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Involvement of 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase in the Regulation of Sesquiterpenoid Phytoalexin Synthesis in Potato[1]

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ABSTRACT

The importance of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) in the regulation of sesquiterpenoid phytoalexin accumulation in potato (*Solanum tuberosum* L. cv Kennebec) was examined. Wounding of potato tubers produced a large temporary increase in HMG-CoA reductase activity of the microsomal and organelle fractions. Treatment of wounded tuber tissue with the sesquiterpenoid phytoalexin elicitor arachidonic acid further increased and prolonged the HMG-CoA reductase activity in the microsomal but not the organelle fraction. Incubation of elicitor-treated tuber tissue in white light reduced organelle and microsomal HMG-CoA reductase activity to 50% and 10%, respectively, of the activity of tissues held in darkness. Constant light also reduced overall phytoalexin accumulation 58% by greatly reducing levels of lubimin. Rishitin accumulation was not significantly altered by light. Application of nanomolar amounts of mevinolin, a highly specific inhibitor of HMG-CoA reductase, to elicitor-treated tuber tissue produced a large decline in lubimin accumulation and did not markedly alter rishitin accumulation. These results indicate that HMG-CoA reductase has a role in the complex regulation of sesquiterpenoid phytoalexin accumulation in potato.

The induction of phytoalexins by elicitors is useful for biochemical studies of the regulation of plant disease resistance metabolism. Elicitors are substances which can trigger the accumulation of phytoalexins in plants in the absence of an attacking microorganism (27). Arachidonic and eicosapentaneoic acids are natural components of the lipids from *Phytophthora infestans*, a pathogen of potato (*Solanum tuberosum*), and each elicits the accumulation of SSM[3] when applied to the cut surface of potato tubers (4, 5, 10). Many of the SSM are phytoalexins and accumulation of these fungitoxic sesquiterpenes is correlated with disease resistance in potato (17, 18). In addition, elicitor activity of the fatty acids is enhanced several-fold by soluble β-glucan preparations from *P. infestans* mycelium, although the glucans alone have no sesquiterpene eliciting activity (23).

Little is known about the mechanisms involved in the regulation of SSM accumulation in potato (19). This is mostly due to the relatively few studies that have been done on the regulatory enzymes of isoprenoid metabolism in plants, despite the importance of isoprenoid compounds in plant growth and development, photosynthesis, and disease resistance (9, 13). A key enzyme controlling the isoprenoid pathway in animals and fungi is HMG-CoA reductase (EC 1.1.1.34) (11). This enzyme is also considered to be a key regulatory enzyme of isoprenoid biosynthesis in plants (13). In the pathway from acetate to mevalonate, HMG-CoA reductase catalyses the conversion of 3-hydroxy-3-methylglutaryl coenzyme A to mevalonate, which is the first committed step in the synthesis of isoprenoid compounds.

Induction of HMG-CoA reductase activity in potato is likely to play a role in sesquiterpenoid phytoalexin accumulation. In *Phaseolus vulgaris*, accumulation of isoflavonoid phytoalexins is due to the induction of biosynthetic enzyme activity (20). A similar induction of biosynthetic activity is implicated in the accumulation of a sesquiterpenoid phytoalexin in sweet potato roots; accumulation of ipomeamarone is preceded by a rapid rise in HMG-CoA reductase activity (25). HMG-CoA reductase is probably subject to complex control. Subcellular fractionation studies have shown that HMG-CoA reductase is a membrane-bound enzyme localized in the plastids, mitochondria, and microsomes, suggesting compartmentation of parallel biosynthetic pathways (3, 7). Also, HMG-CoA reductase is under phytochrome-mediated control (8). This study determines the effects of AA on HMG-CoA reductase activity in tuber tissue and examines the role of this enzyme in the regulation of sesquiterpenoid phytoalexin accumulation.

MATERIALS AND METHODS

Plant and Fungal Material. Certified seed potatoes (*Solanum tuberosum* L. cv Kennebec) were stored at 4°C for 1 month or more until 24 h prior to use when they were placed in darkness at room temperature. Due to the inhibitory effects of laboratory lights on HMG-CoA reductase activity, manipulation of tubers and enzyme extraction were carried out under a green safelight comprised of two green fluorescent tubes (Sylvania F40-G) wrapped with one sheet each of Roscolene filter No. 877 (medium blue green) and No. 874 (medium green). Tubers were washed, surface sterilized in 70% ethanol for 3 min, then disks (22 × 5–7 mm) were cut from the central parenchymous tissue with a sterile cork borer. The disks were washed twice with sterile distilled water and four or five were placed on sterile filter paper in a Petri dish. All treatments and inoculations were applied to the upper surface of tuber disks as 100 μl aqueous samples. Sporangial suspensions of *Phytophthora infestans* (Mont.) deBary race 0 and 1.4 were obtained from 10- to 14-day-old cultures grown on lima bean agar at 20°C (14). Aliquots containing 10^5 sporangia were applied to a tuber disk.

Chemicals. Arachidonic, linoleic, and mevalonic acids were

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[3] Abbreviations: SSM, sesquiterpenoid stress metabolites; HMG-CoA reductase, 3-hydroxy-3-methylglutaryl coenzyme A reductase; SGA, steroid glycoalkaloids; AA, arachidonic acid.

purchased from Sigma. Immediately before application to tuber disks, a fatty acid solution in hexane:isopropanol (3:2, v/v) was dried under N_2 and resuspended in sterile water by brief sonication. The amount of AA usually applied to tuber disks (50 μg/disk) produces approximately 50% of the maximum response for sesquiterpenoid phytoalexin accumulation (5). Glucans that enhanced arachidonic elicitor activity were prepared from *P. infestans* race O mycelia as described by Bostock *et al.* (6). Mevinolin was a generous gift from Dr. C. A. Stone, Merck Sharp & Dohme Research Laboratories. The Na^+ salt of mevinolin (monacolin K) was prepared by saponification as described (12).

Quantitation of Phytoalexins. The sesquiterpenoid phytoalexins were quantified by a semimicro method (15). Triplicate samples each containing 1 g of tissue from the upper 1 mm of tuber disks were removed and extracted for sesquiterpenes. Concentrations of lubimin and rishitin, the major sesquiterpeniod phytoalexins in potato, were determined by gas chromatography.

Enzyme Isolation. HMG-CoA reductase activities of both the organelle and the microsomal fractions were determined for the upper 1 mm of treated tuber disks by a procedure modified from Bach and Lichtenthaler (2). Six g of tissue were homogenized with a prechilled mortar and pestle in 8 ml of ice-cold extraction buffer containing 0.2 M K-Phosphate (pH 7.5), 0.35 M sorbitol, 5 mM $MgCl_2$, 10 mM Na_2EDTA, 20 mM 2-mercaptoethanol, and insoluble PVP (5 g/100 ml). The homogenate, plus 4 ml of extraction buffer used to rinse the mortar, were filtered through four layers of cheesecloth. The filtrate was centrifuged first at 1,200*g* for 5 min, then at 16,000*g* for 40 min, and finally at 105,000*g* for 60 min, all at 4°C. The 16,000*g* pellet (*i.e.* the organelle-enriched fraction), and the 105,000*g* pellet (*i.e.* the microsomal fraction), were each resuspended in 600 μl of freshly prepared buffer containing 42 mM DTT and 33 mM K-phosphate (pH 7.5). After addition of the resuspension buffer, enzyme preparations were allowed exposure to laboratory lights. The pellets were kept on ice and stirred with a pipet tip to resuspend them and then were frozen at −20°C. There was minimal loss of activity over 1 week of storage. Protein content of the enzyme preparations was estimated by the Bio-Rad dye-binding method following the manufacturer's directions and using BSA as the standard.

Enzyme Assay. The activity of HMG-CoA reductase was determined by a radiolabel assay modified from Huber *et al.* (16). To a 1.5 ml microfuge vial containing a reaction mixture of 20 μmol K-phosphate (pH 7.5), 2.5 μmol Na_2EDTA, 5.0 μmol DTT, and 1 mg BSA was added 2.1 nmol (0.025 μCi) of DL-3-[glutaryl-3-^{14}C]-hydroxy-3-methylglutaryl coenzyme A (New England Nuclear). Included in the vial was a NADPH generation system composed of 0.2 μmol $NADP^+$, 2 μmol glucose 6-P, and 0.1 unit of glucose 6-P dehydrogenase (Sigma). A 100 μl aliquot of the undiluted enzyme preparation (150–400 μg of protein) was added to the vial to bring the total volume to 700 μl. After a 20 min incubation at 30°C the reaction was terminated by addition of 100 μl of 3 N HCl; one mg of carrier mevalonate was also added. The vials stood at room temperature for at least 30 min to ensure complete lactonization of the labeled mevalonate, then the contents were transferred to test tubes and 4 to 5 g of anhydrous Na_2SO_4 were added. The tubes were allowed to stand at room temperature overnight. The Na_2SO_4 was extracted with 5 ml of diethyl ether followed by four more extractions with 2.5 ml. The pooled extracts were then dried under N_2 and resuspended in 0.5 ml of water. Labeled 3-hydroxy-3-methylglutarate was removed by eluting aqueous samples with water through 1 ml of Dowex-1 resin (Cl-form, 100–200 mesh, 8% XL). The first 2.5 ml of eluant was collected for liquid scintillation counting of labeled mevalonolactone and concentrations were calculated using a value of 2.64×10^4 dpm per nmol for HMG-CoA. Enzyme activity was expressed as nmol mevalonate produced/h·mg protein.

Light Treatment. The tuber disks were each treated with 50 μg of arachidonic acid and incubated for 24 h under one of two different light regimes: constant irradiation 19 cm under white fluorescent tubes (General Electric F20T12.CW) or constant darkness. The enzyme was extracted within 30 min after the end of the light treatments. All manipulations of the tuber disks were carried out under a green safelight, except for the indicated light treatments.

RESULTS

Time Course of Enzyme Activity Induction. Changes in the activity of HMG-CoA reductase were followed over time after treatment of tuber tissue with AA. Cell-free extracts from the treated tissue were assayed for enzyme activity *in vitro*. Enzyme activity of the microsomal fraction is presented in Figure 1. Both the tuber disks treated with AA and the controls treated with water rapidly increased in activity. At 12 h after treatment, the activity of HMG-CoA reductase had risen over 8-fold. However, by 24 h the activity of the controls had declined sharply, while the activity of tuber disks treated with AA had continued to increase to 4.93 nmol mevalonate/h·mg protein. Microsomal enzyme activities of both treatments had declined by 48 h and were at initial levels 72 h after treatment. Arachidonic acid treatment resulted in a higher and more prolonged increase in enzyme activity than was seen in water-treated controls. The burst in activity at 12 h for controls was apparently in response to wounding, *i.e.* preparing tuber disks.

The HMG-CoA reductase activity of the organelle-enriched fraction was not enhanced by AA treatment (Fig. 2). Although

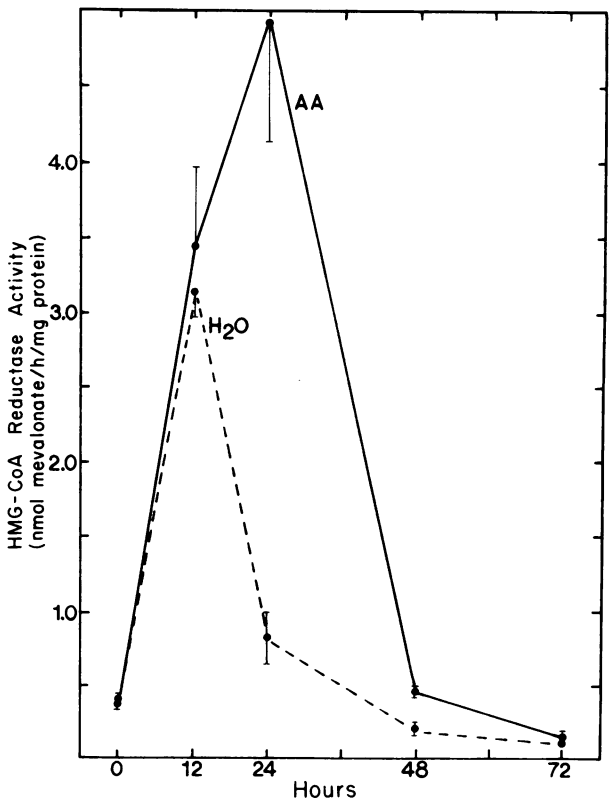

FIG. 1. Time course of microsomal HMG-CoA reductase activity from potato tuber disks. Individual tuber disks were treated with 100 μl of sterile water or with 50 μg of AA in water immediately following the cutting of the disks (O h). Each point is the mean of two determinations from a representative experiment. Bars indicate SE.

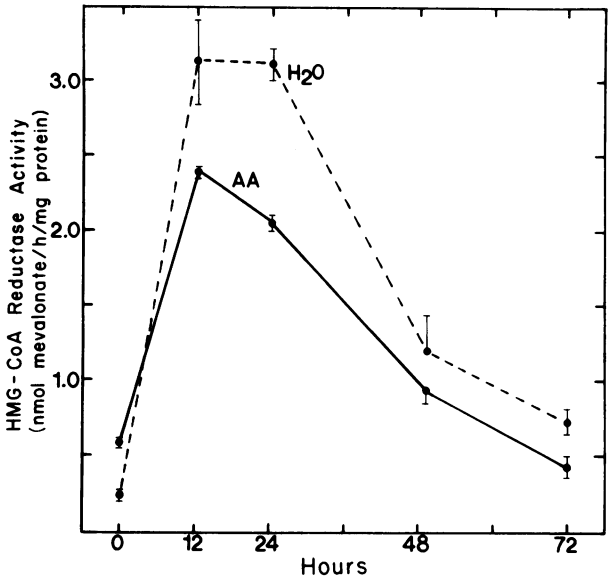

FIG. 2. Time course of HMG-CoA reductase activity of the organelle-enriched fraction from potato tuber disks. Individual tuber disks were treated with 100 μl of sterile water or with 50 μg of AA in water immediately following the cutting of the disks (O h). Each point is the mean for two determinations. Bars indicate SE.

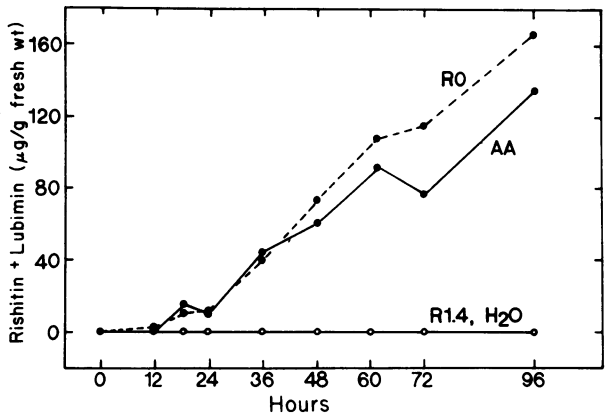

FIG. 3. Time course of sesquiterpenoid phytoalexin accumulation in potato tuber disks. Individual tuber disks were treated with 10^5 sporangia from an incompatible race of *P. infestans* (RO), 10^5 sporangia from a compatible race of the fungus (R1.4), 50 μg of AA or H_2O. Each point is the mean of three determinations.

there was an early increase in activity due to wounding, as was seen in the microsomal fraction, AA treatment did not cause a further rise in the HMG-CoA reductase activity of the organelle-enriched fraction.

Time Course of Phytoalexin Accumulation. The amount of sesquiterpeniod phytoalexin in tuber disks was determined at various times after treatment with AA or after inoculation with *Phytophthora infestans* (Fig. 3). The kinetics of accumulation for the phytoalexins were very similar with either AA (50 μg/disk) or the incompatible race O of *P. infestans* (10^5 sporangia/disk) to induce accumulation. Lubimin was detected first at 12 h after treatment and rishitin appeared at 18 h. The levels of these two phytoalexins induced by AA or race O then rose to 136 and 161 μg/g fresh weight by 96 h after induction, respectively. The compatible race 1.4 of *P. infestans* induced less than 1 μg/g fresh weight of rishitin and lubimin at 96 h, and phytoalexins were not detected in the water-treated controls.

Effect of Elicitor Concentration on Enzyme Activity. The HMG-CoA reductase activity of the microsomal fraction was dependent on the quantity of AA applied to tuber tissue (Fig. 4). In contrast, the activity of the organelle-enriched fraction was not appreciably affected by the elicitor. At 24 h after water treatment (O μg AA) the enzyme activity of the organelle fraction was higher than the activity of the microsomal fraction because the activity of the organelle fraction remained elevated longer after wounding. Nevertheless, AA did not further enhance activity of the organelle fraction.

Effect of Light on Enzyme Activity and Phytoalexin Accumulation. Exposure of tuber tissue to 24 h of constant light reduced the activity of organelle and microsomal HMG-CoA reductase to 50% and 10%, respectively, of the activity in tissue held in darkness (Table I). Even a brief, 5-min exposure of tuber disks to white light caused a marked decrease in extractable enzyme activity (data not shown). Constant light also reduced overall

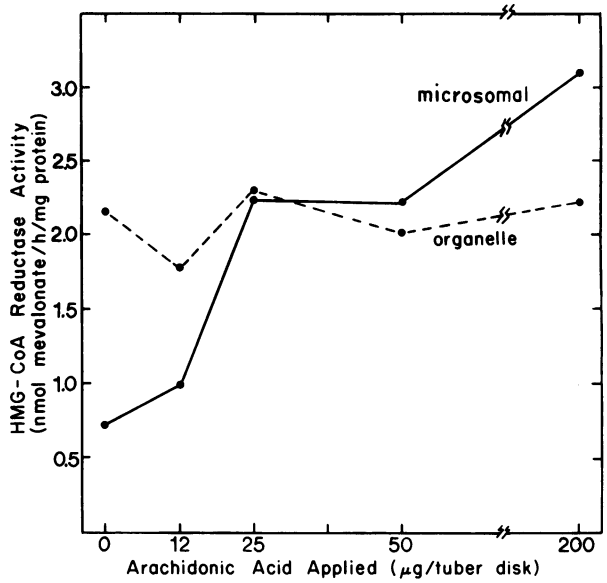

FIG. 4. Effect of the concentration of arachidonic acid applied to potato tuber disks on HMG-CoA reductase activity. Freshly prepared tuber disks were treated with the indicated amounts of AA in 100 μl of sterile water. At 24 h after treatment the disks were harvested and the organelle-enriched and the microsomal fractions were assayed for activity. Each point is the mean of four determinations from two independent experiments.

Table I. *Effect of Light on HMG-CoA Reductase Activity and on Sesquiterpenoid Phytoalexin Accumulation in AA-treated Potato Disks*

Treatment[a]	HMG-CoA Reductase Activity[b]		Rishitin[c]	Lubimin
	Organelle	Microsomal		
	nmol mevalonate/ h·mg protein		*μg/g fresh wt*	
Constant dark	1.87	2.34	95 ± 15 a	186 ± 46 a
Constant light	0.94	0.22	113 ± 29 a	5 ± 3 b

[a] Freshly cut tuber disks were each treated with 50 μg of arachidonic acid and incubated with constant irradiation under white fluorescent lights or constant darkness. [b] Enzyme activity of the organelle and microsomal fractions after 24 h of light treatment. The experiment was repeated four times with similar results. Values are from a representative experiment. [c] Sesquiterpenes were extracted after 96 h of treatment. The experiment was repeated four times with similar results. Values are from a representative experiment and each is the mean ± SE of three determinations. Values within a column followed by the same letter are not significantly different, *t*-test (P = 0.01).

phytoalexin accumulation 58% by greatly reducing the concentrations of lubimin. Rishitin accumulation was not significantly altered by light.

Effect of Linoleic Acid and Mycelial Glucans on Enzyme Activity. Although linoleic acid and glucans lack elicitor activity, they were both capable of significantly increasing microsomal HMG-CoA reductase activity in tuber tissue (Table II). More than a 3-fold increase in enzyme activity was observed 24 h after these substances were applied to tuber disks. However, the stimulation of activity was less than that seen for arachidonic acid treatment. Application of AA plus glucans to tuber disks elicited the highest level of enzyme activity and phytoalexin accumulation.

Reduction of Phytoalexin Levels by Mevinolin. Mevinolin was a potent inhibitor of HMG-CoA reductase. The Ki for mevinolin in the cell-free potato tuber enzyme assay was 8 and 10 nM for the organelle and microsomal fractions, respectively (data not shown). Application of 40 nmol of the Na^+ salt of mevinolin per tuber disk in 10 nmol aliquots at 24 h intervals caused a sharp reduction in the total accumulation of sesquiterpenoid phytoalexins (Table III). Mevinolin reduced the overall phytoalexin accumulation by 60% whether the phytoalexins were induced by *P. infestans* or by AA. However, it was the drop in lubimin levels that accounted for the decrease. Rishitin accumulation was not markedly altered by mevinolin. The inhibitory effect of mevinolin on lubimin accumulation was not significantly reversed by combining 1 μmol of mevalonate with the 10 nmol aliquots of inhibitor. The mevinolin treatments had no apparent effect on the growth of *P. infestans* (data not shown).

DISCUSSION

Wounding of tuber tissue, *i.e.* the preparation of tuber disks, produced a large increase in HMG-CoA reductase activity of both the microsomal and the organelle-enriched fractions. Application of AA to tuber disks caused a further enhancement of enzyme activity in the microsomal fraction. Tuber tissues treated with the elicitor had more than twice the amount of HMG-CoA reductase activity in the microsomal fraction over the 72 h period after treatment. The large increase in enzyme activity of the microsomal and organelle-enriched fractions was followed by the accumulation of sesquiterpeniod phytoalexins, thus suggesting a role for increased HMG-CoA reductase activity in SSM accumulation. Recent work on this enzyme in potato using crude elicitor preparations from the hyphal walls of *P. infestans* demonstrated a similar enhancement of HMG-CoA reductase activity preceding an accumulation of rishitin (22).

Normally, large amounts of SGA are produced at the site of injury in potatoes, and the wound-induced increase in HMG-CoA reductase activity may be involved in the accumulation of SGA in the tuber. However, elicitor treatment of the cut surface of tubers results in a change from SGA to sesquiterpenoid phytoalexin accumulation (26). Biosynthetic studies of tubers infected with *P. infestans* showed that ^{14}C-acetate and ^{14}C-mevalonate were incorporated into rishitin while there was a marked reduction in the incorporation of label in SGA (24). These results suggest that a diversion of the isoprenoid intermediate farnesyl pyrophosphate from sterol to sesquiterpene biosynthesis is at least partially involved in the regulation of sesquiterpenoid phytoalexin accumulation. Evidence presented here supports the idea that increases in HMG-CoA reductase activity alone are not sufficient to cause phytoalexin accumulation. The large increase in enzyme activity after wounding does not produce any phytoalexin accumulation in potato, and further enhancement of HMG-CoA reductase activity by treatment with linoleic acid or glucans does not result in any phytoalexin accumulation.

Nevertheless, two treatments which reduce HMG-CoA reductase activity indicate that this enzyme is an important regulatory component of the mechanism controlling sesquiterpenoid phytoalexin accumulation in potato. Mevinolin is a highly specific inhibitor of microsomal HMG-CoA reductase in plants (1). Application of nanomolar amounts of mevinolin to elicitor-treated tuber tissue produced a large decline in average phytoalexin accumulation, but the effect of mevinolin was not the same for rishitin and lubimin. While rishitin levels did not change much, it was the reduction in lubimin levels that was responsible for the overall drop in phytoalexins observed. The lack of a similar large reduction in rishitin accumulation indicates complex regulation of these sesquiterpenes. Lubimin is thought to be an intermediate in the pathway to rishitin (18, 21). Thus, one possible explanation would be rapid conversion of lubimin to rishitin during biosynthesis so that the reduced flux in the pathway would deplete lubimin pools as rishitin is rapidly formed. An alternative possibility is that there may be distinct pathways for rishitin and lubimin synthesis. Compartmentation of HMG-CoA reductase could result in differences in regulation between the isozymes and produce separately controlled isoprene pools.

Another treatment that inhibits HMG-CoA reductase activity in plants is irradiation of tissue with white light. The activity of this enzyme is light regulated in pea and radish and the incubation of tissues in continous white light reduces the activity of the microsomal fraction to 30% or less of the dark level (3, 8). Results from this paper show that irradiation with white light also causes a dramatic drop in extractable enzyme activity in potato tuber tissue. Furthermore, irradiation produced a similar drop in average phytoalexin accumulation in the tuber tissue.

Table II. *Effect of Fatty Acids and Mycelial Glucans on HMG-CoA Reductase Activity and Phytoalexin Accumulation in Potato Disks*

Treatment[a]	HMG-CoA Reductase Activity 24 h after Treatment	Phytoalexin Levels: Rishitin + lubimin
	nmol mevalonate/h·mg protein[b]	*μg/g fresh wt*[c]
AA (50 μg)	3.83 ± 0.28 a	256 ± 16
AA (50 μg) + glucans (83 μg)	4.67 ± 0.77 a	405 ± 46
Linoleic acid (50 μg)	2.34 ± 0.75 b	0
Glucans (83 μg)	1.99 ± 0.29 b	0
Water	0.58 ± 0.06 c	0

[a] The compounds listed were applied per freshly cut tuber disk in 100 μl of sterile water. [b] Values are the mean ± SE for three experiments. Values followed by the same letter are not significantly different, Duncan's multiple range test (P = 0.05). [c] Sesquiterpenes were extracted 96 h after treatment. Values are from a representative experiment and each is the mean ± SE of three determinations.

Table III. *Effect of Mevinolin and Mevalonic Acid on the Accumulation of Sesquiterpenoid Phytoalexins in Potato Disks Treated with AA or Inoculated with an Incompatible Race of P. infestans*

Mevinolin (10 nmol) in 100 μl of sterile water was applied to a tuber disk ½ h before inoculation (10^5 sporangia of *P. infestans*) or AA treatment (50 μg) and three more 10 nmol aliquots were subsequently applied at 24 h intervals. Where indicated, 1 μmol mevalonate was included with the mevinolin.

Treatment	Phytoalexin Levels		
	Rishitin	Lubimin	Rishitin + Lubimin
	μg/g fresh wt[a]		
Arachidonic acid			
Water	42 ± 5 a	196 ± 15 a	237 ± 19 a
Mevinolin	24 ± 3 b	59 ± 4 b	83 ± 8 b
Mevalonic acid + mevinolin	26 ± 1 b	65 ± 5 b	91 ± 6 b
P. infestans race O			
Water	36 ± 5 b	88 ± 5 a	124 ± 4 a
Mevinolin	42 ± 4 b	12 ± 3 b	55 ± 6 c
Mevalonic acid + mevinolin	65 ± 4 a	15 ± 2 b	80 ± 3 b

[a] Values are the means ± SE of levels 96 h after treatment, each derived from three determinations. Values followed by the same letter within a column for each elicitor treatment (AA or *P. infestans* sporangia) are not significantly different, Duncan's multiple range test (P = 0.05).

Like mevinolin treatment, light treatment had little effect on rishitin levels but caused a large decrease in lubimin concentrations. This supports the idea that light affects the same site as mevinolin, *i.e.* HMG-CoA reductase, and corroborates a role for this enzyme in the biosynthesis and regulation of sesquiterpenoid phytoalexin levels.

In summary, two lines of evidence indicate a role for HMG-CoA reductase in sesquiterpenoid phytoalexin synthesis. First, lubimin accumulation does not occur without a prior large increase in activity of the enzyme. Second, treatments which inhibit HMG-CoA reductase activity also sharply reduce the amount of lubimin that accumulates. These data suggest that sesquiterpenoid phytoalexin accumulation is dependent on the induction of HMG-CoA reductase activity. This enzyme may be part of a coordinated regulatory mechanism involving increased production of mevalonate for phytoalexin synthesis. Later changes in isoprenoid biosynthesis subsequent to farnesyl pyrophosphate are likely sites for additional regulatory point(s) in sesquiterpenoid phytoalexin synthesis. These studies also suggest that compartmentation of isoprene pathways could be an important factor in the phytoalexin response in potato.

LITERATURE CITED

1. BACH TJ, HK LICHTENTHALER 1982 Mevinolin: a highly specific inhibitor of microsomal 3-hydroxy-3-methylglutaryl coenzyme A reductase of radish plants. Z Naturforsch 37c: 46–50
2. BACH TJ, HK LICHTENTHALER 1983 Mechanism of inhibition by mevinolin (MK803) of microsome-bound radish and of partially purified yeast HMG-CoA reductase (EC.1.1.1.34). Z Naturforsch 38c: 212–219
3. BACH TJ, HK LICHTENTHALER, J RETEY 1980 Properties of membrane-bound 3-hydroxy-3-methylglutaryl coenzyme A reductase (EC.1.1.1.34) from radish seedlings and some aspects of its regulation. *In* P Mazliak, P Benveniste, C Costes, R Douce, eds, Biogenesis and Function of Plant Lipids. Elsevier, Amsterdam, pp 355–363
4. BOSTOCK RM, JA KUĆ, RA LAINE 1981 Eicosapentaenoic and arachidonic acids from *Phytophthora infestans* elicit fungitoxic sesquiterpenes in the potato. Science 212: 67–69
5. BOSTOCK RM, RA LAINE, JA KUĆ 1982 Factors affecting the elicitation of sesquiterpenoid phytoalexin accumulation by eicosapentaenoic and arachidonic acids in potato. Plant Physiol 70: 1417–1424
6. BOSTOCK RM, DA SCHAEFFER, R HAMMERSCHMIDT 1986 Comparison of elicitor activities of arachidonic acid, fatty acids and glucans from *Phytophthora infestans* in hypersensitivity expression in potato tuber. Physiol Mol Plant Pathol 29: 349–360
7. BROOKER JD, DW RUSSELL 1975 Subcellular localization of 3-hydroxy-3-methylglutaryl coenzyme A reductase in *Pisum sativum* seedlings. Arch Biochem Biophys 167: 730–737
8. BROOKER JD, DW RUSSELL 1979 Regulation of microsomal 3-hydroxy-3-methylglutaryl coenzyme A reductase from pea seedlings. Rapid posttranslational phytochrome-mediated decrease in activity and in vivo regulation by isoprenoid products. Arch Biochem Biophys 198: 323–334
9. CANE DE 1981 Biosynthesis of sesquiterpenes. *In* JW Porter, SL Spurgeon, eds, Biosynthesis of Isoprenoid Compounds. Wiley, New York, pp 283–374
10. CREAMER JR, RM BOSTOCK 1986 Characterization and biological activity of *Phytophthora infestans* phospholipids in the hypersensitive response of potato tuber. Physiol Mol Plant Pathol 28: 215–225
11. DUGAN RE 1981 Regulation of HMG-CoA reductase. *In* JW Porter, SL Spurgeon, eds, Biosynthesis of Isoprenoid Compounds. Wiley, New York, pp 95–159
12. ENDO A 1981 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors from Penicillium and Monascus species. Methods Enzymol 72: 684–689
13. GARG VK, TJ DOUGLAS 1983 Hydroxymethylglutaryl CoA reductase in plants. *In* JR Sabine, ed, 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase. CRC Press, Boca Raton, FL, pp 29–37
14. HENFLING JWDM, RM BOSTOCK, J KUĆ 1980 Cell walls of *Phytophthora infestans* contains an elicitor of terpene accumulation in potato tubers. Phytopathology 70: 772–776
15. HENFLING JWDM, J KUĆ 1979 A semi-micro method for the quantitation of sesquiterpenoid stress metabolites in potato tuber tissue. Phytopathology 69: 609–612
16. HUBER J, S LATZIN, B HAMPRECHT 1973 A simple and rapid radiochemical assay for 3-hydroxy-3-methylglutaryl coenzyme A reductase. Z Physiol Chem 354: 1645–1647
17. KUĆ J, N LISKER 1978 Terpenoids and their role in wounded and infected plant storage tissue. *In* G Kahl, ed, Biochemistry of Wounded Plant Tissues. De Gruyter, New York, pp 203–242
18. KUĆ J, JS RUSH 1985 Phytoalexins. Arch Biochem Biophys 236: 455–472
19. KUĆ J, E TJAMOS, R BOSTOCK 1984 Metabolic regulation of terpenoid accumulation and disease resistance in potato. *In* WD Nes, G Fuller, L Tsai, eds, Isopentenoids in Plants. Biochemistry and Function. Marcel Dekker, New York, pp 103–126
20. LAMB C, J BELL, P NORMAN, M LAWTON, R DIXON, P ROWELL, J BAILEY 1983 Early molecular events in the phytoalexin defense response. *In* O Ciferri, L Dure III, eds, Structure and Function of Plant Genomes. Plenum, New York, pp 313–327
21. MURAI A, S SATO, A OSADA, N KATSUI, T MASAMUNE 1982 Biosynthesis from solavetivone of the phytoalexin rishitin in potato. Implicit role of solavetivone as an activator. J Chem Soc Chem Commum 1982: 32–33
22. OBA K, K KONDO, N DOKE, I URITANI 1985 Induction of 3-hydroxy-3-methylglutaryl CoA reductase in potato tubers after slicing, fungal infection or chemical treatment, and some properties of the enzyme. Plant Cell Physiol 26: 873–880
23. PREISIG CL, JA KUĆ 1985 Arachidonic acid-related elicitors of the hypersensitive response in potato and enhancement of their activities by glucans from *Phytophthora infestans* (Mont.) deBary. Arch Biochem Biophys 236: 379–389
24. SHIH MJ, J KUĆ 1973 Incorporation of ^{14}C from acetate and mevalonate into rishitin and steroid glycoalkaloids by potato slices inoculated with *Phytophthora infestans*. Phytopathology 63: 826–829
25. SUZUKI H, K OBA, I URITANI 1975 The occurrence and some properties of 3-hydroxy-3-methylglutaryl coenzyme A reductase in sweet potato roots infected by *Ceratocystis fimbriata*. Physiol Plant Pathol 7: 265–276
26. TJAMOS E, J KUĆ 1982 Inhibition of steroid glycoalkaloid accumulation by arachidonic and eicosapentaenoic acids in potato. Science 217: 542–544
27. WEST CA 1981 Fungal elicitors of the phytoalexin response in higher plants. Naturwissenschaften 68: 447–457