

Changes in the Wall-Bound Glycosidase Activities during the Cell Cycle in a Synchronous Culture of *Catharanthus roseus*

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The activities of some of the glycosidases in cell walls were measured during the cell cycle in a synchronous culture of *Catharanthus roseus* (L.) G. Don. The salt-extractable α -galactosidase activity increased during the G2 phase, whereas β -glucosidase and β -galactosidase activities in cell walls increased during the G1 phase after cytokinesis.

Introduction

Cell walls play a number of roles in plant growth. Growth is thought to consist of cell division and cell elongation. Thus, to understand plant growth, cell walls should be investigated separately during these two phases of growth. For this purpose synchronous cell cultures are suitable tools. We reported some dynamic aspects of cell wall metabolism during the cell cycle including changes in cell wall constituents [1], in synthetic activities [2], in UDP-sugar levels [3, 4], and in glucan synthase activities [5] in a synchronous culture system of *Catharanthus* cells [6].

Relationships between cell elongation and wall-bound glycosidases have been suggested by many workers (e.g. [7–12]). Indeed wall-bound glycosidases are responsible for the hydrolysis of the structural polysaccharides of plant cell walls. This hydrolytic activity is thought to participate in the auxin-induced elongation of these cells [13].

In this paper, we report on the changes in some wall glycosidase activities during the cell cycle. These changes are indicative of degradative activity within the cell walls and they provide more detailed evidence for the existence of regulatory mechanisms of cell wall metabolism during the cell cycle.

Materials and Methods

Catharanthus roseus G. Don (*Vinca rosea* L.) cells were subcultured every 7 days in the medium of Murashige and Skoog [14], which contained 2.2 μ M

2,4-dichlorophenoxyacetic acid and 3% (w/v) sucrose. Synchronous cell division was induced by a double phosphate starvation method [6]. The cell cycle was restarted by the second phosphate addition (at 0 time in the figures). Cell numbers were determined as described previously [6].

Preparation of wall glycosidases and assays of enzymatic activities were performed according to the method described previously [11] with minor modifications. Cells in each phase of the cell cycle were collected by filtration, washed with 5 mM Hepes-KOH (pH 6.8), 1 mM EDTA, 1 mM DTT, and homogenized in the same buffer with a glass homogenizer on ice. The homogenate was centrifuged at 700 $\times g$ for 10 min at 4 °C. The pellet was washed three times with 5 mM Hepes-KOH (pH 6.8) at 4 °C and the preparation was designated as cell walls. The cell walls were treated with 2 M NaCl in 10 mM Hepes-KOH (pH 6.8), 0.5 mM DTT for 2 h at room temperature. After centrifugation at 18000 $\times g$ for 20 min, the pellet was washed with same solution of NaCl. The supernatants were combined and used as the salt-extractable glycosidase preparation. The pellet was washed with 5 mM Hepes-KOH (pH 6.8), and then resuspended in the same buffer and used as the tightly bound glycosidase preparations.

Glycosidase activity was followed by the release of *p*-nitrophenol from various artificial substrates, the respective *p*-nitrophenylglycosides. The reaction mixture contained 0.5 ml enzyme preparation and 0.5 ml 10 mM substrate in 0.1 M sodium citrate buffer of pH 4.4. This pH was optimal for the activity of each glycosidase tested. The reaction was allowed to proceed for 20 min at 37 °C and was terminated by

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the addition of 1 ml 0.7 M sodium carbonate. The concentration of liberated *p*-nitrophenol was determined by measuring the absorbance at 410 nm.

Protein was determined by the method of Lowry *et al.* [15] with bovine serum albumin as a standard. The amount of total carbohydrate in the cell wall preparations was estimated after hydrolysis as described previously [1]. All *p*-nitrophenylglycosides were purchased from Sigma Chem. Co., St. Louis, U.S.A. The measurement was performed with two separate samples, and the experiments were repeated three times.

Results and Discussion

Several glycosidase activities, α - and β -glucosidase, α - and β -galactosidase, α - and β -xylosidase, α -

arabinosidase, and β -glucuronidase were investigated. α -Xylosidase and β -glucuronidase activities could not be detected. Changes in α -glycosidase and β -glycosidase activities are shown in Fig. 1 and 2, respectively. Each value of enzymatic activity is expressed as the rate of liberation of *p*-nitrophenol per unit amount of protein in the preparation of cell walls. The protein content in the cell wall preparations was rather constant (137–151 μ g in mg carbohydrate), and changes in activity expressed on the carbohydrate basis show similar patterns. The activities of salt-extractable glycosidases were higher than those of tightly-bound enzymes in the case of α -galactosidase and three β -glycosidases. By contrast, higher levels of α -arabinosidase and α -glucosidase activities remained bound to the cell walls after extraction by 2 M NaCl.

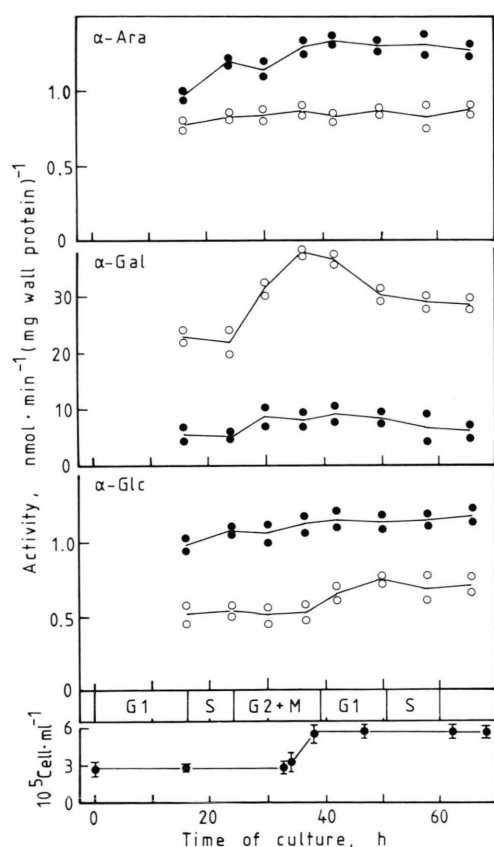


Fig. 1. Changes in α -arabinosidase (α -Ara), α -galactosidase (α -Gal), α -glucosidase (α -Glc) activities and cell number (lower) during the cell cycle. Open circles, salt-extractable activities; closed circles, tightly bound activities. Each phase of the cell cycle is according to the previous report [6].

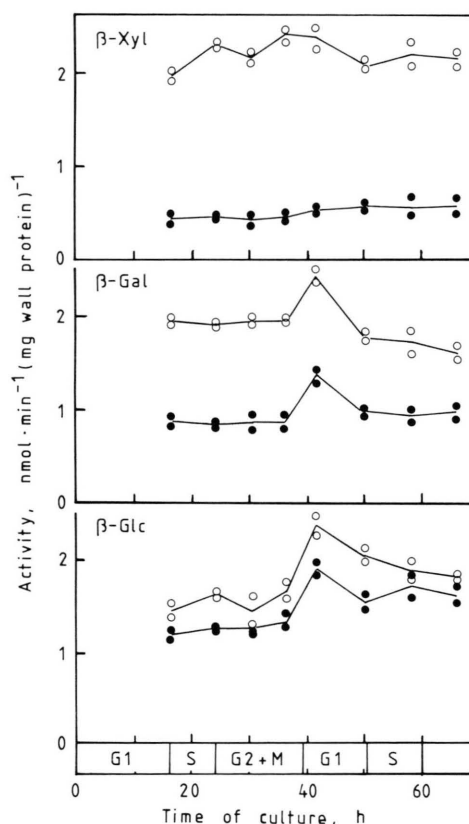


Fig. 2. Changes in β -xylosidase (β -Xyl), β -galactosidase (β -Gal) and β -glucosidase (β -Glc) activities during the cell cycle. Otherwise as for Fig. 1.

When the glycosidase activities are compared, α -galactosidase activity was seen to be about ten times higher than any of the other activities. This high level of α -galactosidase activity may be characteristic of *Catharanthus* cells, because lower level of activity of this enzyme are usually found in other plant sources [7, 9, 16].

We cannot completely exclude the possibility of contamination by cytoplasmic glycosidases, *i.e.*, the cell walls might adsorb cytoplasmic glycosidases non-specifically. However, such non-specifically adsorbed glycosidases is thought to be extracted by 2 M NaCl [11, 16].

The salt-extractable α -galactosidase activity increased during the G2 phase, and β -galactosidase and β -glucosidase activities, both salt-extractable and tightly bound, increased in the G1 phase after cell division. Other glycosidase activities did not change significantly during the cell cycle.

During the G2 phase before the cell division, the level of galactose in the cell walls began to increase [1]. UDP-galactose levels and the rate of synthesis of UDP-galactose also increased [3, 4] during this phase. It is thought that the cell wall polysaccharides which consist dominantly of galactose participate in the process of the cell division, especially cell plate formation [1]. The present results suggest that turn-

over of such cell wall polysaccharides is also active in this period.

In the G1 phase after cell division, expansion of cells and the most active synthesis of cell walls occurred [1, 2]. Levels of substrates and glucan synthase activity also increased during this period [2-4]. Moreover, preliminary experiments revealed that autolytic activity within cell walls peaks during the G1 phase (unpublished results). These suggest that breakage, loosening, synthesis and reconstruction of structural polysaccharides of cell walls are necessary for cell expansion.

In the present experiments, glycosidase activities were measured using synthetic substrates. Therefore, we cannot conclude without reservation that these cell wall-bound glycosidase activities are actually capable of degrading polysaccharides in the cell walls. However, the changes in some glycosidase activities during the cell cycle do correspond the changes observed in cell wall constituents [1], in UDP-sugars [3, 4], and in a polymerization activity of UDP-glucose [5] as mentioned above. In particular, the β -galactosidase and β -glucosidase activities, which increase during the G1 phase, may be involved in the active modification of polysaccharides in the cell walls.

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