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Induction of Alcohol Dehydrogenase and Lactate Dehydrogenase in Hypoxically Induced Barley[1]

Allen G. Good* and William L. Crosby

Molecular Genetics Section, Plant Biotechnology Institute, National Research Council of Canada, Saskatoon, Saskatchewan, S7N OW9, Canada

ABSTRACT

In barley (*Hordeum vulgare* L.), alcohol dehydrogenase (ADH) and lactate dehydrogenase (LDH) are induced by anaerobiosis in both aleurone layers and roots. Under aerobic conditions, developing seeds of cv Himalaya accumulate ADH activity, which survives seed drying and rehydration. This activity consists almost entirely of the ADH1 homodimer. Activity of LDH also increases during seed development, but the level of activity in dry or rehydrated seeds is very low, indicating that this enzyme may not be involved in anaerobic glycolysis during the initial stages of germination. In contrast to ADH, the LDH isozymes present in developing seeds are similar to those found in uninduced and induced roots. Developmental expression of ADH and LDH was monitored from 0 to 24 days postgermination. Neither activity was induced to any extent in the germinating seeds; however, both enzymes were highly induced by anoxia in root tissue during development. Based on gel electrophoresis, this increase in activity results from the differential expression of different *Adh* and *Ldh* genes in root tissue. The changes in ADH and LDH activity levels were matched by changes in the amount of these particular proteins, indicating that the increase in activity results from *de novo* synthesis of these two proteins. The level of inducible LDH activity in an ADH1$^-$ mutant was not found to differ from cv Himalaya. We suggest that although the ADH$^-$ plants are more susceptible to flooding, they are not capable of responding to the lack of ADH1 activity by increasing the amount of LDH activity in root tissue.

The exposure of plants to flooding creates anoxic soil conditions that lead to an alteration of gene expression. This is indicated by a reduction in the number of polypeptides synthesized and an increase in a number of particular anaerobic proteins (21). These anaerobic proteins include ADH,[2] PDC (17), LDH (13), and several other glycolytic enzymes (15, 16). During O_2 deficit, plants produce a number of glycolytic end products including ethanol, lactate, various organic acids, and amino acids (2, 3). Ethanol is produced from pyruvate by PDC and ADH, respectively, whereas lactate is produced in a single step by LDH. Davies *et al.* (4) proposed that during the initial stages of anoxia there is a brief, self-limiting phase of lactate accumulation that serves to lower cytoplasmic pH and thereby activate PDC and ethanol glycolysis in anoxic roots. The *in vivo* NMR data of Roberts *et al.* (20) support this idea. They studied maize root tips during hypoxia and showed that a transient lactic acid fermentation triggered ethanol production by lowering cytoplasmic pH. However, Hoffman *et al.* (13) have shown that in barley, LDH activity increases up to 20-fold during several days of severe hypoxia. This suggests that LDH may have a more important long-term function in anaerobic glycolysis than originally thought (13).

The ADH system of barley shares many features with that of maize (5). Active ADH isozymes are dimers, whose monomer subunits can be the products of the same or different genes. Barley has three *Adh* genes: *Adh*1 and *Adh*2 are tightly linked and sited on chromosome 4, whereas *Adh*3 is sited on chromosome 6 (11). *Adh*1 is constitutively expressed in seeds, whereas anaerobically treated root tissues have all ADH isozymes (11, 18). In barley (*Hordeum vulgare* L.), hypoxically induced LDH activity comprises five isozymes, as do most accessions of its wild relative, *Hordeum spontaneum* (12). These five-banded isozyme profiles are consistent with the presence of two *Ldh* genes, whose products (LDH1 and LDH2) combine to form a set of five tetramers (13).

We are interested in the regulation of *Adh* and *Ldh* during anaerobic stress. Since *Adh* and *Ldh* genes are both induced by hypoxia, they are a valuable system with which to study the coordinate regulation of functionally related genes during environmental stress. This paper examines the degree to which ADH and LDH are coordinately regulated in barley. In this paper we assess the levels of ADH and LDH activity in developing barley seeds and seedlings, the extent to which these enzymes are inducible during development, and finally the levels of enzyme activity compared to absolute ADH and LDH antigen concentrations as measured by immunoassay.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Two varieties of barley (*Hordeum vulgare* L.) were used: cv Himalaya and the *Adh*1-M9 mutant of cv Proctor originally isolated by Harberd and Edwards (10). Barley seeds were surface-sterilized in 1% NaOCl (w/v) for 20 min, rinsed with water, and planted in moist perlite. After 4 d of growth at 20°C, individual seedlings were removed from the perlite and inserted through a hole in a foam stopper. Plants in their foam plugs were placed in holes cut in the top of a 4 L Rubbermaid container. Plants were grown hydroponically with roots in darkness in half-strength Hoagland solution

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[2] Abbreviations: ADH, alcohol dehydrogenase; PDC, pyruvate decarboxylase; LDH, lactate dehydrogenase; FW, fresh weight.

sparged continuously with air. Hypoxically induced plants were placed for 2 d prior to enzyme extraction in an identical container sparged with N_2. The nutrient solution was changed every 6 d. Growth chamber conditions were: d 16 h, 20°C; night 8 h, 20°C; 250 μmol photons m^{-2} s^{-1} photosynthetic photon flux density. For the analysis of developing grains each single ear had the two top and two bottom spikelets discarded. Six spikelets were then removed, three for dry weight determination and three for enzyme activity. To determine the dry weight, grains were frozen overnight at −20°C and dried to a constant weight at 70°C.

Extraction and Assay of ADH and LDH

Extractions were carried out at ice temperature as described previously (13). Roots were rinsed in distilled H_2O, briefly blotted, weighed, and ground with sand in a mortar and pestle in extraction buffer (EB), containing 0.1 M Tris-HCl (pH 8.5) 10 mM Na borate, 10 mM DTT, 15% (v/v) glycerol. The brei was centrifuged for 3 min in a microfuge and the supernatant assayed for enzyme activity. ADH assays were performed in the ethanol → acetaldehyde direction as previously described (8). LDH was assayed in the pyruvate → lactate direction as described previously (13). ADH and LDH activities are reported in IU (μmol/min).

Gel Electrophoresis

Nondenaturing electrophoresis was performed in slab gels (1.5 mm thickness) as described by Hanson and Jacobsen (8) except that the running gel contained 20% sucrose. Samples were homogenized in 2 mL buffer/g FW in EB containing 5 mg/mL BSA. Typically, 90 μL of supernatant plus 15 μL of 90% glycerol containing 0.01% bromophenol blue was loaded. Gels were run in a cold room overnight.

Purification of Barley ADH

This procedure is a modification of that developed for maize ADH (19) and barley ADH (9). All steps were carried out in a cold room. Barley meal was extracted with 0.1 M K-phosphate (pH 7.5) then precipitated in the 40–65% $(NH_4)_2SO_4$ fraction. After chromatofocusing on PBE94 (Pharmacia), fractions containing the ADH activity were loaded directly on to a NAD^+ affinity column (Cibacron Blue 3GA-Agarose, Sigma). This column was washed with 15 mM Na-phosphate (pH 6.5) then 50 mM Na-phosphate (pH 8.0). ADH was specifically eluted by adding 5 mM NAD^+ to the second buffer. The fractions containing ADH activity were pooled, made 10% (w/v) with $(NH_4)_2SO_4$, and loaded directly onto Phenyl Sepharose. After loading, the column was washed with 50 mM Tris-HCl (pH 7.5) containing 10% $(NH_4)_2SO_4$ (4 column volumes). ADH was eluted by applying a 0 to 50% ethylene glycol gradient in 50 mM Tris-HCl (pH 7.5) (4 column volumes). This resulted in a corresponding decrease in $(NH_4)_2SO_4$ to 0% at the completion of the gradient. The fractions containing ADH activity were dialyzed overnight against 10 mM PBS and then concentrated in an Amicon 8050 ultrafiltration cell.

Antiserum Preparation

ADH protein (70 μg) was diluted to 0.5 mL in 10 mM phosphate buffered saline, mixed with 0.5 mL of complete Freund's adjuvant, and injected into New Zealand White rabbits. Booster injections containing 50 μg of ADH in incomplete Freund's adjuvant were made on d 15 and 30. Serum was collected 8 d after the third injection. The antiserum was tested using both native and denaturing Westerns and found to cross-react with all native ADH dimers and the denatured ADH subunits. LDH protein and antiserum were gifts of A. D. Hanson (Michigan State University) (14).

ELISA

ELISAs were performed using a modification of the method of Chibbar *et al.* (1). Dilutions of enzyme supernatant (50 μL) in PBS were allowed to bind to polystyrene 96 well plates (Cooke Microtitre plates) overnight at 4°C. ADH standards (200 pg–50ng per well) were diluted in PBS and included with each plate. The unbound antigen was removed by flicking the plates. The plates were then washed three times with distilled H_2O. The unbound sites on the plastic were blocked with 1% milk powder in PBS by incubating at 37°C for 3 h. After the removal of blocking solution the plates were washed three times with H_2O. Plates were then incubated with 50 μL of primary antibody (1:4000 dilution). Preimmune serum was used as a control to test for unspecific binding. The antibodies were allowed to react for 2 h at 37°C. The unreacted antibodies were removed and the plates washed four times with H_2O. The wells were then incubated with 50 μL of goat anti-rabbit IgGs linked to alkaline phosphatase (1:5000 dilution) for 1 h at 37°C then washed four times. The wells were then incubated with 50 μL of *p*-nitrophenyl phosphate in diethanolamine buffer (pH 9.8) at 37°C in the dark. The reaction was stopped by adding 50 μL of 3 M NaOH. The yellow color developed was read on a microplate reader at 405 nm.

Western and Slot Blots

Western blots were performed using native polyacrylamide gels that had been electroblotted on to nitrocellulose in 5 mM Tris/38 mM glycine (pH 8.3). Slot blots were performed using nitrocellulose and a Schleicher and Schuell Minifold II apparatus. Dilution series of root and seed tissue in EB were made 2 × SSC (3 M NaCl, 0.3 M sodium citrate; pH 7.0), then 50 μL samples were applied to each slot. Both Westerns and slot blots were treated identically thereafter. The nitrocellulose was floated on TBS (10 mM Tris-HCl, 150 mM NaCl; pH 8.0) then incubated in TBS containing 0.05% Tween 20 (TBST) and 4% milk powder for 1 h. The nitrocellulose was then incubated in LDH primary antibody (1:500 dilution in TBST) for 30 min then washed three times in TBST. The blots were then incubated in a 1:5000 dilution of goat anti-rabbit IgGs linked to alkaline phosphatase for 30 min then washed four times in TBST. The blots were then developed in alkaline phosphatase buffer (10 mM Tris-HCl [pH 9.5], 100 mM NaCl, 5 mM $MgCl_2$) containing 0.3 mg/mL nitroblue tetrazolium and 0.2 mg/mL of 5-bromo-4-chloro-3-indolyl phosphate. LDH protein levels were quantified from slot blots by cutting

out each developed slot and then dissolving the nitrocellulose in 250 μL of DMSO. The absorbance was then read at 522 nm against a blank of nitrocellulose lacking protein. The quantification was based on a comparison of the absorbance of purified LDH protein and the dilution series.

RESULTS

Constitutive ADH and LDH Activity in Developing Grains

The constitutive ADH activity of barley grains is known to be almost all ADH1.ADH1 homodimer and accumulates during the later part of grain growth (9). We found similar patterns of ADH accumulation for cv Himalaya; however, in the ADH⁻ mutant, very little ADH activity accumulated (Fig. 1). The isozyme composition of ADH in the mature Himalaya seed was almost entirely ADH1.ADH1 homodimer; however, the ADH⁻ mutant had no ADH1 activity as demonstrated by native gels (Fig. 2).

The levels of LDH activity in maturing grains are shown in Figure 3. The Himalaya grains showed an increase in LDH activity as the grains matured, similar to the increase in ADH activity. The ADH⁻ grains had similar levels of LDH activity as Himalaya, with the exception of the smaller grains. These always had a minimum of 0.019 IU/grain whereas the small Himalaya grains had LDH activities as low as 0.002 IU/grain. This suggests that, in the absence of ADH activity, there is a minimum level of LDH activity that is necessary for developing grains. Mature dry seeds had rather low levels of activity for both the Himalaya and ADH⁻ varieties (Fig. 3; Table I). The lower levels in dried seeds suggest that LDH is not as stable during dehydration and dry storage as is ADH. The

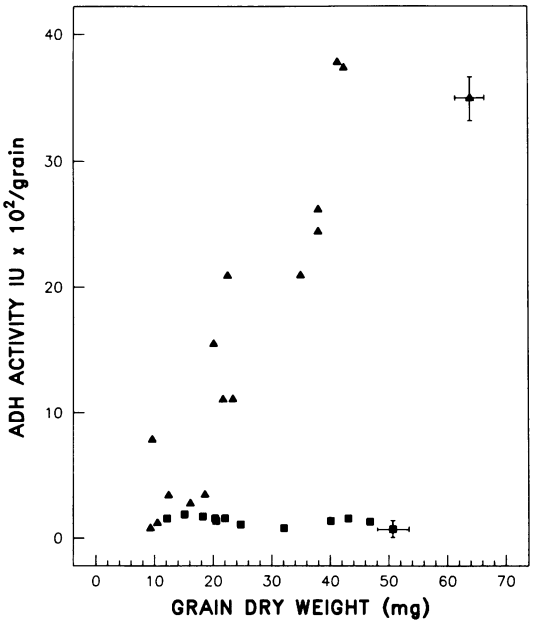

Figure 1. Changes in ADH activity during grain development in barley. Each point represents the value from 6 grains from a single ear. Triangles represent ears of the cv Himalaya; squares, the ADH⁻ mutant. The two points with error bars indicate the values for mature air dry seeds.

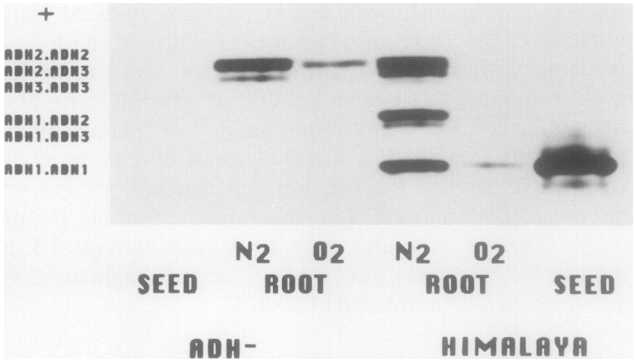

Figure 2. Isozyme composition of ADH in different barley tissues under aerobic and anaerobic conditions. Root samples are equal to 50 mg FW, whereas seed samples are equal to 25 mg FW. The subunit composition of the ADH dimers (7, 10) is shown to the left.

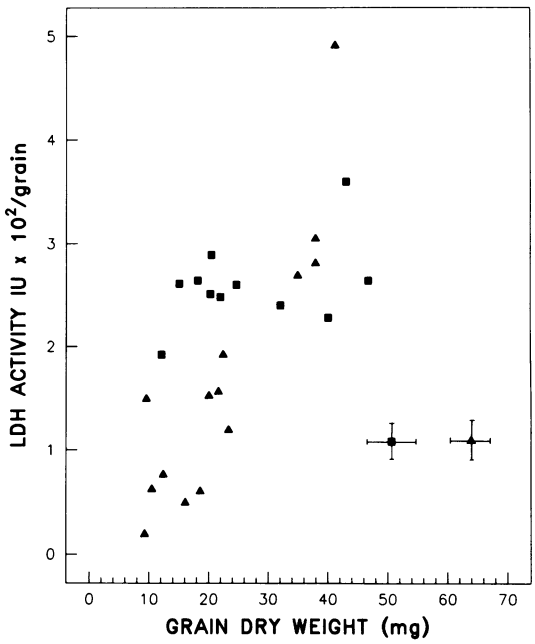

Figure 3. Changes in LDH activity during grain development in barley. Each point represents the value from 6 grains from a single ear. Triangles represent ears of the cv Himalaya; squares, the ADH⁻ mutant. The two points with error bars indicate the values for mature air dry seeds.

LDH isozyme composition of developing grains is shown in Figure 4. Grains from three heads at different stages of maturation all expressed the five LDH isozymes that are found in both uninduced and induced root tissue.

Protein Levels in Developing Grains

The levels of ADH protein in developing seeds are outlined in Table I. ELISAs were performed on protein samples from three separate ears, the corresponding seed dry weight being presented in Table I. The increase in ADH activity in developing Himalaya grains was found to be matched by a corresponding increase in the total amount of ADH protein. Sim-

Table I. *Comparison of ADH and LDH Protein Levels in Developing Barley Heads*

Ear	Himalaya			ADH⁻		
	Average Seed wt	ADH protein	LDH protein	Ear	Average Seed wt	ADH protein
	mg	*μg/grain*			*mg*	*μg/grain*
1	22	16.0	1.7	1	15	2.4
2	38	40.2	2.7	2	32	3.4
3	42	74.4	4.3	3	43	2.8
Mature seed	64	98.2	1.2	Mature seed	51	1.7

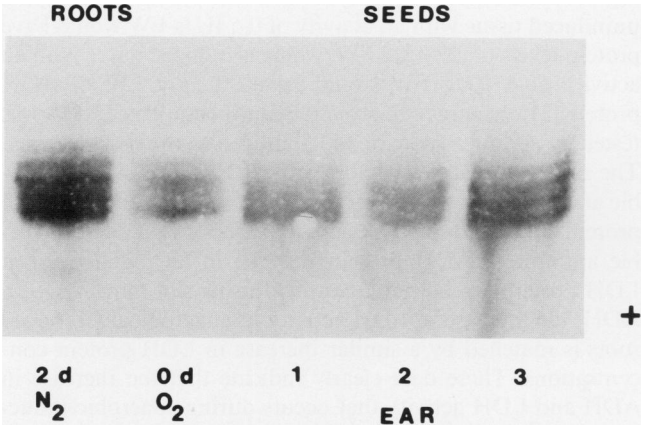

Figure 4. Isozyme composition of LDH in different barley tissues. Samples were run on native PAGE gels, Western blotted onto nitrocellulose, then probed with an anti-LDH antibody. The three seed samples (1, 2, and 3) are the same ears from cv Himalaya as in Table 1.

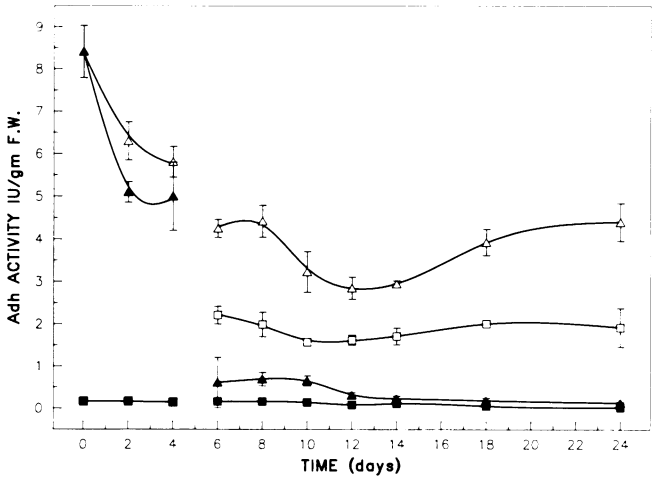

Figure 5. Developmental expression of ADH activity in aerobic and anaerobically induced barley seedlings. Triangles represent samples of cv Himalaya; squares, the ADH⁻ mutant. The open symbols represent tissue that has been anaerobically induced for 2 d, solid symbols are uninduced tissue. Each point represents the mean of three samples (±SE).

ilarly, the low levels of ADH activity found in developing ADH⁻ mutant grains were matched by low levels of ADH protein. This suggests that the ADH⁻ mutant either produces much lower levels of ADH protein, or the protein produced is neither enzymically active nor cross-reacts with polyclonal ADH antibodies. We found that we were unable to quantify LDH protein levels using ELISAs. Instead, we used slot blots and quantified the amount of color from the alkaline phosphatase reaction (see "Materials and Methods"). The level of LDH protein in developing seeds was much lower than the level of ADH protein, with the level of protein showing similar trends to the level of LDH activity (Table I).

ADH and LDH Activity Levels in Developing Seedlings

The levels of ADH activity in developing seedlings are detailed in Figure 5. Activity levels from 0 to 4 d after germination are from the entire seedling, whereas from d 6 through 24 they are for root tissue. During the initial stages of germination, the Himalaya seedlings have very high levels of ADH activity, which decrease during the first 4 d after imbibition. Induced seedlings did show slightly higher levels of ADH activity, suggesting that at least part of the tissue in these seedlings may be inducible. As expected, the ADH⁻ seeds had virtually no ADH activity after imbibition. Neither was there any increase in ADH activity in induced seedlings during the first 4 d of development.

Six d after the start of germination, the root tips in both varieties were long enough to allow the measurement of enzyme activity. During the early stages of root development the level of uninduced ADH activity in Himalaya decreased from 0.6 IU/g FW to 0.13 IU/g FW after 24 d of development (Fig. 5). The level of ADH activity in the ADH⁻ mutant started at a lower initial level (0.16 IU/g FW) but showed a similar trend, decreasing to 0.05 IU/g FW 24 d after imbibition. Both varieties showed large increases in ADH activity after 2 d of anaerobic induction. Under aerobic conditions, Himalaya roots make predominantly the ADH1 subunit; however, when anaerobically induced, the ADH1 and ADH2 subunit are produced at much higher levels. ADH3 subunits are also produced at higher levels but do not show the degree of induction that ADH1 and ADH2 do. Under aerobic conditions the ADH⁻ mutant, which is incapable of making ADH1 subunits, compensates for this mutation by producing ADH2 subunits. The production of ADH2 subunits did not occur in cv Proctor, the variety from which the *Adh*1-M9 mutant was derived (data not shown). Upon induction, the ADH⁻ mutant produces large amounts of the ADH2 subunit (Fig. 2). For Himalaya, the lowest level of inducibility occurred at 12 d, increasing slightly until 24 d after imbibition.

The level of activity after induction for the ADH⁻ mutant stayed relatively constant throughout the first 24 d after germination at approximately 2 IU/g FW.

The level of LDH activity during development is shown in Figure 6. This figure presents the data for the variety Himalaya. The levels of LDH activity for the ADH⁻ mutant did not differ significantly at any developmental stage from that of Himalaya. After initiation of germination the level of LDH activity stayed fairly constant at approximately 0.25 IU/g FW. Placing the developing seeds under anaerobic conditions had no significant effect on the level of LDH activity. The levels of LDH activity in uninduced root tissue showed a similar trend to that found for ADH activity. The level of activity started at 0.37 IU/g FW then decreased slowly as the root tissue aged to 0.16 IU/g FW. The level of LDH activity after induction stayed fairly constant during development at approximately 1.1 IU g/FW. Figure 7 illustrates the induction of LDH activity after 2 and 4 d of anaerobiosis. After 2 d there is more LDH activity, but it is primarily the LDH 2

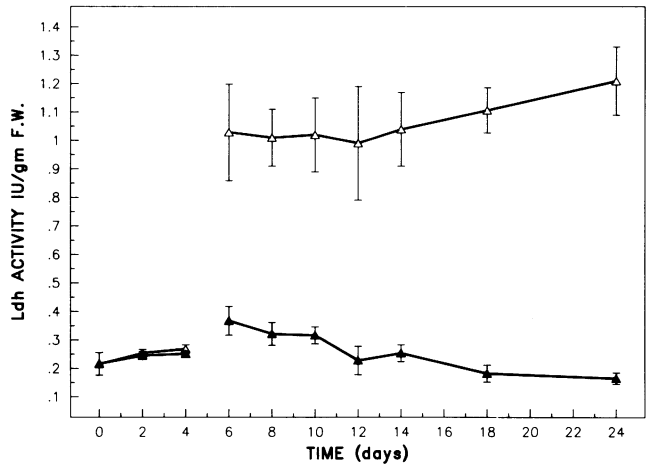

Figure 6. Developmental expression of LDH activity in aerobic and anaerobically induced barley (cv Himalaya) seedlings. The open symbols represent tissue that has been anaerobically induced for 2 d, solid symbols are uninduced tissue. Each point represents the mean of three samples (±SE).

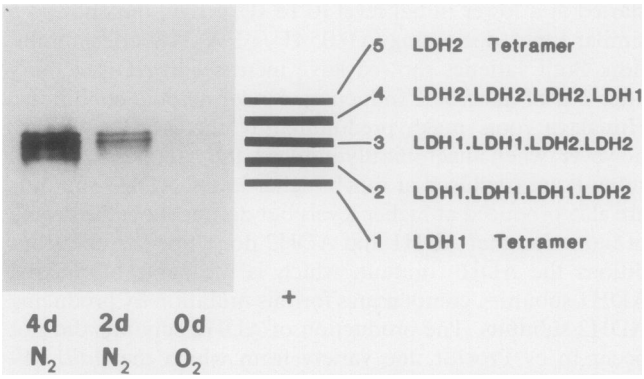

Figure 7. Isozyme composition of LDH in barley root tissue. Roots were induced for 2 or 4 d and the run on native PAGE gels then stained for LDH activity. Isozyme subunit composition is that of Hoffman *et al.*. (13).

homotetramer and the LDH 2.2.2.1 heterotetramer. This indicates that there is much more of the LDH2 subunit present after 2 d of anaerobiosis than the LDH1 subunit.

ADH and LDH Protein Levels in Developing Seedlings

The developmental patterns and pattern of induction found for activity measurements were also found for ADH protein levels (Table II). The level of protein found in these samples was higher than would be predicted, based on protein purification and activity levels. Pryor and Huppatz (19) and Hanson *et al.* (9) found that purified ADH had an activity of 225 IU/mg protein. Based on these results, one would predict that uninduced tissue with an activity of 0.5 IU/g FW would have protein levels of 2.2 μg/g FW, whereas induced tissue with an activity of 4 IU/g FW would have 20 μg/g FW of ADH protein. The accuracy of protein quantification by ELISA was tested by adding a spike of ADH antigen to the tissue extract. The ELISA test accurately quantified this spike in both aerobic and anaerobic tissue extracts. Therefore, the level of ADH protein compared to ADH activity suggests that an appreciable amount of ADH protein may be in an inactive form. LDH protein levels were quantified using slot blots. As with ADH, the increase in LDH activity in anaerobically induced roots is matched by a similar increase in LDH protein concentrations. These data clearly indicate that the increase in ADH and LDH activity that occurs during anaerobic induction results from an increase in protein levels, presumably as a result of *de novo* synthesis of these proteins.

DISCUSSION

The present study indicates that ADH and LDH are both strongly induced under anaerobic conditions, and that these two enzymes show very similar patterns of developmental expression and induction during seedling development.

During grain development there is a rapid increase in ADH activity resulting primarily from the accumulation of ADH1:ADH1 homodimers (9). We found a similar pattern of accumulation of ADH activity which was matched by an increase in steady state ADH antigen as measured by ELISA. A corresponding increase in ADH activity was not observed in the ADH⁻ mutant. Since this mutant is defective in producing functional ADH1 subunits (10), our data provide further evidence that the accumulation of ADH activity in developing seeds results primarily from products of the *Adh1* gene. On the basis of sequence analysis, Trick *et al.* (22) have suggested that the ADH⁻ mutant phenotype is a result of a splice site mutation in the first intron of the *Adh1* gene. In this case the ADH⁻ mutant would produce a truncated mRNA transcript, and presumably would lack any ADH1 subunits. We found that the level of ADH protein in the ADH⁻ mutant seeds was very low, which would support the idea that this mutant line cannot make an ADH1 subunit. It is generally considered that the mature barley grain is preadapted to O_2 deficit as a result of the accumulation of the ADH1.ADH1 homodimer during grain development. However, it is clear that LDH is not accumulated to the same extent as ADH1 during grain development, and this enzyme may be less stable to seed dehydration and/or storage in

Table II. *Comparison of ADH and LDH Protein Levels Over Time in Barley Root Tissue under Aerobic and Anaerobic Conditions*

Time	ADH Levels				LDH Levels	
	cv Himalaya		cv ADH⁻		cv Himalaya	
	Unind[a]	Ind[b]	Unind	Ind	Unind	Ind
d after germination	*μg/g FW*					
6	19.2	59.4	7.6	29.4	3.1	7.1
10	14.6	69.8	6.4	30.8	2.8	8.9
14	13.6	70.8	5.0	22.4	3.4	9.4
24	6.2	78.2	2.8	26.4	1.6	8.2

[a] Uninduced. [b] Induced.

comparison to ADH1. Moreover, the *Ldh* genes that are expressed in developing seeds are similar to those expressed in root tissue. Thus, LDH lacks the tissue specific gene expression characteristic of ADH during grain development and the early stages of germination. ADH may be largely responsible for anaerobic glycolysis during these early stages.

ADH and LDH are both known to be highly induced under anaerobic conditions in maize and barley roots (6, 13, 18). We observed that the level of activity in uninduced tissue for both of these enzymes decreased as the root tissue aged. This result suggests a higher level of constitutive activity in very young seedlings which subsequently decreases with time. The observed decrease in constitutive activity during development was not matched by any reduction in inducibility. Upon induction, the levels of ADH and LDH activity increased substantially; for both proteins this increase in activity corresponded to an increase in detectable antigen. The amount of ADH antigen detected in aerobic and anaerobic root tissue was found to be three to five times higher than would be predicted based on protein purification. Spiking the crude extract with ADH antigen indicated that ELISAs could accurately quantify the spike. Hanson *et al.* (9) identified a species of ADH1 polypeptide in barley aleurone layers they designated ADH1′ and suggested a posttranslational origin for ADH1′. Our data suggest that there may be substantial amounts of inactive ADH antigen present in root tissue.

It has been shown for maize roots and barley aleurone layers (6, 9) that increases in ADH activity are accompanied by an increase in *Adh* mRNA. D Hondred and AD Hanson (personal communication) have also shown an increase in *Ldh* mRNA levels upon anaerobic induction. Given this, and the demonstrated increase in these proteins upon induction, we suggest that the increase in ADH and LDH enzymic activity results from an increased steady state mRNA level, in turn resulting in an increased protein synthesis and enzyme accumulation.

For LDH, the pattern of developmental expression in root tissue was almost identical to that of ADH. This suggests that these two proteins play similar roles during anaerobic stress throughout development of the barley seedling. Both ADH and LDH show degrees of differential regulation under anaerobic induction. *Adh1* is the only *Adh* gene expressed under aerobic conditions in roots (Fig. 2) (7). Under anaerobic conditions both *Adh1* and *Adh2* are induced (Fig. 2); however, there is little induction of *Adh3*. *Adh3* is induced in barley aleurone layers under anaerobic conditions (7). By comparison, LDH1 and LDH2 subunits are produced under both aerobic and anaerobic conditions. However, during hypoxia there is preferential induction of the *Ldh2* gene. One of our reasonings for choosing the ADH⁻ mutant in addition to Himalaya was to determine if the lack of a functional *Adh1* gene would have any effect on the level of expression of *Ldh* genes. This might then allow us to begin to understand the signals that a plant cell uses in turning on anaerobic proteins. A comparison of LDH activity levels in induced root tissue of the two varieties indicated that there was no difference in the degree of induction of LDH. Therefore, although the *Adh1*-M9 mutant has a reduced anaerobic tolerance (10), it cannot respond to anaerobic conditions by further induction of LDH.

Davies *et al.* (3) suggested that during the initial stages of hypoxia there is a brief, self limiting phase of lactate accumulation which serves to lower cytoplasmic pH and thereby activate PDC and ethanol glycolysis in roots. However, our data and the data of Hanson and Jacobsen (8) and Hoffman *et al.* (13) indicate that LDH could play an important and continuing role in anaerobic root tissue during development. In summary, the two major differences between ADH and LDH is first that LDH does not appear to be important in anaerobic glycolysis in developing and mature seeds. Second, although the two *Ldh* genes do show some differences in the degree of expression, they do not show the tissue specific differential regulation seen in the *Adh* genes of maize and barley.

ACKNOWLEDGMENTS

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