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# OCCURRENCE OF GLUCURONOKINASE IN VARIOUS PLANT TISSUES AND COMPARISON OF ENZYME ACTIVITY OF SEEDLINGS AND GREEN PLANTS

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**Key Word Index**—*Phaseolus aureus*; *Lilium longiflorum*; *Nicotiana tabacum*; *Zea mays*; glucuronokinase.

**Abstract**—Glucuronokinase (EC 2.7.1.43) activity was detected in etiolated seedlings of corn, mung bean and soybean. Biosynthesis of glucuronokinase is not limited to seedlings, because expanding green leaves of corn produced almost as much glucuronokinase activity as etiolated seedlings when data were expressed on the basis of soluble protein. The enzyme was also present in extracts of tobacco callus and *Lilium longiflorum* pollen, with more enzyme activity obtained from pollen than any other source. Detection of glucuronokinase in green leaves of of mung bean was precluded by the presence of an enzyme inhibitor.

## INTRODUCTION

Glucuronokinase catalyses the reaction D-glucuronic acid + ATP → D-glucuronic acid-1-phosphate + ADP. The reaction is part of the metabolic pathway by which myoinositol is oxidized to glucuronate and then converted to cell wall uronide and pentose [1]. This pathway occurs in a variety of plant tissues, but there is little information concerning the distribution of glucuronokinase. Glucuronokinase is known to be present in mung bean seedlings (*Phaseolus aureus*), but quantitative data are not available [2]. The present work was undertaken to compare glucuronokinase activity in extracts of etiolated seedlings, green leaves and cultured callus with that of the pollen enzyme which was studied earlier in this laboratory [3, 4].

## RESULTS AND DISCUSSION

Results are given in Table 1, and glucuronokinase activity was found in all seedling tissue tested. It was especially high in 3-day mung bean hypocotyls but was also abundant in roots and shoots of etiolated corn seedlings (*Zea mays*). Similar results were

Table 1. Glucuronokinase activity of extracts from various plant tissues

| Enzyme source | Soluble protein (mg/g) | Enzyme activity (nmol/min/mg protein) | Enzyme activity (nmol/min/g) |
|---|---|---|---|
| Ace lily pollen | 102.5 | 13.6 | 1394.0 |
| Tobacco callus | 2.0 | 6.5 | 13.0 |
| Corn (etiolated, 3-day) | | | |
| Shoot | 8.7 | 1.2 | 10.4 |
| Shoot tip (10 mm) | 6.3 | 0.97 | 6.1 |
| Root tip (5 mm) | 9.7 | 0.72 | 7.0 |
| Corn (green leaves) | | | |
| Third leaf (9-day plants) | 4.8 | 0.80 | 3.8 |
| Sixth leaf (27-day plants) | 5.5 | 0.75 | 4.1 |
| Mung bean hypocotyl | | | |
| 3-day plants | 2.0 | 10.3 | 20.6 |
| 6-day plants | 1.2 | 7.8 | 9.7 |
| Mung bean (green leaves)* | 13.7 | < 0.04 | < 0.5 |
| Soybean (3-day hypocotyl) | 3.8 | 0.79 | 3.0 |

*Second trifoliate leaves were used. No enzyme activity was detected. Numerical values represent the least amount of activity that could have been detected.

obtained using root tips from corn seeds germinated for 3 days in sterile Petri dishes. Glucuronokinase activity of extracts from partly expanded green leaves of corn is not markedly different from that of etiolated seedlings when enzyme activity is expressed per mg protein. Hence, the inositol oxidation pathway probably is functioning in older plants where hexose is the starting point as well as in seedling tissue where myoinositol is available as a result of phytate degradation [1]. Only rapidly growing corn tissues were used in this study, and the time course of glucuronokinase appearance and disappearance for particular tissues has not been established. The lack of detectable enzyme activity in green leaves of mung bean may be due to the presence of an enzyme inhibitor rather than the absence of glucuronokinase. A second mung bean leaf extract was not only enzymically inactive, as was the first (see Table 1), but it eliminated the activity of a hypocotyl preparation when the two were mixed before assay. Finding glucuronokinase activity in the tobacco callus extract is consistent with the earlier observation that this culture can convert radioactive myoinositol into cell wall polysaccharide [5]. It would be of interest to know whether a higher level of enzyme activity could be induced by culturing the cells with myoinositol or glucuronic acid.

The activity of lily pollen glucuronokinase reported here is similar to earlier results [3, 4]. The presence of abundant glucuronokinase is consistent with the observed functioning of the inositol oxidation pathway in germinating lily pollen [6]. Pollen remains the preferred source of glucuronokinase, since these cells yielded more glucuronokinase activity than any other plant tissue tested. Since pollen enzyme was available, its specificity was tested using substrate analogues not available earlier. The strict specificity of the enzyme was confirmed because several sugars were not inhibitory when present at 5 mM in the reaction mixture (10-fold higher than the level of the glucuronic acid substrate). These compounds included D-lyxose (a hexokinase inhibitor), L-lyxose, D-allose, 6-deoxy-D-glucose, 2-deoxy-D-glucose, 3-*O*-methyl-D-glucose, and 5-thio-D-glucose. Also, there was no inhibition when 40 mM 2-deoxy-2-fluoro-D-glucose was present during the standard 10 min assay or was incubated for 15 min with the enzyme before glucuronic acid was added to initiate the reaction.

## EXPERIMENTAL

*Plant material. Lilium longiflorum* pollen (cv Ace) was harvested and stored in liquid $N_2$ until use. Pollen moisture content was 10%. Midway corn seeds were rinsed briefly in 95% EtOH, soaked 15 min in 2% sodium hypochlorite, and germinated at 28° in the dark on paper towels moistened daily with 0.1 mM $CaCl_2$. On the third day, roots were 2–5 cm and shoots 1–4 cm long. Tissue to be used as the source of enzyme was excised, rinsed with $H_2O$, blotted and weighed. Mung bean and soybean hypocotyls were harvested from seedlings germinated in Vermiculite in the dark at 28°. Green leaves were partly expanded and still growing at the time of harvest. The leaves from mung beans and 9-day corn attained *ca* one-third of their final length, and those from 27-day corn were 12–21 cm long. The leaves were cut into strips several mm wide before being homogenized. These plants were greenhouse-grown with supplemental lighting except that 9-day corn was raised in a growth chamber (27°, 14 hr day; 24°, 10 hr night). The tobacco callus was derived from leaves of *Nicotiana tabacum*, cv Xanthi [5]. Cells were in the late log stage at the time of harvest, and had been subcultured regularly for 2 years in Murashige and Skoog medium lacking myoinositol. Cells were rinsed briefly with $H_2O$ on a nylon filter, weighed and immediately homogenized.

*Procedures.* The extraction medium used in the present study (1 mM dithiothreitol, 1 mM EDTA, 10 mM HEPES, pH 7.5) was earlier found to be optimum for isolation of the pollen enzyme [4]. Plant material and ice-cold medium were thoroughly homogenized by grinding for 4 min in a chilled mortar except that tobacco callus was ground for 2 min in a Ten Broeck homogenizer. The ratio of medium to plant material was 1 ml/g except that 1.5 ml/g was used for mung bean leaves. The samples were clarified by centrifugation (10 min, 25 000 *g*), and enzyme activity was determined by the assay procedure described earlier [7]. Briefly, the enzyme converted 1 mM D-[$^{14}$C]glucuronic acid to D-[$^{14}$C]glucuronic acid-1-P at 30°, and pH 7.2. Labelled product was separated from unreacted substrate by anion exchange PC and located with a radiochromatogram scanner. The appropriate section of the chromatogram was cut out and placed in a scintillation bottle. In all cases, a linear relationship was established between amount of radioactive product made and vol. of enzyme added to the reaction mixture; proportionality of product appearance with time (usually 5, 10 and 20 min) was also verified. Paper electrophoresis [7] was used to further confirm the identity of the labelled product made by extracts of mung bean hypocotyls, tobacco callus, and 9- and 27-day corn leaves. The protein content of clarified extracts was determined according to ref. [8] after precipitating the protein with 5% trichloroacetic acid.

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## REFERENCES

1. Loewus, F. A. and Dickinson, D. B. (1982) in *Plant Carbohydrates: Intracellular Carbohydrates* (Loewus, F. A. and Tanner W., eds.) Encycl. Plant Physiol., NS13A. Springer, Berlin (in press).
2. Neufeld, E. F., Feingold, D. S. and Hassid, W. Z. (1959) *Arch. Biochem. Biophys.* **83**, 96.
3. Liebowitz, M. D., Dickinson, D. B., Loewus, F. A. and Loewus, M. W. (1977) *Arch. Biochem. Biophys.* **179**, 559.
4. Gillard, D. F. and Dickinson, D. B. (1978) *Plant Physiol.* **62**, 706.
5. Harran, S. and Dickinson, D. B. (1978) *Planta* **141**, 77.
6. Loewus, M. W. and Loewus, F. A. (1980) *Carbohydr. Res.* **82**, 333.
7. Dickinson, D. B., Hopper, J. E. and Davies, M. D. (1973) in *Biogenesis of Plant Cell Wall Polysaccharides* (Loewus, F. A., ed.) pp. 29–48. Academic Press, New York.
8. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265.