GENETICS OF LEUCINE AMINOPEPTIDASE ISOZYMES IN MAIZE¹

L. BECKMAN², J. G. SCANDALIOS, AND J. L. BREWBAKER

Department of Genetics and Department of Horticulture, University of Hawaii, Honolulu

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BY a combination of starch gel electrophoresis and various enzyme staining techniques multiple molecular forms of enzymes have been demonstrated in a large variety of organisms. MARKERT and MØLLER (1959) introduced the concept of an isozyme, defined as one of the molecular forms of an enzyme.

In maize some highly interesting results have been found concerning esterase (SCHWARTZ 1960a, 1962a, b, 1964) and alkaline phosphatase (SCHWARTZ 1960b). This report deals with the genetic control of leucine aminopeptidase (LAP) isozymes in maize. The genetics of this enzyme system has not been studied very extensively and to our knowledge there have been no previous studies of LAP in plants. The only organism in which genetic variations in this enzyme have been demonstrated is *Drosophila melanogaster* (BECKMAN and JOHNSON 1964).

MATERIALS AND METHODS

Maize ears were harvested on the 16th day after pollination. The fresh liquid endosperm from individual kernels was squeezed onto a piece of filter paper (5×5 mm) to be inserted into a starch gel. Kernels stored up to one month under refrigeration provided usable samples. Homogenates were also prepared from leaf, husk, pollen and young seedlings. Starch gel electrophoresis was carried out in a discontinuous buffer system, pH 8.6, (ASHTON and BRADEN 1961) until the brown borate front zone visible in this system had migrated about 7 cm from the sample slot. Following the electrophoretic separation each gel was sliced lengthwise and incubated at 37°C for 40 minutes in a stain solution consisting of 40 mg L-leucyl-beta-naphthylamide-HCl (substrate) and 50 mg Black K salt (diazonium dye coupler) in 100 ml 0.2 M Tris-maleate buffer, pH 6.0. The Black K salt gives a deep purple stain which renders the enzyme bands visible within 40 minutes after incubation.

RESULTS

Electrophoretic variants of leucine aminopeptidase: Four electrophoretic LAP zones were seen in extracts from maize kernels. They were called A, B, C and D in order of decreasing mobility towards the anode (see Figure 1). The B and C zones were close together and could not be separated clearly in short electrophoretic runs. In extracts from leaves, husk and seedlings the D band was absent. There were variations between different inbred lines of maize with respect to the electrophoretic patterns. To demonstrate the inheritance of these variations

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¹ Portion of a Ph.D. dissertation being submitted to the Department of Genetics, University of Hawaii, by J. G. SCANDALIOS.

² Present address: Institute for Medical Genetics, University of Uppsala, Sweden.

FIGURE 1.—Schematic drawing showing the electrophoretic LAP patterns in maize endosperm. 1 = the P39 strain, 3 = the AA4 strain and 2 = F_1 hybrids between AA4 and P39. The arrow shows the direction of migration towards the anode.

two inbred lines were used in crossing experiments: AA4 (U. Hawaii) and P39 (Purdue U.). In the AA4 line both the LAP A and LAP D zones had a faster mobility than the corresponding zones in the P39 line. The results of the crosses have been summarized in Tables 1 and 2.

Genetic control of LAP D enzymes: In the F_1 hybrids between AA4 and P39 both parental LAP D variants ($F + S$) were present (see Figure 2). In backcrosses to AA4 half of the offspring had the fast LAP D enzyme and half had both zones (Table 1). Backcrosses to P39 showed equal proportions of S and ($F + S$) offspring. In the F_2 generation all three types were present in proportions showing a close fit to a 1:2:1 distribution. These results would justify the following interpretation. The electrophoretic variation of the LAP D enzyme in maize is controlled by two alleles acting without dominance. Individuals homozygous for the LpD^F allele display only the fast moving enzyme variant, and homozygotes for the LpD^S allele show only the slow component. In the hetero-

TABLE 1

Results of crosses made to demonstrate the genetic control of the LAP D enzyme variants. The genotypes noted within brackets are deductions fitting the results

Female parent	Male parent	LAP D zones in offspring			Total
		F (F/F)	F+S (F/S)	S (S/S)	
AA4 (F/F)	AA4 (F/F)	30	0	0	30
P39 (S/S)	P39 (S/S)	0	0	30	30
AA4 (F/F)	P39 (S/S)	0	30	0	30
P39 (S/S)	AA4 (F/F)	0	30	0	30
(AA4×P39) (F/S)	AA4 (F/F)	16	14	0	30
AA4 (F/F)	(AA4×P39) (F/S)	12	18	0	30
(AA4×P39) (F/S)	P39 (S/S)	0	12	18	30
P39 (S/S)	(AA4×P39) (F/S)	0	13	17	30
(AA4×P39) (F/S)	(AA4×P39) (F/S)	16	29	15	60

TABLE 2

Results of crosses made to demonstrate the genetic control of the LAP A enzyme variants. The genotypes noted within brackets are deductions fitting the results

Female parent	Male parent	LAP A zones in offspring			Total
		F (F/F)	F+S (F/S)	S (S/S)	
AA4 (F/F)	AA4 (F/F)	30	0	0	30
P39 (S/S)	P39 (S/S)	0	0	30	30
AA4 (F/F)	P39 (S/S)	0	30	0	30
P39 (S/S)	AA4 (F/F)	0	30	0	30
(AA4×P39) (F/S)	AA4 (F/F)	16	14	0	30
AA4 (F/F)	(AA4×P39) (F/S)	17	13	0	30
(AA4×P39) (F/S)	P39 (S/S)	0	10	20	30
P39 (S/S)	(AA4×P39) (F/S)	0	12	18	30
(AA4×P39) (F/S)	(AA4×P39) (F/S)	20	22	18	60

zygous triploid endosperms ($LPD^F/LpD^F/LpD^S$ and $LpD^F/LpD^S/LpD^S$) both enzymes are present.

The LAP D components were well separated on the starch gel and the classification generally met no difficulties. However, an increase of temperature during the electrophoresis caused smearing, especially of the slow LAP zones. There was no clear evidence of strength variations suggestive of gene dosage in the 3n endosperm. Thus in the heterozygotes (Figure 2) the LpD^F allele was contributed by the maternal parent, which might have resulted in a heavier concentration of the fast D zone in the triploid tissue.

Genetic control of LAP A enzyme: The variations of the LAP A enzyme were

FIGURE 2.—Photograph of starch gel showing the LAP enzymes of single seed extracts. 1 = 2 seeds of the P39 strain, 3 = 2 seeds of the AA4 strain and 2 = 6 seeds of the F_1 hybrid between AA4 and P39. The LAP D enzymes are marked by black dots. The LAP A variations are not clearly distinguishable in this relatively short run.

FIGURE 3.—Photograph and schematic drawing of starch gel showing the independence of the LAP A and LAP D variations in the backcross; $(AA4 \times P39) \times AA4$. Samples 3 and 5 are *LpA* heterozygotes and samples 2, 5 and 6 are *LpD* heterozygotes.

not as easily classified as the LAP D variations. The LAP A band was occasionally weak and the difference in electrophoretic mobility between the fast and slow variants was not very great, which can be seen in Figure 2. Figure 3 shows an electrophoretic run with a longer separation time. Samples 3 and 5 have two LAP A bands and the others have the fast variant only. Independent LAP A classifications made by different investigators were always in full agreement.

The results of the crosses are found in Table 2. These crosses involve the same kernels analyzed for the LAP D results in Table 1. In analogy with findings concerning the LAP D enzyme variations, the variations of the LAP A zones can be

TABLE 3

*Summary of backcross data showing recombination between *LpA* and *LpD* genes*

Backcross	Non-crossover	Recombinants		Total
		Number	Percent	
$(AA4 \times P39) \times AA4$	41	19	32	60
$(AA4 \times P39) \times P39$	57	3	5	60
$(AA4 \times P39) \times AA4$ (second sample)	48	6	11	54
Total	146	28	16	174

interpreted in the following way. The electrophoretic variants are controlled by two alleles, acting without dominance. Homozygotes for the *LpA^F* allele show only the fast LAP A band, homozygotes for the *LpA^S* allele have only the slow variant, and heterozygotes have both bands.

There is recombination between the *LpA* and *LpD* loci (see Figure 3). Backcross data (Table 3) showed that the *LpA* and *LpD* genes are linked; out of 174 offspring in the backcross 28 (16 percent) were recombinants.

DISCUSSION

Leucine aminopeptidase is evidently distributed widely in both the plant and animal kingdoms. Multiple molecular forms of LAP have been found previously in human serum (SMITH, PINEDA and RUTENBURG 1962; SMITH and RUTENBURG 1963) and in homogenates of pupae of *Drosophila melanogaster* (BECKMAN and JOHNSON 1964). SMITH and RUTENBURG have demonstrated that the additional LAP enzyme observable in human serum in metastatic cancer of the liver is synthesized in the cancer tissue and also that the extra LAP pregnancy enzyme of serum has its origin in the placenta. In *Drosophila*, BECKMAN and JOHNSON (1964) found one leucine aminopeptidase (called LAP A) occurring in all developmental stages and a series of extra LAP enzymes occurring only in the pupa stage. The *Drosophila* LAP A and one pupal aminopeptidase (LAP D) both showed electrophoretic variations which were under genetic control of two very closely linked loci.

As in man and *Drosophila*, the different multiple molecular forms of LAP in maize seem to occur in particular tissues or in particular physiological stages. The LAP D enzyme was found only in endosperm tissue. It is not known yet what function this enzyme may have in the endosperm.

There were some variations in the proportions of the two enzyme components of the *LpA* and *LpD* heterozygotes, but a strict classification into two types of heterozygotes (due to gene dosage) was not possible. A heterozygous pattern having only the two parental enzymes is much less informative concerning gene dosage than a pattern showing both the parental enzymes and a hybrid enzyme (cf. SCHWARTZ 1960a). In the first case, the expected proportions of the parental enzymes in the endosperm are 2:1, whereas in the latter case it is 4:1. Still more sensitive to gene dosage is a heterozygous pattern formed through random tetramerization of two electrophoretically different gene products (cf. BECKMAN, SCANDALIOS and BREWBAKER 1964). Here the expected proportion of the parental enzymes is 16:1.

Although more detailed work on the linkage relations of the LAP enzymes in maize remains to be done, results from the present backcrosses seem to leave no doubt about the linkage between the *LpA* and *LpD* alleles. It is interesting to note that in *Drosophila* two different LAP systems were closely linked (BECKMAN and JOHNSON 1964). The studies of isozyme linkage are still too scanty to allow any generalizations but the continued work on the chromosomal localization of isozyme nonalleles may be of great importance for the understanding of the evolution of isozymes.

SUMMARY

Four different molecular forms of leucine aminopeptidase (LAP) were found in maize endosperm (zones A, B, C and D) by means of starch gel electrophoresis. The LAP A and LAP D enzymes show electrophoretic variations. Each enzyme is controlled by a pair of alleles acting without dominance. The two loci, *LpA* and *LpD*, are linked.

LITERATURE CITED

- ASHTON, G. C., and A. W. H. BRADEN, 1961 Serum β -globulin polymorphism in mice. *Austr. J. Biol. Sci.* **14**: 248-254.
- BECKMAN, L., and F. M. JOHNSON, 1964 Genetic control of aminopeptidases in *Drosophila melanogaster*. *Hereditas* (in press).
- BECKMAN, L., J. G. SCANDALIOS, and J. L. BREWBAKER, 1964 Catalase hybrid enzymes in maize. *Science* (in press).
- MARKERT, C. L., and F. MØLLER, 1959 Multiple forms of enzymes: Tissue, ontogenic and species specific patterns. *Proc. Natl. Acad. U.S.* **45**: 753-763.
- SCHWARTZ, D., 1960a Genetic studies on mutant enzymes in maize: synthesis of hybrid enzymes by heterozygotes. *Proc. Natl. Acad. Sci. U.S.* **46**: 1210-1215. — 1960b Electrophoretic and immunochemical studies with endosperm proteins of maize mutants. *Genetics* **45**: 1419-1427. — 1962a Genetic studies on mutant enzymes in maize. II. On the mode of synthesis of the hybrid enzymes. *Proc. Natl. Acad. Sci. U.S.* **48**: 750-756. — 1962b Genetic studies on mutant enzymes in maize. III. Control of gene action in the synthesis of pH 7.5 esterase. *Genetics* **47**: 1609-1615. — 1964 Genetic studies on mutant enzymes in maize. IV. Comparison of pH 7.5 esterases synthesized in seedling and endosperm. *Genetics* **49**: 373-377.
- SMITH, E. E., E. P. PINEDA, and A. M. RUTENBURG, 1962 Localization of serum leucine aminopeptidase activity by paper electrophoresis. *Proc. Soc. Exptl. Med.* **110**: 683-687.
- SMITH, E. E., and A. M. RUTENBURG, 1963 Electrophoretic behavior of an aminopeptidase of human tissues and serum. *Nature* **197**: 800-801.