

be branched as hydrolyzed amylose does not have any effect.

The protein component has been characterized and partially purified. It is heat labile and has a molecular weight of approximately 120,000.

Inactivation has been shown to involve only the active site of the enzyme without altering the overall configuration of the molecule, since the inactivated enzyme retains its antigenic specificity. The inactivation is reversible; 70-80% of initial activity can be recovered by 2-3 hours incubation at 55°C. This reactivation may result from destruction of the heat labile protein component in the complex.

This two factor system might be involved not only in the control of ADH activity in the endosperm, but also in inactivation of ADH in other tissues, such as the root and the plumule during germination.

The role which this system plays in vivo is being tested by the use of a mutant which does not synthesize the protein factor in the embryo.

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3. Genetic differences between ADH₁ isozymes revealed by dissociation and reassociation experiments.

Two unlinked alcohol dehydrogenase (ADH) genes, Adh₁ and Adh₂, are found in maize (Schwartz, 1966, Freeling and Schwartz, 1973). ADH₁-FF and ADH₁-SS, the products of two alleles at the Adh₁ locus, differ in their electrophoretic mobilities.

A dissociation and reassociation procedure (freezing in high salt followed by thawing and dialysis) described by Hart (1971) has been adopted recently for dissociation and reassociation studies of ADH in maize. These studies lend further support for the dimeric structure of these isozymes, as concluded from genetic and electrophoretic analysis (Schwartz and Endo, 1966).

If reassociation is a random process, mixtures of crude extracts with equal ADH activities from Adh₁^F/Adh₁^F and Adh₁^S/Adh₁^S kernels should yield dimers in a ratio 1 FF:2 FS:1 SS upon dissociation and reassociation, comparable to in vivo subunit assembly in Adh₁^F/Adh₁^S heterozygotes.

In zymograms of such reassociated extracts a deviation from this expected ratio is observed. The isozyme band pattern obtained approximates a ratio of 4 FF: 4 FS: 1 SS, as if only one half of the ADH₁^S

monomers recombined into active enzyme. This result suggests that the ADH_1^S monomers are less stable than ADH_1^F monomers under the conditions of treatment.

The addition of Zn^{++} during dialysis, when the subunits are re-associating, has a striking effect in shifting the ratio back to 1 FF: 2 FS: 1 SS, and increasing the amount of activity which is restored. Without Zn^{++} , the average activity recovered for Adh_1^S/Adh_1^S extracts was 12% of the undissociated control and 75% for the Adh_1^F/Adh_1^F extracts. With the addition of Zn^{++} , the activities recovered for Adh_1^S extracts increased to 60% while the Adh_1^F recovery was unchanged. Our results indicate that Zn^{++} is necessary for the reassociation of ADH_1^S monomers to form active enzyme.

Preliminary results obtained with a modification of this procedure, which yields almost 100% active enzyme upon reassociation, suggest that dissociated F monomers bind Zn^{++} more strongly than do the S monomers.

Experiments are currently underway to determine whether or not zinc plays a role in the dimerization process itself.

References:

- Hart, G. E., (1971) *Molec. Gen. Genetics* 111:61.
 Freeling, M. and D. Schwartz, (1973) *Biochemical Genetics* 8:27.
 Schwartz, D., (1966) *Proc. Nat. Acad. Sci.* 56:1431.
 Schwartz, D. and T. Endo, (1966) *Genetics* 53:709.

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4. The products specified by two, unlinked alcohol dehydrogenase genes in maize are immunologically similar.

One way to identify similarities in the primary structures of two different enzyme subunits is to ascertain whether any antibody specified against one subunit will cross-react with the other subunit. The antigen-antibody reaction is extremely specific (see Reichlin, 1972, *J. Mol. Biol.* 64, 485). There are two unlinked alcohol dehydrogenase (Adh genes; ADH enzyme, EC 1.1.1.1.) genes in maize: Adh₁ and Adh₂. Their products dimerize into three electrophoretically separate enzymes of the same molecular size: Set I (ADH1•ADH1), Set II (ADH1•ADH2) and Set III (ADH2•ADH2)