Theoretical and Applied Genetics 46, 353-358 (1975)

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-hydroxylgroup of isovitexin is governed by a series of 4 multiple alleles: g^G, g, g^X, and $g^{X'}$. Gene g^G is the structural gene for UDP-glucose: isovitexin 7-0-glucosyltransferase; the alleles g^X and $g^{X'}$ 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^G and its allele g^X the product of gene g^X (isovitexin 7-0-xyloside) is not detectable. In this respect gene g^G is dominant over its allele g^X. In petal extracts of these g^G/g^X 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 V_{max} 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^Xg^X and isovitexin 7-0-glucoside in g^Gg^G plants are about the same, whereas in g^Gg^X plants isovitexin 7-0-xyloside escapes detection. This competition model is supported by the enzyme kinetic results found with the co-dominant allele $g^{X'}$.

Introduction

In *Melandrium* five genes, g^G, g^X, gl^A, gl^R, and Fg, have been identified which govern the glycosylation of the flavone-aglycone isovitexin. The genes g^G and g^X respectively control the binding of glucose and xylose to the 7-hydroxyl group of isovitexin. The genes gl^A, gl^R and Fg 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-hydroxyl- and the 6-C-glucosylsubstitutions 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^G, is detectable on the chromatogram when both the 7-hydroxylglycosylation genes g^G and g^X are present. In the presence of gene g^G the action of gene g^X seems to be suppressed (van Brederode and van Nigtevecht, 1972a). Genetic coupling studies showed that both the genes g^G and g^X and the genes gl^R and gl^A behave as alleles. In 5000 plants, the offspring of several crosses of heterozygous g^Gg^X plants with recessive gg plants, no crossover was detected. Nor were crossovers scored in the offspring of heterozygous gl^Rgl^A plants crossed with recessive glgl plants, although 3000 plants were investigated.

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

There are several possible levels at which the suppression of the manifestation of gene g^X 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^G no product of the structural gene g^X is formed. As gene g^X 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^X by the endproduct of gene g^G; isovitexin 7-0-glucoside. This can be tested by determining the changes in maximal activity or K_m 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_m, 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_1 can influence the action of another enzyme E_2 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

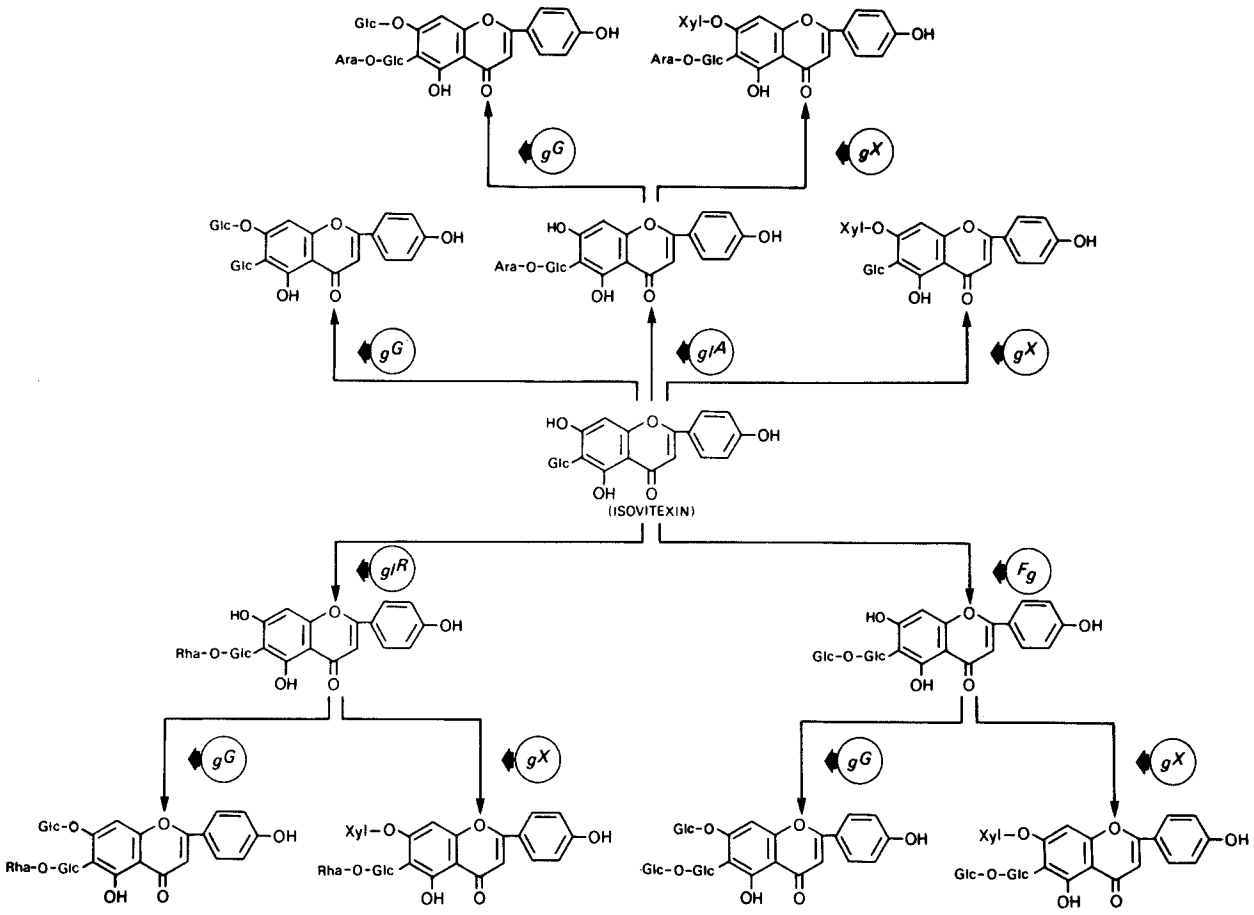

Fig.1. The control of isovitexin-glycosylation in the petals of *Melandrium* by the genes g^G, g^X, gl^A, gl^R, and F_g

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 - independently 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

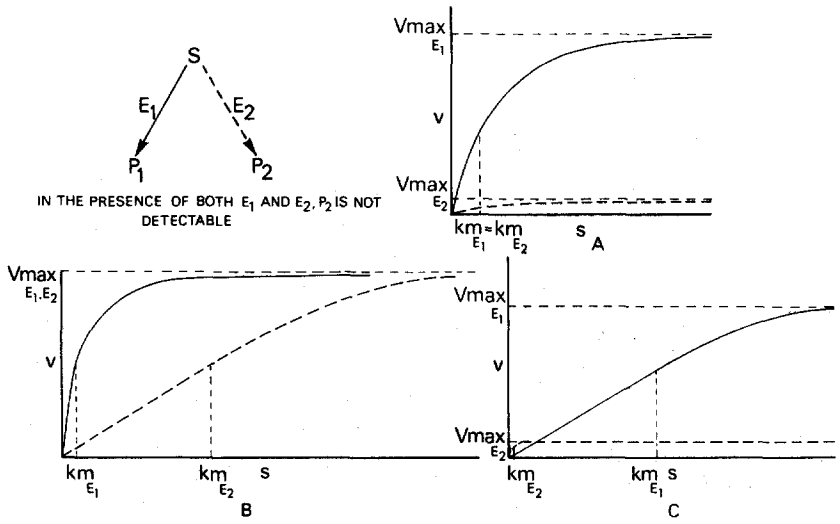

Fig.2. Possible K_m and V_{max} combinations, of two for the same substrate S competing enzymes E_1 and E_2, which result in a reduced synthesis of P_2 the product of E_2

at the translational and/or transcriptional level, for in that case no xylosyltransferase activity should be detectable. The dominance cannot be explained by a change in the genetical background in g^G/g^X plants either. There is no difference in xylosyltransferase activity in extracts obtained from g^Xg^X, g^Xg and g^Xg^G plants (van Brederode and van Nigtevecht, 1974a). Neither could the inhibition of the xylosyltransferase by the end-product (isovitexin 7-0-glucoside) of the gene g^G-controlled 7-0-glucosyltransferase be demonstrated. The various isovitexin 7-0-glucoside concentrations tested had no influence upon the V_{max} or the "apparent K_m" values for UDP-xylose and isovitexin of the xylosyltransferase (van Brederode and van Nigtevecht, 1974c). The dominance can not be explained by differences in affinity for the common substrate isovitexin. To our surprise the "true K_m" for isovitexin (i.e. the K_m for isovitexin determined by extrapolation to infinite concentrations of the second substrate UDP-xylose) was so low, $\ll 0.04$ mM, that it could not be determined exactly in our test system. The "true K_m" value of the glucosyltransferase was much higher (0.17 mM). The maximal velocity of the glucosyltransferase however, was eight times higher than the maximal velocity of the xylosyltransferase in the same plant (van Brederode and van Nigtevecht, 1974d). These results suggest that in g^G/g^X plants the isovitexin concentration is high enough to saturate both the xylosyl- and the glucosyltransferase (Fig.2C). Because of the slower formation of the isovitexin 7-0-xyloside, the common substrate isovitexin is consumed by the glucosyltransferase before a detectable amount of isovitexin 7-0-xyloside has been formed (paperchromatographic analysis).

In petals of g^Xg^X and g^Xg plants, the total amount of isovitexin 7-0-xyloside present corresponds with the total amount of isovitexin 7-0-glucoside present in petals of g^Gg^G, g^Gg, and g^Gg^X plants (van Brederode and van Nigtevecht, 1974c). This discrepancy in rate of synthesis of the different glycosides and the equal amounts of glycosides present in the various genotypes can be explained if we assume that during the development of *Melandrium* petals a certain amount of isovitexin is formed during a restricted period of time, whereas each single glycosylationenzyme is present long enough to glycosylate all the isovitexin because there is no competition. But when both glycosyltransferases are present together, the faster glucosyltransferase will exhaust the supply of isovitexin before a detectable amount of the xyloside has been formed. A schematic representation of this competition model is given in Fig.3. It is supported by the following data: 1) the glycosyltransferases are present in petals of both buds and mature flowers of *Melandrium;* 2) the amount (mg/gr dry weight) of isovitexin present in petals of plants in which recessive alleles are present on all isovitexin glycosylation loci, and the total amount of glycosides present when one or more dominant glycosylation genes are present, are about the same; 3) there is evidence that the enzymes involved in the metabolism of the flavonoid-skeleton are present for a restricted period of time (Hagen, 1966; Bibb and Ha-

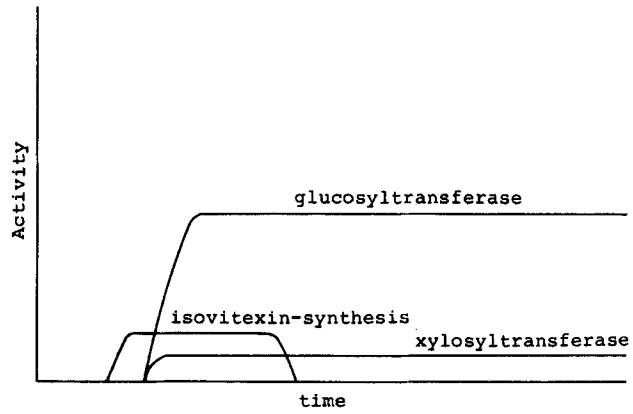

Fig. 3. Schematic representation of the competition model used to explain the dominance relationships between the allelic genes g^G and g^X in *Melandrium*

gen, 1972; Hahlbrock and Wellmann, 1970; Hahlbrock et al. 1971; Wiermann, 1973); 4) in a hybrid swarm of *M. album* and *M. dioicum* from Western Germany a plant was found in which both the 7-O-xyloside and 7-O-glucoside of isovitexin were present in about equal amounts, i.e. the dominance of g^G over g^X had changed into co-dominance. By means of genetical and biochemical studies, it was demonstrated that in this plant gene g^G had not changed (van Brederode and van Nigtevecht, 1974c).

There was no change in the affinity of the xylosyltransferase for both substrates isovitexin and UDP-xylose. However, the maximal activity of the xylosyltransferase in this plant was nine times higher than in the normal g^Xg^X plants (Table 1 last line; van Brederode and van Nigtevecht, 1974d). This increase in activity made the xylosyltransferase equivalent to the glucosyltransferase as a competitor for the common substrate isovitexin. In this way the dominance of g^G over g^X has been changed into co-dominance of g^G and the allele $g^{X'}$, controlling the xylosyltransferase with the higher activity.

Discussion

The concerted action of two alleles is described in terms of dominance. We call an allele A_1 dominant over A_2 when we are unable to discriminate between A_1A_1 and A_1A_2. If the phenotype of A_1A_2 is situated exactly between that of A_1A_1 and A_2A_2, there is no dominance. Partial dominance occurs when the phenotype of A_1A_2 lies closer to one of the homozygotes A_1A_1 or A_2A_2. If the effects of both alleles are detectable in the heterozygote, we call it co-dominance. Overdominance describes the situation where the heterozygote has a higher value than both homozygotes.

An illustrative example is the well documented case of sickle cell anaemia. Persons possessing two Hb^SHb^S sickle cell alleles are strongly anaemic and die at an early age. However, under normal circumstances the heterozygote Hb^SHb^A is not anaemic and can not be distinguished from an Hb^AHb^A individual in this respect. The allele Hb^A is dominant over Hb^S. At low oxygen pressure (flying) the heterozygote develops anaemia which points to partial dominance. However, in regions where certain forms of malaria occur, the heterozygote has a higher "fitness" because of a higher resistance to malaria. At this level there is overdominance. Under the microscope the red blood cells of homozygous Hb^SHb^S persons appear sickle cell shaped. In the heterozygous condition the sickle cell shape appears only at low oxygen pressure. At this level dominance depends upon the circumstances. In the heterozygote both the protein controlled by the allele Hb^A and the protein controlled by the allele Hb^S are detectable (co-dominance). With regard to the concerted action of the two alleles Hb^A and Hb^S we find co-dominance at primary gene product (protein) level, dominance at anaemia and shape of red blood cells level, and overdominance at "fitness" level in regions with certain forms of malaria.

Dominance relationships are not only dependent upon the level of investigation or environmental factors, but also on differences in genetic background.

The inheritance of horns in some breeds of sheep, for instance, is supposed to be controlled by one pair of alleles H and h. The homozygous HH animals are horned in both sexes, the homozygous hh are polled in both sexes, whereas the heterozygous Hh animals are horned in males and polled in females. Apparently the dominance of H is sex controlled, i.e. sex controls or changes the manifestation of characters but not their chromosomal transmission (Serra, 1965a).

Changes in dominance relations have also been described for other changes in the genetic background (Michaelis, 1950; Hallquist, 1953; Serra, 1965b; Hesemann, 1973).

The introduction of electrophoretic and histochemical techniques in genetics offered the geneticist the opportunity to detect allelic isozymes which differed in electrophoretic mobility. In several of these studies it could be shown that differences in the internal milieu of cells at various states of development or from different organs could influence the dominance relationships between al-

Table 1. Comparison of the kinetic parameters of the glycosyltransferases controlled by the allelic genes $g^{X'}$, g^{X} and g^{G}

Enzyme	Controlling gene	Substrate	"True" Michaelis constant (mM)	V_{max} nmoles/min/mg protein
Xylosyltransferase	$g^{X'}$	UDP-xylose	1.8	1569
		isovitexin	$\ll 0.01$[a]	
Xylosyltransferase	g^{X}	UDP-xylose	0.77	184
		isovitexin	$\ll 0.04$	
Glucosyltransferase	g^{G}	UDP-glucose	1	1393
		isovitexin	0.17	

[a] See text

lelic isozymes in these cells (Schwartz, 1964; 1971; Efron, 1973).

It follows that the concept of dominance is hard to define and depends upon the way the problem is approached.

An investigation of the properties of the primary gene product i.e. the enzyme, and of its interaction with changes in the internal and external milieu of the cell, might make it possible to explain the dominance relations in a single framework. Pleiotropic effects, which often accompany dominance, then also might be explained in terms of accumulation or absence of products due to differences in enzyme properties.

In petals of *M.* gene g^{G} is dominant over its alleles g^{X} and g, and codominant with its allele $g^{X'}$. Biochemical studies showed that the allele g^{G} controls an isovitexin 7-0-glucosyl-, and that the alleles $g^{X'}$ and g^{X} control isovitexin 7-0-xylosyltransferases. In homozygous gg plants no active glycosyltransferase is detectable. As in $g^{G}g^{X}$ plants, both the glucosyl- and the xylosyltransferase are detectable, the dominance is not a consequence of transcriptional and/or translational control, but depends solely upon the kinetic properties of the enzymes. From the kinetic properties of the enzymes controlled by the various alleles a competition model could be proposed that explained the dominance relations between these alleles.

The remarkable phenotype of *M.album* petals, when only the recessive alleles of all isovitexin glycosylation genes are present (Brederode and Nigtevecht, 1972a), can be explained in terms of accumulation of a product (isovitexin) which is not further converted. Both *in vitro* and *in vivo* studies have shown that flavonoid-aglycones can influence many physiological processes in the plant (for review, see Brederode, 1974e). This influence is strongly diminished or abolished by glycosylation of the aglycone.

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Received June 9, 1975
Communicated by H. Stubbe

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