INDUCTION BY ETHYLENE OF CYANIDE-RESISTANT RESPIRATION

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Summary: Ethylene and cyanide induce an increase in respiration in a variety of plant tissues, whereas ethylene has no effect on tissues whose respiration is strongly inhibited by cyanide. It is suggested that the existence of a cyanide-insensitive electron transport path is a prerequisite for stimulation of respiration by ethylene.

Introduction: Through the years two apparently unrelated basic problems in plant physiology have been the focus of unabated investigation. On one hand intense interest has centered on the cause and nature of the burst of respiration - the climacteric - which attends ripening in a great variety of fruits (1, 2). Quite separately, the phenomenon of cyanide-insensitive electron transport in certain plant tissues and plant mitochondria has received ever-increasing attention (3). Our studies point to an intimate relationship between the two phenomena.

Material and Methods: Citrus and avocado fruits were obtained from the University orchard. Other fruits, roots, tubers and seeds were purchased. Ethylene and cyanide were applied as previously described (4). Mitochondria were isolated, and their activity assayed, according to published methods (5). Sweet potato slices prepared with a hand slicer were rinsed with distilled water and suspended in air-saturated 0.1 mM $CaSO_4$ for respiratory measurements. Oxygen uptake by slices was measured with a Clark oxygen electrode. Mitochondrial respiration was measured with a Rank (Bottisham, Cambs. U.K.) oxygen electrode.

Results and Discussion: We have described the similar effectiveness of ethylene and cyanide in initiating seemingly identical physiological and

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biochemical changes in avocado fruits and potato tubers (4, 6). Since the enhancement of respiration in each case is seemingly due to the realization of pre-existing enzymatic capacity rather than to de novo synthesis of respiratory enzymes (4, 6), it is assumed that both agents similarly release one or more restraints on respiration.

Cyanide, as an inhibitor of cytochrome oxidase, may be expected to simulate anaerobiosis in tissues where cytochrome oxidase is the main terminal oxidase, and in fact cyanide is known to invoke a conventional Pasteur effect in such tissues (7). The existence of a cyanide-insensitive electron path in certain plants, however, permits the aerobic oxidation of respiratory substrates in the presence of cyanide (3). Cyanide may even stimulate respiration (4, 6, 7, 8). Thus, it is generally recognized that in resistant tissue cyanide evokes the cyanide-resistant, or alternate path (3). If ethylene acts similarly, it should induce cyanide-resistant respiration. Accordingly, tissues whose respiration is stimulated by ethylene should be similarly affected by cyanide, while tissues whose respiration is strongly inhibited by cyanide should be unaffected by ethylene. That is, for ethylene to stimulate plant respiration the alternate path must be present. Fig. 1A depicts the effects of cyanide on the oxygen uptake of fresh green peas, where cyanide strongly inhibits oxygen uptake, and ethylene has no effect. Similar observations with respect to azide and ethylene have been reported for segments of pea internodes (9). Fig. 1B shows that both ethylene and cyanide enhance the rate of respiration of intact red sweet potatoes. Finally Table 1 compares the effects of cyanide and ethylene on a variety of plant tissues. The respiration of lemons and grapefruits is stimulated both by ethylene and by cyanide. Cyanide induces the climacteric in cherimoyas and apples as readily as does ethylene, and the respiration of rutabagas, beet roots, carrots, sweet potatoes and potatoes is stimulated by both cyanide and ethylene. Whereas the enhancement of respiration by cyanide is commonly associated with the evolution of ethylene, ethylene evolution usually lags behind. Furthermore, with rutabagas, and in one

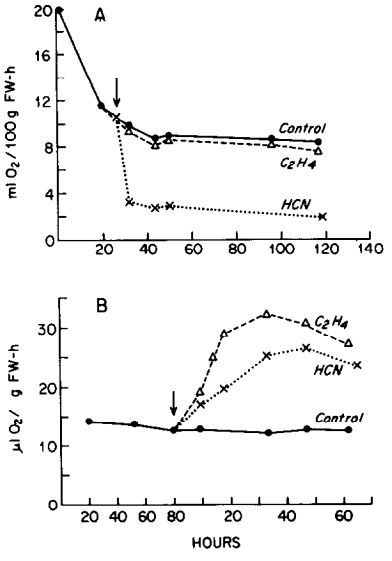

Fig. 1. The effect of ethylene and cyanide respectively on the respiration rate of fresh peas and intact red sweet potato roots.

A. Fresh peas: 60 µl/l ethylene or 100 µl/l HCN provided at arrow.

B. Sweet potato root: 17 µl/l ethylene or 400 µl/l HCN provided at arrow.

experiment with lemons, no detectable ethylene evolution accompanied the cyanide-induced respiration rise - suggesting that the effect of cyanide on respiration may be independent of ethylene production, and that ethylene production is a secondary effect of cyanide action. Table 1 further shows that where cyanide partially inhibits respiration in six-day-old germinating peas, ethylene has but a marginal stimulatory effect. In this case, as with green peas, cyanide results in a large accumulation of glycolytic end-products and in high RQ values (e.g. 1.6-2). The data invite the presumption that where ethylene enhances respiration, the cyanide-resistant electron path must be operative. In consequence it is proposed that ethylene evokes or implements the cyanide-resistant path. The evocation of the alternate path apparently decontrols both aerobic respiration and glycolysis despite the frequent increase in the levels of ATP associated with the climacteric (10), and with

Table 1. The effects of cyanide and ethylene on the respiration rate of a variety of plant tissues.

Tissue	Respiration Rate					
	Control		C_2H_4		HCN	
	O_2	CO_2	O_2	CO_2	O_2	CO_2
	$\mu\ell$/g fr wt-hr					
Apples	6	6	16	16	18	23
Avocados	35	35	150	140	150	140
Cherimoyas	35	35	160	150	152	145
Lemons	7	7	16	15	21	20
Grapefruit	11	11	30	29	40	40
Beetroots	11	11	22	22	24	28
Carrots	12	10	20	19	30	41
Potatoes	2.5	2.5	14	11	14	11
Sweet Potatoes	18	17	22	22	24	28
Rutabagas	9	9	18	15	23	20
Green Peas	8	8	7.5	7.5	2	2
6-day-old pea seedlings	110	110	150	150	60	85

Ethylene and HCN provided at levels evoking optimal response in each case.

the rise in respiration in potato tubers treated with ethylene (6). In our studies with cherimoyas we have found that the levels of ATP increase appreciably with the rise in respiration in response to both cyanide and ethylene treatments (11).

Our data suggest that a primary effect of ethylene on plant respiration is the determination of the electron transport path. Bahr and Bonner (12) have suggested that the apportioning of electrons between the cytochrome path and the alternate path is regulated by an equilibrium mechanism. In this view the restriction of electron flow through cytochrome oxidase evokes the

alternate path. However, ethylene does not inhibit cytochrome oxidase, and whereas diversion of electrons in response to cytochrome oxidase inhibition can explain resistance to cyanide, it cannot account for either the effect of ethylene or the stimulation of respiration by cyanide.

It is noteworthy that both electron paths are in limited use in intact sweet potatoes ("yams") - as evidenced by the fact that the respiration rate of fresh slices, whether in the presence of cyanide or m-chlorobenzhydroyamic acid (m-CLAM), an inhibitor of the alternate path (12), is almost 10 times that of the intact organ (Table 2, experiments 4 and 5). Thus, the restriction of electron flow through cytochrome oxidase does not in itself fully activate the alternate path *in vivo*. In consequence the *stimulation* of root, and occasionally slice, respiration by cyanide and ethylene (Table 2, Fig. 1B) must be taken into account in any full understanding of the role of cyanide (and ethylene) in cyanide-insensitive respiration.

Cyanide markedly increases the respiration of *Chlorella protothecoides* whereas antimycin, an equally effective inhibitor of electron transport through the cytochrome system, has no effect (13). The failure of antimycin to produce a sustained increase in respiration where cyanide does, rules out glycolytic substrate mobilization in response to a Pasteur effect as the main explanation of cyanide stimulation, and points to an additional role of cyanide beyond the inhibition of cytochrome oxidase. In this connection we have found that CO at concentrations far too low to inhibit cytochrome oxidase (viz. 400 µl/l) stimulates potato tuber respiration (Solomos and Laties, unpublished). CO enhances fruit respiration as well (9). Furthermore, azide stimulates respiration in avocado slices (14) and in Chlorella cells (13) - thus ruling out cyanohydrin formation as an explanation for cyanide stimulation.

The similarity of the synergistic effect of cyanide and m-CLAM on slice and mitochondrial respiration (Table 2, experiments 1, 2, 3, 4) establishes the mitochondrion as the locus of the alternate path in red sweet potato. To show inhibition by either cyanide or m-CLAM singly, both

Table 2. The effects of cyanide and m-CLAM on the respiration of mitochondria, tissue slices and intact roots of red sweet potato.

Experiment number	Experimental material	Additions	Respiration rate	Inhibition	ADP/O
			natoms oxygen/mg mitochondrial nitrogen/min	percent	
1		Control (17 mM malate)	520		1.50
		0.2 mM KCN	475	9	.53
2	Mitochondria	0.5 m-CLAM	450		1.56
		0.5 m-CLAM + 0.2 mM KCN	90	80.	0
3		Control (17 mM succinate)	810		1.1
		0.5 mM m-CLAM	730	10	1.2
		0.2 mM KCN	340	58	0
		0.5 mM m-CLAM + 0.2 M KCN	150	81	0
			$\mu\ell$ O_2/g F.W./h		
4	Slices (1 mm thick)	none	98		
		0.2 mM KCN	99		
		3 mM m-CLAM	98		
		0.2 mM KCN + 3 mM m-CLAM	15	85	
5	Intact roots	Control	10		
		180 $\mu\ell/\ell$	31		

paths must be operating at full capacity simultaneously or there must be considerable disparity of the two paths, so that electrons diverted from one path cannot be accommodated by the other. For stimulation by cyanide to take place, on the other hand, the rate of electron transport without cyanide must be less than the potential capacity of the alternate path, and cyanide must enhance or activate the latter.

In tissues where both ethylene and cyanide enhance respiration, glycolysis is activated under conditions which cannot be construed in terms of a conventional Pasteur effect, since the ATP levels are either unaltered or elevated (4, 6). On the other hand in tissues where respiration is strongly (e.g. green peas, Fig. 1A) or partially inhibited (e.g. pea seedlings, Table 1) ethylene either fails to affect, or marginally affects respiration. In these tissues cyanide stimulates anaerobiosis insofar as it evokes an extensive accumulation of ethanol and lactate, decreases ATP and doubles the rate of glucose breakdown. Ethylene, on the other hand, causes neither the accumulation of glycolytic end-products nor changes in the levels of glycolytic intermediates and ATP (11). These observations suggest that where respiration is stimulated by ethylene the "activation" by ethylene of the alternate path is the basis for the decontrol - hence enhancement - of both aerobic respiration and glycolysis. Glycolysis decontrol involves the activation of two rate-controlling enzymes - viz. phosphofructokinase and pyruvate kinase. The lack of adequate cyanide-insensitive electron transport capacity leads to the evocation of a conventional Pasteur effect by cyanide.

The frequent increase in ATP levels educed by cyanide and ethylene (6, 11) seemingly contradicts the expected reduction in phosphorylative efficiency due to the by-pass of the cytochrome path. However, while the alternate path *per se* is not coupled to phosphorylation, site I of the conventional respiratory chain continues to operate in the presence of cyanide (15). Thus in tissues whose respiration is enhanced by cyanide, phosphorylative efficiency may drop sharply while the absolute levels of ATP

may not only fail to drop, but may in fact increase, because of the increased electron flow through site I, together with an increase in substrate level phosphorylation. The foregoing proposition is supported by measurements of both [^{32}P] incorporation and by the persisting levels of ATP in tissues whose respiration is enhanced by cyanide (11, 14). The location of the branch-point somewhere beyond the first, and before the second, phosphorylative site is reaffirmed by the effect of cyanide on the ADP:O ratio attending malate and succinate oxidation respectively. In the first instance the ADP:O ratio is decreased by two-thirds (Table 2, experiment 1). In the latter case the ratio drops to zero (Table 2, experiment 3).

Since the respiration of mitochondria isolated from potato tubers or fresh slices is often strongly inhibited by cyanide (16, 17) while tuber respiration per se is stimulated by cyanide (6, 8) the question is raised as to whether cyanide-stimulated respiration in intact tubers is mitochondrial. In this connection the evidence indicates that mitochondrial cyanide-resistant respiration is labile. Thus, slicing alone makes potato and beet root tissues cyanide sensitive (16, 18). The loss of CN resistance is associated with marked membrane phospho- and galactolipid breakdown (Theologis and Laties, unpublished). On the other hand, aging of potato slices restores cyanide resistance in the latter, and to a considerable degree in mitochondria isolated therefrom (16, 17). Whereas the lability of the alternate path alluded to above precludes the regular demonstration of cyanide resistance in mitochondria from cyanide-resistant intact tissues, the fact that resistance to cyanide can be demonstrated in intact tissues, tissue slices and mitochondria in several plant tissues, suggests that cyanide (and ethylene)-stimulated respiration is mitochondrial in origin.

In summary our results show that ethylene stimulates respiration only in plant tissues in which cyanide acts similarly. That is, the presence of an operative alternate path is a prerequisite for ethylene enhancement of plant respiration. Furthermore, the ability of ethylene to enhance plant

respiration is widespread and not confined to climacteric fruits. Whether ethylene "activates" the alternate path directly or indirectly is not clear. Neither is the mechanism by which the evocation of the alternate path acts as a positive feedback modulator of key glycolytic enzymes.

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