Phytochemistry, 1979, Vol. 18, pp. 655–656. ©Pergamon Press Ltd. Printed in England. 0031-9422/79/0401-0655 $02.00/0

THE pH DEPENDENT SUBSTRATE SPECIFICITY OF UDP-GLUCOSE: ISOVITEXIN 2″-*O*-GLUCOSYLTRANSFERASE IN *SILENE ALBA*

JAN VAN BREDERODE*, JEAN CHOPIN†, JOHN KAMSTEEG*, GERRIT VAN NIGTEVECHT* and RIA HEINSBROEK*

*Department of Population and Evolutionary Biology, University of Utrecht, Padualaan 8, Utrecht, The Netherlands; †Laboratoire de Chimie Biologique, Université Claude Bernard—Lyon, 43 Bd du 11 Novembre 1918, 69621 Villeurbanne, France

(*Received* 22 *August* 1978)

Key Word Index—*Silene alba*; Caryophyllaceae; flavone *C*-glucosides; glucosyltransferases; isovitexin; pH specificity; chemical genetics.

Abstract—In *Silene alba* plants the dominant allele of gene Fg controls an enzyme which catalyses the formation of isovitexin 2″-*O*-glucoside both in petals and green parts. Both isovitexin and isoorientin can act as substrate. K_m values for the isovitexin glucosylation are 0.09 mM for isovitexin and 0.3 mM for UDP-glucose, V_{max} 0.17 nmol min^{-1} mg $protein^{-1}$. For the isoorientin glucosylation K_m values of 0.45 mM for isoorientin, of 0.75 mM for UDP-glucose and V_{max} of 0.27 nmol min^{-1} mg $protein^{-1}$ are found. The pH optima for both substrates differ markedly. For the substrate with one hydroxyl in the B-ring, isovitexin, the pH optimum is pH 8.5. For isoorientin, which has two hydroxyls in the B-ring, a pH optimum of 7.5 is found. These results suggest that the B-ring hydroxylation pattern influences the pH at which the substrate has optimal affinity for the enzyme. The location of the carbon-carbon bound glucose on a the flavonoid skeleton is of importance for enzyme activity as well. Vitexin, which has glucose at the 8-position, was not a substrate. The glucosylation of vitexin could, however, be demonstrated in enzyme extracts of petals of plants, grown from seed collected in Armenia; in these petals apart from isovitexin glycosides, vitexin glycosides are found as well.

INTRODUCTION

In petals of *Silene alba* the carbon–carbon bound glucose of isovitexin can be substituted with either rhamnose or glucose. In petals of *S. dioica* this glucose is substituted with arabinose. It has been shown that in all cases the 2″-hydroxyl group of the carbon-carbon bound glucose is substituted [1]. The binding of these glycosides is controlled by the genes gl^R [2], Fg [3] and gl^A [2], respectively. The genes gl^A and gl^R behave as alleles [4]; gene Fg segregates independently from the gl locus. For all three genes it has been demonstrated that they are structural genes for the various UDP-glycosyl: isovitexin 2″-*O*-glycosyltransferases [3, 5, 6] which catalyse these substitutions. Isovitexin glycosyltransferase enzymes are present in the green parts of the plant as well [3, 5, 6]. In these other parts, as yet unidentified flavone glycosides are present. Preliminary investigations suggest that in some of these compounds the B-ring possesses two hydroxyl groups. In order to obtain insight into the significance of the activity of the glycosyltransferases in the leaves, the properties of these leaf enzymes have to be investigated. In this paper, the kinetics, influence of hydroxylation pattern on B-ring on pH optimum and the activity towards the 8-carbon-carbon glucosyl-substituted substrate vitexin of the Fg controlled isovitexin 2″-*O*-glucosyltransferase will be described. The genetic control and optimum conditions for activity in the petals of this enzyme have been investigated and described before [3].

RESULTS AND DISCUSSION

In protein extracts of both petals and leaves of *S. alba* plants with a dominant allele of the gene Fg, the formation of isovitexin 2″-*O*-glucoside could be demonstrated. The pH optima coincided and there were no differences in kinetic behaviour. In fg/fg plants this activity was absent. It can therefore be concluded that gene Fg is the structural gene for the UDP-glucose: isovitexin 2″-*O*-glucosyltransferase and that it expresses its activity both in petals and leaves of the plants.

Incubation of the protein fraction isolated from the leaves with isoorientin led to ambiguous results. There was some incorporation into isoorientin 2″-*O*-glucoside but this never exceeded 3–4 times the background. Saleh *et al.* [8] have found that the hydroxylation pattern of the aromatic ring of the substrates has a strong influence upon the pH at which the flavanone synthases present in cell suspension cultures of *Haplopappus gracilis* and parsley express their maximal activity. We therefore tested the isoorientin 2″-*O*-glucoside formation at various pHs. From Fig. 1 it can be concluded that the maximal isoorientin 2″-*O*-glucoside formation takes place at one pH value less than the formation of isovitexin 2″-*O*-glucoside. These results suggest that the ionisation grade of the substrate influences its binding to the 2″-*O*-glucosyltransferase. The hypothesis that the formation of both products, despite the differences in pH optima, is catalysed by the same enzyme is supported by the genetical results: fg/fg plants are neither able to catalyse the formation of the 2″-*O*-glucoside of isovitexin nor that of isoorientin.

Both substrates, tested at the appropriate pH values, exhibited Michaelis–Menten kinetics. However, at the substrate concentrations used, the 'apparent K_m' value for one substrate depended upon the concentration of the second substrate. We therefore determined the 'true K_m' values according to Florini and Vestling [10]. For. the isovitexin 2″-*O*-glucosylation K_m values for

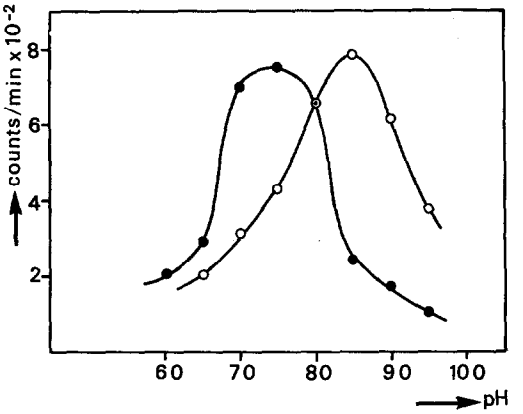

Fig. 1. pH Dependence of isovitexin and isoorientin glucosylation. The reaction mixture contained, in a total volume of 46 μl, 12 μg protein, 100 nmol potassium sodium phosphate, 40 nmol β-mercaptoethanol, 20 nmol UDP-glucose (D-glucose-[^{14}C(U)]) (S.A. 6 Ci/mol), 120 nmol $MnCl_2$, 2 μl of either 1% isovitexin or 1% isoorientin in EGME, 16 mmol glycylglycine buffer. Incubation mixtures prepared in parallel (UDP-glucose, aglycone ommited) were used to determine the pH.

isovitexin of 0.09 mM and for UDP-glucose of 0.3 mM were found. For the isoorientin 2″-*O*-glucosylation K_m values of 0.45 mM for isoorientin and of 0.75 mM for UDP-glucose were found. The V_{max}'s were respectively 0.17 and 0.27 nmol mg min^{-1} protein^{-1}. From this it can be concluded that the flavone 2″-*O*-glucosyltransferase has a higher affinity for the substrate isovitexin, which is normally found in petals of *Silene* plants than for isoorientin. Nevertheless, the affinity for isoorientin is quite high and at enzyme saturating conditions both substrates are glucosylated with about the same velocity.

In petals of *S. alba* plants grown from seed which had been collected in Armenia, apart from isovitexin-glycosides, also vitexin and a derivative of it, in which another glucose molecule is bound to the carbon-carbon bound glucose, are present. However, it is not known at which hydroxyl group of the carbon-carbon bound glucose this glucose is substituted and whether this glucosylation is also catalysed by the Fg controlled 2″-*O*-glucosyltransferase. We therefore tested vitexin as a substrate for the formation of vitexin X″-*O*-glucoside both with protein extracts isolated from petals of these Armenian plants and with protein extracts in which the dominant allele of Fg is present. In the test system, with a protein extract isolation from the Armenian plants, we did find radioactivity on the vitexin X″-*O*-glucoside position (pH optimum 7.5) after 2D chromatography in BAW and H_2O. This activity is absent in the test system with a protein extract of Fg plants, which contain solely isovitexin and its glycosides in the petals. So in *S. alba* different enzymes are responsible for the glucosylation of vitexin and isovitexin, whereas the internal conditions of the plant can determine whether isovitexin or isoorientin is preferentially glucosylated. In *Silene*, so far 3 different sugars (glucose, rhamnose and arabinose) have been found to be attached to the 2″-position of isovitexin, whereas glucose and xylose are known to be bound to the carbon-carbon bound glucose of vitexin. The sugars glucose, xylose [4] and galactose [9] have been identified to be bound to the 7-hydroxyl group. The various isovitexin glycosides are, however, not universally present in *Silene*. There are striking differences, both geographical and interspecies, in the distribution of these compounds. This suggests some physiological and therefore selective significance in the flavone glycoside pattern in *Silene*. However, it cannot be excluded for the moment that these differences are the result of genetic drift.

EXPERIMENTAL

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 [3]. UDP-glucose (D-glucose [^{14}C(U)]) (S.A. 230 Ci/mol) was brought from the Radiochemical Centre, Amersham and diluted as described [3]. Vitexin was obtained from Roth. Isovitexin and vitexin glycosides were isolated from *Silene* plants with the appropriate genotypes. Isoorientin and isoorientin 2″-*O*-glucoside were a gift from Prof. Chopin. Protein was determined according to Lowry *et al.* [7].

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