An *In Vitro* Control Mechanism for Potato Stress Metabolite Biosynthesis

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ABSTRACT

Ethylene/oxygen (E/O_2) elevates sesquiterpenoid stress metabolite (SSM) levels in potato (*Solanum tuberosum* L.) tuber tissue which is reacting hypersensitively. To determine whether E/O_2 retards SSM turnover, a measured amount of rishitin was applied to tuber tissue which was then incubated in air or E/O_2, and rishitin disappearance was monitored. No difference in the rate of rishitin disappearance was detected between air and E/O_2 incubations. However, tissue treated with rishitin and incubated in E/O_2 accumulated intermediates of the katahdinone and phytuberin pathways. This was not the case in rishitin-air treatments. These results suggest the dual involvement of ethylene and SSM intermediates in the regulation of the biosynthesis of SSM, compounds which may serve as phytoalexins.

Potato tubers infected by an incompatible race of *Phytophthora infestans* produce SSM[2] as part of the hypersensitive response (17). The primary SSM produced, katahdinone (solavetivone) (3), lubimin (11, 12), rishitin (9, 19), and phytuberin (6, 21), possess fungistatic and/or fungitoxic properties. The inhibition of fungal growth in hypersensitive interactions has been attributed to the presence of these SSM in host tuber tissue. For this reason, they are sometimes termed phytoalexins (17).

The hypersensitive response is elicited in potato tuber tissue by treatment with a cell-free extract from *P. infestans* (20). Using this approach, we have reported that SSM levels are elevated and hypersensitive symptomology is made more severe by atmospheres containing low levels of ethylene (1). E/A elevated rishitin levels by 25% over those produced by treatment in air alone. E/O_2 elevated rishitin levels by an additional 120% over the increase caused by E/A. Since SSM levels in challenged tissue incubated in 100% O_2 were no higher than in air-incubated tissue, the elevation of SSM levels by E/A and E/O_2 was demonstrated to be an ethylene effect which is sensitive to O_2 concentration.

It was unclear whether the elevated SSM levels caused by E/A and E/O_2 were due to stimulation of SSM biosynthesis, retardation of SSM turnover, or both. We sought to discriminate among these possibilities by examining the effects of ethylene on rishitin metabolism (2).

Kalan and Osman (8) have demonstrated that potato tuber slices metabolize exogenously applied katahdinone to isolubimin, an SSM subsequently discovered in fungal-infected potatoes (18) and proposed as an intermediate between katahdinone and lubimin (8). Ward *et al.* (22) demonstrated the conversion of exogenously applied rishitin to 13-hydroxyrishitin and of exogenously applied lubimin to unidentified products by potato cell suspensions. Murai *et al.* (13) reported the appearance of two rishitin metabolites in potato tuber slices, and Ishiguri *et al.* (7) in potato tuber discs treated with rishitin.

Our experimental approach was to monitor the rate of rishitin disappearance, the rate of rishitin metabolite appearance, and new biosynthesis of rishitin precursors as well as SSM from the phytuberin pathway in tuber slices treated with rishitin and incubated in air or E/O_2. Since E/O_2 consistently produced higher SSM levels in challenged tuber tissue than E/A (1), E/O_2 was used to determine whether elevated rishitin levels are due to increased biosynthesis, retarded turnover, or both.

MATERIALS AND METHODS

Biological Material. Tubers of white potato (*Solanum tuberosum* L.) cv. Houma, Katahdin, Kennebec, Merrimac, and Wauseon were obtained from the University of Maine Aroostock Farms, Presque Isle; and cv. Atlantic and Sebago from a commercial supplier in Florida. Tubers were stored at 10°C until treated. Tubers aged for 5 months or longer postharvest were utilized in this study.

Treatment of Tubers. Tubers were treated by a modification of a previously published method (1). Potato tubers were washed, temperature-acclimated at 20°C, surface-sterilized, and sliced. Slices (3–5 mm thick, 3 per trial, 60 g wet weight) were placed in a modified 7-liter pressure cooker at 20°C and were ventilated overnight with various gas mixtures at a flow-rate of 10 l/h at atmospheric pressure. Rishitin (0.5 mg in 0.5 ml of a 1:1 solution of methanol/H_2O) was then applied to each slice. Slices were then returned to the pressure cooker for additional ventilation and were extracted for SSM at specified time intervals. E/O_2 composition was 10 μl ethylene/liter (1% [v/v] N_2; balance O_2). E/O_2 was obtained from Air Products and Chemicals and included a certified standard.

Purified SSM Preparations. Rishitin and katahdinone were extracted from hypersensitive potato tuber tissue by a previously published method (1) and were purified by TLC and column chromatography. Purity and concentrations of these SSM were determined by GLC (5).

SSM Extraction. This protocol was previously described (1).

Chromatography. This protocol was previously described (1, 5). Authentic katahdinone, lubimin, rishitin, and phytuberin of known concentration were individually co-chromatographed with tuber tissue extracts to establish the identity of SSM GLC peaks and to provide a basis for SSM quantitation.

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Abbreviations: SSM, sesquiterpenoid stress metabolite(s); E/A, ethylene in air; E/O_2, ethylene in oxygen; RM, rishitin metabolite.

RESULTS AND DISCUSSION

Figure 1 shows the rate of rishitin disappearance in Kennebec slices after incubation in air. By 24 h over 60% of the rishitin was metabolized, by 48 h about 80%, and by 72 h over 90%.

Table I is a comparison of the rate of rishitin disappearance in three different cultivars in air and E/O_2. The rates of disappearance in both atmospheres were in close agreement. Similar data from the four other cultivars tested firmly established that rishitin disappearance was unaffected by E/O_2. Thus, we turned our attention to the effect of E/O_2 on SSM biosynthesis.

The TLC plates of extracts from tuber slices treated with rishitin-E/O_2 showed detectable levels of katahdinone and lubimin, both rishitin precursors (8), as well as phytuberin, a compound of very different structure (6). Katahdinone, lubimin, and phytuberin did not appear until between 48 and 72 h after treatment and only in rishitin-E/O_2 treatments. Quantitation of these three SSM by GLC in three different cultivars 72 h after rishitin-air and rishitin-E/O_2 treatments is shown in Table II. E/O_2 by itself did not cause the appearance of katahdinone, lubimin, rishitin, phytuberin, or any other identifiable SSM.

That this was *de novo* synthesis of katahdinone, lubimin, and phytuberin and not direct conversion of rishitin to the above-mentioned SSM is supported by two considerations: (a) rishitin conversion to katahdinone, lubimin, and phytuberin would require that rishitin reacquire the carbon previously lost during conversion from a 15-carbon sesquiterpene skeleton to a 14-carbon norsesquiterpene skeleton (15), a reaction that seems unlikely and has never been demonstrated; and (b) by 24 h most of the rishitin had already disappeared, presumably converted to the three rishitin metabolites mentioned below, whereas katahdinone, lubimin, and phytuberin were not detected until 48 to 72 h after treatment. This time course suggests that the metabolism of exogenously applied rishitin is directed toward rishitin conversion products and that katahdinone, lubimin, and phytuberin originate from some common precursor of these SSM.

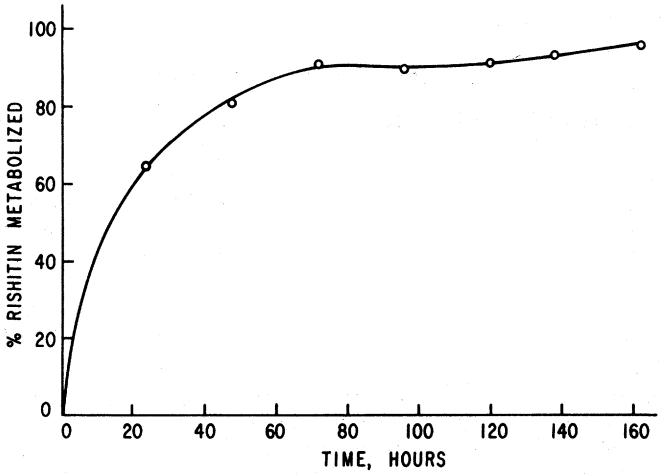

FIG. 1. Time course of rishitin metabolism in Kennebec slices incubated in air (0.5 mg rishitin/slice, 3 slices, 60 g wet weight at each time point, temp 20°C).

Table I. *Effect of Air and E/O_2 on the Metabolism of Rishitin by Potato Tuber Slices from Three Different Cultivars*

The concentration of rishitin was 1,500 μg/3 slices, (60 g wet weight, temp 20°C).

Time	Cultivar					
	Atlantic		Houma		Kennebec	
	Air	E/O_2	Air	E/O_2	Air	E/O_2
h	*% rishitin metabolized*					
24	68.0	78.2	68.4	69.9	65.9	67.3
48	84.1	87.7	82.8	84.6	78.0	75.7
72	92.1	81.3	91.4	83.0	89.6	88.9

Table II. *Formation of Sesquiterpenoid Stress Metabolites in Potato Tuber Slices After 72 h of Treatment with Rishitin-E/O_2*

The concentration of rishitin was 1,500 μg/3 slices, (60 g wet weight, temp 20°C).

SSM	Cultivar					
	Atlantic		Houma		Kennebec	
	Air	E/O_2	Air	E/O_2	Air	E/O_2
	μg SSM/60 g wet wt potato tissue					
Katahdinone (solavetivone)	0	41.9	0	9.8	0	28.2
Lubimin	0	160.2	0	62.0	0	148.1
Phytuberin	0	34.0	0	38.3	0	104.8

Similar experiments were repeated with exogenous application of katahdinone instead of rishitin. In this case, lubimin, rishitin, and phytuberin were detected at 48 h after katahdinone-E/O_2 treatment.

Rishitin metabolism was also followed by monitoring via TLC the appearance and intensity of three compounds which arose in extracts from tuber tissue treated with rishitin, which had an R_F of 0.39. RM I had an R_F of 0.26 to 0.30 and showed TLC color reactions identical to those of rishitin, staining red with saturated $SbCl_3$ in $CHCl_3$ at room temperature and turning violet after being heated for 3 to 5 min at 110°C. RM II and RM III had R_F values of 0.16 to 0.18 and 0.12 to 0.14, respectively, and were detected via TLC as violet spots only after being heated. RM II and RM III were detected in seven different potato cultivars tested: Atlantic, Houma, Katahdin, Kennebec, Merrimac, Sebago, and Wauseon; RM I was detected only in Kennebec and Merrimac. It has not been determined whether this is a qualitative cultivar difference or whether RM I was indeed present in the five other cultivars but at concentrations too low to be detected by TLC.

The structures of RM I, RM II, and RM III are unidentified to date. On the basis of chromatographic behavior, RM II and RM III may be similar or identical to the rishitin metabolites described by Murai *et al.* (13).

To summarize, the presence of an SSM intermediate, either rishitin or katahdinone, together with E/O_2 induced the *de novo* synthesis of compounds in the katahdinone and phytuberin pathways. Neither an SSM intermediate nor E/O_2 alone was sufficient to accomplish this; both were necessary. This leads us to propose that in our previous study (1), ethylene, a well-known plant hormone and a modulator of potato tuber respiration (10), as well as SSM intermediates themselves, together elevated SSM levels via increased SSM biosynthesis. This is the first demonstration, to our knowledge, of a presumed means for control of biosynthesis along the SSM pathway.

The postulation of such a mechanism's playing a role *in vivo* requires that certain conditions be demonstrated: the cooccurrence of the modulators, ethylene and SSM, in infected tuber tissue; the build-up of endogenous tissue ethylene to a concentration allowing effective regulation of pathway activity; and functional efficiency of such a mechanism at endogenous tissue O_2 levels. Ethylene resulting from infection is most often localized at the site of the lesion (14) as are SSM (16). Additionally, ethylene produced by infected potato tuber tissue increases to a concentration which we have shown to be within the range of *in vitro* effectiveness in

modulating SSM biosynthesis (4). We are planning experimentation to test the activity of this mechanism at various O_2 concentrations, including ambient O_2 levels found in infected tissue. Identification of biosynthetic control mechanisms may be of great importance to our understanding of the role of SSM in hypersensitivity and disease resistance.

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