Ontogeny and Biosynthesis of Isovitexin 7-O-Galactoside in a Mutant of *Silene pratensis* Unable to Glycosylate this Compound in the Petals

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Enzyme activity responsible for the biosynthesis of isovitexin-7-O-galactoside from isovitexin and UDP-galactose can be demonstrated in protein extracts of cotyledons and rosette leaves from *Silene pratensis* plants. This galactosyltransferase activity is absent in stem leaves and petals. "True Km" values of 0.0025 mM for isovitexin and of 8.2 mM for UDP-galactose were found. At infinite concentration of both substrates the "true V_{max}" value was 0.11 mM/min/mg protein. The galactosyltransferase activity was optimal at pH 7.0; the divalent ions Zn and Hg were inhibitory, whereas Ca, Co, Mg, Mn and EDTA were not inhibitory.

Introduction

The glycosylation pattern of the flavone isovitexin in the petals of *Silene pratensis* is determined by the presence of alleles from three different independent loci: *g*, *gl* and *fg* (Brederode *et al.;* Mastenbroek *et al.* [1, 2]). Three, three and two alleles are known respectively for these loci. The dominant alleles of the g-locus, gG and gX, control the binding of glucose and xylose respectively to the free 7-OH group of isovitexin; the recessive g is the third allele (Brederode and Nigtevecht [3, 4]). The recessive allele of the *gl* locus is *gl* and the two dominant alleles *gl R* and *gl A* control the binding of rhamnose and arabinose respectively to the 2″-OH group of isovitexin (Besson *et al.* [5]). Only two alleles have been identified for the *fg* locus: *fg*, the recessive and *Fg*, the dominant, which controls the binding of glucose to the 2″-OH group of isovitexin (Brederode and Nigtevecht [6]). When the recessive alleles are present in the homozygous state at all three loci, only isovitexin is found in the petals. However, when we screened the cotyledons and the rosette leaves of these homozygous recessive lines for flavones, we detected various flavone glycosides, whose chromotographic behaviour resemble that of the flavone glycosides formed in the presence of the dominant alleles *gG* and *glA*. Either loci other than *g*, *gl* and *fg* are involved in the flavone glycosylation in the cotyledons and rosette leaves, or the loci *g*, *gl* and *fg* regulate the presence of the enzymes that catalyze the various glycosylations steps, *i.e. g*, *gl* and *fg* are not structural loci. Steyns *et al.* (preceding paper) showed that the 7-O-glycoside, whose chromatographic behaviour resembles that of isovitexin 7-O-glucoside, was the 7-O-glycoside epimere isovitexin 7-O-galactoside. In the present paper we will describe the biosynthesis of this compound.

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Experimental

Silene pratensis code number 56D (genotype *gg glgl fgfg*, with plants unable to glycosylate isovitexin in the petals) was used for the characterization of the isovitexin-7-O-galactosyltransferase. Determination of K_m and V_{max} was according to van Brederode & van Nigtevecht [3], except that UDP-galactose (0.83 Ci/mol) was used in stead of UDP-glucose.

Results

Incubation of protein extracts of cotyledons of plants *gg*, *glgl*, *fgfg* together with isovitexin and UDP-galactose, resulted in the synthesis of a product which co-chromatographed with isovitexin-7-O-glucoside. Hydrolysis of the formed glycoside, however, revealed that galactose was incorporated at the 7-position and that no epimerisation to glucose had taken place. The reaction proceeded linearly in time for 15 min and a pH optimum was found at pH 7.0. At a final concentration of 2 mM, the divalent cations

Zn and Hg were inhibitory (25% of original activity left), whereas the divalent cations Ca, Co, Mg and Mn and EDTA were not inhibitory. The "apparent K_m" value for UDP-galactose was dependent on isovitexin concentration, the "apparent K_m" value for isovitexin also changed when the UDP-galactose concentration was changed. By extrapolation "true K_m" values of 0.0025 mM for isovitexin and of 8.2 mM for UDP-galactose were found. The "true maximal activity", *i.e.* maximal activity at infinite concentration of both substrates (obtained by extrapolation), was 0.11 mM/min/mg protein. The galactosyltransferase activity changed during the ontogeny of the plant, being most active in the cotyledons with some activity still present in the first to third leaves to develop. No activity could be detected in leaves that developed later. These results are comparable with the results of the ontogenetic studies of Steyns *et al.* on the appearance of flavoneglycosides (Steyns *et al.*, preceding paper). The presence of isovitexin 7-O-galactosyltransferase activity has been tested for in cotelydons of genotypes, which are able to glycosylate isovitexin. These tests, however, are hampered by the epimerase activity which is present. In protein extracts of cotyledons of plants *gG. glgl fgfg*, high radioactivity was present at the isovitexin 7-O-glycoside position upon incubation with isovitexin and UDP-galactose. However, when the 7-O-glycoside formed was hydrolysed the radioactivity was predominantly detected in glucose and not in galactose (only 10–20%), *i.e.* most of the UDP-galactose has been epimerized to UDP-glucose before the sugar is transferred to the 7-position of isovitexin. This epimerase is active in presence of a glucose transferring protein. In the cotyledons with the genotype *gg, glgl,FgFg*, a high incorporation had taken place into an isovitexin 2″-O-glycoside. When this compound was hydrolysed no radioactive galactose but only radioactive glucose was liberated. This binding of glucose to the 2″-OH is controlled by the locus *Fg* (v. Brederode & v. Nigtevecht [6]). At the 7-OH position galactose, but not glucose was incorporated. The incorporation at the 7-OH position is 5–10 times lower than the incorporation at the 2″-OH position.

In cotyledons of a genotype in which no glucose transferring protein is present, as in genotype *gg gl Rgl R fg fg*, no incorporation had taken place into the isovitexin 2″-O-glycoside, whereas the radioactivity present at the isovitexin-7-O-glycoside position had been incorporated exclusively into isovitexin-7-O-galactoside.

Discussion

Enzyme activity responsible for the biosynthesis of isovitexin-7-O-galactoside can be demonstrated in cotyledons and rosette leaves of mutants of *Silene pratensis*, unable to glycosylate isovitexin in the petals. This galactosyltransferase activity is also present in cotyledons of genotypes containing a dominant allele of the isovitexin glycosylating loci *g*, *gl* and *fg*, although its demonstration can be complicated by epimerase activity. The presence of the isovitexin-7-O-galactoside, however, is not regulated by the locus *g*, which controls the binding of glucose or xylose to the 7-OH position of isovitexin (Steyns *et al.*, preceding paper) and until now no gene has been described in *Silene* that controls the binding of galactose to the 7-OH in mature organs, *i.e.* stem leaves and petals. Of course the possibility cannot be excluded that in our population genetic studies we failed to detect the isovitexin-7-O-galactoside because it is in its chromatographic properties difficult to discriminate from the 7-O-glucoside. However, galactosyltransferase activity was absent in stem leaves of the genotypes tested, which is consistent with the abscence of isovitexin-7-O-galactosides in stem leaves (J. M. Steyns, unpublished). It appears therefore that the galactosyltransferase is typical of early ontogenetic stages (cotyledons, rosette leaves).

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