of the tuber	No. plants with tubers, % of total number	Content of starch, mg/g dry wt
WT	35.1 ± 3.0	1.20 ± 0.20	65.5 ± 7.1	510.5 ± 25.65
rolB	44.5 ± 2.4	1.09 ± 0.05	86.2 ± 4.8	481.5 ± 15.04
rolC	67.2 ± 5.4	2.71 ± 0.31	47.0 ± 4.2	437.8 ± 14.84

Table 2. Thermodynamic characteristics of melting of starch samples from tubers of WT and transgenic (*rolB* and *rolC*) plants of potato

Plant material	T_{mlt}, °C	ΔH_{mlt}, kJ/mol	ΔH^{VH}, kJ/mol	ν, relative units	L_{crl}, nm
WT	65.9 ± 0.2	3.6 ± 0.1	44.9 ± 0.1	14.5 ± 0.3	5.1 ± 0.1
rolB	64.9 ± 0.1	3.2 ± 0.3	45.5 ± 0.3	13.5 ± 0.3	4.7 ± 0.2
rolC	69.1 ± 0.2	1.0 ± 0.1	15.9 ± 0.1	17.9 ± 0.2	6.3 ± 0.1

Note: T_{mlt}—temperature of melting of crystalline lamellae; ΔH_{mlt}—enthalpy of melting; ΔH^{VH}—van't Hoff enthalpy; ν—cooperative unit of melting; L_{crl}—thickness of crystalline lamella in investigated starches.

where R—universal gas constant; T_{mlt}—melting temperature of starch crystalline lamella; C_p —ordinate peak on the DSC-thermogram; ΔC_p —difference between heat capacities of melted and native starch dispersions.

Deconvolution of melting thermograms of the investigated starch samples was conducted using a Peak Fit software product (Gandel Scientific Software).

Scanning electron microscopy. Morphology of starch granules was studied by scanning electron microscopy. Ultrathin sections of potato microtubers (3–4 μm) were prepared using an UltracutE microtome (Reichert-Jung, Germany). The sections were placed on a copper grids and coated with a thin layer of gold using a Jeol JEE-400 vacuum vaporizer. The samples prepared in that way were examined under a Jeol 5200 scanning electron microscope at accelerating voltage of 10 kV.

X-ray scattering. Native starch was investigated by the method of X-ray scattering [19], using an X-ray difractometer equipped with a linear coordinate detector. Debye–Scherrer X-ray optical circuit was employed. X-ray emission was produced by a BSV33Cu sharp-focused X-ray tube with a nickel filter (radiation CuK_α, $\alpha = 0.154$ nm). Before measurement, starch was saturated with distilled water.

RESULTS

Table 1 shows growth characteristics of the tubers of WT, *rolB*, and *rolC* plants and total content of starch therein. The same as in our previous works [12, 13], tuber-specific expression of *rol* transgenes was accompanied by changes in the pattern of tuber formation, including the shape of tubers, their size and number. The tubers of *rolC* transformants were elongated, larger in size, and less numerous than in WT and *rolB* plants. Rounded tubers of *rolB* plants were more numerous than in WT and *rolC* plants. These results show that, in respect of the effect on tuber formation in transgenic potato, the used transgenic lines *rolB* and *rolC* are typical. As to starch content calculated on dry weight basis, there were no appreciable differences between WT and *rolB* plants, whereas, in the tubers of *rolC* plants, the level of starch slightly decreased (on the verge of reliability). The same as in our previous experiments [7], expression of *rol* transgenes considerably affected the size of starch granules in tubers (Fig. 1). In the tubers of *rolC* plants, the granules were smaller, and in the tubers of *rolB* transformants, they were larger.

Figure 2 shows DSC-thermograms and Table 2—thermodynamic parameters of melting of crystalline lamellae of the investigated starch samples. The obtained results showed that melting temperature of starch crystalline lamellae in *rolB* plants was much lower and in *rolC* plants much higher than in WT potato. Enthalpy of starch melting in *rolC* plants was much lower than in *rolB* and WT plants. The thickness of crystalline lamella in *rolB* plants was somewhat less and in *rolC* plants much greater than in starch of WT potato.

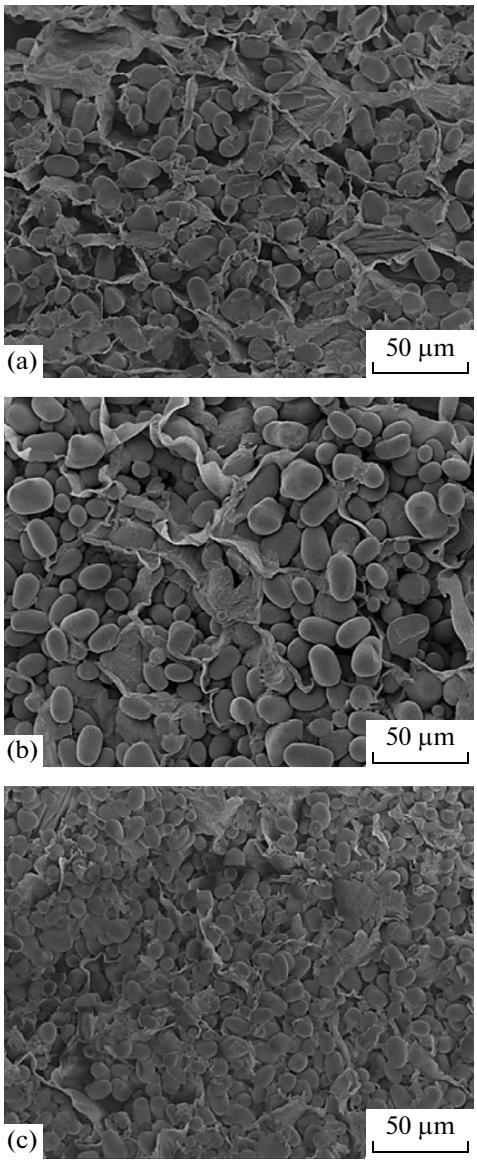

Fig. 1. Starch granules (scanning electron microscopy images) from potato tubers.

(a) Tubers of nontransformed plants; (b) tubers of transgenic *rolB* plants; (c) tubers of transgenic *rolC* plants.

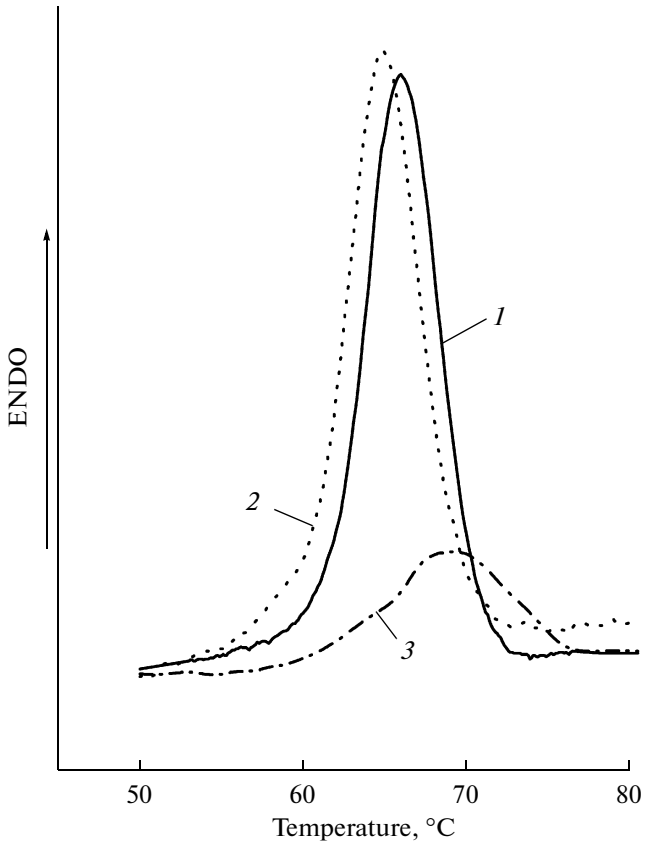

Fig. 2. DSC-thermograms of melting of water dispersions of starch samples (concentration of 0.15%) from potato tubers.

(*1*) Tubers of nontransformed plants; (*2*) tubers of transgenic *rolB* plants; (*3*) tubers of transgenic *rolC* plants. ENDO—consumption of heat energy associated with melting of starch crystalline lamellae.

Thermograms in Fig. 2 show that melting curves of starch samples in all the types of plant material display typical of native potato starch thermal transitions reflecting melting of crystalline lamellae of amylopectin [18]. The graphs also show that, in starch samples isolated from the tubers of *rolB* and WT plants, the melting curves were rather symmetric relative the melting peak. In contrast, in starch of *rolC* plants, the thermogram of crystalline lamella melting was much less symmetric. Such an asymmetry of the calorimetric melting peak is most likely accounted for by the presence in the starch from *rolC* tubers of two or more independent well-ordered structures with different thermostability of crystalline lamellae differing in the thickness. Depending on type A or B of polymorphic organization, high-amylose starch can contain from 2 to 3 types of crystalline structures, which melt independently in the same temperature interval [19, 20]. As a rule, starch from potato tubers belongs to type B; however, our experimental conditions, such as the procedure of transgenic plant production and subsequent long culturing in vitro, could modify the type of starch polymorphic structure in microtubers. In order to elucidate a possible number of crystalline structures, which could cause asymmetry of DSC thermogram describing *rolC* starch melting, we conducted the experiments designed to determine the type of polymorphic organization of starch in the investigated material. To this end, we analyzed the melting of starch from WT and transgenic (*rolB*) potato in 0.6 M KCl (Fig. 3). In the presence of KCl, the melting temperature of crystalline lamella is to become higher [18], with melting temperature of A-polymorphic structure of starch usually increasing by 7–12°C and in the case of B-polymorphic structure—by only 1–

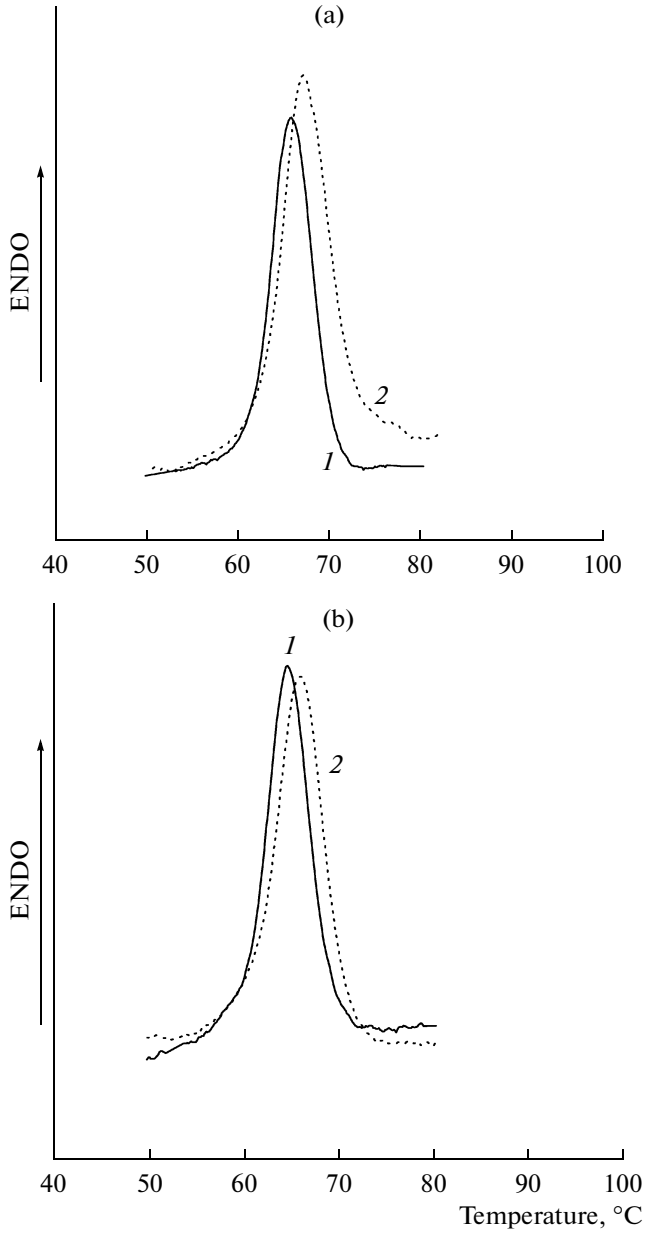

Fig. 3. DSC-thermograms of melting of water suspension of starch samples from potato tubers (concentration of 0.15%) in water (*1*) and 0.6 M KCl (*2*).

(a) Tubers of nontransformed plants; (b) tubers of transgenic *rolB* plants. ENDO—see Fig. 2.

4°C [21]. Figure 3 shows that, as compared with water suspension, the melting temperature of starch samples in KCl solution increased by 1.1–1.3°C, which is characteristic of polymorphic structure of type B.

The obtained calorimetric results were confirmed by determination of the type of starch polymorphic structure using the method of X-ray scattering (Fig. 4). The type of crystalline structure was determined by the location of crystalline reflexes, i.e., by the scattering angle corresponding to the greatest rate of X-ray scattering [22]. For the starch samples under investigation, reflexes were observed at 2Θ values equal to 6.1, 13.5, 21.7, 27.8, and 34.4°. Difractograms of X-ray scattering showed that location of the reflexes for the investigated starch samples corresponded to polymorphic structure of type B.

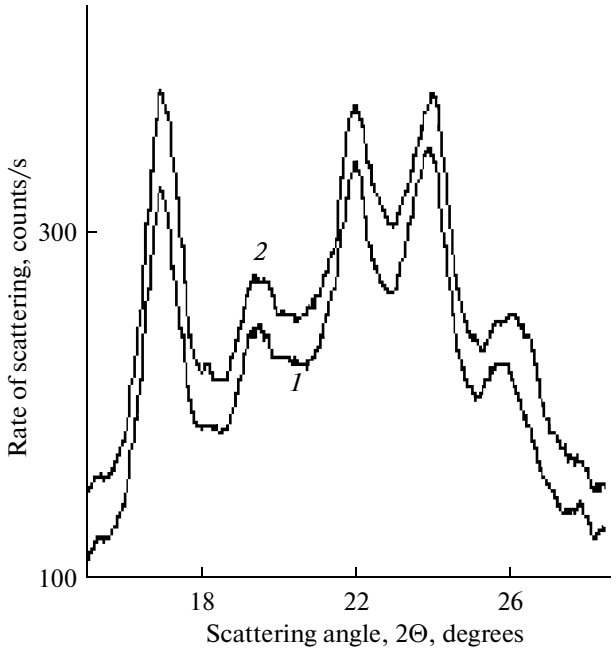

Fig. 4. Difractograms of X-ray scattering for starch samples from potato tubers.

(*1*) Tubers of nontransformed plants; (*2*) tubers of transgenic *rolB* plants.

On the basis of obtained results, melting of starch from the tubers of *rolC* plants shown in Fig. 2 may be regarded as independent melting of two different crystalline structures. Deconvolution of DSC thermogram describing melting of starch granules from *rolC* plants is shown in Fig. 5, where, for convenience, the scale of thermal energy consumed for starch melting (ENDO) is exaggerated as compared with the curves in Fig. 2. Figure 5 shows that the thermogram of the melting of starch from *rolC* tubers may be considered as the curve describing melting of two crystalline structures, with the calculated proportion of low-temperature structure (T_{mlt} = 65°C) being 24.4% and that of high-temperature structure (T_{mlt} = 69.8°C) being 75.6%.

DISCUSSION

Our experiments showed that tuber-specific expression of *rol* genes considerably affected structural and thermodynamic properties of starch in the amyloplasts from tubers of transformed potato. Transgenes *rolB* and *rolC* induced different changes in crystalline organization of starch. For instance, melting temper-

ature of crystalline lamellae of starch from the tubers of *rolB* plants was somewhat lower and in *rolC* tubers higher than in nontransformed control material. Thickness of starch crystalline lamellae calculated on the basis of single-stage model of melting was greater in *rolC* plants and less in *rolB* transformants than in WT plants.

Considerable elevation of starch melting temperature in *rolC* tubers probably depended on numerous disturbances in the structure of crystalline lamellae therein. As a rule, melting of crystalline lamellae starts from a destruction of imperfect areas in starch crystals, such as the ends of chain and loop segments [11]. Therefore, elevated temperature of starch melting in *rolC* tubers suggests that there were the greatest number of imperfections in this starch, which was accompanied by a reduction in the value of melting enthalpy and an increase in the thickness of crystalline lamellae. In addition, in contrast to starch from WT and *rolB* plants, melting thermogram of crystalline lamellae in *rolC* starch was extended and asymmetric (Fig. 2), which is characteristic of high-amylose starch [23]. It is possible that the tubers of *rolC* plants contain storage starch most rich in amylose among all investigated samples. Thermograms of melting in 0.6 M KCl and the results of X-ray scattering confirmed that polymorphic structure of the investigated starch samples belongs to type B. This made it possible to interpret the asymmetry of the melting peak on the DSC-thermogram of starch from the tubers of *rolC* plants as a result of the presence in this starch of two independent crystalline structures with differing melting temperatures. Deconvolution calculations done for starch of B type showed reliably (correlation coefficient $R^2 = 99.4\%$) the presence of two different crystalline structures in the storage starch of *rolC* tubers. One of them (with melting temperature of 65.0°C) accounted for 24.4% and the other (with melting temperature of 69.8°C) for 75.6%.

On the whole, the obtained results revealed a considerable effect of *rolB* and *rolC* transgenes on starch metabolism in tubers. So far, it is difficult to identify exact pathways of this influence, but one can speculate about possible mechanisms of the effect of investigated transgenes on production and deposition of storage starch. It is known for sure [10] that biosynthesis and deposition of storage starch in potato tubers involve at least four main groups of enzymes: ADP-glucose pyrophosphorylases (AGP), starch synthases (SS), enzymes catalyzing the branching of glucan chains (BE), and debranching enzymes (DBE). AGP catalyze production of ADP-glucose (this reaction restricts the total rate of starch biosynthesis in tubers) [24]. Because expression of *rolB* and *rolC* genes did not induce considerable changes in the total content of starch in tubers of transformed plants (Table 1), one can assume that these transgenes do not affect the initial stage of starch biosynthesis associated with AGP activity. Changes in the activity of the enzymes

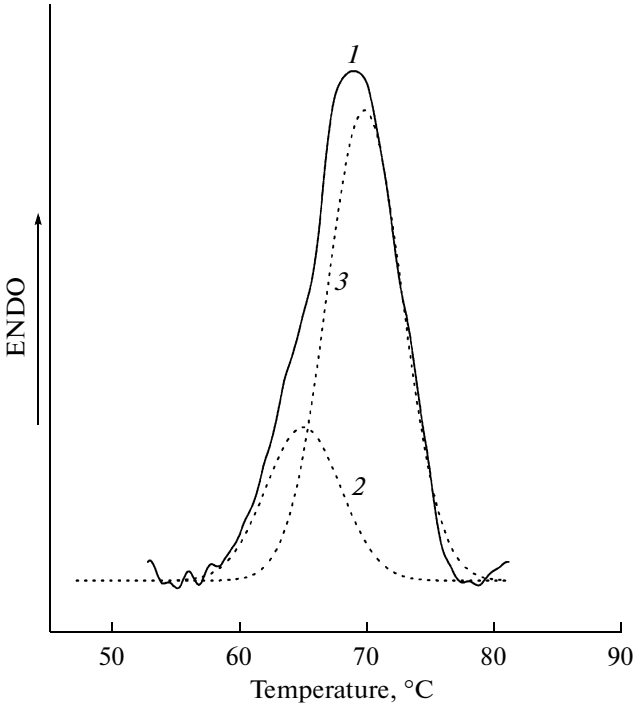

Fig. 5. Deconvolution of DSC-thermogram of starch from tubers of transgenic *rolC* plants of potato.
(*1*) Experimental DSC-thermogram; (*2, 3*) theoretical DSC-thermograms, where the proportion of low-temperature ordered structures (*2*) with $T_{mlt} = 65°C$ is 24.4% and the proportion of high-temperature ordered structures (*3*) with $T_{mlt} = 69.8°C$ is 75.6%. ENDO—see Fig. 2.

belonging to the rest three groups playing an important role in the determination of starch structure could account for the revealed structural features of starch and starch granules in transformed plants.

For instance, Fig. 1 shows that, as a result of expression of *rol* transgenes, dimensions of starch granules changed: in the tubers of *rolB* plants, they became larger and in *rolC*-tubers smaller than in WT plants. Presently, the processes influencing the size of starch granules are poorly investigated [9]; however, there are data pointing to a possible participation of the enzyme BE-I in the regulation of these processes [10]. It is worth noting that the average size of starch granules in the tubers of potato transformed with an antisense construction of BE-I considerably increased [25]. These data suggest that changes in the activity of BE-I enzyme can modify dimensions of starch granules in *rolB* and *rolC* plants of potato as compared with control material.

As it was noted above (Fig. 2, Table 2), elevated melting temperature of crystalline lamella and low melting enthalpy of starch in *rolC* plants suggest a high content of imperfect structural areas in this starch. Presently, there accumulate data indicating that one of the functions of DBE is breakdown of wrong structures of starch impeding normal granule growth [14].

It is possible that accumulation of imperfect structures in starch from *rolC* plants is related to the reduced activity of DBE in tubers of these transformants, which could result in the formation of smaller starch granules.

In conclusion, we should stress that further thorough investigations are needed to elucidate specific biochemical mechanisms modifying the structure of storage starch in the tubers of *rolB* and *rolC* transformants of potato.

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REFERENCES

1. Hannapel, D.J., Signaling the Induction of Tuber Formation, *Potato Biology and Biotechnology: Advances and Perspectives*, Vreugdenhil, D., Ed., Amsterdam: Elsevier, 2007, pp. 237–256.
2. Ooms, G., Twell, D., Bossen, M.E., Hoge, J.H.C., and Burrell, M., Developmental Regulation of Ri T_1-DNA Gene Expression in Roots, Shoots and Tubers of Transformed Potato (*Solanum tuberosum* cv. Diseree), *Plant Mol. Biol.*, 1986, vol. 6, pp. 321–330.
3. Schmülling, T., Fladung, M., Grossmann, K., and Schell, J., Hormonal Content and Sensitivity of Transgenic Tobacco and Potato Plants Expressing *rol* Genes of *Agrobacterium rhizogenes* T-DNA, *Plant J.*, 1993, vol. 3, pp. 371–382.
4. Fladung, M., Ballvora, A., and Schmülling, T., Constitutive or Light Regulated Expression of the *rolC* Gene in Transgenic Potato Plants Has Different Effects on Yield Attributes and Tuber Carbohydrate Composition, *Plant Mol. Biol.*, 1993, vol. 23, pp. 749–757.
5. Romanov, G.A., Konstantinova, T.N., Sergeeva, L.I., Golyanovskaya, S.A., Kossmann, J., Willmitzer, L., Schmülling, T., and Aksenova, N.P., Morphology and Tuber Formation of *In Vitro* Grown Potato Plants Harboring the Yeast Invertase Gene and/or the *rolC* Gene, *Plant Cell Rep.*, 1998, vol. 18, pp. 317–324.
6. Grishunina, E.V., Effects of *rol* Genes on Carbohydrate Metabolism during Tuberization in Potato Plants, *Cand. Sci. (Biol.) Dissertation*, Moscow: Inst. Plant Physiol., Russ. Acad. Sci., 2006.
7. Gukasyan, I.A., Golyanovskaya, S.A., Grishunina, E.V., Konstantinova, T.N., Aksenova, N.P., and Romanov, G.A., Effect of *rol* Transgenes, IAA, and Kinetin on Starch Content and the Size of Starch Granules in Tubers of *In Vitro* Potato Plants, *Russ. J. Plant Physiol.*, 2005, vol. 52, pp. 809–813.
8. Jobling, S., Improving Starch for Food and Industrial Applications, *Curr. Opin. Plant Biol.*, 2004, vol. 7, pp. 210–218.
9. Yuryev, V.P., Kozlov, S.S., Noda, T., Bertoft, E., and Blennow, A., Influence of Different GBSSI and GWD Combinations on the Amylose Localization within Wheat and Potato Starch Granules, *Starch: Achievements in Understanding of Structure and Functionality*, Yuryev, V., Tomasik, P., and Bertoft, E., Eds., New York: Nova Sci. Publ., 2007, pp. 1–47.
10. Davis, J.P., Supatcharee, N., Khandelwal, R.L., and Chibbar, R.N., Synthesis of Novel Starches in Plants: Opportunities and Challenges, *Starch/Starke*, 2003, vol. 55, pp. 107–120.
11. Jenkins, P.J., Cameron, R.E., and Donald, A.M., A Universal Feature in the Structure of Starch Granules from Different Botanical Sources, *Starch/Starke*, 1995, vol. 45, pp. 417–420.
12. Aksenova, N.P., Konstantinova, T.N., Golyanovskaya, S.A., Schmülling, T., Kossmann, J., Willmitzer, L., and Romanov, G.A., *In vitro* Growth and Tuber Formation by Transgenic Potato Plants Harboring *rolC* or *rolB* Genes under Control of the Patatin Promoter, *Russ. J. Plant Physiol.*, 1999, vol. 46, pp. 513–519.
13. Aksenova, N.P., Konstantinova, T.N., Golyanovskaya, S.A., Schmülling, T., Kossmann, J., Willmitzer, L., and Romanov, G.A., Transformed Potato Plants as a Model for Studying the Hormonal and Carbohydrate Regulation of Tuberization, *Russ. J. Plant Physiol.,* 2000, vol. 47, pp. 370–379.
14. Sergeeva, L.I., de Bruijon, S.M., Koot-Grosveld, E.A.M., Navratil, O., and Vreugdenhil, D., Tuber Morphology and Starch Accumulation Are Independent Phenomena: Evidence from *ipt*-Transgenic Potato Lines, *Physiol. Plant.*, 2000, vol. 108, pp. 435–443.
15. Rikhter, M., Augustat, Z., and Shirbaum, F., *Izbrannye metody issledovaniya krakhmala* (Selected Methods for Starch Investigations), Moscow: Pishchevaya. Promyshlennost', 1975.
16. Billarderis, C.G., Page, C.M., Slade, L., and Sirett, R.R., Thermal Behaviour of Amylose–Lipid Complexes, *Carbohydr. Polymers,* 1985, vol. 5, pp. 367–371.
17. Privalov, P.L. and Potekhin, S.A., Scanning Microcalorimetry in Studying Temperature-Induced Changes in Proteins, *Methods Enzymol.*, 1986, vol. 131, pp. 4–51.
18. Yuryev, V.P., Wasserman, L.A., Andreev, N.R., and Tolstoguzov, V.B., Structural and Thermodynamic Features of Low- and High-Amylose Starches. A Review, *Starch and Starch Containing Origins – Structure, Properties and New Technologies*, Yuryev, V.P., Cesaro, A., and Bergthaller, W., Eds., New York: Nova Sci. Publ., 2002, pp. 23–56.
19. Yuryev, V.P., Krivandin, A.V., Kiseleva, V.I., Wasserman, L.A., Genkina, N.K., Fornal, J., Blaszczak, W., and Schiraldi, A., Structural Parameters of Amylopectin Clusters and Semi-Crystalline Growth Rings in Wheat Starches with Different Amylase Content, *Carbohydr. Res.*, 2004, vol. 339, pp. 2683–2691.

20. Gernat, Ch., Radosta, S., Anger, H., and Damaschun, G., Crystalline Parts of Three Different Conformations Detected in Native and Enzymatically Degraded Starches, *Starch/Starke*, 1993, vol. 45, pp. 309–314.
21. Bogracheva, T.Ya., Morris, V.J., Ring, S.G., and Hedley, C.L., The Granular Structure of C-Type Starch and Its Role in Gelatinization, *Biopolymers*, 1998, vol. 45, pp. 323–332.
22. Cairs, P., Bogracheva, T., Ring, S.G., Hedley, L.L., and Morris, V.J., Determination of the Polymorphic Composition of Smooth Pea Starch, *Carbohydr. Polymers*, 1997, vol. 31, pp. 275–282.
23. Matveev, Y.I., van Soest, J.J.G., Nieman, C., Wasserman, L.A., Protserov, V.A., Ezernitskaja, M., and Yuryev, V.P., The Relationship between Thermodynamic and Structural Properties of Low and High Amylose Maize Starches, *Carbohydr. Polymers*, 2001, vol. 44, pp. 151–160.
24. Sweetlove, L.J., Müller-Röber, B., Willmitzer, L., and Hill, S.A., The Contribution of Adenosine 5'-Diphosphoglucose Pyrophosphorylase to the Control of Starch Synthesis in Potato Tubers, *Planta*, 1999, vol. 209, pp. 330–337.
25. Flipse, E., Suurs, L., Keetels, C., Kossmann, J., Jacobsen, E., and Visser, R., Introduction of Sense and Antisense cDNA for Branching Enzyme in the Amylose-Free Potato Mutant Leads to Physico-Chemical Changes in the Starch, *Planta*, 1996, vol. 198, pp. 340–347.