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THE 2″-*O*-GLUCOSYLATION OF VITEXIN AND ISOVITEXIN IN PETALS OF *SILENE ALBA* IS CATALYSED BY TWO DIFFERENT ENZYMES

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Key Word Index—*Silene alba*; Caryophyllaceae; isovitexin 2″-*O*-glucoside; vitexin 2″-*O*-glucoside; MS fragmentation; glucosyltransferases; biosynthesis.

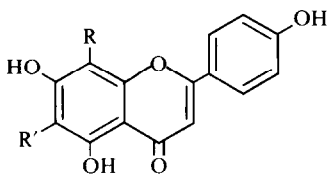
Abstract—Two separate genes, Fg and Vg, which govern the presence of isovitexin 2″-*O*-glucoside and vitexin 2″-*O*-glucoside respectively in the petals of *Silene alba* control different glucosyltransferases. In Vg/Vg,fg/fg plants no isovitexin 2″-*O*-glucosyltransferase was present and in vg/vg,Fg/Fg plants no vitexin 2″-*O*-glucosyltransferase activity could be detected. The Fg-controlled UDP-glucose: isovitexin 2″-*O*-glucosyltransferase has a pH optimum of 8.5, while the Vg-controlled vitexin 2″-*O*-glucosyltransferase has a pH optimum of 7.5. Both glucosyltransferases are stimulated by the divalent cations Ca^{2+}, Co^{2+}, Mn^{2+} and Mg^{2+}. For isovitexin 2″-*O*-glucosylation, however, much higher concentrations are needed than for vitexin 2″-*O*-glucosylation. For UDP-glucose a 'true K_m' value of 0.3 mM with the Fg-controlled and of 0.2 mM with the Vg-controlled enzyme was found. For isovitexin and vitexin these values are respectively 0.09 and 0.01 mM.

INTRODUCTION

Population genetic studies of the petal flavonoids in *Silene dioica* and *S. alba* have revealed a bewildering variation with geography and between species. This is the more remarkable, since the main flavonoid in the petals of both species is always isovitexin [1–4]. In Armenian populations of *S. alba*, however, a different basic flavonoid is present. In this paper, the structure elucidation, genetic control and biosynthesis of one of the compounds present in Armenian populations will be described.

RESULTS AND DISCUSSION

Chromatographic studies on the flavonoid glycosylation pattern of petals of Armenian populations of *S. alba* revealed a compound, **1**, which differed in its chromatographic and other properties from the isovitexin glycosides previously found in petals of *S. alba*. Its chromatographic behaviour suggested it was a flavone glycoside. The methanolic UV spectra, determined according to Mabry *et al.* [5], both in the presence and absence of diagnostic reagents, indicated it was either vitexin or its *O*″-glycoside, i.e. a vitexin derivative in which a second sugar moiety is bound to the carbon–carbon bound glucose. Hydrolysis for 1 hr in 1 N HCl liberated glucose and a mixture of vitexin and isovitexin in the ratio 4:1. The R_f values and the amount of glucose liberated (determined according to Morris [6]) confirm the presence of one glucose molecule in this vitexin derivative. It is therefore an *O*″-glucoside of vitexin. This was confirmed by the mass spectrum of the permethyl derivative M^+ 734 (4%), SO 515 (65%), S 499 (22%), i 355 (16%), j 341 (100%), which showed the fragmentation pattern expected for a permethylvitexin 2″-*O*-hexoside [7]. Co-chromatography of **1** and vitexin 2″-*O*-glucoside from *Cannabis sativa* [8] in five solvents showed complete identity. The 2″-*O*-glucoside of isovitexin, **2**, has been demonstrated before in petals of *S. alba*, its formation being governed by gene Fg [4, 9].



1 R = Glc-β1 → 2-Glc; R′ = H
2 R = H; R′ = Glc-β1 → 2-Glc

The finding of vitexin 2″-*O*-glucoside in the Armenian populations suggests that gene Fg might only be specific for the position of the OH group on the carbon bound glucose but not for the position of attachment of this glucose molecule to the flavonoid nucleus. Thus the only difference between the Armenian and other *S. alba* populations might be the presence of vitexin instead of isovitexin in the petals. In order to test this hypothesis, we tested the vitexin 2″-*O*-glucoside containing plants for isovitexin 2″-*O*-glucosyltransferase activity. To our surprise we failed to demonstrate the conversion of isovitexin into isovitexin 2″-*O*-glucoside in the Armenian plants. Neither were we able to detect the formation of vitexin 2″-*O*-glucoside with vitexin as substrate in other populations of *S. alba*. The formation of vitexin 2″-*O*-glucoside from vitexin and UDP-glucose in Armenian plants, however, proceeded with the same efficiency as the

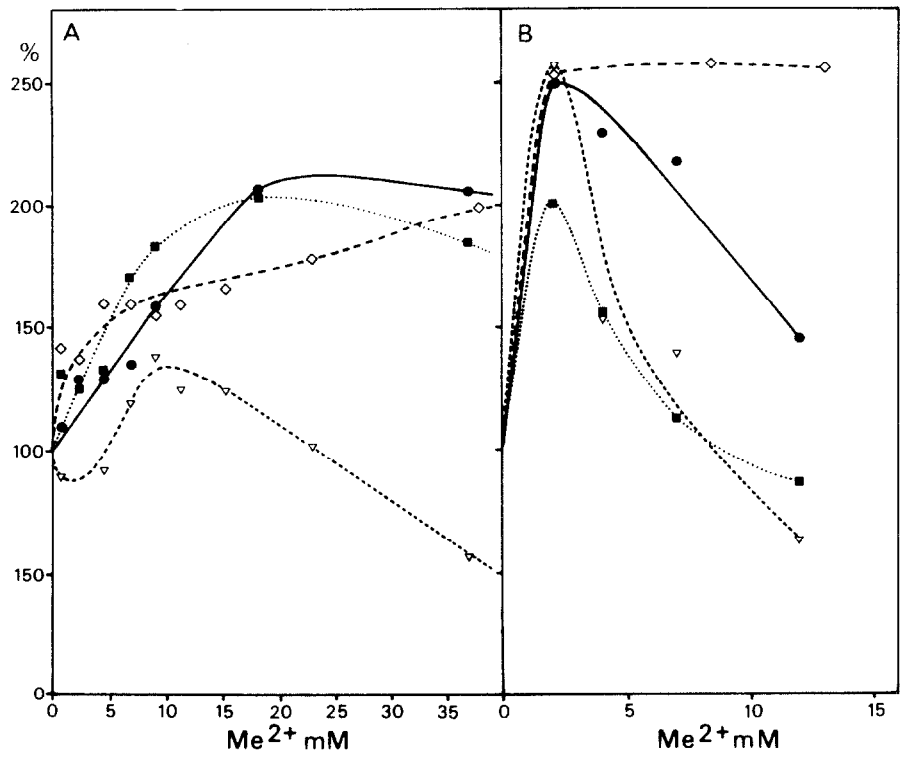


Fig. 1. Effect of divalent metal ions on UDP-glucose: isovitexin 2″-*O*-glucosyltransferase (A) and on UDP-glucose: vitexin 2″-*O*-glucosyltransferase (B). Apart from the indicated additions the reaction conditions were as described in ref. [4]. (◇ ··· ◇) Mg^{2+}, (▽ – – ▽) Mn^{2+}, (■ —— ■) Co^{2+}, and (● ··· ●) Ca^{2+}.

formation of isovitexin 2″-*O*-glucoside from isovitexin and UDP-glucose in plants from other geographical areas. The V_{max}'s of the two enzymes, determined according to Florini and Vestling [10] by extrapolation to infinite concentrations for the two substrates, were 3×10^{-3} and 4×10^{-3} nmol/min, mg protein for isovitexin and vitexin 2″-*O*-glucosylation respectively. The 'true K_m' values were 0.09 and 0.01 mM for isovitexin and vitexin respectively; for UDP-glucose these values were 0.3 mM for isovitexin 2″-*O*-glucosylation and 0.2 mM for vitexin 2″-*O*-glucosylation. Both vitexin and isovitexin 2″-*O*-glucosylation were stimulated by the divalent cations Ca^{2+}, Co^{2+}, Mn^{2+} and Mg^{2+}. The vitexin 2″-*O*-glucosylation showed a sharp optimum at 2 mM for the metal ions Ca^{2+} and Mn^{2+} (2.5-fold stimulation) and Co^{2+} (2-fold stimulation). With Mg^{2+} a saturation curve was obtained (2.5-fold stimulation) (Fig. 1B). For the isovitexin 2″-*O*-glucosylation much higher metal ion concentrations were needed and—apart from Mn^{2+}—no sharp optima were obtained (Fig. 1A). Maximal vitexin 2″-*O*-glucosylation took place at pH 7.5, maximal isovitexin 2″-*O*-glucosylation at pH 8.5.

Thus it can be concluded that the 2″-*O*-glucosylations of isovitexin and vitexin are catalyzed by different enzymes, which are governed by different genes. The gene which controls the vitexin 2″-*O*-glucosylation has been assigned the symbol Vg. However, apart from their differences in substrate specificity, these enzymes are quite similar in their kinetic properties and cofactor requirements. Crosses are in progress to determine the origin and relationships of these two glucosylation genes.

EXPERIMENTAL

Seed of the Armenian *Silene alba* populations was obtained via Dr. E. A. Mennega, Institute of Systematic Botany, University of Utrecht. Collection of plant material, enzyme preparation and enzyme assay conditions were performed as described before. For all assays the PVP/G-50 eluate was used [4]. UDP-glucose ([^{14}C(U)] D-glucose S.A. 230 Ci/mol) was bought from the Radiochemical Centre, Amersham and diluted as described [4]. Vitexin was obtained from Roth. Isovitexin and isovitexin 2″-*O*-glucoside were isolated from *Silene* plants of the appropriate genotypes.

The vitexin *O*″-glucoside was isolated by PC of the MeOH petal extract in H_2O and after elution with 70% MeOH further purified by PC in BuOH–HOAc–H_2O (4:1:5, upper phase) (hR_f 48). The main component was identified as a vitexin *O*″-glucoside by PC in 15% HOAc (hR_f 54) and H_2O (hR_f 46) and by UV spectrophotometry. The spectral data in MeOH and the diagnostic shifts with NaOAc, NaOAc + H_3BO_3, $AlCl_3$, $AlCl_3$ + HCl and MeONa were in each case the same as those given by vitexin. The glycoflavone and sugar liberated after 1 hr hydrolysis in 1 N HCl in a boiling water bath were identified according to [2]. The location of the *O*″-bound glucose was determined by permethylation of the vitexin *O*″-glucoside and recording of the MS of this permethylated compound according to [7, 9] and by co-chromatography with an authentic sample of vitexin 2″-*O*-glucoside from *Cannabis sativa* [8].

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