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BIOSYNTHESIS AND GENETIC CONTROL OF ISOVITEXIN 2″-*O*-ARABINOSIDE IN PETALS OF *SILENE DIOICA*

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Abstract—In petals of *Silene dioica* plants, an enzyme has been demonstrated which catalyses the transfer of the arabinose moiety of UDP-arabinose to the hydroxyl group on the 2″-position of the carbon-carbon bound glucose of isovitexin. The presence of this arabinosyltransferase activity is controlled by the dominant allele gl^A. Maximal activity takes place at pH 7.2–7.5; the reaction is stimulated by the divalent metal ions Mg and Mn. For optimal solubilization of the enzyme, Triton X-100 is necessary. Substrate specificity and kinetic behaviour have been investigated.

INTRODUCTION

Several genes are involved in the glycosylation of isovitexin in the petals of *Silene dioica* and *S. alba*. The genes g^X and g^G [1] control the binding of xylose and glucose respectively to the 7-hydroxyl group of isovitexin. The genes gl^A [1], gl^R [2] and Fg [3] are involved in the arabinosylation, rhamnosylation and glucosylation respectively of the 2″-hydroxyl group of the carbon-carbon bound glucose at the 6-position of the flavone skeleton. Both the genes g^X, g^G and the genes gl^A and gl^R [4] behave as alleles. The loci g, gl, and Fg, however, are not linked [1, 3]. The distributions of the various isovitexin glycosylation genes show remarkable differences between the two species. The genes g^X and gl^A are typical for *S. dioica*, the genes g^G and gl^R for *S. alba* [3]. Gene Fg is found in some Hungarian populations of *S. alba*. As part of our investigations on the biosynthesis and genetic control of the various isovitexin glycosides, we here describe the biosynthesis and genetic control of isovitexin 2″-*O*-arabinoside.

RESULTS AND DISCUSSION

After incubation of isovitexin, UDP-arabinose C-14 labelled in the arabinose moiety and Mg^{2+} with an enzyme preparation isolated from petals of *S. dioica* plants, which possess the dominant allele of gene gl^A, a labelled product was formed which could not be distinguished from authentic isovitexin 2″-*O*-arabinoside by 2D PC in *n*-BuOH–HOAc–H_2O (4:1:5, upper phase) and 1% HCl. The amount of 2″-*O*-arabinoside formed was proportional to added protein and to time for incubation periods up to 10 min. Triton X-100 (0.01% v/v) is necessary for solubilization of the enzyme; in its absence, more than 80% of the total activity in the crude homogenate precipitated after centrifugation for 10 min at 38000 *g*. In the presence of this compound, only 20% stays in the pellet. Removal of inhibitory phenolic compounds by chromatography over a PVP column led to a rise in total activity of about four times. $(NH_4)_2SO_4$ fractionation followed by Sephadex G-50 chromatography gave a 3-fold rise in specific activity. The arabinosyltransferase exhibited maximal ativity at pH 7.2–7.3 (half maximal activities at pH 6.0 and 8.5) both in glycylglycine and in phosphate buffer. The enzyme was stimulated by the divalent ions Mn^{2+} and Mg^{2+} (Fig. 1). High phosphate concentrations (200 mM), Ca^{2+} and EDTA inhibited the reaction. The inhibition by EDTA suggests that the enzyme binds Me^{2+} ions which are not easily removed by $(NH_4)_2SO_4$ precipitation and Sephadex chromatography.

Substrate specificity

Both isovitexin and isovitexin 7-*O*-xyloside can act

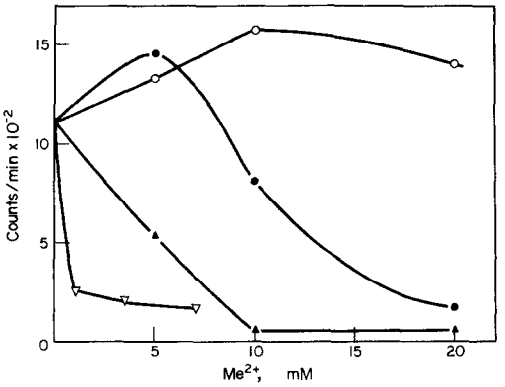

Fig. 1. Effect of divalent metal ions and EDTA on UDP-arabinose; isovitexin 2″-*O*-arabinosyltransferase. Apart from the indicated additions, the reaction conditions were as described in the Experimental. ○—○, Mg^{2+}; ●—●, Mn^{2+}; ▲—▲, Ca^{2+}; ▽—▽, EDTA.

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Table 1. Substrate specificity and genetic control of UDP-arabinose: isovitexin 2″-*O*-arabinosyltransferase

Genotype	Substrate	Sugar donor	cpm	Incorporation into product
gl^A/gl^A	isovitexin	UDP-arabinose	1450	isovitexin 2″-*O*-arabinoside
gl/gl	isovitexin	UDP-arabinose	none	isovitexin 2″-*O*-arabinoside
gl^A/gl^A	isovitexin	UDP-glucose	none	isovitexin 2″-*O*-glucoside
gl^A/gl^A	isovitexin	UDP-rhamnose	none	isovitexin 2″-*O*-rhamnoside
gl^A/gl^A	isovitexin 7-*O*-xyloside	UDP-arabinose	550	isovitexin 7-*O*-xyloside, 2″-*O*-arabinoside
gl^A/gl^A	isovitexin 7-*O*-glucoside	UDP-arabinose	none	isovitexin 7-*O*-glucoside, 2″-*O*-arabinoside
gl^R/gl^R	isovitexin	UDP-arabinose	none	isovitexin 2″-*O*-arabinoside

Counts/min incorporated into product. The Sephadex G-50 eluate fraction was used with the gl^A/gl^A genotypes. With the other genotypes, the 38000 *g* supernatant of the crude homogenate was used. Apart from the variation in the substrates used, the enzyme activities were tested as described in the Experimental. The final UDP-glucose, UDP-arabinose and UDP-rhamnose concentrations were respectively 0.76 mM, 2.5 μM and 3 μM. For isovitexin and the isovitexin 7-glycosides used, 1 % w/v solution in ethylene glycol monomethyl ether was used.

as substrate for the arabinosyltransferase (Table 1). In contrast, isovitexin 2″-*O*-arabinoside-7-*O*-glucoside, a compound found in plants possessing genes g^G and gl^A cannot be synthesized from isovitexin 7-*O*-glucoside by the arabinosyltransferase. It had been shown before, however, that the by gene g^G controlled isovitexin 7-*O*-glucosyl transferase [5] is able to catalyse the formation of the herefore mentioned compound from isovitexin 2″-*O*-arabinoside. The arabinosyltransferase can use neither UDP-glucose nor UDP-rhamnose as substrate: the products: isovitexin 2″-*O*-glucoside and 2″-*O*-rhamnoside are found in Fg and gl^R plants, respectively.

Genetic control

In protein extracts of plants homozygous for the recessive allele gl, no arabinosyltransferase is present. This is not the case when homozygous dominant gl^R alleles are present on the gl locus (Table 1).

Enzyme kinetics

The arabinosyltransferase exhibited Michaelis–Menten kinetics for both isovitexin and UDP-arabinose. Due to the limited amount of UDP-arabinose available (55 nmol in all), all curves intercepted both axes too close to the origin to allow either V_{max} or K_m to be determined accurately. For isovitexin an apparent K_m value of 3 μM was found in the presence of 11 μM UDP-arabinose. This high affinity for isovitexin agrees with our earlier finding on the affinity of the UDP-xylose isovitexin 7-*O*-xylosyltransferase for isovitexin in *S. dioica*, which is also very high [4].

EXPERIMENTAL

Plant material. S. dioica was grown in the open in the experimental garden of the Department of Population and Evolutionary Biology, University of Utrecht. The plants used were grown from seed collected from a *S. dioica* population in western Germany. For collection and storage of petals see ref. [1].

Chemicals. UDP-arabinose (L-arabinose-^{14}C(U)) (S.A. 183 Ci/mol) was supplied by New England Nuclear. Unlabelled UDP-arabinose is not commercially available and we therefore used the compound with the S.A. indicated. The radiochemical purity was checked by PC on Whatman No. 1 in EtOH–1M $CH_3CO_2NH_4$ and in *n*-BuOH–HOAc–H_2O (4:1:5, upper phase) and was in all cases larger than 98%. UDP-glucose (D-glucose-^{14}C(U)) (S.A. 233 Ci/mol) was supplied by the Radiochemical Centre, Amersham. The specific activity was adjusted by addition of carrier to 6 Ci/mol. UDP-rhamnose (L-rhamnose-^{14}C(U)) was prepared according to Kamsteeg *et al.* [7]. A specific activity of 200 Ci/mol was used. For determination of specific activity and preparation of isovitexin and isovitexin glycosides see ref. [5].

Enzyme preparation. All preparations were carried out at 0–4°. 6 g petals were homogenized in an all glass Potter Elvehjem homogenizer in 10 ml 20 mM β-mercaptoethanol, 5% PVP (MW 44000) 0.01% Triton X-100, 50 mM Na–K phosphate buffer pH 7.2 and centrifuged for 20 min at 38000 *g*. The pellet was again extracted with 5 ml of the same buffer and centrifuged for 20 min at 38000 *g*. The supernatants were combined and applied on a PVP-column (MW 700000) 1.5 × 30 cm which was equilibrated and eluted with a 20 mM β-mercaptoethanol–50 mM phosphate buffer pH 7.5 to remove phenolics. The protein fractions were concd by precipitating with an equal vol. $(NH_4)_2SO_4$, pH 7.0. After centrifugation for 20 min at 38000 *g*, the pellet was washed with and suspended in 2 ml 20 mM β-mercaptoethanol–50 mM phosphate buffer, pH 7.5. The concd protein fraction was applied on a Sephadex G-50 column equilibrated and eluted with 20 mM β-mercaptoethanol, 50 mM phosphate buffer, pH 7.5 and the eluate containing the protein was collected in 0.5 ml fractions which were stored at −20°. These fractions were used for the enzyme tests.

Protein assay. Protein was determined according to the method of ref [6] using bovine albumin as a standard.

Enzyme assay. The standard reaction mixture consisted of 2 μl 1% isovitexin in ethylene glycol monomethyl ether w/v, 2 μl UDP-arabinose (L-arabinose-^{14}C(U)) 37 μM, 183 Ci/mol and 25 μl protein fraction. The reaction mixture was incubated for 10 min at 30° and stopped by the addition of 50 μl 15% TCA in MeOH. Together with carrier isovitexin 2″-*O*-arabinoside, the reaction mixture was applied as a spot on Whatman 3 chromatography paper and chromatographed two dimensionally in *n*-BuOH–HOAc–H_2O (4:1:5, upper phase) and 1% HCl. The isovitexin 2″-*O*-arabinoside spot was detected under UV, cut out and counted in a toluene based mixture. The zero time control was 30 counts/min with this procedure.

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