GENETIC CONTROL OF LEUCOANTHOCYANIDIN FORMATION IN MAIZE¹

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Received April 17, 1964

IN maize the anthocyanin mutant a_2 accumulates a colorless substance, leucoanthocyanidin, in the aleurone tissue of the kernel. Heating of an acidified-alcoholic mutant extract gives rise to colored anthocyanidin (COE 1955). The biosynthesis of anthocyanin itself is controlled by the complementary factors A_1 , A_2 , C_1 , C_2 , and R . The aleurone tissue is colorless if any one of these factors is homozygous recessive or if dominant inhibitor C^I is present. Pr controls the hydroxylation pattern of the anthocyanin molecule. Bz_1 , Bz_2 , and In control the intensity of anthocyanin in aleurone tissue; the first two result in weak red to bronze color when recessive, and in results in recessive deep purple to black color (COE 1957).

Cross-feeding studies with fresh aleurone tissue of anthocyanin mutants have established a gene-action order for the control of steps in anthocyanin biosynthesis (REDDY and COE 1962; see Table 1A). If the accumulation of leucoanthocyanidin by the a_2 mutant is associated with this pathway, it would be expected that the recessives which block steps prior to that of the a_2 gene should result in the absence of leucoanthocyanidin. Conversely, genes which control steps after a_2 should have no effect on the accumulation of leucoanthocyanidin by the a_2 mutant. This paper presents data supporting close association of leucoanthocyanidin accumulation with the anthocyanin pathway. A preliminary account has appeared in abstract form (REDDY 1963).

MATERIALS AND METHODS

Homozygous double mutant stocks of $c_1 a_2$, $c_2 a_2$, $r a_2$, $in a_2$, $a_1 a_2$, $a_2 bz_1$, $a_2 bz_2$, $a_2 pr$, and $C^I a_2$, and homozygous single mutant stocks of a_1 , a_2 , c_1 , c_2 , r , and C^I were used. The mature dry kernels (10 to 50) of single and double mutants were soaked in water separately for about an hour and the pericarp was removed by peeling. The kernels with exposed aleurone were then soaked in 95 percent ethyl alcohol and shaken on a rotary shaker for 1 to 2 days at room temperature. The resulting extracts were filtered, acidified with 1 percent hydrochloric acid and heated to boiling for 2 to 5 minutes. Whole kernels with exposed aleurone were also heated to boiling in 1 percent acidified-ethanol or acidified-butanol. Depending on the genotype these treatments resulted in the conversion of colorless leucoanthocyanidins to purple or reddish pigments. The measurements of absorption spectra (400 to 600 $m\mu$) of these colored pigments obtained from

¹ This work was carried out at the Department of Botany and Plant Biochemistry, University of California, Los Angeles, while the author was a postdoctoral fellow. Supported by National Science Foundation Grant to Professor B. O. Phinney.

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leucoanthocyanidins were made with a Cary Model 14M recording spectrophotometer. The pigments were further characterized by paper chromatography. Chromatograms were developed on Whatman No. 1 paper in a descending system chromatocab in one dimension with n-butyl alcohol:acetic acid:water (6:1:2). The R_f values were compared with pure cyanidin chloride developed on the same chromatogram.

OBSERVATIONS

The data on accumulation of leucoanthocyanidin in different homozygous mutants are presented in Table 1B. Extracts of $C^1 a_2$, $c_1 a_2$, $c_2 a_2$, $r a_2$, and $a_1 a_2$ remained colorless when boiled with acidified alcohol. However, extracts of $a_2 bz_1$, $a_2 bz_2$, $in a_2$, $a_2 pr$, and a_2 treated similarly, yielded purple or reddish pigment. Previous investigations by COE (1955) showed that $a_2 Pr$ and $a_2 pr$ accumulate leucoanthocyanidin which gives rise to cyanidin and pelargonidin respectively, so these sources can be considered as controls along with cyanidin chloride. Figure 1 shows the absorption spectra of the present pigments and of cyanidin chloride in 1 percent acidified-ethanol; Table 2 presents the absorption maxima and R_f values.

The purple pigments of $a_2 bz_1$, $a_2 bz_2$, $in a_2$, and a_2 gave absorption maxima at 543 to 546 $m\mu$; $a_2 pr$ double mutant gave an absorption maximum at 523 $m\mu$. The $a_2 bz_1$, $a_2 bz_2$, $in a_2$ and a_2 pigments gave average R_f values of 0.43 to 0.46.

FIGURE 1.—Absorption spectra obtained from leucoanthocyanidins after boiling the acidified-alcoholic extracts of aleurone tissue of homozygous mutant kernels: A. $a_2 bz_1$; B. $a_2 bz_2$; C. $a_2 Pr$; D. $a_2 pr$; E. Cyanidin chloride.

TABLE 1
A. Gene-action order in biosynthesis of anthocyanin

	(C ¹)	C ₁	(C ₂)	R	A ₁	A ₂	Bz ₁	Bz ₂	
	→	→	→	→	→	→	→	→	Anthocyanin
B. Presence (+) or absence (−) of leucocyanidin in homozygous double-mutant aleurone tissue									
Genotype	C ¹ a ₂	c ₁ a ₂	c ₂ a ₂	r a ₂	a ₁ a ₂	a ₂ bz ₁	a ₂ bz ₂		
Leucocyanidin	—	—	—	—	—	+	+		

TABLE 2
Identification of anthocyanidins obtained from the leucoanthocyanidins

Homozygous mutants	Average R _f value (n-butanol:acetic acid:water)	Absorption maxima, mμ
a ₂ bz ₁	0.43	543
a ₂ bz ₂	0.45	543
in a ₂	0.43	542
a ₂ Pr	0.46	544
a ₂ pr	0.52	523
Cyanidin chloride	0.46	546

The cyanidin chloride on the same chromatograms gave an average R_f value of 0.46.

Quantitative studies with *in a₂* versus *In a₂* for the accumulation of leucoanthocyanidin showed that the double recessive mutant *in a₂* accumulated five times as much as *In a₂*. The genotypes *in A₂* and *In A₂* were compared for the quantitative production of anthocyanin itself; *in A₂* contained about 15 times more anthocyanin than *In A₂* in the aleurone tissue.

DISCUSSION

Leucoanthocyanidins are compounds which can be converted to corresponding anthocyanidins by boiling extracts with dilute hydrochloric acid (CLARK-LEWIS 1962). This criterion is used for the detection of these compounds in tissue extracts. The a₂ Pr and the double recessive a₂ pr aleurone tissue accumulate leucocyanidin and leucopelargonidin, respectively, as reported previously by COE (1955). The a₂ bz₁, a₂ bz₂, and *in a₂* double recessives accumulate leucoanthocyanidins in aleurone tissue that upon conversion give pigments with spectral and chromatographic properties identical to those from a₂ Pr and cyanidin chloride, demonstrating that these purple pigments are cyanidin. The absorption maximum of a₂ pr pigments is distinctly different from that of all the other mutants and indicates that this mutant accumulates leucopelargonidin.

The recessive gene *in* enhances the production of anthocyanin and leucoanthocyanidin. The mechanism by which the recessive intensifier *in* controls the level of pigment synthesis in the aleurone tissue is obscure. However, the quantitative increase by about fivefold of leucocyanidin in *in a₂* and by about 15-fold

anthocyanin in *in* A_2 aleurone tissues over their *In* counterparts suggests that *in* might act by regulating production of a common precursor of these pigments.

The factors A_1 , C_1 , C_2 , and R must be present in dominant condition for the production of leucoanthocyanidin in a_2 mutant aleurone, since double recessives $a_1 a_2$, $c_1 a_2$, $c_2 a_2$, and $r a_2$ lack this substance. Doubles recessives $a_2 bz_1$ and $a_2 bz_2$, however, accumulate leucocyanidin. This is what would be expected if the bz_1 and bz_2 blocks are sequentially after the a_2 block and the c_1 , c_2 , r , and a_1 blocks before the a_2 block. The absence of leucocyanidin in $C^1 a_2$ shows that the inhibitor (C^1) also blocks its synthesis.

The common requirement of the basic genes A_1 , C_1 , C_2 , and R in dominant condition and the common effects of C^1 , *in*, and Pr factors in control of the production and chemical nature of both leucoanthocyanidin and anthocyanin suggest a close biosynthetic relationship between these two compounds. In cotton flower petals, pseudoalleles G and S were thought to be responsible for the production of leuco-substance and its eventual utilization in anthocyanin synthesis but the later studies, however, have failed to support this hypothesis (STEPHENS 1951). A correlation between the disappearance of leucoanthocyanin and appearance of anthocyanin in the flower color petals of *Impatiens* has led to the conclusion that leucoanthocyanin may act as a precursor in anthocyanin synthesis in this system (ALSTON and HAGEN 1955). Some later studies have, however, suggested that the two pigments are produced simultaneously and there is no apparent correlation (ALSTON and HAGEN 1958). Studies with seed coats of *Phaseolus* have also shown that there is no correlation between the formation of anthocyanin and decrease in leucoanthocyanidins (FEENSTRA 1960).

The present studies clearly show that there is a common gene-controlled pathway for the synthesis of leucoanthocyanidin and anthocyanin. In the absence of more direct evidence, the biosynthetic relationship of leucoanthocyanidin to anthocyanin cannot be stated certainly but appears close.

The author is greatly indebted to DR. B. O. PHINNEY, University of California, Los Angeles for his encouragement and suggestions during the course of this work, and to DR. E. H. COE, U.S. Department of Agriculture and University of Missouri, for the supply of double mutant stocks and for criticism of the manuscript. He thanks DR. T. A. GEISSMAN, University of California, Los Angeles, for the sample of cyanidin chloride.

SUMMARY

The accumulation of leucoanthocyanidin in a_2 mutant aleurone tissue of maize was studied in relation to the established gene-action order for the biosynthesis of anthocyanin pigment. Double recessive combinations of $a_2 bz_1$, $a_2 bz_2$, and *in* a_2 accumulate leucocyanidin, while it is absent in double mutants of $C^1 a_2$, $c_1 a_2$, $c_2 a_2$, $r a_2$, and $a_1 a_2$ aleurone tissue, showing that bz_1 , bz_2 , and *in* do not interfere with this synthesis, whereas C^1 , c_1 , c_2 , r , and a_1 block it. These observations are exactly those predicted by the gene-action order if leucoanthocyanidin formation is closely related to the anthocyanin pathway. The biosynthetic relationship of leucoanthocyanidin to anthocyanin is discussed briefly.

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