Short Communication

Upsurge in Respiration and Peroxide Formation in Potato Tubers as Influenced by Ethylene, Propylene, and Cyanide

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ABSTRACT

A continuous application of ethylene (10 μl/l) and propylene (500 μl/l) to potato tubers (Solanum tuberosum L.) resulted in an upsurge of respiration and a concomitant rise in peroxides. When applied in 100% O2, the effect of ethylene and propylene on respiration and peroxide formation was augmented. Hydrogen cyanide (500 μl/l) mimicked the action of ethylene and propylene inducing a respiratory rise and a corresponding increase in peroxides. As with ethylene, the effect of HCN was augmented in high O2 tensions. The results support the suggestion that ethylene activates the cyanide-insensitive respiratory pathway.

Previous studies showed that applied ethylene stimulates peroxide formation in ripening pears (3), suggesting that the action of ethylene consists, in part, of inducing peroxide formation in tissues. However, in climacteric fruit, including pear (3) and tomato (8), both ethylene and peroxides increase concomitantly, and for that reason it is difficult to ascertain the interrelation between the compounds.

Application of ethylene to potato tubers induces a respiratory upsurge (14) resembling the rise in respiration displayed by climacteric fruit (2). However, the respiratory rise in potatoes, unlike fruit, can be studied as related directly to ethylene action since it is not spontaneous, and clearly depends on the application of ethylene (14). In addition, the ethylene-induced respiratory rise in potatoes can be studied as an independent senescence process. For that reason, we employed potato tubers in order to study the action of ethylene as it pertains to the stimulation of respiration and the changes in peroxides.

In the present work, we show that the respiratory upsurge induced by ethylene and its analogue, propylene, is accompanied by a similar upsurge in the formation of peroxides. High O2 tensions enhanced respiration and the corresponding rise in the formed peroxides. Cyanide mimicked the action of ethylene.

MATERIALS AND METHODS

Locally grown potato tubers (Solanum tuberosum L. var. Northchip) were preconditioned at room temperature for 2 weeks following harvest. Whole potato tubers, each weighing approximately 150 g, were placed in 4-liter jars and ventilated continuously with different gas mixtures, at a flow rate of 400 ml/min.

The employed gas mixtures consisted of air (21% O2) and 100% O2. Each O2 regime was supplemented with either zero, 10 μl ethylene, 500 μl propylene, or 500 μl cyanide/l ventilating gas, as previously described (9). The ambient temperature was maintained at 21 C for the duration of the experiment.

Samples consisting of 1 kg on the average were used for the measurement of CO2 evolution (10) and peroxide levels as previously described using a titanium reagent (3). These measurements were performed at intervals for 36 hr. All determinations were run in duplicate.

RESULTS AND DISCUSSION

Figure 1 shows the effect of ethylene and propylene on CO2 evolution by potatoes tubers (1A) and the corresponding changes in peroxide levels (1C) as related to time of treatment. Potatoes kept in air show a steady state of respiration. The addition of ethylene to the ventilating gas resulted in a respiratory upsurge, as previously observed (14). The rise in respiration, after a lag period, reached a peak 2- to 3-fold the initial rate, followed by a decline. The application of propylene induced a similar response.

The induced upsurge in respiration was accompanied by a corresponding increase in peroxides (1C). Both ethylene and propylene induced an almost identical change in peroxides. The present results confirm previous observations showing that the changes in ethylene are accompanied by corresponding changes in peroxides. Application of ethylene stimulated the upsurge of peroxides in fruit (3). In climacteric fruit, the onset of ethylene evolution is accompanied by a similar upsurge in peroxides as shown in pear (3) and tomato (8), whereas in ripening strawberries, a nonclimacteric fruit, the continuous decline in ethylene is accompanied by a corresponding decline in peroxides (8). Collectively, the data suggest that the changes in ethylene levels in storage tissues, as occurring naturally or upon the application of exogenous ethylene, can not only induce changes in respiration but also corresponding changes in peroxides.

In a previous study (9) involving the effect of ethylene and high O2 tensions on lycopene formation in the nonripening rin tomato mutant, it was shown that lycopene formation, as induced by ethylene, is markedly enhanced by high O2 tensions. In the present study, we used the synergistic effect of O2 to ascertain further that both respiration and peroxide formation are a function of ethylene action. Ethylene and propylene were applied in combination with 100% O2 and induced a marked increase in respiration (Fig. 1B). Whereas in air the ethylene- or propylene-induced respiration was two to three times the initial rate, in O2 the respiration was almost 10-fold greater. Likewise, ethylene and propylene in O2 induced higher levels of peroxides (Fig. 1D) as compared with ethylene and propylene in air (1C). The results show that the effect of ethylene and propylene on respiration and peroxide formation is augmented in high O2.
Fig. 1. Effect of ethylene and propylene on CO₂ evolution (A and B) and peroxide formation (C and D) in air (21% O₂) and 100% O₂. Employed concentrations of ethylene were zero (○) and 10 μl/l (●). Concentration of propylene (△) was 500 μl/l.
tensions, suggesting that both processes are catalyzed by enzyme(s) with high $K_m$ ($O_2$).

It is not clear whether the respiration and peroxides are induced independently, or sequentially, by ethylene. Solomos and Laties (18) observed that plant tissues in which ethylene induces a respiratory rise also exhibit a cyanide-insensitive respiration. Both ethylene and cyanide had a similar effect on the turnover of metabolites including the accumulation of end products of glycolysis and increase in ATP levels (17). On the basis of these and other results, these authors proposed that ethylene stimulates an alternate respiratory pathway which is also triggered by cyanide and other respiratory poisons (17, 18). We used this concept by employing cyanide to examine whether the upsurge in respiration and peroxides reflect the activation of a similar path or are a function of the trigger compound employed. Figure 2A shows that the application of HCN in air induced a respiratory upsurge, as previously observed (16), and that the rise in respiration was accompanied by an increase in peroxides.

Fig. 2. Effect of HCN on CO$_2$ evolution (A and B) and on peroxide formation (C and D) in air (21% O$_2$) and 100% O$_2$. Employed concentrations of HCN were zero (○) and 500 μl/l (●).
Cyanide in 100% O₂ induced a further rise in respiration (2B) and a corresponding enhancement in the formation of peroxides (2C). These results show that although the trigger compound (ethylene or cyanide) is different, it induced a similar and concomitant upsurge in respiration and peroxide formation. Furthermore, as with ethylene and propylene, the magnitude of these processes was a function of the O₂ tensions employed. These data indicate that the increase in respiration and peroxide levels results essentially from the activation of the cyanide-insensitive respiratory path, and, moreover, support the view (17, 18) that ethylene triggers the cyanide-insensitive respiration.

Hamilton (13) argued that the single most important reason for the low kinetic reactivity of O₂ is that molecular O₂ exists in the ground triplet state. The direct reaction of a triplet molecule (O₂₃) with a singlet (organic metabolites) to give a singlet product, namely, oxidized metabolites, is a spin forbidden process and thus will not occur readily. This is so because the time required for the electron spin inversion of a triplet to a singlet is far greater relative to the time in which molecular collision and, thereby, chemical reactions, occur. By comparison, the stable reduction products of O₂, i.e., peroxides, exist in the singlet form (11, 12) and therefore, may readily attack cellular constituents. From that standpoint, the formation of peroxides, and possibly other partially reduced O₂ intermediates (11–13), represents the onset of oxidative processes and, thereby, the loss of the relative immunity of tissues toward the action of O₂.

The concept that the action of ethylene may consist of reducing the resistance of tissues to the action of O₂ is supported by the results (Fig. 1) showing that although O₂ tensions are rate-limiting, the onset of senescence processes in potato cannot be triggered by O₂ alone, even at high tensions. The initiation of these processes requires the presence of ethylene. Likewise, the rate of lycopene synthesis in the nonripening rin tomato mutant is a function of the O₂ tension but only in combination with ethylene (9). However, in climacteric fruit, including banana (1) and pear (5, 6), high O₂ tensions were sufficient to promote ripening processes since this class of fruit is capable of synthesizing adequate levels of ethylene (4). Ethylene may function by stimulating the formation of peroxides and possibly other reduced O₂ intermediates. In this way, ethylene could initiate oxidative processes and thereby, the onset of senescence. This concept is in keeping with previous suggestions viewing fruit-ripening as an oxidative phenomenon (5–7). Ethylene analogues, including propylene (Fig. 1) and vinyl chloride (Chin and Frenkel, manuscript in preparation), likewise can induce the formation of peroxides. The stimulation of peroxide levels by cyanide as shown in the present work may explain how the compound can mimic the action of ethylene as for example in the stimulation of ripening (3). Cyanide and other respiratory poisons can block the Cyt-mediated electron flow and thereby shift the flow of electrons from the complete toward the partial reduction of molecular O₂, as shown by the formation of peroxides by isolated mitochondria (15) and in intact tissue (Fig. 2). However, the action of cyanide may also consist of preventing the breakdown of peroxides as previously shown (3).

Currently we are attempting to identify the enzyme system(s) which catalyze the formation of peroxides in plants and examine its regulation by ethylene.

LITERATURE CITED

7. FRENSKEL C 1976 Regulation of ripening in Bartlett pears with sulfhydryl reagents. Bot Gaz 137: 154–159