Plant Physiol. (1993) 101: 1163–1168

Long-Term Anaerobic Metabolism in Root Tissue[1]

Metabolic Products of Pyruvate Metabolism

Allen G. Good* and Douglas G. Muench

Department of Genetics, University of Alberta, Edmonton, Alberta, Canada, T6G 2E9

The onset of anaerobiosis in barley root tissue (*Hordeum vulgare* L. cv Himalaya) results in the following metabolic responses. There are rapid increases in the levels of pyruvate, lactate, and ethanol. Malate and succinate concentrations increase over the first 12 h, after which they return to the levels found in oxygenated root tissue. Alanine concentration increases over the first 12 h, and this is matched by a corresponding decrease in aspartate. The initial stoichiometric decline in aspartate and increase in alanine suggests that the amino group of aspartate is conserved by transaminating pyruvate to alanine. Aspartate catabolism also probably provides the initial source of carbon for reduction to succinate under anoxic conditions. Under long-term anaerobiosis (>24 h), there is no further accumulation of any of the fermentative end products other than ethanol, which also represents the major metabolic end product during long-term anaerobiosis. Although a number of the enzymes involved in fermentative respiration have been found to be induced under anaerobic conditions, neither aspartate aminotransferase nor malate dehydrogenase is induced in barley root tissue. The observations suggest that the long-term adaptations to hypoxic conditions may be quite different than the more well-characterized short-term adaptations.

Under hypoxic or anaerobic conditions, there are changes in the levels of glycolytic intermediates in roots and an increased glycolytic flux (the Pasteur effect) (Faiz-ur-Rahman et al., 1974; Davies, 1980). The Pasteur effect is believed to occur so that the cell can meet its requirements for ATP despite the much lower efficiency of ATP production by fermentation. The increase in glycolytic flux during hypoxia is accompanied by the accumulation of a number of glycolytic end products including ethanol, lactate, Ala, and various organic acids and amino acids (Effer and Ranson, 1967b; Gfeller and Gibbs, 1984; Roberts et al., 1984; Hoffman et al., 1986; Roberts et al., 1992). A number of alternative pathways to lactate and ethanol fermentation have been discovered in animals with high resistance to anoxia. These animals accumulate combinations of a number of end products including Ala, octopine, alanopine, acetate, propionate, 2-methyl-butyrate, and succinate (Fields, 1983). The advantages of these alternative strategies for anaerobic fermentation are based on an increased ATP production per mol of substrate fermented, the production of NAD^+ to provide a net sink for reductant, and the maintenance of the cellular pH.

Vanlerberghe et al. (1990) have recently shown that the green alga *Selenastrum minutum* accumulates succinate under anaerobic conditions. They demonstrate that the OAA required for reduction to succinate is initially provided by transferring the amino group of aspartate to pyruvate to produce Ala. The advantage of this pathway is that it would provide a 2-fold increase in ATP yield per hexose as compared with ethanol or lactate, and at the same time providing 2 NAD^+. Once the aspartate has been depleted, the OAA is synthesized via carboxylation of phospho*enol*pyruvate to produce 2 ATP and 2 NAD^+ per hexose. Succinate has been shown to accumulate in a number of higher plants under oxygen deficiency; however, in the case of rice this accumulation occurred in the shoots but not the roots (Menegus et al., 1988, 1989).

One of the difficulties with most of the studies on anaerobic metabolism in plants is that they are restricted to short time intervals, usually a maximum of 12 to 24 h (Effer and Ranson, 1967a, 1967b; Hoffman et al., 1986; Vanlerberghe et al., 1990; Roberts et al., 1992). However, since barley (*Hordeum vulgare* L.) and maize can usually survive 3 to 5 d of anaerobic stress (Harberd and Edwards, 1982; Lemke-Keyes and Sachs, 1989), it may be the long-term adaptations to anaerobic stress that are more important in determining sensitivity to flooding.

In this study, we looked at the long-term effects of anaerobiosis on glycolytic metabolism to see how they differ from short-term effects. We show that although a number of metabolic products, lactate, Ala, malate, and succinate, accumulate during short-term anaerobiosis, they do not continue to increase during long-term anaerobiosis. We also examined the effect on the accumulation of metabolites of reducing both the level of available nitrogen and the accumulation of ethanol by using an ADH^- mutant. Finally, we looked at some of the enzymes involved in aspartate and succinate production to determine whether any of these enzymes are induced under anaerobic conditions.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

Barley seeds (*Hordeum vulgare* L. cv Himalaya and an ADH^- mutant isolated by Harberd and Edwards [1982]) were

[1] This research was supported by a grant to A.G.G. from the Natural Sciences and Engineering Research Council of Canada.

* Corresponding author; fax 1–403–492–1903.

Abbreviations: ADH, alcohol dehydrogenase; AlaAT, alanine aminotransferase; AlaDH, alanine dehydrogenase; AspAT, aspartate aminotransferase; LDH, lactate dehydrogenase; MDH, malate dehydrogenase; OAA, oxaloacetate.

surface sterilized in 1% NaOCl, rinsed with water, and planted in vermiculite. After 4 to 6 d of growth at 20°C, individual seedlings were removed and transferred to hydroponic tanks as described (Good and Crosby, 1989a). Plants were grown hydroponically with the roots submerged in half-strength Hoagland solution sparged with air for 15 to 25 d. Anaerobic conditions were achieved by sparging the tank with N_2. The growth conditions were as described previously (Good and Crosby, 1989a).

Nutrient Solutions and Media Experiments

To determine the levels of metabolites outside of the roots, plants were grown in 30-mL test tubes plugged with cotton and containing a medium consisting of half-strength Hoagland solution plus 70 μg/mL of chloramphenicol to limit bacterial growth. Plants were transferred to test tubes containing fresh medium (aerobic or anaerobic) every 24 h. Therefore, the measure of metabolite levels in the medium reflects the cumulative measure over a series of 24-h periods. The medium was assayed directly for the various metabolite levels. The nitrate-free nutrient solution was identical to that described previously (Good and Crosby, 1989b). Plants used in the N-free experiments were transferred from the half-strength Hoagland solution into the N-free media at the start of anaerobic induction.

Enzyme Assays

Extractions were carried out on ice as described previously (Good and Crosby, 1989a). Roots were rinsed in distilled H_2O, briefly blotted, weighed, and ground with sand in a mortar and pestle in extraction buffer containing 0.1 M Tris-HCl (pH 8.5), 10 mM DTT, 15% glycerol. The extract was clarified by centrifugation, and the supernatant was assayed for enzyme activity. AlaAT assays were performed in the Ala → pyruvate direction, whereas ADH was assayed in ethanol → acetaldehyde as described previously (Good and Crosby, 1989a, 1989b). AspAT and MDH activity were assayed by monitoring the oxidation of NADH at 340 nm as described by Griffith and Vance (1989) and Savithiry et al. (1992), respectively. AlaDH activity was assayed at 23°C by monitoring the change in A_{340}. Assays were run in both the forward and reverse directions at different pH values (pH 7–10) using oxidized and reduced forms of NAD and NADP as coenzymes. Assay mixtures for the forward (aminating) reaction consisted of 0.1 M Tris-HCl, 0.03 μmol of NADH or NADPH, 3 μmol of Na-pyruvate, and 200 μL of enzyme extract. To initiate the reaction, 0.1 mM NH_4Cl was added to the reaction. The reverse (deaminating) reaction contained 0.1 M Tris-HCl, 1 mM NAD or NADP, and 200 μL of enzyme extract. The reaction was started by adding 25 mM Ala. In all cases, the assay buffers were tested using bacterial AlaDH (Sigma, A1638).

Assay for Metabolite Levels

Root tissue (1 g/5 mL) was weighed and then immediately ground in an ice-cold mortar and pestle with 0.6 M $HClO_4$ and a pinch of sand. The extract was microcentrifuged, the supernatant was transferred to a new tube, and the pH of the supernatant was adjusted to 4.5 with 3 M KOH. The extract was then recentrifuged and the supernatant collected, sealed in tubes, and stored at −70°C until it was assayed. Ala, aspartate, ethanol, lactate, malate, pyruvate, and succinate concentrations were assayed enzymically according to Bergmeyer (1974). To determine metabolite losses due to the extraction, root extracts were spiked with the appropriate metabolite and the recovery of added compound was determined.

RESULTS

Enzyme Induction

The effect of anaerobiosis on ADH, AspAT, and MDH activity levels in barley roots is shown in Figure 1. ADH activity increased over the 4 d of anaerobic induction, as has been shown previously (Good and Crosby, 1989a). In contrast, AspAT and MDH activity were not anaerobically induced in roots. Both enzymes remained relatively constant in activity during the first 24 h of induction, after which the levels of activity decreased for the next 3 d. The absence of NO_3^- from the nutrient solution had no effect on the pattern of activity of either AspAT or MDH (data not shown). In bacteria and algae, Ala can be formed from pyruvate either by AlaAT or AlaDH (Rowell and Stewart, 1976; Goodwin and Mercer, 1983). We tested the possibility that AlaDH was converting pyruvate to Ala by assaying for AlaDH activity in aerobic and anaerobic root tissue extracts. Using different pH values (pH 7–10) and different cofactors (NAD, NADP), we were unable to detect AlaDH activity in any of the tissue extracts, including leaf extracts.

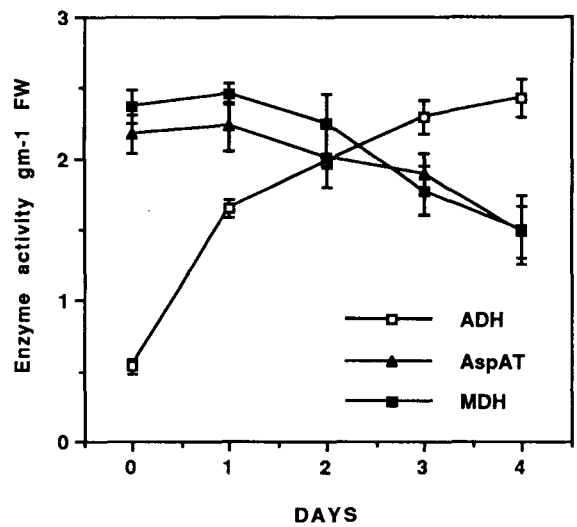

Figure 1. Induction of ADH, AspAT, and MDH activity in anaerobic roots. Plants were sparged with air until 2 weeks old; N_2 then replaced air for the N_2 and NO_3^- plants. Enzyme activities are expressed as IU g^{-1} fresh weight with the exception of MDH, which is IU × 10^{-1} g^{-1} fresh weight. Activities are the mean of three plants, bars represent the SE.

Fermentation Products

With the onset of hypoxia, there is a rapid increase in pyruvate in root tissue. In the Himalaya plants anaerobically induced in the presence or absence of nitrate, the level of pyruvate rose to twice that of the aerobic control within 24 h and remained unchanged for the remaining 72 h (Fig. 2). The level of pyruvate in the ADH$^-$ plants increases much more rapidly than in Himalaya, indicating that in the absence of a functional *Adh1* gene (Harberd and Edwards, 1982), the decreased rate of flux of pyruvate to ethanol results in an accumulation of pyruvate (Fig. 2). However, after 24 h the level of pyruvate began to decrease until it was at or below the control levels of pyruvate (Fig. 2).

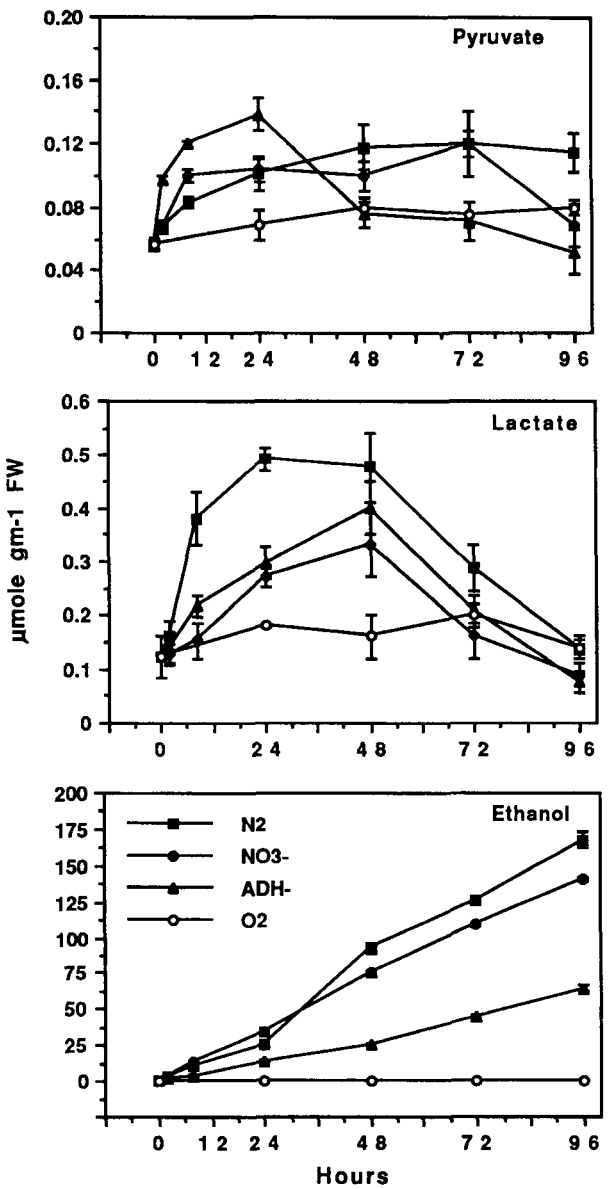

Figure 2. Pyruvate, lactate, and ethanol levels in anaerobically induced barley root tissue. Metabolite levels were measured in root tissue ("Materials and Methods") or media from plants sparged with air (O) or N_2 (■) or grown in a nitrate-free nutrient solution sparged with N_2 (●). ▲, ADH$^-$ plants. Levels are the mean of three plants, bars represent the SE.

Lactate and Ethanol

Lactate and ethanol are both important fermentative end products (Fig. 2). Lactate increased 4-fold within the first 24 h of anaerobiosis. Subsequently, the levels decreased until, after 96 h, they had returned to those of the control plants. The rate of increase for the plants in the N-free media and the ADH$^-$ plants occurred more slowly than for Himalaya; however, the pattern of rapid increase followed by a gradual decline in lactate levels remained the same.

The total ethanol accumulation (tissue and media) under anaerobic conditions is shown in Figure 2. Ethanol accumulated at an average rate of 42 μmol g^{-1} fresh weight d^{-1}. In the absence of an N source, the rate of ethanol accumulation was slightly lower than the Himalaya plants in Hoagland solution. The level of ethanol accumulation in the ADH$^-$ plants was only 16 μmol g^{-1} fresh weight d^{-1}, less than 40% of that found in the wild-type plants. Although none of the other metabolites were detected outside of the root in the medium, almost all of the ethanol that accumulated during anaerobiosis was present in the medium after diffusing out of the root tissue. The concentration of ethanol in root tissue was at most 2% of that found in the medium. Ethanol was the predominant product of long-term anaerobic glycolysis, occurring at levels of up to 50 times higher than any of the other metabolites.

Ala and Aspartate

There is a rapid increase in Ala during the first 24 h of anaerobiosis, followed by a leveling off through to 96 h (Fig. 3). Aspartate concentrations decreased rapidly almost immediately after exposure to anaerobic conditions (Fig. 3). Vanlerberghe et al. (1991) have argued that during the early stages of anaerobiosis, the amino group of aspartate is conserved by transaminating pyruvate to Ala. Similarly, we found that the decrease in aspartate over the first 8 h can account for the increase in Ala, since during this time a stoichiometry of approximately 1.08 mol of aspartate depleted per mol of Ala accumulated was observed. After 8 h of anaerobiosis, the level of Ala continued to increase another 2-fold. The level of aspartate, however, did not decrease further after the first 8 h. Instead, it leveled off and then rose slowly (Fig. 3). This pattern of aspartate depletion was similar for the ADH$^-$ plants and plants in the nitrate-free medium.

Although the levels of Ala in the ADH$^-$ plants rose more slowly than those in the Himalaya plants, they eventually reached similar concentrations near the end of the induction period (Fig. 3). The plants grown in a nitrate-free medium had levels of Ala that continued to increase for 48 h but then decreased to control levels. This clearly indicates that after 8 h the amino group of Ala is no longer provided by aspartate, and to maintain Ala levels a medium that contains a supply of N is required.

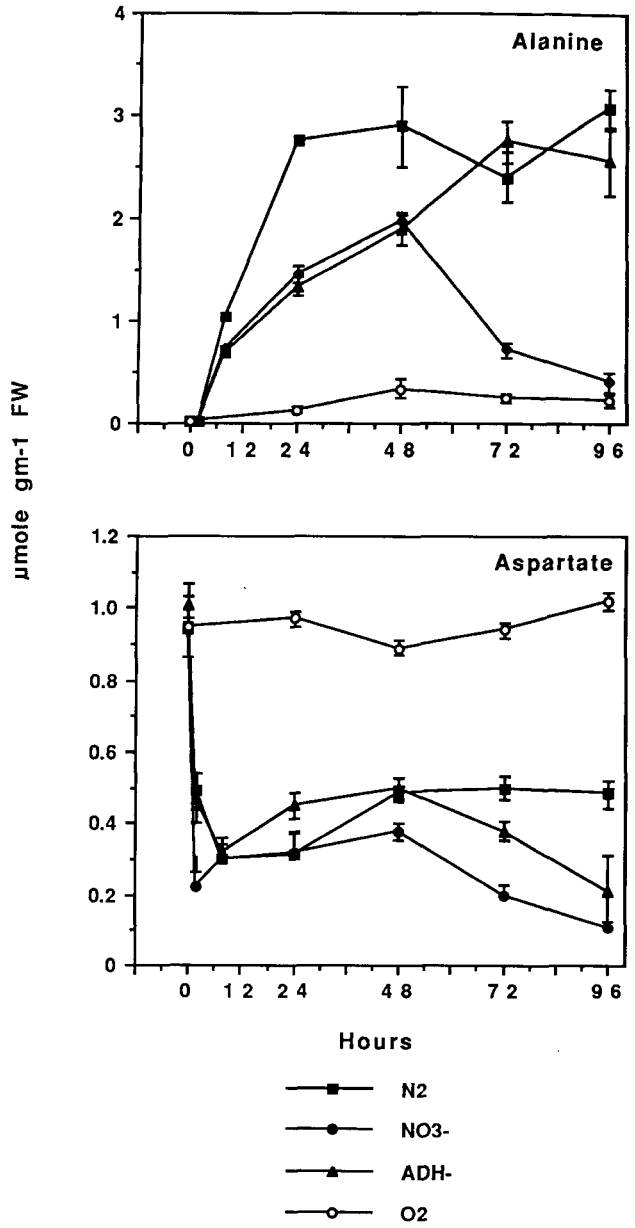

Figure 3. Ala and aspartate levels in anaerobically induced barley root tissue. Ala and aspartate levels were measured in root tissue ("Materials and Methods") from plants sparged with air (○) or N_2 (■) or grown in a nitrate-free nutrient solution sparged with N_2 (●). ▲, ADH^- plants. Levels are the mean of three plants, bars represent the SE.

Malate and Succinate

Malate showed an initial increase during the first 8 h of anaerobiosis, followed by a gradual long-term decline (Fig. 4). This occurred for both genotypes and in the presence or absence of NO_3^-. Succinate increased approximately 1.5 times during the first 8 h, followed by a gradual decrease back to control levels after 24 h. Thereafter, the concentration of succinate remained stable (Fig. 4). The ADH^- plants had a higher initial level of succinate, which increased during the first 8 h of hypoxia and then gradually declined to a level similar to that of the Himalaya plants.

DISCUSSION

Enzyme Induction

The onset of anaerobiosis in root tissue has been shown to result in increases in a number of glycolytic enzymes, including ADH, LDH, and AlaAT (Kelley and Freeling, 1984a, 1984b; Good and Crosby, 1989a, 1989b). One of the interesting observations from this work is that whereas both LDH and AlaAT activity levels continue to accumulate for up to 96 h (Good and Crosby, 1989a, 1989b), the end products (lactate and Ala, respectively) reach their maximum levels

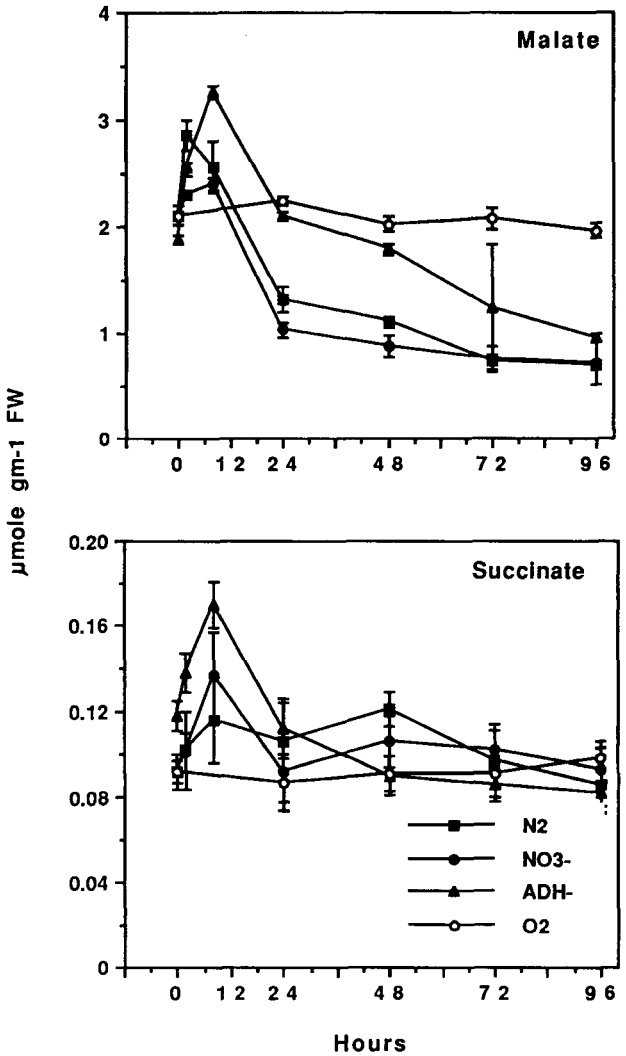

Figure 4. Malate and succinate levels in anaerobically induced barley root tissue. Malate and succinate levels were measured in root tissue ("Materials and Methods") from plants sparged with air (○) or N_2 (■) or grown in a nitrate-free nutrient solution sparged with N_2 (●). ▲, ADH^- plants. Levels are the mean of three plants, bars represent the SE.

after the first 24 h (Figs. 2 and 3). Both AspAT and MDH remained constant in activity during the first 24 h, followed by a long-term decline in activity levels. The end products aspartate and malate, however, are not maintained at a steady state. Under anaerobic conditions, aspartate concentration drops rapidly, whereas the concentration of malate showed a transient increase followed by a steady decrease. The final enzyme involved in the synthesis of succinate, succinate dehydrogenase, is not induced under anaerobic conditions (Kennedy et al., 1987). These data suggest a correlation between the anaerobic induction of an enzyme and an increase in the level of product for at least the first 24 h of anaerobiosis. However, there are several other enzymes (e.g. AlaAT and LDH) that do not appear to need further induction under long-term anaerobiosis, since the products of these enzymes no longer increase in concentration. The evolutionary and physiological significance of this response is not known.

The possibility that Ala is formed from pyruvate by AlaDH seems unlikely, since AlaDH activity was undetectable in any of the tissues assayed. Although AlaDH occurs in bacteria and algae (Rowell and Stewart, 1976; Goodwin and Mercer, 1983), to the best of our knowledge the only report of AlaDH activity in higher plants was in mulberry (Talyshinskii, 1983).

Fermentation Products

The accumulation of pyruvate in anaerobic root tissue presumably results from the Pasteur effect, which is an increase in glycolysis so that the cell can meet its ATP utilization requirements. The Pasteur effect has been shown to occur in both higher plants and algae (Faiz-ur-Rahman et al., 1974; Peavey et al., 1983; Vanlerberghe et al., 1989). Associated with this increase in pyruvate is the continual production of ethanol, most of which moves from the root tissue into the medium (Fig. 2). The amount of ethanol produced by the ADH^- mutant was only 40% of that produced by the ADH^+ genotype. This corresponds to the difference in ADH activity in these plants (Good and Crosby, 1989a), indicating that the rate of ethanol accumulation is determined by the level of ADH activity. Lactate and Ala are also important metabolic end products during the first 24 h of anaerobiosis (Fig. 2); however, they do not accumulate further during long-term anaerobiosis.

The comparison of Ala and aspartate levels during the first 8 h of anaerobiosis supports the hypothesis of Vanlerberghe et al. (1990). They argue that during short-term anaerobiosis, aspartate provides the OAA for reduction to succinate. The amino group of aspartate is conserved by transaminating pyruvate to Ala. We have found a 1:1 relationship between the production of Ala and the depletion of aspartate during the first 8 h of anaerobiosis. After that point, the level of Ala continued to increase for the next 16 h. Therefore, during this stage of anaerobiosis the amino group of Ala clearly does not come from aspartate. Because Ala synthesis requires N, it has been suggested that the N status of a tissue may be important in determining whether Ala is a significant fermentation end product (Hoffman et al., 1986; Good and Crosby, 1989b). Vanlerberghe et al. (1991) have recently shown that under anaerobic conditions almost all of the assimilated N accumulates in Ala. Similarly, we found that in the absence of an N source and under anaerobic conditions, the Ala concentration did not reach the level found in plants grown in an N-rich medium, and decreases after 48 h. Presumably, the increase in Ala seen between 8 and 48 h is a result of the endogenous N supply, since our plants were not maintained in an N-limited environment prior to anaerobic induction.

Aspartate levels have been shown to decrease dramatically during the onset of hypoxia, in both higher plants and algae (Vanlerberghe et al., 1990; Roberts et al., 1992). We found a similar pattern of aspartate depletion; however, in the Himalaya plants, the level of aspartate rose slowly during the next 72 h. As with Ala, aspartate was the only other metabolite that required an external N source to be maintained at higher levels.

The pattern of malate and succinate accumulation during short-term anaerobiosis in higher plants and algae has recently been characterized (Vanlerberghe et al., 1991; Roberts et al., 1992). Malate has been shown in some studies to accumulate during anoxia (Davies, 1980), whereas in other studies there has been no evidence of malate as a significant fermentative end product (Vanlerberghe et al., 1991; Roberts et al., 1992). Consistent with what has been found in corn and *S. minutum* (Vanlerberghe et al., 1991; Roberts et al., 1992), we found that there is a short-term increase in malate, followed by a long-term decline (Fig. 4).

Although it has been argued that succinate accumulation plays a role in resistance to hypoxia (Menegus et al., 1988, 1989), Roberts et al. (1992) found that succinate does not accumulate in corn root tips. We found a similar pattern, with succinate showing a short-term increase in levels followed by a long-term decline. Presumably, the short-term increase in succinate levels results from the deamination of aspartate. However, the complete fate of the OAA produced in the deamination of aspartate during the early stages of anaerobiosis is not clear in this system, since the ratio of aspartate depletion to succinate accumulation is not 1:1.

One of the mechanisms by which plants might be able to survive long-term anaerobiosis would be to utilize alternative fermentative pathways that would be capable of increased ATP production per mol of substrate fermented. Although it has been argued that the formation of succinate might be an alternative strategy, these data indicate that whereas succinate and malate are products of anaerobic fermentation in the short term, in the long term they are not (Fig. 4). In addition, none of the enzymes involved in aspartate deamination and succinate production show any form of anaerobic induction (Fig. 1; Kennedy et al., 1987). The predominant long-term anaerobic product in this system is clearly ethanol. It has been argued that LDH and ADH activities do not seem to be directly involved in the mechanisms of resistance to anoxia (Roberts et al., 1989; Xia and Saglio, 1992). However, the only known mutation that has been shown to affect survival during anaerobic stress is an ADH^- mutant (Harberd and Edwards, 1982). Clearly, there are other aspects of anaerobic metabolism that must play an important role in determining long-term survivability.

Received September 10, 1992; accepted December 2, 1992.
Copyright Clearance Center: 0032-0889/93/101/1163/06.

LITERATURE CITED

Bergmeyer H-U (1974) Methods of Enzymatic Analysis, Ed 2. Academic Press, New York

Davies DD (1980) Anaerobic metabolism and the production of organic acids. *In* DD Davies, ed, The Biochemistry of Plants, Vol 2. Academic Press, New York, pp 581–611

Effer WR, Ranson SL (1967a) Respiratory metabolism in buckwheat seedlings. Plant Physiol **42:** 1042–1052

Effer WR, Ranson SL (1967b) Some effects of oxygen concentration on levels of respiratory intermediates in buckwheat seedlings. Plant Physiol **42:** 1053–1058

Faiz-ur-Rahman ATM, Trewavas AJ, Davies DD (1974) The Pasteur effect in carrot root tissue. Planta **118:** 195–210

Fields JHA (1983) Alternatives to lactic acid: possible advantages. J Exp Zool **228:** 445–457

Gfeller RP, Gibbs M (1984) Fermentative metabolism in *Chlamydomonas reinhardii*. I. Analysis of fermentative products from starch in dark and light. Plant Physiol **47:** 841–843

Good AG, Crosby WL (1989a) Induction of alcohol dehydrogenase and lactate dehydrogenase in hypoxically induced barley. Plant Physiol **90:** 860–866

Good AG, Crosby WL (1989b) Anaerobic induction of alanine aminotransferase in barley root tissue. Plant Physiol **90:** 1305–1309

Goodwin TW, Mercer EI (1983) Introduction to Plant Biochemistry, Ed 2. Permagon Press, New York

Griffith SM, Vance CP (1989) Aspartate aminotransferase in alfalfa root nodules. I. Purification and partial characterization. Plant Physiol **90:** 1622–1629

Harberd NP, Edwards KJR (1982) The effect of a mutation causing alcohol dehydrogenase deficiency on flooding tolerance in barley. New Phytol **90:** 631–644

Hoffman NE, Bent AF, Hanson AD (1986) Induction of lactate dehydrogenase isozymes by oxygen deficit in barley root tissue. Plant Physiol **82:** 658–663

Kelley PM, Freeling M (1984a) Anaerobic expression of maize glucose phosphate isomerase I. J Biol Chem **259:** 673–677

Kelley PM, Freeling M (1984b) Anaerobic expression of maize fructose 1,6-diphosphate aldolase. J Biol Chem **259:** 14180–14183

Kennedy RA, Fox TC, Siedow JN (1987) Activities of isolated mitochondria and mitochondrial enzymes from aerobically and anaerobically germinated barnyard grass (*Echinochloa*) seedlings. Plant Physiol **85:** 474–480

Lemke-Keyes CA, Sachs MM (1989) Genetic variation for seedling tolerance to anaerobic stress in maize germplasm. Maydica **34:** 329–337

Menegus F, Cattaruzza L, Chersi A, Fronza G (1989) Differences in the anaerobic lactate-succinate production and in the changes in cell sap pH for plants with high and low resistance to anoxia. Plant Physiol **90:** 29–32

Menegus F, Cattaruzza L, Chersi A, Selva A, Fronza G (1988) Production and organ distribution of succinate in rice seedlings during anoxia. Physiol Plant **74:** 444–449

Peavey DG, Gfeller RP, Gibbs M (1983) Presence of a Pasteur effect in algae. Physiol Veg **21:** 124–128

Roberts JKM, Callis J, Wemmer P, Walbot V, Jardetzky O (1984) Mechanism of cytoplasmic pH regulation in hypoxic maize root tips and its role in survival under hypoxia. Proc Natl Acad Sci USA **91:** 3379–3383

Roberts JKM, Chang K, Webster C, Callis J, Wemmer P, Walbot V (1989) Dependence of ethanolic fermentation, cytoplasmic pH regulation, and viability on the activity of alcohol dehydrogenase in hypoxic maize root tips. Plant Physiol **89:** 1275–1278

Roberts JKM, Hooks MA, Miallis AP, Edwards S, Webster C (1992) Contribution of malate and amino acid metabolism to cytoplasmic pH regulation in hypoxic root tips studied using nuclear magnetic resonance spectroscopy. Plant Physiol **98:** 480–487

Rowell P, Stewart WPD (1976) Alanine dehydrogenase of the N_2-fixing blue-green alga, *Anabeana cylindrica*. Arch Microbiol **107:** 115–124

Savithiry S, Wert V, Ries S (1992) Influence of 9-β-L(+)-adenosine on malate dehydrogenase activity in rice. Physiol Plant **84:** 460–466

Talyshinskii GM (1983) Electrophoretic study of amylase and alanine dehydrogenase in leaves of male and female polyploid mulberries. Izv Akad Nauk Kaz SSR Ser Biol Nauk **4:** 53–56

Vanlerberghe GC, Feil R, Turpin DH (1990) Anaerobic metabolism in the N-limited alga *Selenastrum minutum*. I. Regulation of carbon metabolism and succinate as a fermentation product. Plant Physiol **94:** 1116–1123

Vanlerberghe GC, Horsey AK, Weger HG, Turpin DH (1989) Anaerobic carbon metabolism by the tricarboxylic acid cycle. Evidence for partial oxidative and reductive pathways during dark ammonium assimilation. Plant Physiol **91:** 1551–1557

Vanlerberghe GC, Joy KW, Turpin DH (1991) Anaerobic metabolism in the N-limited alga *Selenastrum minutum*. III. Alanine is the product of anaerobic ammonium assimilation. Plant Physiol **95:** 655–658

Xia J-H, Saglio PH (1992) Lactic acid efflux as a mechanism to hypoxic acclimation of maize root tips to anoxia. Plant Physiol **100:** 40–46