COMPARISONS OF RELATIVE ACTIVITIES OF MAIZE *Adh*$_1$ ALLELES IN HETEROZYGOTES—ANALYSES AT THE PROTEIN (CRM) LEVEL*

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ABSTRACT

The method of high-resolution electrophoresis was employed to compare the relative amounts of enzyme produced by the *Adh*$_1^F$ and *Adh*$_1^S$ alleles in heterozygotes at different stages of development. The results are in complete agreement with those obtained from enzymatic analyses and support the competition hypothesis for the regulation of the alcohol dehydrogenase gene.

ANALYSIS of the alcohol dehydrogenase (ADH) system in maize led to the formulation of the competition hypothesis for the regulation of gene activity (SCHWARTZ 1971). The evidence indicated that the level of ADH activity is set by the limited concentration of a factor which is essential for gene activation. Various alleles of the *Adh*$_1$ gene differ in their ability to compete for the limited activation factor, and the relative competitive abilities of these alleles may vary with stage of development. *Adh*$_1^F$ and *Adh*$_1^S$ compete equally well in the embryo of the maturing kernel and the three ADH dimer isozyme bands of the heterozygote occur in an intensity ratio of 1 FF: 2 FS: 1 SS. However upon germination there is a shift in competitive abilities to the point where the *Adh*$_1^F$ allele competes twice as well as *Adh*$_1^S$ for the limited factor. The two-fold difference in the number of F to S protomers produced is reflected in the 4 FF: 4 FS: 1 SS ratio of isozyme band intensities observed in the zymograms.

The results reported in the previous paper were based entirely on measurements of enzymatic activity. Relative ADH isozyme activities in heterozygotes was determined from densitometric tracings of zymograms and total ADH activities in extracts were calculated from the rate of reduction of NAD in reaction solutions as measured by the rate of increase of absorbancy at 340 nm. The conclusions were based on the assumption that there was a close correlation between enzyme activity and actual level of ADH protein. Since it is unlikely that the specific activities of enzymes produced by two alleles of the same gene would change, and moreover in an uncoordinated fashion, at different stages of development, the above assumption is probably valid. A similar analysis at the protein rather than at the enzymatic activity level is ordinarily very difficult. The allelic isozymes can only be separated on the basis of net charge differences.

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ADH protein bands cannot be detected in gels unless the extracts are concentrated and fractionated prior to electrophoresis, by procedures which result in substantial ADH loss. However I have developed a technique of high resolution immunoelectrophoresis of ADH isozymes which circumvents the above-mentioned difficulties and permits comparison of allelic isozyme concentrations at the protein (CRM) level (SCHWARTZ 1972). The technique involves cross electrophoresis in starch gels of an ADH test sample in the first dimension and of anti-ADH antisera and a marker ADH in the second dimension. In the second dimension electrophoresis, the antiserum and marker ADH migrate toward each other until they meet, and since the ADH-antibody complex is enzymatically active the precipitation line can be revealed by ADH staining. At specific points the migration of the ADH antibodies will be retarded by the ADH isozyme band(s) of the test sample which was previously run in the first dimension (90° shift). The retardation is observed as a deviation from the straight line of antibody-marker enzyme precipitation and peak height is correlated with isozyme concentration in the band. Advantages of the technique, in addition to the high resolution, are that low enzyme concentrations can be detected and neither the antisera nor ADH extract need be pure.

Embryo and seedling extracts of Adh_1^S/Adh_1^F heterozygous material were prepared as described (SCHWARTZ 1971). The extracts were analyzed by the high resolution immunoelectrophoresis technique for comparison of ADH isozyme concentrations, at the protein (CRM) level, in the two stages of development. The results, presented in Figure 1, are in complete agreement with the results obtained from the enzymatic analyses and clearly show differences in the ratio of Adh_1^F and Adh_1^S gene products. In the embryo extract the FS heterodimer

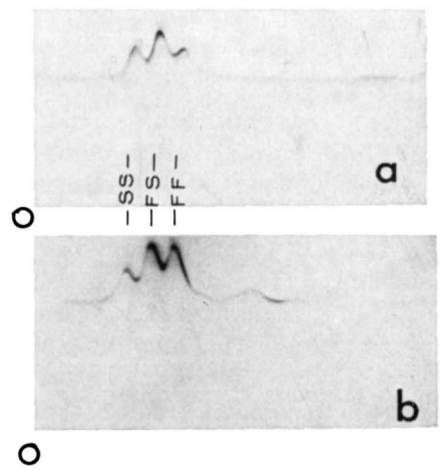

FIGURE 1.—Immunoelectrophoretograms of ADH isozymes in (a) embryo and (b) seedling exartcts. The dimer isozymes, designated SS, FS, and FF, migrated to the right in the first dimension from the origin, O (see text). The anti-ADH antiserum was obtained by immunizing rabbits with purified ADF^{FF} enzyme. ADH^{CC}, which is the fastest migrating isozyme, is routinely used as marker ADH.

peak is the highest and the FF and SS homodimer peaks are lower and about equal. In the seedling extract the FF and FS peaks are about equal in height and the SS peak is much reduced. This method is only semi-quantitative but the differences in the concentration of allele products in the two extracts are clear and consistent with dimer ratios of 1 FF: 2 FS: 1 SS and 4 FF: 4 FS: 1 SS.

LITERATURE CITED

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