The Distribution and Localization of an UDP-Glucose: Flavonol 3-O-Glucosyl Transferase Activity in Pollen

R. Wiermann * and M. Buth-Weber

Botanisches Institut, Westfälische Wilhelms-Universität, Münster

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Summary

The occurrence of UDP-glucose: flavonol 3-O-glucosyltransferase activity in pollen extracts of various plant species was tested. In case of Alnus, Quercus, Narcissus and Tulipa pollen high enzyme activity could be detected. The high level of enzyme activity in Tulipa pollen made short time extraction experiments possible, which showed that the O-glucosyltransferase activity might be located in the pollen wall, possibly in the exine.

Keywords: Pollen wall proteins; UDP-glucose: flavonol 3-O-glucosyl-transferase activity; Tulipa.

Abbreviations: UDP-glucose, uridine diphospho-D-glucose; UDP-rhamnose, uridine diphospho-L-rhamnose; DTE, dithioerythritol.

1. Introduction

Microspores represent an important and, in the case of higher plants, an ubiquitously extensive accumulation area for phenylpropanoids, especially for flavonol glycosides (Sosa and Percheron 1970, Pratviel-Sosa and Percheron 1972, Stanley and Linskens 1974, Wiermann 1979, Wollenweber and Wiermann 1979). In this connection, it is of importance that in maize both, with intact pollen grains as well as with pollen extracts the enzymatic formation of quercetin 3-O-glucoside has been demonstrated from UDP-glucose and quercetin (Larson 1971, Larson and Lonegran 1972). In the course of our studies on the phenylpropanoid metabolism during pollen development in Tulipa cv. Apeldoorn we could demonstrate the separation of two different O-glycosyltransferases and their occurrence in enzyme preparations of the tapetum fraction of the anthers as well as of the mature

* Correspondence and Reprints: Botanisches Institut, Westfälische Wilhelms-Universität, Schlossgarten 3, D-4400 Münster, Federal Republic of Germany.
pollen (KLEINEHOLLENHORST et al., in preparation). In the present paper the localization of one of the two enzymes, the UDP-glucose: flavonol 3-O-glucosyltransferase in mature Tulipa pollen and, furthermore, the distribution of this transferase activity in pollen of other plant species will be described.

2. Materials and Methods

2.1. Plant Material

Pollen of Tulipa cv. Apeldoorn were obtained from plants grown in the greenhouse of the Botanical garden in Münster/Westf. The pollen of the other plant specimens were collected in the area of Münster/Westf.

2.2. Radiochemicals

UDP-D-[U-14C] glucose was purchased from Amersham Buchler, Braunschweig. Quercetin and rutin were obtained from Roth, Karlsruhe; quercetin 3-O-glucoside was a generous gift from Prof. H. D. Zinsmeister, Saarbrücken.

2.3. Enzyme Extraction and (NH₄)₂SO₄ Precipitation

Mature pollen (100 mg/sample) was stirred for various times (0.5, 1, 2, 15 minutes) in 10 ml of a 0.2 M glycine/NaOH buffer (pH 8.75; containing 11 µM DTE and 0.4 M sucrose). Immediately after the exposure to the buffer medium, the suspension was filtered through a GFA filter (Whatman, retention efficiency 1.5 µm). The protein of the filtrate was precipitated with ammonium sulfate (60% saturation) and collected by centrifugation at 42,000 × g for 15 minutes. Each precipitate was dissolved in 2 ml of a 0.2 M glycine/NaOH buffer (pH 8.75; containing 11 µM DTE) and used for the enzyme test (= diffusate; sample 1). The filtered residue was homogenized in a mortar with quartz sand and Polyclar AT in 10 ml of the same buffer (containing 10 µM DTE and 0.4 M sucrose). This homogenate was filtered and the filtrate treated as described above (= residue; sample 2).

2.4. Enzyme Assays

2.4.1. Standard Assay for the Formation of Quercetin 3-O-Glucoside

The incubation assay contained 33.5 nmoles of quercetin (dissolved in 10 µl ethylene glycol monomethylether), 100 nmoles of UDP-D-glucose (containing 0.05 µCi or 0.05 µCi of U-14C glucose), 41 µmoles of glycine/NaOH and 3 µmoles of DTE in a total volume of 240 µl. The reaction was started by the addition of 100 µl protein solution (up to 1 mg/ml). The mixture was incubated at 30 °C for 30 minutes. The reaction was stopped by adding 10 µl glacial acetic acid, and the total solution was applied to Whatman 3 MM paper. The paper was routinely developed in 15% acetic acid. After viewing the paper under ultraviolet light, the product zone was cut out and counted in a toluene scintillation fluid in a liquid scintillation spectrometer (betasint BF 5000; Fa. Berthold, Wildbad, Schwarzwald). The reaction product was identified as in KLEINEHOLLENHORST et al. (in preparation).

2.4.2. Standard Incubation for the UDP-Rhamnose: Flavonol 3-O-Glucoside Rhamnosyltransferase

The standard assay and the identification of the reaction product was carried out as described in KLEINEHOLLENHORST et al., (in preparation).