(2) only degradation (or inactivation) without further formation of active enzyme molecules.

We have noticed that the time-course of ADH activity after germination remains unchanged in the presence of a protein synthesis inhibitor (cycloheximide, 10ug/ml) or an RNA synthesis inhibitor (actinomycin D, 50ug/ml). This suggests that the control of ADH activity is independent of transcription and translation. Furthermore, by employing density labeling techniques we found that ADH molecules from seeds germinated in 70% D_2O and 10 mM $^{15}NH_4Cl$ for 36 hrs have exactly the same buoyant density as the ADH from seeds germinated in H_2O and $^{14}NH_4Cl$, clearly showing that there is no turnover of ADH molecules (at least for ADH-2, which is the most dominant ADH isozyme after germination). Thus, we have established that there is no de novo synthesis of active ADH molecules after germination. What then is the mechanism controlling the decrease in ADH activity? Is it due to degradation by a protease or the activation of a specific inhibitor? In reciprocal mixing experiments using the scutellar extract from early stages (e.g., 2 hr imbibed) and that from later stages of germination, we observed a significant decrease in the original ADH activity in the early stage extract, indicating the presence of an inhibitory substance(s) generated in the later stages of germination. The inhibition can be prevented by beta-mercaptoethanol; this in addition to other experiments eliminated the possibility that the inhibitor is a non-specific protease. The amount of inhibitor, assuming it is proportional to the extent of inhibition, increases steadily after 24 hrs of germination; simultaneously, ADH activity begins to decline. This fact leads us to propose that the generation of this inhibitor actually accounts for the decrease in ADH activity following germination.

The inhibitor is being purified by affinity chromatography, and preliminary data indicate that it is a protein of small molecular weight. A detailed account of the inhibitor and its role in ADH regulation is being published elsewhere.

D.T.H. Ho* and J.G. Scandalios

*Washington University, St. Louis, Missouri

Substrate specificities and kinetic data for genetically defined maize aminopeptidases — Aminopeptidase isozymes from maize have been investigated with respect to substrate specificity and apparent K_ms. Four aminopeptidases — LAP-A, LAP-B, LAP-C and LAP-D — exist in maize and are resolved by starch gel electrophoresis; the maize aminopeptidases had earlier been designated by the restrictive name leucine aminopeptidase. A fast and a slow variant have been found for each isozyme. The direction of decreasing migration to the anode is A to D at pH 7.0.

Formal genetic analysis has shown that the LAP-A variants and the LAP-D variants are controlled by the LpA and LpD loci (Ed. note — Lp and Lp2 loci, respectively), and that the LpD and LpA genes are linked (Beckman, Scandalios and Brewbaker, Genetics 50:899-904, 1964).

Qualitative studies of the substrate specificities of the aminopeptidases were made by staining gel slices with various amino acid-naphthylamides as substrates. The source of enzyme was immature liquid endosperm from maize kernels collected 19 days after pollination. The LAP-A band preferentially cleaves the naphthylamide derivatives of the basic amino acids arginine and lysine. The B and C bands cleave the non-polar methionine, leucine and phenylalanine naphthylamide derivatives to the greatest extent as judged by the staining intensities of the zymogram bands. All other amino acid-naphthylamides are cleaved to lesser extents by one or more of the aminopeptidase forms except for the derivatives of histidine and the acidic amino acids and their amides, which are not hydrolysed.

In order to make quantitative measurements of aminopeptidase activity, the isozyme bands were cut individually from the starch gel; a 2mm gel slice was stained for aminopeptidase activity and then used as a template by placing it over the rest of the starch gel and cutting the stained regions out with a sharp scalpel. The starch strips were pressed through a syringe into a centrifuge tube and then spun at 35,000 g for 30 minutes. The supernatant was used as the source of aminopeptidase activity, which is assayed by a modification of the Goldbarg-Rutenburg method (Goldbarg and Rutenburg, Cancer 11:283-291, 1958).

Table 1-A shows the relative activities of the aminopeptidase isozymes separated in this manner toward arginyl-, leucyl- and alanyl-naphthylamide derivatives. For each isozyme the activity with the substrate most hydrolysed is given an arbitrary value of 100, and the activities toward the other two substrates are expressed relative to 100. The isozymes B_S and C_S run close together, and it is difficult to avoid cross-contamination completely when cutting the bands from the gels. This is probably the reason for the higher relative activity of the B_S variant toward leucine-NA and alanine-NA than B_F. Only data for the C_S variant are given because of lack of sufficient material from those lines having the C_F variant.

Table 1-B gives the apparent K_ms of the aminopeptidase forms. Where data are not available, the activity obtainable from the gel eluant was not high enough to make an accurate K_m determination for a particular substrate. The best fit linear regression line of substrate concentration^{-1} versus velocity^{-1} was used to calculate the apparent K_m; in most cases the values of two independent determinations are listed.

Table 1.

A. Relative activities of aminopeptidase isozymes toward three amino acid naphthylamides. The highest activity with a given substrate is expressed arbitrarily as 100 and the activities with the other substrates are given in values relative to 100.

B. Apparent K_ms for aminopeptidase isozymes for the given amino acid-naphthylamide.

A. % Relative Activity				B. Apparent K_ms		
Arginyl-NA	Leucyl-NA	Alanyl-NA	Isozyme*	Arginyl-NA	Leucyl-NA	Alanyl-NA
100.0	8.06	3.30	A_F	3.39×10^{-5} $2.58 \times$ "	---	---
100.0	7.66	4.93	A_S	$3.71 \times$ " $2.24 \times$ "	---	---
20.8	100.00	3.76	B_F	31.2×10^{-5} $22.4 \times$ "	8.14×10^{-5} $12.3 \times$ "	---
67.9	100.00	34.3	B_S	$29.3 \times$ " $28.2 \times$ "	$8.07 \times$ "	---
50.8	100.00	20.3	C_S	$29.3 \times$ " $26.4 \times$ "	$10.4 \times$ " $9.12 \times$ "	---
100.0	28.5	80.0	D_F	6.00×10^{-5} $4.81 \times$ "	---	57.9×10^{-5}
100.0	35.2	97.0	D_S	$5.88 \times$ "	---	$98.6 \times$ "

*The subscripts F and S refer to the fast and slow variants of the A, B, C and D aminopeptidases.

The aminopeptidases fall into three main groups. The LAP-A fast and slow variants, which hydrolyse arginine-NA and lysine-NA to the greatest extent, have a comparatively low K_m for arginine-NA. The B and C bands, which preferentially cleave non-polar amino acid-NA's, have consistently lower K_ms for leucine-NA than for the arginine derivative. The third group, LAP-D_F and LAP-D_S, have approximately equal maximal velocities for arginine-NA and alanyl-NA, but the K_m for alanyl-NA is considerably higher than for arginine-NA.

In addition to the relative velocities and K_ms for various substrates, we are also investigating the heat stabilities, pH optima and molecular weights of the isozymes and the effect of various chemicals on the aminopeptidases. The data obtained in these studies will be correlated with information concerning the genetic control of the aminopeptidases.

The LAP-A and LAP-D variants have a low K_m for arginyl-NA. This fact suggests that the A and D aminopeptidases possibly have a substrate-binding site that has

a high affinity for the basic amino acids. On the other hand, the B and C forms have lower K_ms and higher affinities for the non-polar side chains of some of the neutral amino acids. As mentioned previously, the LpA and LpD genes are linked (approximately 13 map units apart). The linkage relationships of the B and C loci and of the B and C loci to the A and D loci will be investigated, and we plan to determine the chromosomal locations of the aminopeptidase genes. The maize aminopeptidase system represents a potentially good system for correlating structural, biochemical and genetic information in an effort to understand possible evolutionary relationships between the aminopeptidase loci.

Lila A. Ott and John G. Scandalios

Quantitative catalase inhibitor variants — We have previously reported on a catalase-specific inhibitor in maize scutella which is involved in catalase regulation

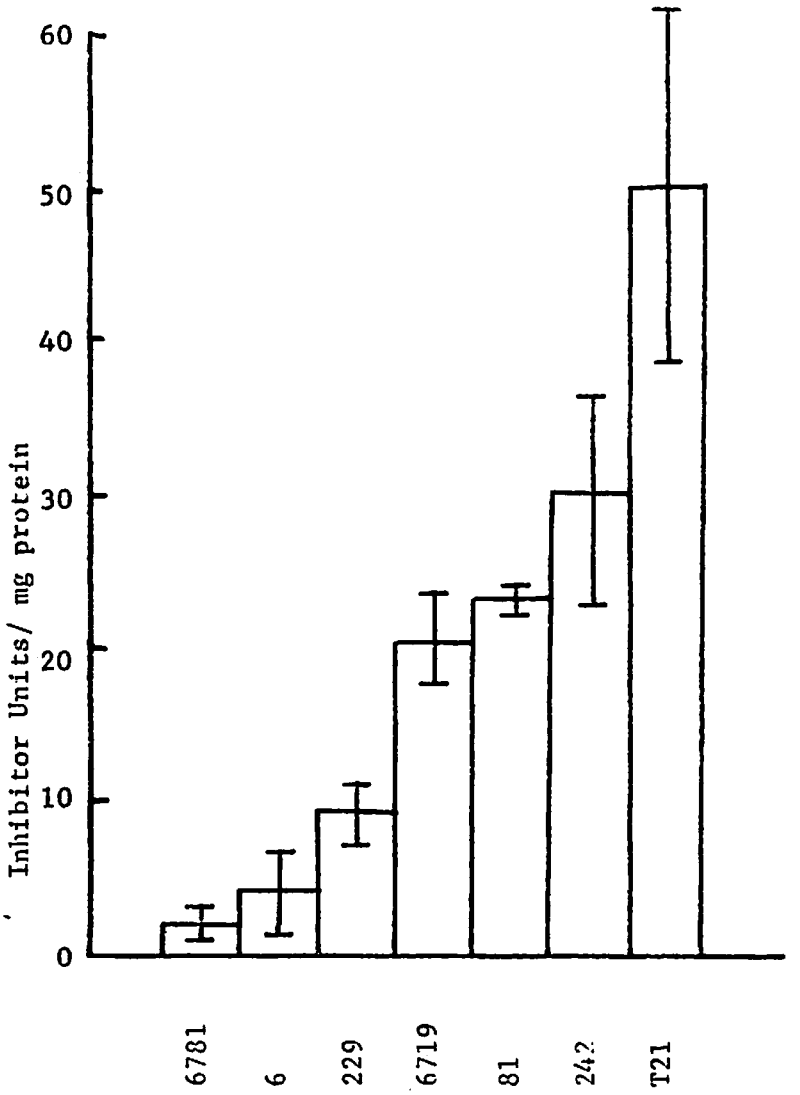

Fig. 1. Levels of inhibitor in various inbred maize strains. Error bars represent standard deviations of at least four replicates in two or three independent experiments.