and NADH of each isozyme were determined at pH 7.5, pH 8.5 and pH 9.5. In general the K_ms for OAA increase when pH increases, but the K_ms for OAA of the s-MDHs increase to a much greater extent than those of the m-MDHs. The K_ms for NADH are less dependent on pH. The K_ms for malate decrease when pH increases; however, the K_ms of m-MDHs are more affected by higher pH. For s-MDHs the K_ms for NAD^+ are almost independent of pH, but those of m-MDHs increase when pH increases. High concentrations of OAA (250 um) preferentially inhibit s-MDHs, but NAD^+ at high concentrations (500 um) seems to inhibit both s-MDHs and m-MDHs similarly. Both inhibitions are pH-dependent.

s-MDHs and m-MDHs are quite different in their capacity to use three NAD analogs as coenzymes (Table 1). Citric acid and isocitric acid inhibit only

Table 1. Catalytic activity of maize malate dehydrogenase isozymes in the presence of NAD or NAD analogs.

Coenzyme	Isozymes (Relative activity)*						
	s-MDH[1]	s-MDH[2]	m-MDH[1]	m-MDH[2]	m-MDH[3]	m-MDH[4]	m-MDH[5]
NAD^+	100	100	100	100	100	100	100
Deam-NAD^+	54.6	44.8	20.7	28.5	24.4	26.5	21.1
3-Ap-NAD^+	96.9	100.6	188	200	216.8	192.3	182.8
TN-NAD^+	241	253	18.3	21.4	21.8	21.1	21.5

*The data are the percentage of the reaction rates relative to NAD^+. Spectrophotometric assays were conducted with 0.75 mM NAD^+ or analog at 5 mM malate, 0.025 M Glycylglycine buffer pH 8.5.

m-MDHs; however, cis-aconitic acid and alpha-ketoglutaric acid inhibit both s-MDHs and m-MDHs. Reducing agents, chelating agents and monovalent metal ions are not required for the enzymatic activities of either s-MDHs or m-MDHs, but divalent metal ions may enhance MDH activities, especially for m-MDHs.

N.S. Yang* and J.G. Scandalios

*Roche Institute of Molecular Biology
Nutley, N.J.

On the regulation of alcohol dehydrogenase — The activity of many enzymes increases sharply after germination of maize seeds. However, alcohol dehydrogenase (ADH; EC 1.1.1.1) activity in the scutellum declines rapidly during this developmental period, indicating that there might be a unique mechanism for the control of ADH activity. Two possible ways for this to occur are (1) faster degradation (or inactivation) than formation of active enzyme molecules or

(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.