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Metabolic Control of Anaerobic Glycolysis[1]

Overexpression of Lactate Dehydrogenase in Transgenic Tomato Roots Supports the Davies-Roberts Hypothesis and Points to a Critical Role for Lactate Secretion

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Roots of all plants examined so far have the potential for both ethanol and lactate fermentation. A short burst of lactate fermentation usually occurs when plant tissues are transferred from normoxic to anoxic conditions. According to the Davies-Roberts hypothesis, the consequent pH drop both initiates ethanol fermentation and blocks further production of lactate by inhibiting lactate dehydrogenase (LDH). However, the role of LDH in this pH control mechanism is still a matter of debate. To perturb the control system in a defined way, a barley LDH cDNA under the control of the cauliflower mosaic virus 35S promoter was introduced into tomato (*Lycopersicon esculentum* Mill. cv VFMT) using *Agrobacterium rhizogenes*. The transgenic root clones expressed up to 50 times the LDH activity of controls. The fermentative metabolism of these clones was compared using roots grown previously in normoxic conditions or roots given a 3-d hypoxic pretreatment. During the transition from normoxia to anoxia, lactate accumulation was no faster and no more extensive in transgenic roots than in controls. Similarly, during prolonged anoxia the flux of ^{14}C from [U-^{14}C] glucose to lactate and ethanol was not modified by the expression of the transgene. However, in both transgenic and control roots, hypoxic pretreatment increased the flux to lactate and promoted lactate export to the medium. These results show that LDH has a very low flux control coefficient for lactate fermentation, consistent with the Davies-Roberts hypothesis. Moreover, they suggest that lactate secretion exerts major control over long-term lactate glycolysis in vivo.

When plant roots are completely deprived of oxygen, they typically rely on ethanol fermentation for the production of metabolic energy (Davies, 1980). Nevertheless, all plants studied so far possess LDH enzymes and thus have the potential for lactate fermentation. Current views on the role of LDH are based on data obtained in vitro with extracts of parsnip roots and pea seeds (Davies et al., 1974) and in vivo with excised maize root tips (Roberts et al., 1984b). These studies indicated that lactate glycolysis is limited to the transition phase between aerobic metabolism and ethanolic fermentation, and led to the hypothesis schematized in Figure 1. This hypothesis (Davies et al., 1974) states that the NADH generated by glycolysis is initially oxidized by LDH, which is usually present at a low level under aerobic (normoxic) conditions. The resulting lactate accumulation lowers the cytoplasmic pH; this acidification progressively inhibits LDH and activates the first enzyme of ethanol fermentation, PDC. This drop in cytoplasmic pH also enhances the potent inhibition of LDH by ATP (Davies and Davies, 1972). Roberts et al. (1984a, 1984b) further proposed that when lactate accumulation is not stopped, runaway cytoplasmic acidification can lead to cell death.

Two types of observations appear to conflict with the Davies-Roberts hypothesis. First, in some cases lactate accumulation seems not to be coupled to cytoplasmic acidification or to the onset of ethanol production. Specifically, in maize root tips, lactate accumulation can lag well behind the rate of cytoplasmic acidification (Saint-Ges et al., 1991); in rice embryos, lactate accumulation is undetectable and cytoplasmic acidification is very limited, but ethanol fermentation is active (Rivoal et al., 1989; Menegus et al., 1991). Second, LDH may be more important after the transition between normoxic and anoxic metabolism than during the transition. Thus, in roots of barley and other species, LDH is induced by long-term hypoxia (Hoffman et al., 1986; Hanson et al., 1987; Rivoal et al., 1991). In barley aleurone layers (Hanson and Jacobsen, 1984) and roots of some *Limonium* species (Rivoal and Hanson, 1993), lactate fermentation is sustained during prolonged anoxia.

It is important to note that the evidence for and against the Davies-Roberts hypothesis is largely correlative, and that the physiological and biochemical data involve different species, organs, and experimental conditions. Such dispersion seems likely to account for much of the uncertainty over the roles of lactate accumulation and LDH induction in the control of fermentation. Therefore, we took a genetic approach, overexpressing a barley LDH cDNA in tomato (*Lycopersicon esculentum* Mill.) root cultures and then measuring the repercussions on fermentative metabolism.

We chose to engineer high levels of LDH expression because LDH activity increases in many species in response to oxygen deprivation. Barley LDH was chosen because it is the

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Abbreviations: ADH, alcohol dehydrogenase; LDH, lactate dehydrogenase; MSLS, Murashige-Skoog (MS) medium modified by lowering the MS salts to 1 g L^{-1}; MYA, mannitol/yeast extract/ammonium sulfate medium; PDC, pyruvate decarboxylase.

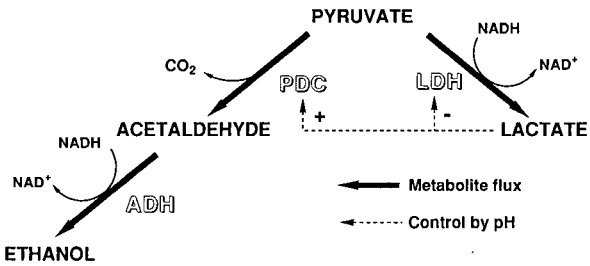

Figure 1. The control architecture of lactate and ethanol fermentation in roots according to the Davies-Roberts hypothesis.

best-characterized plant LDH (Hondred and Hanson, 1990) and is the only one definitely shown to be associated with a high capacity for lactate production in vivo (Hanson and Jacobsen, 1984). We used *Agrobacterium rhizogenes* carrying a binary expression vector to produce transgenic tomato root cultures (Morgan et al., 1987). Such cultures have the advantages of being genetically stable and self-cloning, since each root originates from a single cell (Tepfer, 1983). They also allow radiotracer experiments without interference from microorganisms and simplify the rigorous control of oxygen levels.

We studied two physiologically relevant sets of conditions: roots grown under normoxic conditions before being subjected to anoxia, and roots that received hypoxic pretreatment prior to anoxia. Such hypoxic treatment, termed induction or acclimation (Saglio et al., 1988), improves survival under anoxia (Johnson et al., 1989). The approach of Metabolic Control Analysis (for a recent review, see Fell, 1992) was used to interpret the results because it provides a framework for measuring the degree of control exerted by a specific enzyme on overall flux through a metabolic pathway.

We set out to answer two questions: (a) would an increase in LDH activity change the rates or amounts of lactate and ethanol production during the transition phase between normoxia and anoxia? and (b) would it increase the flux to lactate during long-term anoxia? If the Davies-Roberts hypothesis is correct, lactate fermentation should still be transient (although the initial rate and final extent of lactate production might increase), and there should be no increase in the long-term flux to lactate.

MATERIALS AND METHODS

Plant Material, Bacterial Strains, and Plasmids

Tomato plants (*Lycopersicon esculentum* Mill. cv VFMT) for transformation were grown in a glasshouse until they reached the age of 3 weeks. *Agrobacterium rhizogenes* wild-type strain A4 (Moore et al., 1979) was from Dr. D. Tepfer (Institut National de la Recherche Agronomique, Versailles, France) and was maintained on MYA medium (Petit et al., 1983). *Escherichia coli* MC1000 carrying the plant expression plasmid pGA643 (An et al., 1988) was from Dr. G. An (Washington State University, Pullman) and was maintained on Luria broth medium (Sambrook et al., 1989) containing 10 mg L^{-1} kanamycin and 2 mg L^{-1} tetracycline. *A. rhizogenes* A4 carrying pGA643 was grown on MYA containing 25 mg L^{-1} kanamycin and 5 mg L^{-1} tetracycline.

Construction of Plasmids

Standard procedures (Sambrook et al., 1989) were used to prepare two constructs from the barley LDHA cDNA clone (Hondred and Hanson, 1990). An *Eco*RI fragment (containing the entire 1305-bp cDNA) and a *Eco*RI-*Sca*I fragment (lacking 122 bp of the 3′ untranslated region) were isolated. After filling the recessed termini using the Klenow fragment, these fragments were cloned into the *Hpa*I site of pGA643; correct orientation was confirmed by restriction digests. The two constructs were transferred to *A. rhizogenes* A4 by direct transformation (An et al., 1988).

Petiole Transformation and Root Cultures

Freshly cut tomato petioles were soaked in commercial bleach diluted 1:20 (v/v) with water (500 mL for 10 petioles) for 10 min and washed with 5 × 500 mL of sterile water. The petioles were then cut into 4-cm segments that were inserted apical end down in MS medium (Murashige and Skoog, 1962; prepared with Gibco MS salt mixture) containing 0.6% (w/v) Phytagar (Gibco) and 3% (w/v) Suc. The upper cut ends were infected with *A. rhizogenes* A4 carrying pGA643 with or without an LDH insert. After 10 d, transformed roots appearing at the infection site were excised and subcultured on MSLS containing carbenicillin (500 mg L^{-1}) to prevent growth of *A. rhizogenes* (Petit et al., 1983). The roots were progressively freed of remaining microorganisms as described (Morgan et al., 1987). They were routinely subcultured every month on solid MSLS medium. Material used in labeling experiments and in metabolite and enzyme determination was obtained by subculturing the roots in 50-mL Erlenmeyer flasks containing 15 mL of liquid MSLS medium. The flasks were continuously sparged with humidified, filter-sterilized air or N_2 containing 3 kPa O_2, depending on the experiment.

Enzyme Extraction and Activity Measurement

Operations were carried out on ice. For LDH and ADH, roots were homogenized in the buffer described by Hoffman et al. (1986), and for PDC they were homogenized in the buffer described by Rivoal et al. (1990). LDH activity was measured in the pyruvate-to-lactate direction in the presence of 4-methylpyrazole to inhibit interference from the coupled action of PDC and ADH (Hoffman et al., 1986). For measurements of kinetic properties, LDH was partially purified from tomato root clones or hypoxically induced barley roots by affinity chromatography on Affi-Gel Blue agarose (Bio-Rad) (Hondred and Hanson, 1990). The specific activity of the partially purified fractions from tomato roots was 10 to 30 μmol min^{-1} mg^{-1} protein. ADH activity was measured in the ethanol-to-acetaldehyde direction as described (Shimomura and Beevers, 1983). PDC activity was measured spectrophotometrically in a coupled assay as described (Rivoal et al., 1990). Protein was determined by the method of Bradford (1976).

Immunoscreening and Western Blot Analysis

For the screening of transgenic roots, extracts were prepared as described above for LDH except that BSA was omitted from the extraction buffer. Samples of crude extracts containing 10 μg of protein were applied to nitrocellulose filters; LDH peptides were detected using rabbit antiserum raised against barley LDH (Hondred and Hanson, 1990) in a coupled immunoreaction with alkaline phosphatase-labeled anti-rabbit IgG (Bio-Rad). For western blot analysis, crude extracts were prepared in LDH extraction buffer. The proteins were separated by SDS-PAGE and transferred to nitrocellulose as described by Hoffman and Hanson (1986). LDH peptides were detected as described above.

^{14}C Labeling of Roots and Analysis of Labeled Metabolites

Transgenic roots grown in liquid cultures were prepared as follows: 24 h before the labeling, the MSLS medium with 3% Suc was replaced by MSLS medium containing 0.3% Suc to partially deplete the internal sugar pools and so enhance the metabolism of added tracer. Just before the labeling, the roots were removed from their medium and washed with 3 × 25 mL of labeling medium (1 g L^{-1} MS salts, pH 5.7). Batches (250 mg) of roots were then incubated at 25 ± 2°C in 25-mL vials closed with a rubber stopper and containing 1 mL of labeling medium with carbenicillin (final concentration 500 mg L^{-1}). The vials were flushed with pure N_2 (containing <0.3 Pa O_2) for 1 h and then 1 mL of N_2-purged labeling medium containing 74 kBq of [U-^{14}C]Glc (11 GBq/mmol, New England Nuclear) was injected. Incubation was for 8 h. Analysis of labeled metabolites was as described (Rivoal and Hanson, 1993).

Extraction and Enzymatic Determination of Lactate and Ethanol

Roots were prepared as described above and placed in 5-mL vials containing 1 mL of degassed medium (1 g L^{-1} MS salts, pH 5.7). Vials were closed with a rubber stopper, and anoxia was imposed by flushing for 5 min with pure N_2. The vials were then placed in a sealed container flushed with pure N_2 and incubated at 25 ± 2°C. Incubation was stopped after 1 or 9 h by freezing the vials in liquid N_2. Lactate and ethanol were determined enzymatically in neutralized perchloric acid extracts as described (Rivoal et al., 1989).

RESULTS

Production of Transgenic Roots

Tomato petioles were transformed with two expression constructs that contained the entire coding sequence of barley LDHA (Hondred and Hanson, 1990) and differed only in whether the 3′ untranslated region had been partly removed. Transformed clones were screened immunologically for the presence of the barley LDH peptide, and positive clones were confirmed by assaying LDH activity. From a total of 32 independent clones expressing high levels of LDH activity, three with different levels of LDH were chosen for further study. LDH1 carried a cDNA lacking part of the 3′ untranslated region; LDH2 and LDH3 contained the entire LDH cDNA.

Characterization of Selected Clones

Figure 2 shows extractable LDH activities in clones LDH1, LDH2, and LDH3 (henceforth, transgenic roots) and those for clones transformed with the vector alone (henceforth, control roots). LDH activities were 10 to 50 times higher in the transgenic roots and were comparable to those in hypoxically induced barley roots (Hoffman et al., 1986). The proteins extracted from control and transgenic roots were subjected to western blot analysis (Fig. 2, inset). The intensity of the 40-kD band corresponding to the LDH peptide (Hondred and Hanson, 1990) was well correlated with extractable LDH enzyme activity. The two smaller immunoreactive peptides detected in transgenic roots were taken to be degradation products of LDH because their intensity was proportional to that of the 40-kD band.

Because the Davies-Roberts hypothesis depends heavily on the kinetic properties of LDH, we verified that the enzyme expressed in tomato roots had normal kinetic parameters. The K_m values (mean ± SE) for pyruvate (0.72 ± 0.06 mM) and NADH (15 ± 2 μM) measured for LDH from the three transgenic clones were indistinguishable from those for the barley root enzyme. The pH optimum was also the same (7.8–8). All these values are in accord with those published for plant LDHs (Asker and Davies, 1984; Hoffman and Hanson, 1986).

Fermentative Enzyme Levels in Hypoxically Induced Roots

During exposure to hypoxia or anoxia, there is usually a substantial induction of LDH (Hoffman et al., 1986), ADH (Freeling, 1973), and PDC (Kelley, 1989). Therefore, we measured the activities of these enzymes in roots grown under normoxic conditions (henceforth, noninduced roots) and during a 3-d hypoxic induction treatment (henceforth, hypoxically induced roots) (Fig. 3). In the case of LDH, a

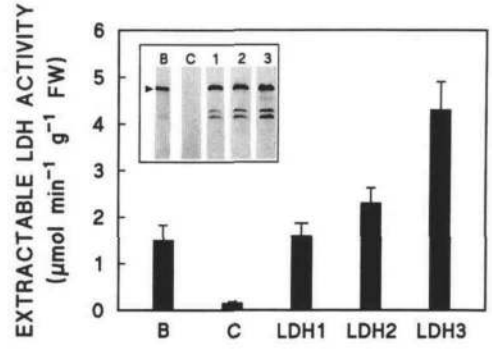

Figure 2. LDH activities and protein levels in transgenic tomato roots. Proteins were extracted from control (C) and transgenic (LDH1–LDH3) roots and used for enzyme assays (main panel) or western blot analysis (inset). Data for hypoxically induced barley roots (B) are included for comparison. Bar graphs are means ± SE for at least three independent experiments. Western blot data are from one representative set of extracts. The arrowhead in the inset marks the position of the 40-kD barley LDH peptide.

small induction of the endogenous tomato enzyme was apparently added to the constitutive expression of the transgene. Expression of the LDH transgene did not alter that of ADH and PDC, both of which were strongly induced by the hypoxic treatment. Thus, after hypoxic induction, the catalytic potential for lactate fermentation remained different between control and transgenic roots, whereas that for ethanol fermentation was the same.

Lactate and Ethanol Accumulation during Anoxia

The transgenic root clone expressing the highest LDH level (LDH3) and control roots were grown in normoxic conditions or were hypoxically induced for 3 d; lactate and ethanol production under anoxia were then measured (Fig. 4). There was no difference between transgenic roots and controls; in both there was a burst of lactate production within the 1st h and a continuous production of ethanol throughout the experiment. The kinetics of lactate accumulation during the 1st h of anoxia were hard to establish because the amounts of lactate produced approached the sensitivity limit of the lactate assay. However, there were no apparent differences between transgenic and control roots (not shown). Between 1 and 9 h, hypoxically induced roots produced more lactate and ethanol than noninduced roots, showing that hypoxic induction caused an increase in the rate of fermentation. The amounts of lactate plus ethanol produced were around 10 μmol h^{-1} g^{-1} fresh weight, which is equivalent to a glycolytic flux of 5 μmol hexose h^{-1} g^{-1} fresh weight. This value is close to that observed for freshly excised maize root tips (15 μmol hexose h^{-1} g^{-1} fresh weight) (Xia and Saglio, 1992), when allowance is made for the higher degree of vacuolation in whole transgenic roots. Therefore, despite preculturing on low-sugar medium, the roots showed no evidence of sugar starvation.

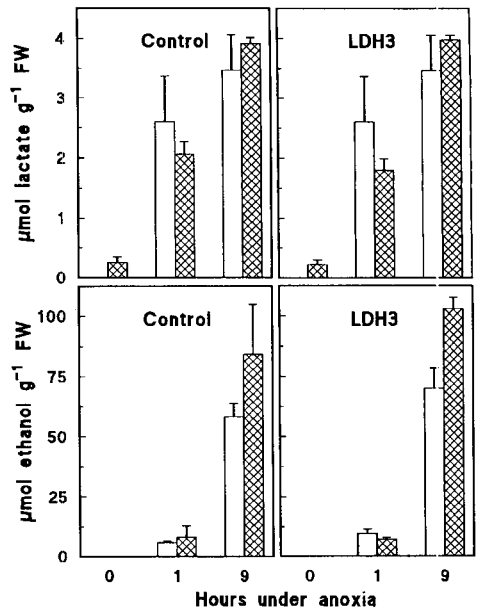

Figure 4. Lactate and ethanol accumulation in control and transgenic roots after transfer to anoxic conditions. Batches (250 mg) of noninduced roots (open bars) or roots given a 3-d hypoxic induction (hatched bars) were placed in vials containing 1 mL of medium. Anoxia was initiated at time 0 by flushing the vial with pure N_2. After 1 or 9 h, the roots were frozen in liquid N_2. Ethanol and lactate were measured enzymatically in perchloric acid extracts. Results are means ± SE for triplicate samples.

[^{14}C]Glc Metabolism during Long-Term Anoxia

To confirm and extend the above results, we used labeling with [U-^{14}C]Glc to examine the glycolytic flux to fermentative end products in clones expressing different levels of LDH. Root cultures were transferred to anoxia for 1 h before adding label to avoid interference by the initial burst of lactate synthesis. Noninduced control and transgenic roots behaved identically, showing a large ^{14}C flux to ethanol and a small flux to lactate (Fig. 5A). There was also significant ^{14}C accumulation in amino acids (mainly Ala). When the same experiment was performed with hypoxically induced roots, there was again no difference between transgenic and control clones (Fig. 5B). However, the proportion of ^{14}C metabolized to lactate was approximately double that observed in noninduced roots. ^{14}C labeling of amino acids (again, mainly Ala) was lower. Allowing for numbers of carbon atoms, the lactate:ethanol ratios in Figure 5 follow the same trends as those in Figure 4, but are about 4- to 9-fold higher in both control and transgenic roots. This would be explained if more lactate relative to ethanol fermentation would occur in some subset of root cells that have a better access to added [^{14}C]Glc than the remainder of the tissue. Such within-tissue heterogeneity in fermentative pathways is consistent with the cell-specific expression of the *Adh-1* gene reported in maize roots (Rowland et al., 1989).

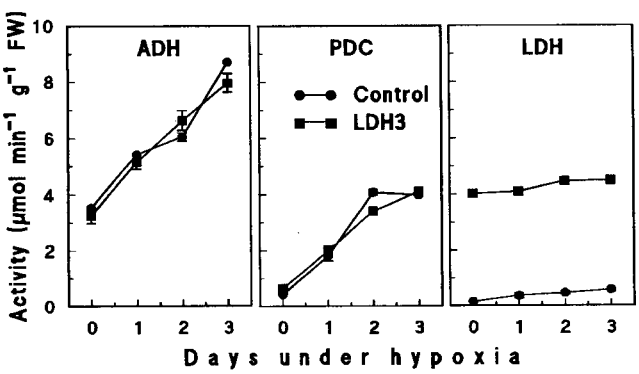

Figure 3. Effect of hypoxia on the extractable activity of ADH, PDC, and LDH in control and transgenic roots. Control and transgenic (LDH3) roots were cultured in liquid medium sparged with air. At time 0 they were subjected to hypoxia by changing the sparging gas to N_2 containing 3 kPa O_2. Enzyme activities were assayed spectrophotometrically on crude extracts. Data are means ± SE for triplicate samples; where no error bars appear they were smaller than the symbols. Cultures maintained in medium sparged with air did not show induction of ADH, PDC, or LDH. Results were similar with clones LDH1 and LDH2.

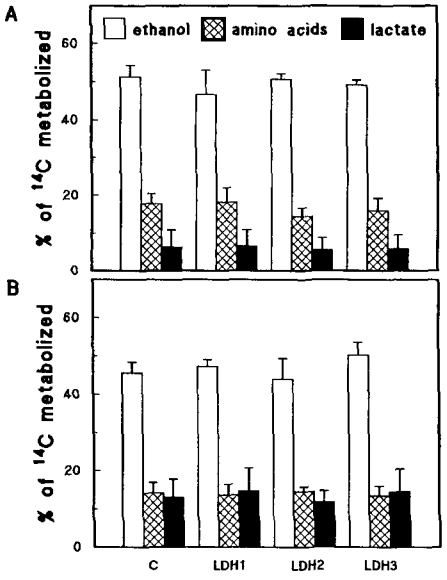

Figure 5. The ^{14}C flux to ethanol, amino acids, and lactate in noninduced (A) and hypoxically induced (B) control and transgenic roots labeled with [U-^{14}C]Glc. A, Roots were incubated under anoxia for 1 h; [U-^{14}C]Glc (74 kBq) was then added and the anoxic incubation continued for 8 h. The amount of tracer metabolized ranged from 25 to 35% of the input. Recovery of ^{14}C was typically approximately 90%. Radioactivity that was not recovered as ethanol, lactate, or amino acids was distributed among CO_2, insoluble matter, and organic acids other than lactate. B, For 3 d before the labeling, the roots were cultured in N_2 containing 3 kPa O_2. Other details are as in A. Bars are means ± SE for three independent experiments. C, Control roots transformed with vector alone; LDH1–3, transgenic roots.

Estimation of the Flux Control Coefficient of LDH for Lactate Fermentation

The flux control coefficient of an enzyme is a quantitative expression of the control that it exercises on the metabolic flux in a whole system. It is defined as the fractional change of flux that results from a fractional change in enzyme activity (Kacser, 1987). LDH activity values were taken from Figures 2 and 3. Glycolytic flux rates to lactate were estimated as explained in the legend to Figure 6. Flux control coefficients were then calculated graphically (Fell, 1992) from the slopes of the response curves shown in Figure 6. For noninduced and hypoxically induced roots, the slopes (flux control coefficients) were 0.02 and 0.01, respectively. This means that LDH accounts for only 1 to 2% of the total control of lactate fermentation and that at least 98% of the control is exerted by other enzymes or transport proteins. It should be noted that our estimates of lactate production were subject to several assumptions. However, considerable errors in these estimates would not materially affect the above conclusion.

Lactate Export from Roots

The transport of lactate out of root cells has been observed in other plants (Hoffman et al., 1986; Xia and Saglio, 1992; Rivoal and Hanson, 1993) and could potentially contribute to the overall control of lactate fermentation. Therefore, we assessed its importance in our system by measuring the distribution of [^{14}C]lactate between the tissue and the medium after an 8-h incubation with [^{14}C]Glc under anoxic conditions (Fig. 7). In noninduced control and transgenic roots, [^{14}C]-lactate was found principally inside the tissue at the end of the incubation. With hypoxically induced roots of both types, there was much greater [^{14}C]lactate production and most of this was recovered from the medium. The efflux of lactate observed in induced roots was apparently a specific process rather than a general leakage of metabolites because most of the ^{14}C-amino acids were retained in the tissue (not shown).

DISCUSSION

To probe the in vivo function of LDH in plant tissues, we overexpressed a cDNA coding for the anaerobically inducible barley LDH in tomato root cultures. Constitutive expression of the transgene had no appreciable effect on fermentative metabolism under anoxia in either induced or noninduced roots. Thus, the kinetics of accumulation of lactate and ethanol followed a similar pattern in control and transgenic clones, with lactate production slowing markedly after the 1st h of anoxia. These results appear to be broadly consistent with the Davies-Roberts hypothesis that lactate fermentation in plant roots is a self-limiting process controlled by a pH-stat mechanism (Davies et al., 1974; Roberts et al., 1984b). It is nevertheless surprising that we could find no effect whatsoever of the transgene expression on lactate production. In the simplest case, noninduced roots containing up to 50 times

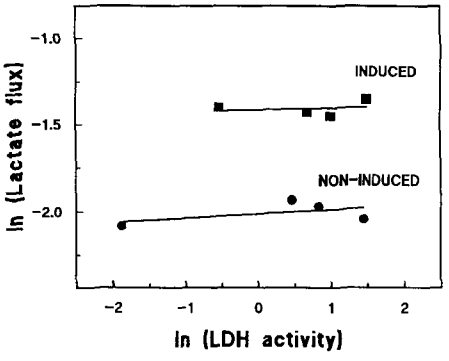

Figure 6. Enzyme activity-flux relationship for LDH and lactate fermentation. Data refer to noninduced roots or to roots hypoxically induced for 3 d. Values for LDH activities in noninduced roots were taken from Figure 2; LDH activities for induced control and LDH3 roots were from Figure 3. Activities for induced LDH1 and LDH2 roots were measured in a separate experiment. Average fluxes to lactate in units of μmol h^{-1} g^{-1} fresh weight were calculated from the 1- and 9-h ^{14}C data of Figure 5 using specific activity values of 5.28 and 6.88 kBq μmol^{-1} for noninduced and induced roots, respectively. These specific activity values were derived from the experimental data obtained for control and transgenic roots in Figures 4 and 5. Although lactate glycolysis was probably more active in a subset of the root cells (see text), this should not have affected the above calculations because there was no evidence that expression of the transgene influenced this phenomenon.

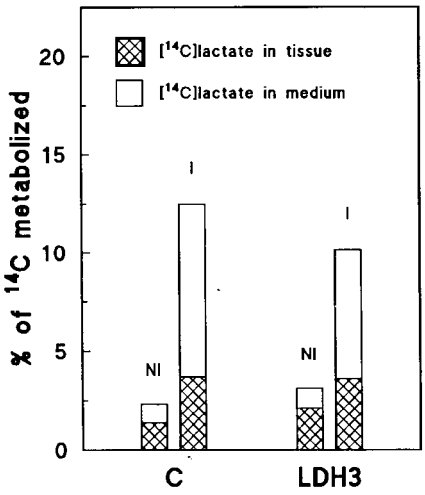

Figure 7. Distribution of [^{14}C]lactate between the roots and the medium after labeling with [^{14}C]Glc for 8 h under anoxic conditions. Control (C) or transgenic (LDH3) noninduced roots (NI) or hypoxically induced roots (I) were incubated in anoxic conditions with [^{14}C]Glc as described in Figure 5. After incubation for 8 h, the medium and roots were analyzed separately. Other details were as in Figure 5. Results were similar with clones LDH1 and LDH2.

more LDH would be expected to show an altered partitioning of the glycolytic flux between lactate and ethanol during or after cytoplasmic acidification. We can suggest two explanations for why this did not occur. First, cytoplasmic pH may have fallen further in the transgenic roots, thereby reducing the activity of the introduced LDH to a greater extent. Second, the LDH transgene product may have been more sensitive than the tomato LDH to a drop in pH (in conjunction with the concentration of effectors or substrates). We favor the latter alternative because the former implies enhanced transient lactate production, which was not observed. However, a definitive answer must wait until measurements of intracellular pH in control and transgenic roots are made.

To quantify the minimal influence of LDH on lactate production, we adopted the formal approach of Metabolic Control Analysis. We recognize that this approach must be applied carefully when changes in the amounts of enzymes are very large (Kaczer, 1987; Fell, 1992). However, it seems impossible to escape the conclusion that, because the flux control coefficient of LDH for lactate glycolysis is very low, LDH has very little control over flux to lactate. Similar conclusions have been reported for other glycolytic enzymes; overexpression of phosphofructokinase (Heinish, 1986; Davies and Brindle, 1992) and phosphoglycerate kinase (Brindle, 1988) had little effect on glycolytic flux.

Overall, Metabolic Control Analysis brings into sharp focus the question, if LDH level does not control lactate fermentation, what does? Our data strongly implicate the capacity to secrete lactic acid to the medium as one controlling factor. Such secretion is not envisioned by the Davies-Roberts hypothesis and would, in fact, interfere with the pH-stat mechanism that is central to it. Hypoxically induced tomato roots secreted lactic acid more efficiently than roots grown under normoxic conditions and showed double the glycolytic carbon flux to lactate. There is already some evidence that hypoxic pretreatment of maize root tips induces the secretion of lactate into the medium (Xia and Saglio, 1992). Also, the roots of some *Limonium* species have an unusually sustained glycolytic flux to lactate, and this is correlated with their ability to export lactate into the medium (Rivoal and Hanson, 1993). Therefore, we hypothesize that the activity of a lactate transporter in the plasma membrane contributes significantly to the control of lactate fermentation in higher plants, and that this transporter is hypoxically inducible in at least some species.

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