Genetic regulation of the catalase developmental program in maize scutellum: Identification of a temporal regulatory gene

gene expression/enzyme turnover/additive trans-acting gene


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ABSTRACT Genetic and biochemical analyses suggest that the developmental program of catalase (H₂O₂/H₂O₂ oxidoreductase, EC 1.11.1.6) activity in maize scutella is controlled by a temporal regulatory gene (Carl) that is distinct from the structural genes thus far identified. Recombination data show that Carl is located about 37 map units from the Cat2 structural gene on the chromosome 15. Turnover studies indicate that Car1 may act by regulating the rate of catalase synthesis.

The enzymes in specific tissues of an organism usually express a particular developmental pattern (1). Developmental mutants altering the timing for the expression of a given enzyme encoded by a specific gene can be used to study the genetic control of gene expression in eukaryote development. Such mutants have been identified in mice and other organisms (2–6).

In maize, catalase (H₂O₂/H₂O₂ oxidoreductase, E.C. 1.11.1.6) is an enzyme for which the genetic background and developmental program have been thoroughly characterized (7–9). Catalase in maize scutella is coded by two loci, Cat1 (expressed during kernel maturation) and Cat2 (expressed in the scutellum during germination) (9, 10). The expression of these two structural genes is regulated by several factors (10, 11); among these are different synthesis and degradation rates for the catalase gene products (CAT-1 and CAT-2) of Cat1 and Cat2 (10). One inbred line of maize, R6-67, has been found to express an altered developmental program for catalase in the scutellum as compared with the standard profile exemplified by the inbred strain W64A. Herein, we report on the genetic analysis and regulation of the catalase developmental program in maize scutellum.

MATERIALS AND METHODS

The highly inbred maize strains W64A, R6-67, and R6-43 were used in these experiments; these lines are maintained by our laboratory. Seeds were surface-sterilized by soaking in 1% sodium hypochlorite solution for 10 min and were germinated on moist germination paper at a constant temperature of 25°C.

Isolated scutella were ground with a mortar and pestle in 25mM glycylglycine buffer (pH 7.4). The homogenate was centrifuged at 17,000 × g for 30 min.

Enzyme Assays. The supernatant fraction was used to assay for catalase (12), malate synthetase (13), isocitrate lyase (13), aminopeptidase (14), endopeptidase (15), alcohol dehydrogenase (16), malate dehydrogenase (17), glutamic oxaloacetic transaminase (18), and superoxide dismutase (19) activity. Protein concentration was determined by the method of Lowry (20). Starch-gel electrophoresis and staining procedures were performed as described (8).

Determination of Catalase Synthesis and Degradation Rates. Five scutella were incubated in 5 ml of 0.08 M 2-allyl-2-isopropylacetamide/0.2 M sodium acetate, pH 5.6, for 24 hr. As a control, five scutella were incubated in the same buffer without the 2-allyl-2-isopropylacetamide. Both incubation mixtures were supplemented with 100 units of penicillin and 20 mg of mycostatin per ml to prevent microbial contamination and were shaken at room temperature.

2-Allyl-2-isopropylacetamide is an inhibitor of catalase synthesis (21). The degradation rate of catalase can be calculated by measuring the decrease in catalase activity during the period that the scutella were incubated in the 2-allyl-2-isopropylacetamide solution. The amount of catalase synthesis can...
be obtained from the sum of net synthesis and degradation during the incubation period. The rate constants of synthesis ($k_s$) and degradation ($k_d$) were determined as described (22).

**Rocket Immunoelectrophoresis.** The technique of rocket immunoelectrophoresis (23) was modified as follows: Fifty milliliters of 1% Agarose (SeaKem, Rockland, ME)/0.05 M Tris-HCl, pH 8.6, was boiled and then cooled to 57°C, and 25 $\mu l$ of CAT-2 antiserum was added. The gel was poured onto a $23 \times 13$ cm glass plate. Samples (5 $\mu l$) were added to 1-mm-diameter wells. Electrophoresis was conducted at 4°C with 60 V for 24 hr. At the end of the run, the gel was pressed and washed (but not dried) as described (23), except that the electrophoresis buffer was used for the washes and the whole pressing/washing procedure was conducted at 4°C to avoid enzyme inactivation. The gel was stained for catalase (8) by taking advantage of the fact that immunoprecipitated catalase is still active in decomposing $\text{H}_2\text{O}_2$.

**Statistical Procedure.** Frequency distributions expected for the contrasting homozygous genotypes and their heterozygotes were computed for normal distribution from the parental lines and their $F_1$ progeny means and standard deviations. Using these calculated values, points of minimum overlap among the distributions representing these three genotypes were identified as boundaries for classification (24). From these expected frequencies based on the normal distributions of the parental lines and the $F_1$ crosses, expected frequencies were computed for the $F_2$ and backcross generations. The $F_2$ and backcross data were compared with the expected frequencies by goodness-of-fit $\chi^2$ tests.

**RESULTS**

**Catalase Time Course in Inbred Lines R6-67 and W64A.** The catalase activity in scutella of W64A and R6-67 differed significantly. Unlike its activity in W64A, which peaked at days 4–5, catalase activity in R6-67 scutella continued to increase beyond day 5 after germination and maintained an even higher level at later days (Fig. 1A and B). When the catalase activity was expressed as units per scutellum, the activity of catalase in R6-67 after 7 days was about 3-fold that in W64A (Fig. 1A). Because the protein content in R6-67 scutella was higher than in W64A, the specific activity of catalase in R6-67 after 7 days...
was about twice that in W64A (Fig. 1B). Electrophoretic, immunoprecipitative, and genetic analyses indicated that R6-67 and W64A have the same alleles at both structural loci—namely, Cat1' and Cat2Z (data not shown).

The isozyme pattern shift from CAT-1 to CAT-2 was identical in R6-67 and W64A, because they both possessed the same allele for Cat1 and Cat2Z (data not shown). As has been reported elsewhere, this shift is due to differences in the turnover rates between the two gene products, leading to the elimination of CAT-1 and the predominant expression of CAT-2. Furthermore, data from rocket immunoelectrophoresis with CAT-2 antibody showed that the increase in CAT-2 activity was due to an increase in CAT-2 protein rather than to mere activation of the CAT-2 enzyme (Fig. 2), which suggests that line R6-67 was producing more CAT-2 protein than W64A was during the period examined. Immunoelectrophoresis with CAT-1 antibodies showed very low and declining CAT-1 protein (data not shown).

**Constants of Synthesis and Degradation of Catalase.** Six days after imbibition, the degradation rate constants of catalase in the scutella of W64A and R6-67 were similar, but the synthesis rate constants for these two lines showed very significant differences (Fig. 3). These results suggest that the higher level of catalase activity in R6-67 scutella after 6 days was due to a higher $K_s$ value that found with W64A and not due to a smaller $K_d$ value than that found with W64A. In other words, the catalase molecules in R6-67 scutella were synthesized faster than in W64A scutella, but their degradation rate was similar to that in W64A scutella.

**Expression of Other Enzymes in W64A and R6-67 Scutella.**

The developmental patterns of eight other enzymes were examined in W64A and R6-67 scutella. These were the glyoxysomal enzymes isocitrate lyase and malate synthetase (11); superoxide dismutase; alcohol dehydrogenase; malate dehydrogenase; glutamate oxaloacetic transaminase; aminopeptidase; and endopeptidase. The developmental profiles for all these enzymes were very similar.
enzymes (except alcohol dehydrogenase) were identical between the two lines (data not shown). These results also eliminate the possibility that R6-67 possesses a general protease variant.

Genetic Studies. The time course of catalase activity in progeny from reciprocal F1 crosses between W64A and R6-67 were intermediate to those of the two parental levels (Fig. 4). Since the difference in catalase activity between W64A and R6-67 at 10 days was significant, the level of catalase 10 days after germination was used as a marker for genetic studies. Scutella isolated from 10-day-old seedlings of W64A, R6-67, R6-43, R6-67 × W64A, W64A × R6-67, R6-43 × R6-67, F1, and backcrosses were used to measure catalase activity (Table 1). R6-43 was chosen for linkage studies because it possesses the Cat2P allele but has the catalase developmental program of W64A.

The means of catalase activity in W64A, R6-67, R6-43, R6-67 × W64A, W64A × R6-67, and R6-43 × R6-67 illustrate that the pattern of scutellar catalase activity is inherited additively. The F1 scutella tested showed activity levels between those of parental types. Furthermore, the rocket immunoelectrophoresis data with CAT-2 antibody clearly showed that the F1 was intermediate to the parental lines (Fig. 2), which supports the hypothesis that the alleles are additive. By using the statistical approach described, approximate points of minimal overlap were determined for the crosses involving R6-67 and W64A or R6-67 and R6-43 (Figs. 5 and 6). The expected distributions generated to test the hypothesis involved assumptions that the alleles are additive and that errors of measurement of catalase activity are randomly distributed variables (Table 1). The χ²-analysis (Table 2) supports the hypothesis that a major part of the developmental activity program differences between W64A and R6-67, and between R6-43 and R6-67, is due to allelic differences at a single locus.

Inbred line R6-43 has the P allele (electrophoretically distinct from the Z allele) at the Cat2 locus and exhibits the same level
of catalase activity as the standard line W64A (Table 1). The F₁ hybrid, and the segregating generations (F₂ and backcrosses) were analyzed for zymogram phenotypes and for catalase activity (Fig. 6). The zymogram analysis suggested that the P and Z Cat-2 forms were affected similarly. Distributions of data clearly showed a preponderance of parental types when the Cat2 genotype and catalase activity were considered jointly. Goodness of fit χ² tests indicated that independent assortment between these loci is unlikely. Analysis of the data showed that the two genes (Carl and Cat2) may be loosely linked with a recombination value of approximately 37% (Table 3). This was also supported by data based upon units of catalase activity per mg (fresh weight) of scutellum (data not shown).

**DISCUSSION**

The developmental profile of catalase activity in the scutellum of W64A and R6-67 differ significantly (Fig. 1). This is due to a higher activity, as a result of increased synthesis, of the CAT-2 isozyme in R6-67 (Figs. 2 and 3).

Examination of the time courses of other enzymes indicated that only catalase, and to a lesser degree alcohol dehydrogenase, express altered developmental activity profiles in the scutellum of R6-67. In addition, the possibility that R6-67 is a mutant expressing a low protease activity was eliminated. We also have recently demonstrated that the increased alcohol dehydrogenase activity in R6-67 is due to a regulatory gene (Adrl) that acts independently and is unlinked to Carl (25). The identification of Carl and Adrl in R6-67 was merely fortuitous, because we have identified two other inbred strains (S9 and D10) with high catalase activity, like R6-67, but with normal (low) alcohol dehydrogenase activity levels, similar to W64A. The finding that isocitrate lyase and malate synthetase do not vary between the two lines suggests that the higher catalase activity in R6-67 is not due to the promotion of glyoxysome development in the scutellum.

Genetic studies showed that the inheritance of the factor controlling catalase activity was additive. The time course of catalase activity in the F₁ crosses W64A × R6-67 and R6-67 × W64A is intermediate to the two parental levels after the 6th day of germination (Fig. 4). In addition, the means of the F₁ progeny of the crosses R6-67 × W64A, W64A × R6-67, and R6-43 × R6-67 are intermediate to the parental means (Table 1). This is illustrated further by the distribution of F₁ progeny in Figs. 5 and 6. In addition, rocket immunoelectrophoresis with CAT-2 antibody confirmed that the F₁ CAT-2 protein level was between the parental protein levels (Fig. 2).

The crosses were analyzed statistically as described. The values obtained support the hypothesis that the developmental activity program differences between W64A and R6-67 and between R6-43 and R6-67 are due largely to a single gene (Table 2, Figs. 5 and 6). Linkage analysis showed that Carl is approximately 37 map units from the Cat2 structural gene (Table 3).

The fact that Carl and Cat2 appear to be linked further indicates that there is only one gene coding for the higher catalase activity in the day 10 scutellum of R6-67. We have located the Cat2 gene on the distal-half of chromosome 15 (unpublished data). Carl is not on chromosome 1L, since Carl is not linked to Ampl (data not shown), which is located approximately 27 map units from the centromere on chromosome 1L (26).

Thus, our results show that Carl is linked with Cat2 and is located on chromosome 15. Since the regulatory gene is not located adjacent to the structural gene, its action on Cat2 gene expression may be mediated by diffusible substances (trans-acting) which are produced by the regulatory gene.

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