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Activities of Isolated Mitochondria and Mitochondrial Enzymes from Aerobically and Anaerobically Germinated Barnyard Grass (*Echinochloa*) Seedlings[1]

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ABSTRACT

Activity of mitochondria isolated from whole seedlings of *Echinochloa crus-galli* (L.) Beauv. var *oryzicola* germinated under aerobic and anaerobic conditions for 5 to 7 days was investigated. Mitochondria from both treatments exhibited good respiratory control and ADP/O ratios. Although O_2 uptake was low in anaerobic mitochondria, activity rapidly increased when the seedlings were transferred to air. Mitochondria from both aerobically and anaerobically grown seedlings of *E. crus-galli* var *oryzicola* maintained up to 66% of their initial respiration rate in the presence of both cyanide and salicylhydroxamic acid, and the inhibitory effects of cyanide and azide were additive. In addition, antimycin A was not an effective inhibitor of respiration. Reduced-minus-oxidized absorption spectra revealed that cytochromes *a*, a_3, and *b* were reduced to a greater extent and cytochrome *c* was reduced to a lesser extent in anaerobically germinated seedlings relative to that in aerobically germinated seedlings. An absorption maximum in the cytochrome *d* region of the spectrum was reduced to the same extent under both germination conditions and an absorption maximum at 577 nm was present only in anaerobically germinated seedlings. Anaerobically germinated seedlings contained 70% of the cytochrome *c* oxidase activity found in air grown seedlings. Upon exposure to air, the developmental pattern of this enzyme in anaerobically germinated seedlings was similar to air controls. Succinate dehydrogenase activity in anaerobic seedlings was only 45% of the activity found in aerobically germinated seeds, but within 1 hour of exposure to air, the activity had increased to control levels. The results suggest that mitochondria isolated from *E. crus-galli* var *oryzicola* differ from other plants studied and that the potential for mitochondrial function during anaerobiosis exists.

Echinochloa crus-galli (barnyard grass) is a noxious weed in virtually all crops throughout the world. For several years, we have been studying one variety of this species, *E. crus-galli* var *oryzicola* (hereafter, oryzicola). It too is a noxious weed, but its distribution is limited to rice fields in California and the Far East. As a group, barnyard grass species reduce the yield of rice more than any other stress factor (2).

We have been especially interested in the ability of oryzicola to germinate and grow anaerobically. In the laboratory, this plant provides a unique system to study metabolic adaptations to low oxygen. Compared to rice, oryzicola germinates better at lower temperatures with or without O_2 (12). Oryzicola also germinates better under flooded conditions and the resulting seedlings are taller than those of rice. Indeed, after 10 d, oryzicola can germinate, produce a seedling, and emerge through 25 cm of water while rice seedlings are only 10 to 15 cm tall. These two environmental conditions, low O_2 and low temperature, are the same conditions that exist in rice fields at time of sowing.

While fermentative metabolism is an important feature of anaerobic metabolism in oryzicola, other metabolic characteristics such as the oxidative pentose phosphate pathway (13, 18, 19) and lipid metabolism (4, 14, 19, 24) appear to play equally important roles. Mitochondrial electron transport may so be an important metabolic consideration not only during anoxia, but also during the transition from anaerobic to aerobic conditions. Reduction of an alternate electron acceptor, such as nitrate (6) or organic acids (5, 8), could permit electron transport in the absence of O_2. In support of this hypothesis, we have found that nitrate reserves are depleted in oryzicola seeds during germination (13). Once germinated, the ability to rapidly shift from an anaerobic mode to an aerobic one may be an important metabolic feature during early establishment of this flood tolerant species.

In previous studies, we noticed that, unlike rice, germination of oryzicola is inhibited by cyanide and azide (13). In addition, tricarboxylic acid cycle intermediates are produced and acetate is readily metabolized under anaerobic conditions (19). Finally, unlike reports for other flood tolerant species (16, 23, 24, 26), mitochondrial ultrastructure in seedlings germinated under N_2 were indistinguishable from those grown in air (11). These results suggested to us that, at least in oryzicola, mitochondria may function to some extent even under anaerobic conditions. While much research remains to be done on mitochondrial structure and function in all plants, especially electron transport, almost no research has been done concerning the capability of mitochondria under low or nonoxygenic conditions. Here, we show the activity of mitochondria isolated from anaerobically grown seedlings and their development upon transition to air.

MATERIALS AND METHODS

Plant Material. Seeds of *Echinochloa crus-galli* (L.) Beauv. var *oryzicola*, mung bean (*Vigna radiata* L.) and rice (*Oryza sativa* L.) were surface sterilized with 2.5% NaOCl for 15 min, washed with tap water for 20 min, and imbibed on moist filter paper. Aerobically germinated seedlings were grown in air in the dark for 7 d. Anaerobically germinated seedlings were grown in an anaerobic chamber (Forma Scientific, Inc.) for 5 to 7 d before

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transfer to air or extraction.

Mitochondrial Isolation. Mitochondria were isolated from 7 d old oryzicola seedlings or excised mung bean hypocotyls by differential centrifugation modified from Ikuma and Bonner (10). For anaerobically grown seedlings, tissue homogenization was performed in an anaerobic chamber. The remaining steps were carried out in air; the preparation was exposed to air for 20 to 30 min before measurements were made. Aerobically grown seedlings or seedlings transferred from anaerobic to aerobic conditions were isolated in air. For oryzicola, the grinding medium (pH 7.5) consisted of 0.45 M mannitol, 20 mM Hepes, 0.1 mM EGTA, 0.3 mM sodium metabisulfite ($Na_2S_2O_5$), 5.0 mM DTT, 1% (w/v) BSA, and 1% (w/v) PVPP; the washing medium (pH 7.5) contained 0.45 M mannitol, 20 mM Hepes, 0.1 mM EGTA, and 0.1% (w/v) BSA. For mung bean, the grinding medium (pH 7.5) was composed of 0.3 M mannitol, 20 mM Hepes, 1.0 mM EDTA, 0.3 mM $Na_2S_2O_5$, and 0.1% (w/v) BSA; the washing medium (pH 7.5) consisted of 0.3 M mannitol, 20 mM Hepes, 1.0 mM EDTA, and 0.1% (w/v) BSA. The tissue was cut into 2 cm segments, suspended in the appropriate grinding medium, and homogenized with two 10 s bursts in a Waring Blendor. The brei was filtered through four layers of cheesecloth and centrifuged for 4 min at 2,000*g*. The pellet was discarded and the supernatant centrifuged for 6 min at 14,500*g*. The supernatant was discarded; the pellet was gently resuspended in 15 to 20 ml washing medium, and centrifuged for 4 min at 2,000*g*. The pellet was discarded and the supernatant centrifuged for 6 min at 14,500*g*. The supernatant was discarded and the pellet was resuspended in 1 ml washing medium.

Activity of isolated mitochondria was assessed in a 1 ml reaction mixture (pH 7.5) containing 0.3 M mannitol, 10 mM K-phosphate, 10 mM KCl, 5 mM $MgCl_2$, 150 μM ATP, mitochondria containing 0.15 to 0.25 mg protein, and one or more of the following substrates: 25 mM L-malate, 10 mM succinate, 1.0 mM NADH, 20 mM L-glutamate. Subsequently, ADP and metabolic inhibitors were added to the reaction mixture to yield the following concentrations: 50 to 165 μM ADP, 250 μM KCN, 1 μM SHAM,[2] 250 μM rotenone, 6 μM antimycin A, and 250 μM NaN_3. Oxygen utilization was measured polarographically with a Clark-type electrode in a 1 ml water jacketed plexiglass reaction cell maintained at 25°C. Oxygen content of air saturated media was taken as 250 μM O_2. Protein was determined by the BioRad protein assay.

Degree of intactness of isolated mitochondria was estimated from the ratio of succinate: Cyt oxidoreductase activity in isotonic and hypotonic buffers (3). The 3 ml reaction mixture consisted of 5.0 mM K-phosphate (pH 7.2) containing either sucrose or 0.015% (v/v) Triton X-100, 50 μM Cyt *c*, 1.0 mM KCN, 10 mM succinate, and 100 μl isolated mitochondria. The increase in absorbance at 550 nm was monitored following addition of succinate.

Reduced-Minus-Oxidized Spectra. Isolated mitochondria (0.15–0.25 mg protein) were suspended in 3 ml of 50% glycerol and 50% buffer (0.3 M mannitol, 10 mM K-phosphate, 10 mM KCl, and 5 mM $MgCl_2$; pH 7.2). The cuvettes were subjected to three freeze/thaw cycles using liquid N_2. The base line for the samples was determined and subsequently subtracted from the difference spectrum. Either 15 mM sodium dithionite (sample cuvette) or 15 mM potassium ferricyanide (reference cuvette) was added to the cuvettes and the difference spectrum was obtained with a Hitachi 100-80 spectrophotometer with the cuvettes cooled to −140°C.

Enzyme Assays. One to 5 g of seedlings were ground in 10 ml extraction medium (50 mM Hepes buffer [pH 7.5], 10 mM mercaptoethanol, 5% [w/v] PVPP) at 4°C with mortar and pestle. The homogenates were filtered through cheesecloth and centrifuged at 14,500*g* for 20 min at 4°C. The supernatant was analyzed for Cyt *c* oxidase and succinate dehydrogenase activities at 25°C. For Cyt *c* oxidase (7), the reaction mixture contained 46 mM Hepes (pH 7.5), 0.1% (w/v) digitonin, 50 μM Cyt *c*, and 100 μl extract in a final volume of 3 ml. The decrease in absorbance at 550 nm was monitored following addition of Cyt *c*. For succinate dehydrogenase (20), the reaction mixture was composed of 40 mM Hepes (pH 7.5), 2 mM KCN, 100 mM NEM, 20 mM succinate, 0.002% (w/v) DCIP, 0.033% (w/v) PMS, and 100 μl extract in a final volume of 3 ml. The decrease in absorbance at 600 nm was monitored following addition of PMS.

RESULTS

Functionally competent mitochondria were isolated from both aerobically and anaerobically grown oryzicola seedlings (Fig. 1). The mitochondria exhibited good respiratory activity, especially for N_2 grown plants. Intactness for mitochondria isolated from either tissue was approximately 70%. Moreover, "aerobic mitochondria' had good RC (ratio of State 3 to State 4 respiration rates) and ADP/O ratios, comparing well with mitochondria isolated from mung bean (Table I; Ref. 3). For "anaerobic mitochondria,' RC values were generally lower than those from air grown plants for all substrates tested, but still indicated control by ADP. ADP/O ratios and rates of O_2 consumption, however, were much lower in N_2 grown tissues when compared to those grown in air.

The most striking difference between mitochondria from oryzicola and mung bean was in their residual respiration (O_2 uptake remaining after the sequential addition of KCN and SHAM to the reaction medium). Whereas mung bean showed little or no residual respiration (Table I), mitochondria isolated from oryzicola exhibited much greater resistance to KCN and SHAM. Depending on substrate, residual respiration in aerobic mitochondria ranged from 13 to 40%, while that in anaerobic mitochondria varied from 20 to 66%.

Mitochondrial response to various electron transport inhibitors are presented in Figure 2. With malate as substrate, residual respiration for both air and N_2 grown mitochondria were 32 and 52%, respectively. Also, sequential addition of cyanide plus azide (or conversely, azide plus cyanide) gave additive effects. Finally, the addition of antimycin A prior to the addition of cyanide did not reduce the inhibition due to cyanide significantly, whereas addition of rotenone prior to KCN greatly reduced the amount of inhibition resulting from cyanide.

Differences in electron transport chain between aerobically and anaerobically grown seedlings were determined by analyzing the reduced-minus-oxidized spectra of isolated mitochondria (Fig. 3). The spectra were qualitatively similar with one exception. Anaerobic mitochondria exhibited a peak at 577 nm that was not present in aerobic mitochondria. In addition, quantitative differences in the absorbance of several cytochromes were observed (Table II). Although a peak in the Cyt *d* region of the spectrum was similar in both aerobic and anaerobic tissues, Cyt $a \cdot a_3$, and *b* were reduced to a greater extent in anaerobic tissue. Cyt *c*, on the other hand, was reduced to a greater extent in mitochondria isolated from aerobic tissue.

The development of mitochondrial function was determined by the activity of mitochondria isolated from oryzicola seedlings that were grown in N_2 for 5 d and subsequently exposed to air for varying periods of time (Fig. 4). During the first 48 h of exposure to air, RC decreased while ADP/O ratios increased slightly. During the same period, the respiration rate increased sharply, with the largest increase (16-fold) taking place during the first 30 min in air.

[2] Abbreviations: SHAM, salicylhydroxamic acid; DNP, 2,4-dinitrophenol; NEM, *N*-ethylmaleimide; DCIP, 2,6-dichlorophenolindolphenol; PMS, phenazine methosulfate; RC, respiratory control.

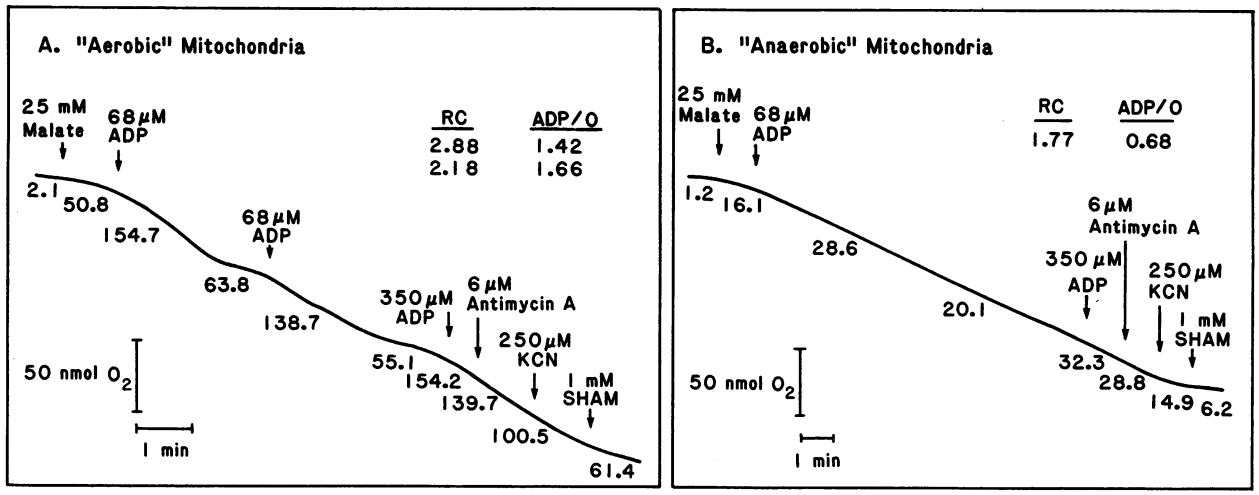

FIG. 1. Representative tracings of O_2 uptake in mitochondria isolated from (A) aerobically and (B) anaerobically germinated seedlings of *E. crus-galli* var *oryzicola*. Isolation procedure and assay conditions are described in "Materials and Methods."

Table I. *Respiratory Properties of Mitochondria Isolated from 7-d-old Seedlings of E. crus-galli var oryzicola and Mung Bean Grown under Aerobic or Anaerobic Conditions*

Values are means ± SE of six replications for oryzicola and three replications for mung bean.

Condition	Intactness	Substrate	RC	ADP/O	O_2 Consumption			
					Rate	Inhibition		
						KCN	SHAM	Residual
	%				*nmol min^{-1} mg^{-1} protein*		%	
Oryzicola								
O_2	72.4 ± 3.34	Malate, 25 mM	1.86 ± 0.12	1.55 ± 0.09	228.4 ± 13.7	38.5 ± 3.5	30.4 ± 13.0	31.1
		Succinate, 10 mM	1.55 ± 0.02	0.96 ± 0.07	306.5 ± 12.2	52.9 ± 7.3	34.4 ± 9.2	12.7
		NADH, 1 mM	1.38 ± 0.06	1.54 ± 0.09	224.0 ± 31.7	48.5 ± 8.5	29.0 ± 11.5	22.5
		Malate, 25 mM; succinate, 10 mM; glutamate, 20 mM	1.82 ± 0.06	1.42 ± 0.07	172.0 ± 16.3	29.6 ± 4.1	41.7 ± 13.2	28.7
N_2	68.0 ± 2.34	Malate, 25 mM	1.56 ± 0.22	0.65 ± 0.03	34.8 ± 4.6	36.6 ± 13.3	26.8 ± 11.6	59.8
		Succinate, 10 mM	2.17 ± 0.41	0.69 ± 0.06	28.5 ± 1.4	11.8 ± 6.9	21.8 ± 3.8	66.4
		Malate, 25 mM; succinate, 10 mM; glutamate, 20 mM	1.92 ± 0.22	0.57 ± 0.05	30.6 ± 6.2	35.4 ± 5.0	25.2 ± 8.3	39.4
Mung Bean								
O_2		Malate, 25 mM	2.51 ± 0.35	2.48 ± 0.10	145.8 ± 10.5	51.2 ± 6.0	44.5 ± 2.5	4.3
		Succinate, 10 mM	1.77 ± 0.08	1.54 ± 0.13	169.3 ± 18.2	61.0 ± 3.7	38.2 ± 3.1	0.8
		NADH, 1 mM	1.67 ± 0.16	1.42 ± 0.10	100.0 ± 6.2	62.0 ± 5.0	35.4 ± 2.9	2.6

Similar developmental comparisons were made with two mitochondrial marker enzymes from seedlings of both oryzicola and rice that were germinated in N_2 for 5 d before transfer to air. During the first 24 h of exposure to air, Cyt *c* oxidase activity of oryzicola seedlings germinated under N_2 increased 2-fold (Fig. 5). The developmental pattern of Cyt *c* oxidase activity in anaerobically germinated oryzicola seedlings closely paralleled that of aerobically germinated seedlings of the same age. On an absolute basis, however, Cyt *c* oxidase activity in anaerobically germinated seedlings was only 70% of that of aerobically germinated seedlings. Succinate dehydrogenase activity increased 2.4-fold within 1 h of exposure to air, whereas aerobically germinated seedlings exhibited no change in the activity of this enzyme during the same developmental stage. Exposure of seedlings to light after 24 h in air caused a slight increase in Cyt *c* oxidase activity and a marked decrease in succinate dehydrogenase activity.

For 5 d old rice seedlings, transfer from anaerobic to aerobic conditions caused Cyt *c* oxidase activity to decline sharply during the initial 1 h in air (Fig. 6). Subsequently, changes in Cyt *c* oxidase activity paralleled that of aerobically grown seedlings, albeit at lower rates. During the initial 24 h in air, succinate dehydrogenase activity decreased in both air grown controls and anaerobically germinated seedlings transferred to air, with the latter declining more rapidly. But, during the next 24 h period, air grown controls continued to lose succinate dehydrogenase activity, while activity in seedlings that had been transferred from N_2 to air increased.

DISCUSSION

Earlier results suggested that, in anaerobically germinated oryzicola, mitochondria may be functional even in the absence of O_2. This evidence included the presence of intact, nondistorted mitochondria (11) and the formation of TCA cycle intermediates when oryzicola seedlings were germinated anaerobically in the

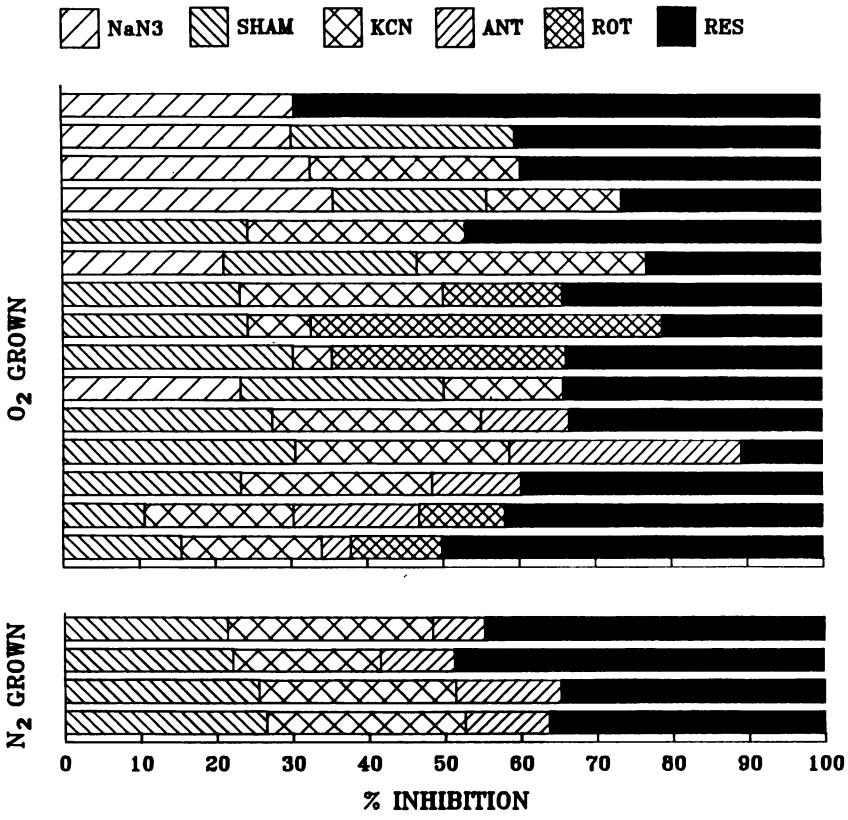

FIG. 2. Effect of inhibitors on O_2 consumption of mitochondria isolated from aerobically and anaerobically germinated seedlings of *E. crus-galli* var *oryzicola*. Concentrations of inhibitors in the reaction mixture were 250 μM NaN_3, 250 μM KCN, 1 mM SHAM, 6 μM antimycin A (ANT), and 250 mM rotenone (ROT). RES, residual respiration.

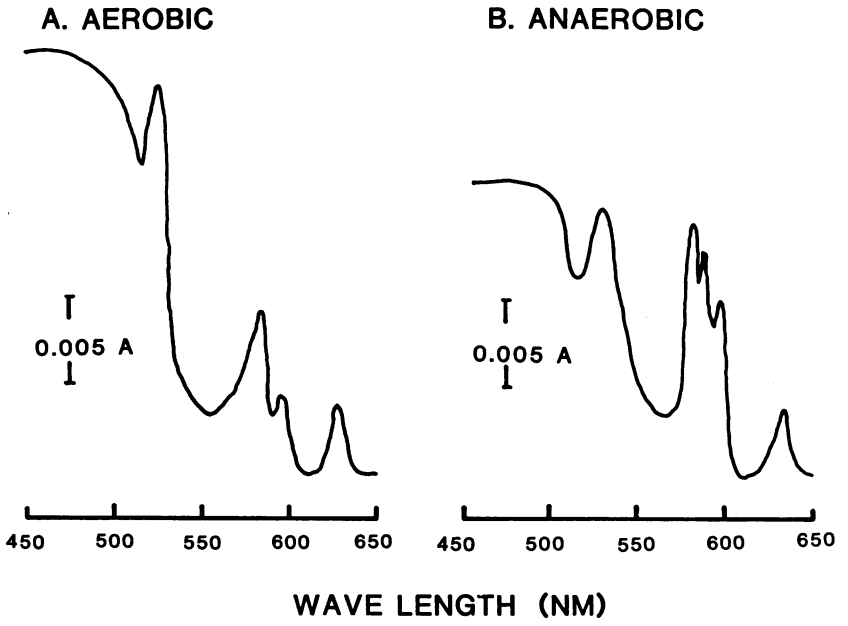

FIG. 3. Reduced-minus-oxidized spectra of mitochondria isolated from (A) aerobically and (B) anaerobically germinated seedlings of *E. crus-galli* var *oryzicola*. The cuvettes contained 0.150 and 0.147 mg protein for aerobic and anaerobic mitochondria, respectively. The spectra were obtained at −140° C and the base line subtracted.

presence of [U-^{14}C]sucrose (19). Here, we demonstrate that although anaerobic mitochondria exhibited much lower rates of O_2 consumption than aerobic mitochondria, mitochondria from both tissues possessed remarkably good RC and ADP/O ratios, indicating control by ADP. Thus, the potential for electron transport even under anoxic conditions exist for this species.

Upon transfer from N_2 to air, mitochondria isolated from oryzicola exhibited rapidly increasing rates of O_2 uptake. In fact, the transition from anaerobic to aerobic metabolism occurs more rapidly in oryzicola than in rice. Vartapetian (25) observed low respiratory activity in anaerobically grown rice coleoptiles which increased only after 30 min exposure to air. Oryzicola exhibited its largest increase in O_2 consumption rate during this period. The rapid development of aerobic respiration may, at least in part, account for the advantage that oryzicola exhibits as a competitive weed species in rice fields.

The mechanism that permits mitochondrial metabolism under anoxia is as yet unknown, but several important differences between oryzicola and other tissues have been observed. The most striking difference is the exceptionally large residual respiration. Whereas the addition of both KCN and SHAM greatly reduces mitochondrial respiration in most other tissues (Table I; Ref. 15), oryzicola maintains up to 66% of its initial respiration rate in the presence of these two inhibitors. Furthermore, anti-

Table II. *Reduced-Minus-Oxidized Spectra of Cytochromes in Mitochondria Isolated from Aerobically and Anerobically Germinated E. crus-galli var oryzicola*

Identification of cytochromes is based on absorbance maxima reported by the indicated investigators.

Cytochrome	λ	Spectra		Reference
		Aerobic	Anaerobic	
		$\Delta A\ mg^{-1}$ protein		
d	629	0.0267	0.0258	Poole (17)
$a \cdot a_3$	598	0.0300	0.0667	Smith (21)
b	577	ND[a]	0.0871	Vartepetian *et al.* (26)
b	565	0.0653	0.0986	Storey (22)
c	536	0.1500	0.1027	Storey (22)

[a] Not detected.

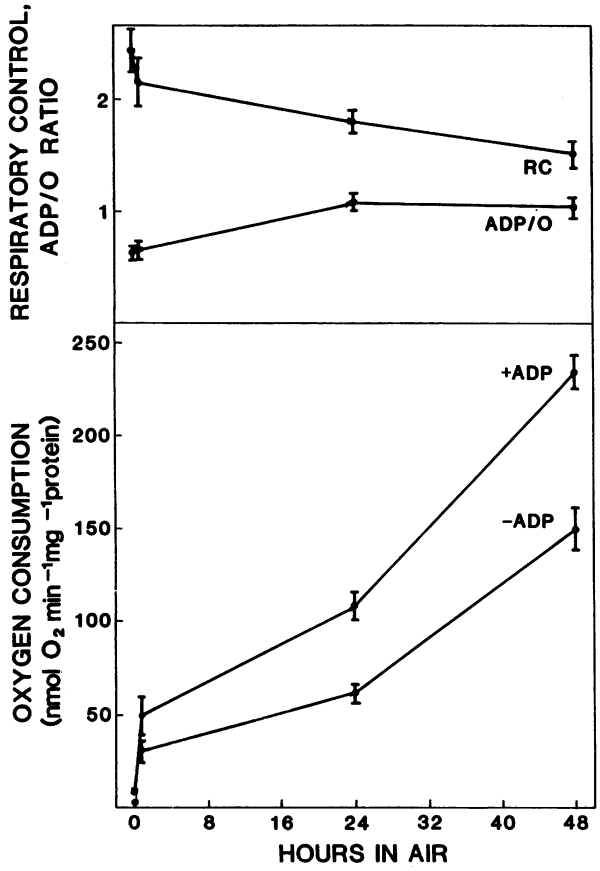

FIG. 4. Changes in respiratory control (RC), ADP/O ratio, and rate of oxygen consumption with and without exogenous ADP in the presence of 25 mM malate. Mitochondria were isolated from *E. crus-galli* var *oryzicola* seedlings germinated under anaerobic conditions for 5 d and transferred to air. For time zero, anaerobic mitochondria were isolated in an anaerobic chamber. For all other times, mitochondria were isolated under aerobic conditions. Values are means ± SE of at least three replicates. SE of points without error bars is smaller than symbol.

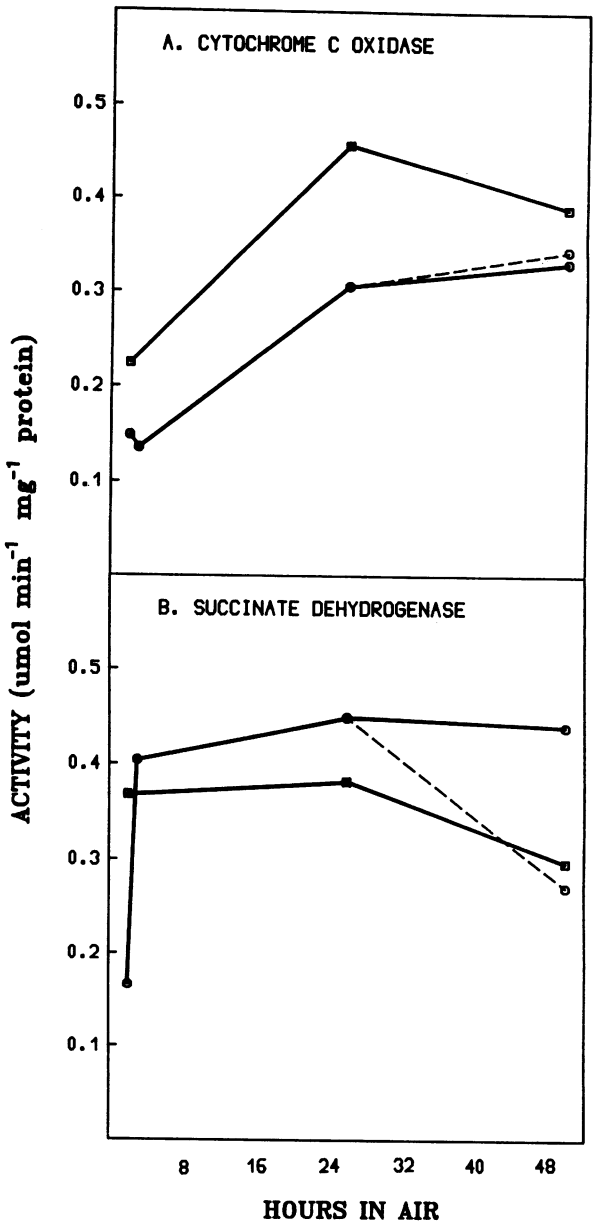

FIG. 5. Development, in air, of (A) Cyt *c* oxidase and (B) succinate dehydrogenase activities in 5 to 7 d old seedlings of *E. crus-galli* var *oryzicola* germinated under aerobic (□) and anaerobic (●, ○) conditions. After 5 d ($t = 0$), anaerobically germinated seedlings were transferred to air in the dark (●) and, after 24 h in air, a subset was exposed to continuous light (○). For time zero, anaerobically germinated seedlings were extracted in an anaerobic chamber. All other extractions were performed in air. Values are means ± SE of three replicates. SE of points without error bars are smaller than symbol.

mycin A is not an effective inhibitor of O_2 uptake and the effects of cyanide and azide are additive, regardless of the order in which the inhibitors are added to the reaction mixture. Finally, reduced-minus-oxidized spectra reveal not only an additional absorbance peak under anoxia, but also differences in the reduction of cytochromes due to treatment. It should be cautioned, however, that the additional Cyt *b* maximum observed here may actually be present in both aerobic and anaerobic tissues, but, under the conditions of our assay, detectable only under anoxia when it is reduced to a greater extent. Storey (22) notes that performing reduced-minus-oxidized spectra at 77 K greatly enhances the ability to resolve individual cytochromes compared to spectra performed at room temperature. Due to physical limitations, we were able to maintain the cuvettes at only 128 K during our measurements. At this temperature, we did achieve better resolution of absorbance maxima relative to difference spectra performed at room temperature, but it seems likely that even better resolution could be obtained at 77 K. Nonetheless, we observed that three cytochromes (b_{577}, b_{565}, and $a \cdot a_3$) were reduced to a greater extent during anoxia (Table II), as would be expected. Curiously, the *d*-type Cyt was unaffected by anoxia and Cyt *c*

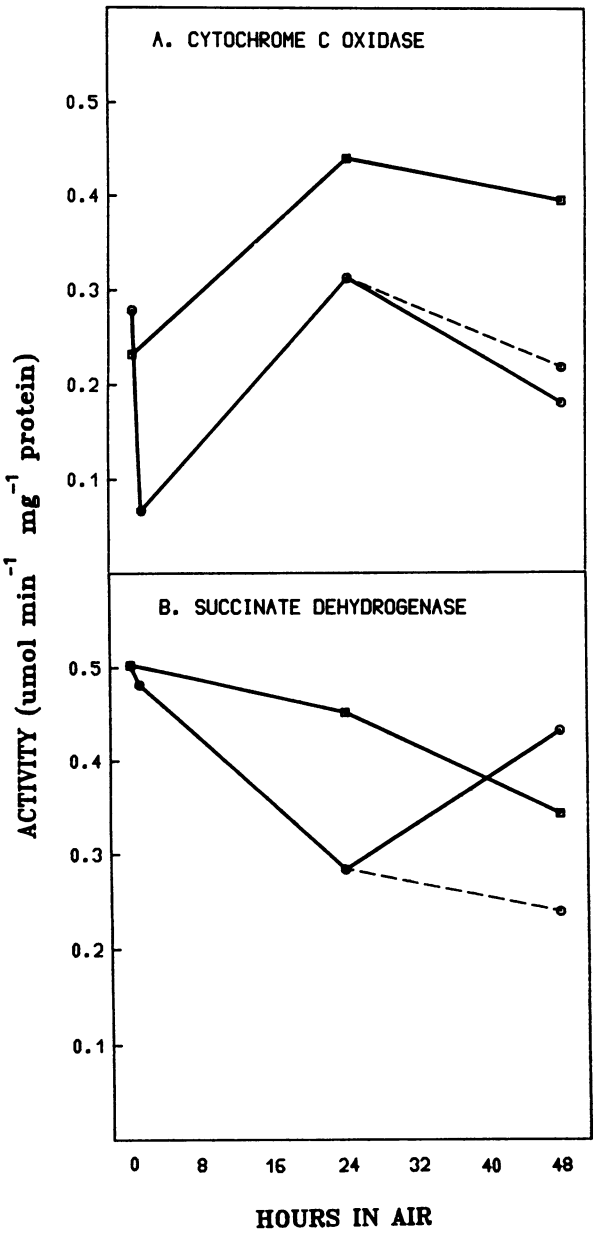

FIG. 6. Development, in air, of (A) Cyt *c* oxidase and (B) succinate dehydrogenase activities in 5 to 7 d old rice seedlings germinated under aerobic (□) and anaerobic (●, ○) conditions. After 5 d (t = 0), anaerobically germinated seedlings were transferred to air in the dark (●) and, after 24 h in air, a subset was exposed to continuous light (○). For time zero, anaerobically germinated seedlings were extracted in an anaerobic chamber. All other extractions were performed in air. Values are means ± SE of three replicates. SE of points without error bars are smaller than symbol.

was reduced to a greater extent in aerobic mitochondria. Thus, in comparison with other plants, it appears that the mitochondrial electron transport system of oryzicola possesses some unique properties that warrant further investigation.

The presence of an absorption peak corresponding to Cyt *d* is an extraordinary feature of the mitochondrial electron transport chain of oryzicola. Cyt *d* is clearly absent from mung bean mitochondria (22). To our knowledge, Cyt *d* has previously been reported only in procaryotes, where it exhibits several interesting properties (17): (a) it functions as a terminal oxidase; (b) it is insensitive to cyanide; (c) it exhibits a high affinity for O_2; (d) its appearance is enhanced by hypoxic or anoxic conditions; (e) it may utilize several N-compounds, particularly nitrite and to a lesser extent nitrate, as terminal electron acceptors; (f) it is involved in sulphite reduction; and (g) similar to the alternative respiratory pathway, it is not involved in energy conservation.

In oryzicola, a *d*-type Cyt could perform similar functions, thereby accounting for some of the unusual features we have observed compared to other plant systems. For example, here we reported on the large amount of residual respiration present in oryzicola (Fig. 2). A *d*-type Cyt would provide a third branch in the electron transport chain that may continue to function when both conventional (KCN-sensitive) and alternative (SHAM-sensitive) electron transport are inhibited. In addition, previous work from our laboratory (13) demonstrated that oryzicola seeds contain considerable quantities of nitrate (4 and 10 times that of rice and wheat, respectively) and that approximately 75% of this nitrate was depleted during the initial 24 h of germination. This observation is consistent with the reduction of nitrate by Cyt *d* as observed in bacteria. Preliminary attempts to detect nitrate reductase activity in oryzicola have been unsuccessful. This is analogous to studies in *Achromobacter*, bacteria in which Cyt *d* binds and reduces nitrite and nitrate; although Arima and Oka (1) postulated a nitrate reductase function for Cyt *d*, they were unable to detect nitrate reductase activity or NO formation. Unlike bacterial systems, however, the *d*-type Cyt does not appear to be enhanced by hypoxia or anoxia, but rather is a constitutitive component of the electron transport system of oryzicola (Table II). Studies designed to clarify the role of this *d*-type Cyt in the electron transport chain of oryzicola are currently underway.

Analysis of mitochondrial enzymes also supports the conclusion that the potential for mitochondrial activity exists under anoxia. Although Cyt *c* oxidase activity was 30% higher in 5 d old aerobically germinated seedlings than in anaerobically germinated seedlings, when transferred to air, the enzyme activity in anaerobically germinated seedlings exhibited similar developmental patterns as aerobically germinated seedlings. The presence of Cyt *c* oxidase during anoxia suggests that the potential for electron transport (foreseeably, for some process other than O_2 reduction) does indeed exist in oryzicola under such conditions. Furthermore, the presence of succinate dehydrogenase activity indicates that at least part of the tricarboxylic acid cycle may be operational under anaerobic conditions. Studies of invertebrates that are facultative anaerobes, such as helminths, demonstrate that part of the tricarboxylic acid cycle plays a significant role in anaerobic metabolism (9). Currently, we are examining the activities of other tricarboxylic acid cycle enzymes in addition to succinate dehydrogenase to determine which reactions of the tricarboxylic acid cycle proceed under anoxia in oryzicola.

In seedlings germinated in N_2 for 5 d, it is interesting to note the differences between oryzicola and rice in the development of Cyt *c* oxidase activity when seedlings are transferred to air. In rice, Cyt *c* oxidase activity was radically depressed within 1 h exposure to air. Subsequently, development of Cyt *c* oxidase activity paralleled that of aerobic controls. In oryzicola, on the other hand, no depression in activity occurred upon transition to air and the pattern of development of activity was very similar to that of aerobic controls. Such differences in development of Cyt *c* oxidase activity between rice and oryzicola may explain the different developmental patterns of O_2 uptake exhibited by mitochondria isolated from these two species. In oryzicola, Cyt *c* oxidase is stable (Fig. 5) and O_2 consumption increases directly upon transition to air (Fig. 4). In contrast, Vartapetian (25) found that the respiration rate in mitochondria isolated from rice coleoptiles increased only after 30 min in air. This corresponds to the decrease in Cyt *c* oxidase activity that we found in rice upon transfer to air (Fig. 6). Thus, at least part of the lag in

the development of rice compared to that of oryzicola may be traceable to specific components of the respiratory process when flooded seedlings are exposed to an aerobic environment.

As suggested by our previous studies (4, 11–14, 18, 19, 24), anaerobic metabolism in oryzicola is not characterized by one predominant metabolic pathway. Here, we have shown that mitochondria, although usually considered only in the context of aerobic respiration, may also contribute to anaerobic metabolism. The present data also suggest that mitochondria in oryzicola may differ in important and fundamental ways from those of other plants investigated thus far.

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