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Short Communication

Mitochondrial Responses to Ethylene and Other Hydrocarbons[1]

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The growing realization over the past few years that ethylene serves as a natural plant hormone has quickened interest in the mechanism of action of this gas. Among the suggestions that have been made is the proposal by Lyons and Pratt (8) that ethylene may increase the permeability of mitochondrial membranes. Their experiments on mitochondrial swelling were repeated by Olson and Spencer (9, 10), who also found a rise in phosphatase when mitochondria were exposed to ethylene. The unique effectiveness of ethylene in biological responses (1, 4) permits a simple inquiry into the question of whether the observed swelling of mitochondria is closely associated with the biological effects of ethylene. We report here that the mitochondrial responses to ethylene are also obtained with other gases including propylene, propane, ethane, acetylene, butane, and especially butene-1.

Mitochondria were prepared from heads of cauliflower (*Brassica oleracea* var. botrytis L.), seedlings of Alaska pea, and fruits of tomato (var. KC 39). Cauliflower heads purchased locally were used to prepare mitochondria as described by Lyons and Pratt (8). Mitochondria from 7-day-old etiolated pea seedlings were prepared following the method of Ikuma and Bonner (6), and preparations were made from mature unripened tomato fruits following the method of Ku *et al.* (7). In each case the final mitochondrial pellets were suspended in 2 to 4 ml of washing medium.

Mitochondrial swelling was measured as the increase in light transmission at 520 nm in a Spectronic 20 spectrophotometer. The reaction medium consisted of 0.125 M KCl or 0.25 M sucrose in 2 mM $MgCl_2$ and 0.01 M tris HCl buffer at pH 7.4. The various hydrocarbons were introduced by the method of Lyons and Pratt (8).

Oxidative activity of the mitochondria was measured by the oxygen electrode method of Chance and Williams (5) as modified by Ku *et al.* (7). Protein content of the mitochondrial suspensions was measured by the method of Biale *et al.* (2).

In an effort to find a suitable reaction medium, mitochondria of pea seedlings and cauliflower buds were suspended in either 0.25 M sucrose or 0.125 M KCl, and their responsiveness to the swelling stimulus of calcium ions was tested. The results were similar to those reported by Lyons and Pratt (8): 1.0 mM inducing swelling in either sucrose or KCl media. However, it was found that ethylene and other hydrocarbons induced swelling of mitochondria only in KCl solution, and therefore 0.125 M KCl was used as the suspending medium in all experiments.

In order to determine whether the mitochondrial swelling response was unique to ethylene, comparisons were run with six other hydrocarbon gases. Two concentrations of each gas were utilized, and both pea and cauliflower mitochondria were treated with each. The results are shown in Table I, where it is evident that all of the gases tested brought about the swelling of mitochondria, and that butene-1 was the most effective. This compound dramatically illustrates the lack of correlation between the mitochondrial swelling response and the biological responses to ethylene, as Burg and Burg (4) have shown butene-1 to be only about one hundred-thousandth as active as ethylene in biological activity. The data on swelling of mitochondria also show another lack of correlation with biological ethylene responses in that the swelling response requires more than 10 μl/liter of ethylene or the other hydrocarbons for the swelling response threshold, whereas biological responses to ethylene ordinarily reach half-saturation at about 0.1 or 0.2 μl/liter.

The rate of swelling response varied between mitochondrial preparations. Comparative responses between the various gases tested, however, were consistent with butene-1 being substantially more rapid than ethylene responses, as illustrated in Figure 1. For example, in this experiment, after 5 min the change in absorbance (Δ) for mitochondria treated with butene-1 was 1.2, and for ethylene was 0.65. At 10 min the ΔA for butene-1 was 1.6, and for ethylene, 1.0.

Experiments on the swelling of mitochondria from tomato fruits were attempted, but the pigmentation of the mitochondrial preparation was too great to permit precise measurement of absorbance changes, even after the mitochondria had been purified on a sucrose density gradient.

Turning to the respiratory response of mitochondria to ethylene, experiments were done principally with preparations taken from

Table I. *Effect of Various Hydrocarbons on Swelling of Mitochondria from Pea and Cauliflower*

Gas Concentration	Δ (A_{520}) 10 Min[1]							
	Pea				Cauliflower			
	0	10	50	100	0	10	50	100
	μl/liter				μl/liter			
Control	0.08				0.20			
Ethylene		0.08	0.12	0.15		0.20	0.25	0.30
Ethane		0.08	0.12	0.14		...	...	...
Acetylene		0.08	0.13	0.14		0.20	0.24	0.26
Propane		...	...	...		0.25	0.30	0.32
Propylene		0.10	0.13	0.18		0.24	0.30	0.33
Butane		0.08	0.12	0.13		...	...	...
Butene-1		0.12	0.14	0.20		0.22	0.35	0.40

[1] Swelling measured as decrease in absorbance at 520 nm in 10 min. Each value is the average of three experiments.

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mature green fruits of tomato. Using the oxygen electrode method of Chance and Williams (5), tomato mitochondria suspended in 0.125 M KCl exhibited respiratory control by ADP, as illustrated in tracing A of Figure 2. When succinate was provided as substrate, it is shown in Figure 2 that the rate of oxygen consumption was markedly accelerated by additons of ADP (state 3) and returned to a slower rate after the ADP had been depleted (state 4). The respiratory control ratio of Chance and Williams (5) has been calculated and entered in table form in Figure 2. This ratio of oxygen consumption by mitochondria in state 3 and state 4 increased gradually from 3.2 to 6.0 as the experiment proceded. The ADP/O ratio ranged between 1.5 and 1.7.

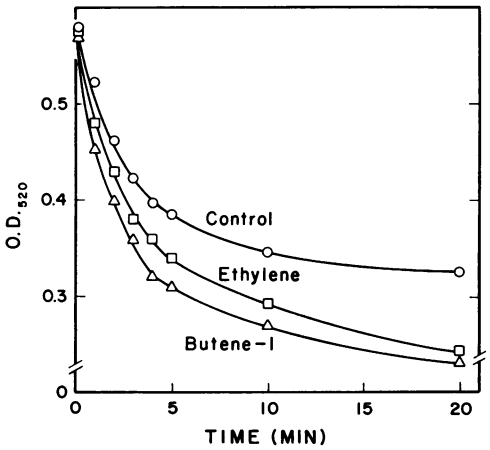

Fig. 1. Effect of ethylene and butene-1 on swelling of isolated pea seedling mitochondria. Mitochondrial fractions were suspended in 0.125 M KCl containing 0.01 M tris-HCl buffer (pH 7.4). The effect of alkene on swelling of isolated mitochondria is indicated by changes in the absorbance (520 nm). The concentration of ethylene and buene-1 were 100 μl/liter

Tracing B in Figure 2 illustrates the effects of ethylene on mitochondrial respiration. Ethylene was introduced at 100 μl/liter shortly after ADP addition, and no alteration of the rate of oxygen uptake in state 3 was observed but there was a marked increase in state 4 respiration. If the ethylene concentration was further increased to 200 μl/liter, the rate of oxygen consumption in state 4 was further increased. The ADP/O ratio was not altered by the ethylene, but the rise in the respiration control ratio was prevented or even reversed by the ethylene.

Tracing C in Figure 2 illustrates that butene-1 shows a similar ineffectiveness on state 3 and a stimulation of respiration in state 4 mitochondria similar to that of ethylene. Examination of other hydrocarbons such as propylene, propane, ethane, and acetylene yielded effects which were similar to those illustrated for ethylene and butene-1, with a stimulation of oxygen uptake in state 4 and no effect on state 3 or on the ADP/O ratio.

Stimulations of respiration (CO_2 evolution) with ethylene have been observed by Simons (11) in preclimacteric but not in postclimacteric tomato fruits. Burg and Burg (3) earlier reported such stimulations in mango fruits. We have found marked stimulations of CO_2 evolution from succinate by ethylene applications to preclimacteric but not postclimacteric fruits (data to be published elsewhere). On the other hand, F. B. Abeles (personal communication) has found that the action of ethylene in causing abscission in bean petiole explants is not accompanied by a respiratory increase, and we have not found increases during the ethylene induced swelling of pea stems or the inhibition of root growth. The lack of a respiratory stimulation in state 3 tomato mitochondria may imply that during the isolation procedure the responsiveness of the mitochondria to ethylene was lost, or that these mitochondria are in fact not the site of ethylene-stimulated respiration.

Collectively, the experiments reported here indicate that mitochondria may not be the site of ethylene regulation of growth and respiratory processes. Whereas ethylene was found to alter mitochondrial swelling as reported by Lyons and Pratt (8), the con-

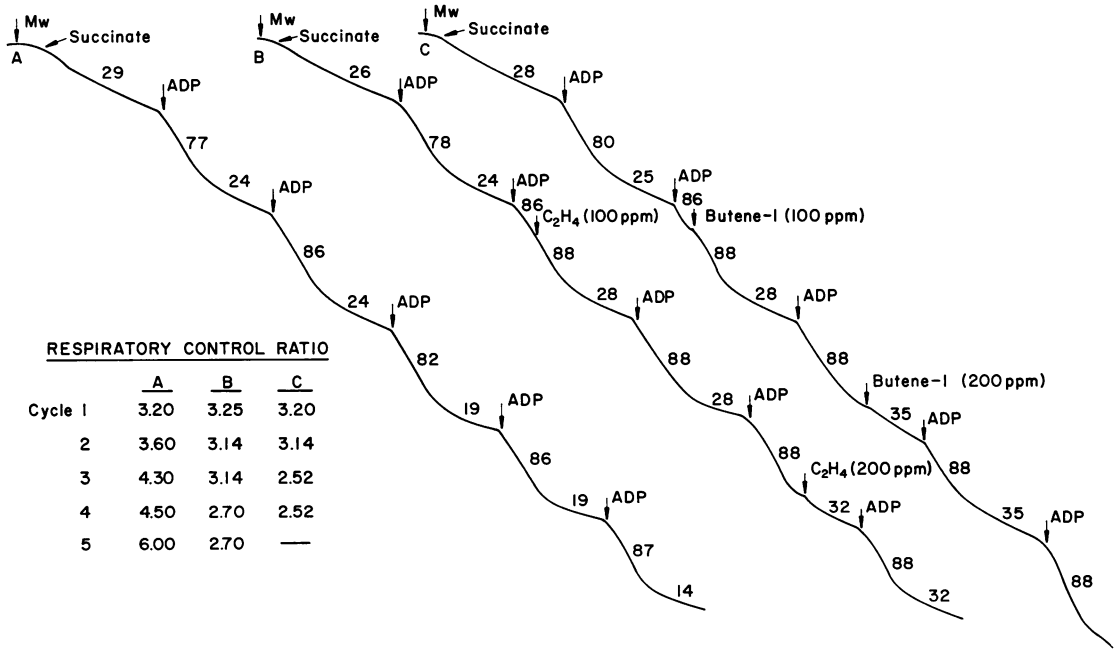

Fig. 2. Effect of ethylene and butene-1 on respiratory activity of tomato fruit mitochondria. Oxygen electrode trace for succinate oxidation by mitochondria isolated from mature green tomato fruit. The medium contained 0.125 M KCl, 10 mM potassium phosphate buffer, 10 mM tris-HCl, 5 mM $MgCl_2$, 0.5 mM EDTA, 0.5 mg bovine serum albumin, pH 7.4. Mitochondria (0.5 mg protein) were added at "Mw"; total volume was 3.0 ml. Other additions were as shown on the figure. Rates were expressed as mμmoles oxygen per minute. A: Control; B: ethylene introduced at 100 or 200 μl/liter; C: butene-1 introduced at 100 or 200 μl/liter.

centration of the gas needed for this response is markedly higher than biologically active concentrations, and alkenes which lack the biological activity of ethylene induced similar swelling responses. The alteration of respiratory rate in state 4 mitochondria is likewise not specific for ethylene.

Note Added in Proof. C. W. Mehard and J. M. Lyons (Plant Physiol. 46: 36–39, 1970) have also reported the nonspecificity of mitochondrial responses to various gases.

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