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BIOSYNTHESIS AND GENETIC CONTROL OF ISOVITEXIN 7-*O*-XYLOSIDE IN THE PETALS OF MELANDRIUM ALBUM

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Abstract—An enzyme was detected in petal extracts of *Melandrium album* which catalyzed the transfer of the xylose moiety of UDP-xylose to the 7-hydroxyl group of isovitexin Genetical analysis revealed that the presence of the dominant allele g^X was necessary for enzymic activity This activity was independent of the residual genetic background Xylosyltransferase activity is also present in extracts of $g^G g^X$ plants, in which the product of the enzyme is not detectable Maximal activity was found between pH 7 0 and 7 5, $MnCl_2$ inhibited this transfer The enzyme had an 'apparent K_m' value of 1 0 mM for UDP-xylose and of 0 04 mM for isovitexin

INTRODUCTION

IN THE petals of *Melandrium*, the glycosylation of isovitexin (6-*C*-glucosylapigenin) is governed by the genes g^X, g^G, gl^G, gl^R and Fg[1–5]. Gene g^G transfers glucose and gene g^X xylose to the 7-hydroxyl group of isovitexin.[1,3] Gene g^G is dominant over g^X; i.e. in the presence of both g^G and g^X only the 7-*O*-glucoside of isovitexin is detectable.[3] Gene gl^A controls the transfer of arabinose, gene gl^R the transfer of rhamnose and gene Fg the transfer of glucose to the 6-*C*-glucose of isovitexin.[2,5] Both the 7-hydroxyl and 6-*C*-glucosyl can carry sugar substitutions at the same time. When isovitexin is not glycosylated there is a pronounced effect on morphology; the flowers are small with slender petals which curl up easily Genetical linkage of the various recessive alleles of the glycosylation genes with hypothetical genes determining this morphological trait has been ruled out.[3]

Both the genes g^G and g^X and the genes gl^A and gl^R behave as alleles.[6,7] Gene g^G controls an UDP-glucose · isovitexin 7-*O*-glucosyltransferase[4], gene Fg an UDP-glucose: isovitexin 6-*C*-glucosyltransferase.[5] In this paper the properties of the enzyme controlled by gene g^X will be described.

RESULTS AND DISCUSSION

Enzymic synthesis of isovitexin 7-O-xyloside

When a petal homogenate of *M. album* possessing the dominant allele g^X was incubated with C^{14}-labelled UDP-xylose and isovitexin a radioactive compound was formed. This

[1] VAN BREDERODE, J and VAN NIGTEVECHT, G (1972) *Genen Phaenen* **15**, 3
[2] VAN NIGTEVECHT, G and VAN BREDERODE, J (1973) *Genen Phaenen* **15**, 9
[3] VAN BREDERODE, J and VAN NIGTEVECHT, G (1972) *Mol Gen Genet* **118**, 247
[4] VAN BREDERODE, J and VAN NIGTEVECHT, G (1973) *Mol Gen Genet* **122**, 215
[5] VAN BREDERODE, J and VAN NIGTEVECHT, G (1974) *Biochem Genet* **11**, 65

product was chromatographically identical with carrier isovitexin 7-*O*-xyloside in BAW (4:1:5), *t*-BuOH-HoAc-H_2O (3:1:1) and in 1% aq. HCl. Also after 2D PC the position of the radioactive compound coincided with that of carrier isovitexin 7-*O*-xyloside. The amount of isovitexin 7-*O*-xyloside formed from UDP-xylose and isovitexin was proportional to added protein and to time for incubation periods up to 25 min. Maximal synthesis took place between pH 7.0 and 7.5. The formation of isovitexin 7-*O*-xyloside was inhibited by the addition of Mn^{2+} (Table 1). EDTA, pH 7.5 in a final concentration of 9.7 mM had no influence upon the reaction velocity. The xylosyltransferase had, in the presence of 1.0 mM isovitexin, an 'apparent K_m' value for UDP-xylose of 1.0 mM and in the presence of 2.3 mM UDP-xylose an 'apparent K_m' value of 0.04 mM for isovitexin.

Table 1. Inhibition of UDP-xylose : isovitexin 7-*O*-xylosyltransferase activity by $MnCl_2$.

Concentration (mM)	cpm Incorporated into isovitexin 7-*O*-xyloside	Concentration (mM)	cpm Incorporated into isovitexin 7-*O*-xyloside
$MnCl_2$		$MnCl_2$ cont'd	
0	192	9.7	none
0.6	192	12.9	none
1.9	147	19.3	none
3.8	111	32.3	none
5.8	33	EDTA (pH 7.5)	
7.7	none	9.7	192

The reaction mixtures were as described in Experimental except that the final volume was 50 µl and that $MnCl_2$ was added in the indicated concentrations. Mixtures were incubated for 20 min at 30° and were assayed for the formation of isovitexin 7-*O*-xyloside.

Substrate specificity of xylosyltransferase

The enzyme is also capable of xylosylating 6-*C*-arabinosyl-, 6-*C*-rhamnosyl- and 6-*C*-glucosylglucosylapigenin to their 7-*O*-xylosides, although at a lower velocity (Table 2). The formation of these 6-*C*-glucosylglycosides in *Melandrium* is controlled by the genes gl^A, gl^R and Fq respectively.[1, 2, 5] Apigenin 7-*O*-xyloside-6-*C*-glucosylglycosides are formed when both g^X and a 6-*C*-glucosylglycosylation gene are present.

The xylosyltransferase failed to transfer the glucose moiety of UDP-glucose to the 7-hydroxyl group of isovitexin. This transfer can be demonstrated, however, in petal extracts of *M. album* plants possessing the dominant gene g^{G}.[4]

Genetic control of xylosyltransferase activity

It has been demonstrated that isovitexin 7-*O*-glucosyltransferase activity is under genetic control.[4] In order to find out whether the xylosyltransferase is also under genetic control, plants with different genotypes were screened for xylosyltransferase activity. Table 3 demonstrates that the presence of the dominant allele of gene g^X is necessary for the transfer of the xylose moiety of UDP-xylose to the 7-hydroxyl group of isovitexin. This table also shows that the activity of the g^X controlled xylosyltransferase is independent of other genes. Also in $g^G g^X$ plants, in which the product of the xylosyltransferase, isovitexin 7-*O*-xyloside, is not detectable in the petals,[3] a normal xylosyltransferase activity is present. The genetical and biochemical basis for this discrepancy will be described elsewhere.[6, 7]

[6] van Brederode, J. and van Nigtevecht, G. (1974) *Genetica*, in press.

[7] van Brederode, J., van Wordragen-Bolkenstein, G. and van Nigtevecht, G. (1974) *Mol. Gen. Genet.* in press.

TABLE 2 SUBSTRATE SPECIFICITY OF UDP-XYLOSE ISOVITEXIN 7-*O*-XYLOSYLTRANSFERASE

Sugar–donor	Acceptor	cpm *	Carrier
UDP-xylose	Isovitexin	192	7-*O*-xylosylisovitexin
UDP–xylose	None	none	7-*O*-xylosylisovitexin
UDP–glucose	Isovitexin	none	7-*O*-glucosylisovitexin
UDP-xylose	6-*C*-glucosylglucosyl apigenin	158	7-*O*-xylosyl-6-*C*-glucosyl-glucosyl apigenin
UDP–xylose	6-*C*-rhamnosylglucosyl apigenin	50	7-*O*-xylosyl-6-*C*-rhamnosylglucosyl apigenin
UDP-xylose	6-*C*-arabinosylglucosyl apigenin	132	7-*O*-xylosyl-6-*C*-arabinosylglucosyl apigenin

* cpm Incorporated into 7-*O*-xyloside of acceptor

The reaction vessel contained in a total volume of 29 μl 500 nmol potassium–sodium phosphate buffer pH 7 5, 100 nmol β-mercaptoethanol, 2 μl 1% solution in ethylene glycol monomethyl ether of the flavone to be tested, 20 nmol of respectively UDP-[U-^{14}C]-xylose (sp. act 6 Ci/mol), or UDP-[U-^{14}C]-glucose (sp act 6 Ci/mol), and 125 μg protein The enzyme was tested as described in Experimental The reaction mixture, together with the carrier 7-*O*-xyloside product, was chromatographed on paper (2D) in 1% aq HCl and BAW The carrier was located under UV, cut out, and the radioactivity in the carrier spot determined

TABLE 3 GENETIC CONTROL OF UDP-XYLOSE ISOVITEXIN 7-*O*-XYLOSYLTRANSFERASE

Genotype	Flavone present in petals	cpm*
g^Xg^X *fgfg glgl*	Isovitexin 7-*O*-xyloside	192
g^Xg *Fg glgl*	6-*C*-glucosylglucosyl-7-*O*-xylosylapigenin	195
g^Xg *fgfg* gl^Agl	6-*C*-arabinosylglucosyl-7-*O*-xylosylapigenin	180
g^Xg *fgfg* gl^Rgl	6-*C*-rhamnosylglucosyl-7-*O*-xylosylapigenin	163
g^Xg^G *fgfg glgl*	Isovitexin 7-*O*-glucoside	187
g^Gg *fgfg glgl*	Isovitexin 7-*O*-glucoside	none
g^Gg *Fg. glgl*	6-*C*-glucosylglucosyl-7-*O*-glucosylapigenin	none
g^Gg *fgfg* gl^Agl	6-*C*-arabinosylglucosyl-7-*O*-glucosylapigenin	none
g^Gg *fgfg* gl^Rgl	6-*C*-rhamnosylglucosyl-7-*O*-glucosylapigenin	none
gg fgfg glgl	Isovitexin	none
g^Gg *Fgfg* $glgl^R$	6-*C*-glucosylglucosyl-7-*O*-glucosylapigenin and 6-*C*-rhamnosylglucosyl-7-*O*-glucosylapigenin	none

* cpm Incorporated into 7-*O*-xylosylisovitexin

One *g* petals of plants with a given genotype was homogenized in 5 ml 20 mM β-mercaptoethanol, 5% PVP, 50 mM sodium–potassium phosphate buffer pH 7 5 and centrifuged for 10 min at 38000 *g* 25 μl of this supernatant was added to 2 μl 1% isovitexin in ethylene glycol monomethyl ether and 2 μl 10 mM UDP-[U-^{14}C]-xylose (sp act 6 Ci/mol) The protein content varied between 6–7 mg/ml

EXPERIMENTAL

Plant material M album was grown in the open in the experimental garden of the Genetical Institute, University of Utrecht Seed collected on various original habitats all over Europe was obtained via Dr E A. Mennega, Institute of Systematic Botany, University of Utrecht Crosses were performed according to Nigtevecht [8] For collection and storage of petals, see Brederode and Nigtevecht [4]

[8] VAN NIGTEVECHT, G (1966) *Genetica* **37**, 281

Chemicals UDP-[U-^{14}C]-glucose (S.A. 233 Ci/mol), was supplied by the Radiochemical Centre, Amersham. UDP-[U-^{14}C]-xylose (198 Ci/mol) by New England Nuclear. UDP-glucose and UDP-xylose were purchased from Sigma. The specific activities of the nucleotide sugars were adjusted by addition of carrier to 6 Ci/mol. For determination of specific activity, checking of radiochemical and chemical purity and preparation of isovitexin and isovitexin-glycosides, see Brederode and Nigtevecht [4].

Enzyme preparation. One gram of petals was homogenized at (0–4°) with an all glass Potter–Elvehjem homogenizer in 5 ml 20 mM β-mercaptoethanol 5% polyvinylpyrrolidone (PVP) 50 mM sodium-potassium phosphate buffer pH 7.5 and centrifuged for 10 min at 38000 *g*. To remove endogenous substrate and PVP, the supernatant was passed through a Sephadex G-25 column (30 × 2.5 cm) previously equilibrated with a 4 mM β-mercaptoethanol 10 mM sodium-potassium phosphate buffer pH 7.5. Unless otherwise noted this eluate was used as enzyme source to determine the enzyme properties.

Protein assay. Was determined using bovine albumin as a standard [9].

Assay of UDP-xylose: isovitexin 7-O-xylosyltransferase. The standard reaction mixture consisted of 25 μl enzyme, 2 μl 1% isovitexin in ethylene glycol monomethyl ether w/v and 2 μl 10 mM UDP-xylose labelled uniformly with ^{14}C in the xylose moiety (S.A. 6 Ci/mol). The reaction mixture was incubated for 20 min at 30°. The reaction was stopped by the addition of an equal vol. 1% trichloroacetic acid in MeOH (w/v). Together with carrier isovitexin 7-*O*-xyloside, the reaction mixture was then applied as a band to a 5 × 36 cm strip of Whatman No. 1 paper and chromatographed for 2.5 hr in 1% HCl. The band corresponding with isovitexin 7-*O*-xyloside was detected under UV, cut out, and placed in a scintillation vial. After addition of 20 ml scintillation solvent, composed of 4 g 2,5-diphenyloxazole, 50 mg 1,4-bis-2-(5-phenyloxazoyl)-C_6H_6 in 1 liter toluene, the vial was counted in a Packard liquid scintillation spectrometer (counting yield approximately 75%). The tests were run in duplicate or triplicate. For the determination of the zero time control trichloroacetic acid was added to the reaction mixture before incubation. The zero-time control was 27–33 cpm. The radiochemical purity of the UDP-xylose was tested by PC on Whatman No. 1 in ethanol–1.0 M ammonium acetate pH 3.8 (5:2, v/v) and was for UDP-xylose in all cases > 95%. The chemical purity was checked by UV spectrophotometry at pH 7.0. Ratio $A_{250}/A_{260} = 0.75 \pm 0.03$, $A_{280}/A_{260} = 0.38 \pm 0.02$, $A_{290}/A_{260} < 0.01$, and by acid hydrolysis for 1 min at 100° in 0.01 M HCl followed by identification of the released sugar. The counting error at a gross count of 10000 was normally ±3 and never exceeded ±7 cpm. There was no difference in incorporation rate into isovitexin 7-*O*-xyloside purified by one- and two-dimensional chromatography.

[9] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265.