Dominance Relationships between Allelic Glycosyltransferase Genes in *Melandrium*: An Enzyme-Kinetic Approach

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Summary. In the petals of *Melandrium* the glycosylation of the 7-hydroxyl group of isovitexin is governed by a series of 4 multiple alleles: g, g, g, and g. Gene g is the structural gene for UDP-glucose: isovitexin 7-0-glycosyltransferase; the alleles g and g are structural genes for UDP-xylose: isovitexin 7-0-xylosyltransferase. Gene g is inactive and does not produce a functional glycosyltransferase. In the presence of both gene g and its allele g the product of gene g (isovitexin 7-0-xyloside) is not detectable. In this respect gene g is dominant over its allele g. In petal extracts of these g/g plants, xylosyltransferase as well as glucosyltransferase, can be detected. The dominance is therefore not a consequence of transcriptional and/or translational control. Enzyme kinetic experiments demonstrated that inhibition of xylosyltransferase by the end product of glucosyltransferase did not occur. Comparison of the enzyme kinetic parameters revealed that dominance is probably caused by differences in Vmax between the two enzymes, both working at saturating isovitexin concentrations. A competition model is proposed which explains why the amounts of isovitexin 7-0-xyloside in g/g and isovitexin 7-0-glucoside in g/g plants are about the same, whereas in g/g plants isovitexin 7-0-xyloside escapes detection. This competition model is supported by the enzyme kinetic results found with the co-dominant allele g.

Introduction

In *Melandrium* five genes, g, g, g, g, and Fg, have been identified which govern the glycosylation of the flavone-aglycone isovitexin. The genes g and g respectively control the binding of glucose and xylose to the 7-hydroxyl group of isovitexin. The genes g and g control respectively the binding of arabinose, rhamnose and glucose to the carbon-bound glucose of isovitexin (van Brederode and van Nigtevecht, 1972a; 1972b; 1974; van Nigtevecht and van Brederode, 1972). The 7-hydroxy- and the 6-C-glycosylsubstitutions can occur together. A summary of the various isovitexin-glycosides which can be formed under the action of the five isovitexin-glycosylation genes is given in Fig. 1.

When various isovitexin 6-C-glucosylglycosylation genes are present, the various 6-C-glucosylglycosides controlled by these genes are formed in about equal amounts. In contrast, only isovitexin 7-0-glucoside, the product of gene g, is detectable on the chromatogram when both the 7-hydroxyglycosylation genes g and g are present. In the presence of gene g the action of gene g seems to be suppressed (van Brederode and van Nigtevecht, 1972a). Genetic coupling studies showed that both the genes g and g and the genes g and g behave as alleles. In 5000 plants, the offspring of several crosses of heterozygous g/g plants with recessive gg plants, no crossover was detected. Nor were crossovers scored in the offspring of heterozygous g/g plants crossed with recessive gil plants, although 3000 plants were investigated.

Enzyme studies showed that the genes Fg, g, g, and g are structural genes. Gene Fg controls an UDP-glucose: isovitexin 6-C-glucosylglycosyltransferase; gene g controls an UDP-xylose: isovitexin 7-hydroxy-xylosyltransferase; gene g controls an UDP-glucose: isovitexin 7-hydroxy-1-glucosyltransferase (van Brederode and van Nigtevecht, 1973; 1974a, b).

There are several possible levels at which the suppression of the manifestation of gene g in the presence of its allele may act:

1. the suppression might act at the translational and/or transcriptional level. In the presence of gene g no product of the structural gene g is formed. As gene g controls an isovitexin 7-0-xylosyltransferase, this activity should then be absent in extracts of these plants.

2. the suppression might act at the enzyme level. In this case there are two alternatives: a) Inhibition of the xylosyltransferase controlled by gene g by the end product of gene g; isovitexin 7-0-glucoside. This can be tested by determining the changes in maximal activity or K of the xylosyltransferase in the presence of various concentrations of isovitexin 7-0-glucoside. In the case of competitive inhibition there would be a change in the K, and in the case of allosteric or non-competitive inhibition a change in the maximal velocity. b) Without the synthesis of an inhibitor, an enzyme E can influence the action of another enzyme E when both enzymes compete for the same substrate. Differences in maximal velocity and/or affinity for the common substrate between the two enzymes can mean that, when both...
enzymes are present, the product of one escapes detection (Fig. 2). In this case three theoretical models are possible: 1) both enzymes possess the same affinity (equal $K_m$) for the common substrate but the maximal activity of $E_1$ (Fig. 2A) is much higher than that of $E_2$. This enables $E_1$ to take away the substrate; at the final gene product level, the gene controlling $E_1$ will be independent of the substrate concentration - dominant over the gene controlling $E_2$. 2) differences in affinity ($K_m$) between the two enzymes for the common substrate can have the consequence that at low substrate concentration all the substrate is taken away by the enzyme with the highest substrate affinity (lowest $K_m$). At saturating substrate concentrations for both enzymes $E_1$ and $E_2$, the dominance will of course be determined by the respective maximal velocities (Fig. 2B). 3) the third possibility is that at high substrate concentration the gene controlling $E_1$ is dominant over the gene controlling $E_2$, whereas at low substrate concentration the opposite is the case. This situation arises when the affinity of $E_2$ for the common substrate $S$ is much higher than the affinity of $E_1$ for $S$, whereas the maximal velocity of $E_2$ is much lower than the maximal velocity of $E_1$. At low substrate concentrations $E_2$ will take away all substrate but, at high substrate concentrations when both enzymes are saturated with substrate, because of the much higher maximal velocity of $E_1$, principally the product of $E_1$ will be detectable (Fig. 2C).

In this paper the results of enzyme-kinetic investigations of the dominance relationships between the allelic genes $g^G$ and $g^X$ are described and a model is proposed to explain the dominance.

Results and Conclusions

Enzyme-kinetic investigations of the isovitexin 7-0-glycosyltransferases revealed that in $g^G/g^X$ Melandrium plants both the 7-0-glucosyl- and the 7-0-xylosyltransferase are present (van Brederode and van Nigtevecht, 1974a, 1974b). Therefore, the suppression cannot act