Structure and Biosynthesis of Vitexin 2″-O-Xyloside in *Silene alba*

Jan van Brederode and Ria Kamps-Heinsbroek

Department of Population and Evolutionary Biology, University of Utrecht, Padualaan 8, Utrecht, The Netherlands

Z. Naturforsch. **36 c,** 484–485 (1981); received February 20, 1981

Silene, Caryophyllaceae, Biosynthesis, Vitexin 2″-O-Xyloside, Xylosyltransferase

In petals of *Silene alba* plants of Armenian populations, vitexin 2″-O-xyloside was demonstrated to be present. The enzyme, catalyzing the transfer of the xylose moiety of UDP-xylose to vitexin, has its optimal activity at pH 7.8. Mg^{2+} and Mn^{2+} stimulated the reaction.

The K_m values were 2×10^{-5} M for vitexin and 3×10^{-5} M for UDP-xylose.

Introduction

With regard to the flavonoid composition of petals of *Silene alba* several chemical races can be recognized. In Western European populations isovitexin 7-O-glucoside is present in the petals, whereas in central and Eastern European populations apart from isovitexin 7-O-glucoside, isovitexin 2″-O-rhamnoside is found. Sometimes isovitexin 2″-O-glucosides are present as well, in Hungarian populations [1–4].

The phenolic composition of Armenian populations differs from the before mentioned populations, as next to isovitexin glycosides, glycosides with another basic skeleton are present. We showed before, that one of these compounds is vitexin 2″-O-glucoside [5]. In this paper the structure elucidation and biosynthesis of another 8-C glycosyl flavone, present in these populations, will be described.

Results and Discussion

Chromatographic studies of petals of Armenian populations showed that next to vitexin 2″-O-glucoside another compound was present as well. The R_f-values were 0.42 in the solvent system BuOH/HOAc/H_2O 4:1:5, upper phase, and 0.39 in 1% HCl.

The spectra of the unknown compound, both with and without diagnostic reagents [6], coincided with those of vitexin 2″-O-glucoside. Acid hydrolysis liberated only xylose and vitexin. From the spectra it could be concluded that all phenolic OH groups were free, so the xylose liberated must have been bound to the C-C bound glucose of vitexin. Cochromatography of the unknown compound with an authentic sample of vitexin 2″-O-xyloside showed identity. So the unknown compound is vitexin 2″-O-xyloside. Its biosynthesis has been elucidated as well. Incubation of vitexin and ^{14}C labeled UDP-xylose with an enzyme preparation isolated from petals of Armenian population resulted in the formation of a compound, which in the solvent systems BuOH/HOAc/H_2O 4:1:5, upper phase, and 1% HCl, cochromatographed with vitexin 2″-O-xyloside.

Maximal incorporation took place at pH 7.8. Of the metal ions tested, Zn^{2+} and Hg^{2+} were inhibitory, Ca^{2+} and Co^{2+} had no effect and Mn^{2+} and Mg^{2+} stimulated the reaction. For Mg^{2+} saturation was obtained at conc. > 2 mM. When Mn^{2+} was used, there was an optimal activity at 2 mM. The true K_m values were 2×10^{-5} M for vitexin and 3×10^{-5} M for UDP-xylose.

By crossing, inbreeding and selection of the appropriate genotypes, both lines with vitexin 2″-O-glucoside and lines with vitexin 2″-O-xyloside in the petals were obtained. Lines with only vitexin 2″-O-xyloside in the petals were unable to catalyze the 2″-O-glucosylation. When the vitexin 2″-O-glucoside plants were tested for xylosyl transferase activity, this activity also appeared to be present, albeit at a reduced rate (25%), as compared with vitexin 2″-O-xyloside plants.

It appears therefore, that either the enzyme catalyzing the glucosylation has a broader substrate specificity, or the gene governing the xylosylation is present in both lines, but as a result of the selection performed, does not come to expression anymore in 2″-O-glucoside lines. Genetic studies are in progress to discriminate between these two possibilities.

Experimental

Plant material. Seed of the Armenian *Silene alba* populations was obtained from Dr. E. A. Mennega, Institute of Systematic Botany, University of Utrecht. From the seeds plants were grown in the experimental garden of the department of Population and Evolutionary Biology, Unversity of Utrecht. Crosses were performed as described before [7].

Reprint requests to Dr. J. van Brederode.

0341-0382/81/0500-0484 $ 01.00/0

Chemicals. UDP-xylose ([U-^{14}C]xylose), 278 Ci/mol, was obtained from New England Nuclear, and deluted with UDP-xylose, bought from Sigma, to 1 Ci/mol. Vitexin was bought from Roth.

Enzyme preparation was the same as described before [8]. The standard reaction mixture consisted of 2 µl vitexin, (1% in EGME), 2 µl UDP-xylose, (10 mM in H_2O, 1 Ci/mol), and 25 µl of the protein fraction. After 1 h incubation the reaction was stopped with 50 µl trichloro acetic acid 15%. The reaction mixture was applied as a spot on Whatman III chromatography paper, together with carrier vitexin 2″-O-xyloside. After two-dimensional chromatography in BuOH/HOAc/H_2O 4:1:5, upper phase, and 1% HCl, the vitexin 2″-O-xyloside spot was cut out and the radioactivity present was counted.

Acknowledgement

The authors are much indebted to Prof. Chopin, Laboratoire de Chimie Biologique, Université de Lyon, 69622, Villeurbanne, France, for the identification of our vitexin O″-xyloside as vitexin 2″-O-xyloside.

[1] J. van Brederode and G. van Nigtevecht, Genen Phaenen **15** (1), 3 (1972).
[2] G. van Nigtevecht and J. van Brederode, Genen Phaenen **15** (1), 9 (1972).
[3] J. van Brederode and G. van Nigtevecht, G. Molec. Genet. **118,** 247 (1972).
[4] J. van Brederode and G. van Nigtevecht, G. Molec. Genet. **112,** 215 (1973).
[5] R. Heinsbroek, J. van Brederode, E. Besson, J. Chopin, G. van Nigtevecht, J. Maas, and J. Kamsteeg, Phytochemistry **19,** 1935 (1980).
[6] T. J. Mabry, K. R. Markham, and M. B. Thomas, The Systematic Identification of Flavonoids, Springer, New York 1970.
[7] G. van Nigtevecht, Genetica **37,** 281 (1966).
[8] J. van Brederode and G. van Nigtevecht, Phytochemistry **13,** 2763, (1974).