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Regulation of Ethylene Biosynthesis in Virus-Infected Tobacco Leaves[1]

II. TIME COURSE OF LEVELS OF INTERMEDIATES AND *IN VIVO* CONVERSION RATES

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ABSTRACT

Ethylene production was stimulated severalfold during the hypersensitive reaction of Samsun NN tobacco to tobacco mosaic virus (TMV). Exogenous methionine or *S*-adenosylmethionine (SAM) did not increase ethylene evolution from healthy or TMV-infected leaf discs, although both precursors were directly available for ethylene production. This indicates that ethylene production is not controlled at the level of methionine concentration or availability, nor at the level of SAM production or concentration. In contrast, 1-aminocyclopropane-1-carboxylic acid (ACC) stimulated ethylene production considerably. Thus, ethylene production is primarily limited at the level of ACC production.

The regulation of ethylene production during the hypersensitive reaction to TMV was further studied by determining the time course of the concentrations of methionine, SAM, and ACC, as well as the course of their *in vivo* conversion rates. Endogenous concentrations of methionine and SAM remained unaffected until late in infection. On the contrary, the peak in ethylene production near the time of local lesion development was preceded by a large increase in ACC production. As a result of this increase, ACC accumulated in the leaf tissue. Only after local lesions became visible, the capacity to convert ACC into ethylene increased severalfold, associated with a sharp decrease in ACC content and a large increase in ethylene production.

Ethylene production in tobacco leaves reacting hypersensitively to TMV is thus regulated at the level of both the production of ACC and its conversion to ethylene.

Infection of *N* gene-containing tobacco cultivars with TMV[2] leads to the formation of necrotic local lesions within 48 h after inoculation (10). Such a hypersensitive reaction is accompanied by a sharp peak in ethylene production near the time of lesion appearance. Virus-stimulated ethylene production gradually subsides during subsequent lesion growth (6, 17).

In a previous paper, we demonstrated that methionine is the precursor of both normal and virus-stimulated ethylene in tobacco leaves (6). This conclusion was based on observations that AVG, known to inhibit methionine-derived ethylene production (9, 14), inhibited both normal and virus-induced ethylene production. Furthermore, after labeling leaves with L-[U-^{14}C]methionine, the specific radioactivity of the ethylene produced was in accordance with that of the methionine pool within the leaves. Moreover, the concentration of the intermediate, ACC (1, 16), increased severalfold 2 days after TMV infection, when ethylene production was maximal.

An increase in ethylene production may be the result of an increase in: (a) the concentration and/or availability of the ethylene precursor(s), and/or (b) the activity of one or more of the enzymes involved in ethylene biosynthesis. It has been suggested that in the biosynthetic pathway for methionine-derived ethylene production, ACC synthesis rather than its conversion to ethylene is the rate-limiting step (3, 11, 25). However, the accumulation of ACC after virus infection (6) indicates that the utilization of ACC may also be limiting. The present study was undertaken to analyze how, during the hypersensitive reaction of Samsun NN tobacco to TMV, the limiting factors change in the pathway from methionine to SAM to ACC to ethylene in order to enable the ethylene outburst.

MATERIALS AND METHODS

Plant Material and Incubation Conditions. Tobacco plants (*Nicotiana tabacum* L. cv. Samsun NN) were grown from seed in a greenhouse at a minimum temperature of 20°C; photoperiod was maintained at 16 h by additional illumination from high pressure halide Philips HPI/T lamps at 18 w/m^2. Almost full-grown leaves from 10- or 11-week-old plants were selected. The leaves were inoculated on the plant by dusting with carborundum, rubbing with either water (controls) or TMV W U1 (100 μg/ml) using a gauze pad, and rinsing with tap water. At specific times leaves were picked off for analyses. Subsequent incubations of the detached leaves were carried out in growth chambers under controlled conditions (20°C, 16 h photoperiod, 70% RH) (20).

Uptake of labeled substrates was accomplished by vacuum infiltration. Leaf discs (40 mm diameter) were immersed for 30 s at 1 mm Hg in aqueous solutions of either L-[U-^{14}C]methionine or SAM-[3,4-^{14}C]methionine, blotted dry with Kleenex tissues, and incubated in water-locked 750-ml Petri dishes (6).

Determination of Methionine Concentration. Leaf material was extracted and the concentration of methionine determined by amino acid analysis as described earlier (6). The specific radioactivity of methionine from [^{14}C]methionine- or [^{14}C]SAM-labeled leaves was determined by liquid scintillation counting of the methionine-containing fractions eluted from the amino acid analyzer. Corrections for counting efficiency were made using an external standard.

Determination of SAM Concentration and *in Vivo* Conversion of Methionine to SAM. Leaf material was frozen in liquid N_2 ground in a mortar with pestle, and 2 ml 6% $HClO_4$/g fresh weight

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[2] Abbreviations: TMV, tobacco mosaic virus; ACC, 1-aminocyclopropane-1-carboxylic acid; AVG, aminoethoxyvinylglycine; SAM, *S*-adenosylmethionine.

were added. The homogenate was stirred for 30 min at 0°C and centrifuged for 10 min at 30,000*g*. The supernatant was adjusted to pH 4.5 at 0°C by slowly adding solid $KHCO_3$.

SAM was separated from other UV-absorbing compounds by subjecting the mixture to sulfopropyl (SP)-Sephadex ion-exchange column-chromatography according to Glazer and Peale (8). A comparison with authentic SAM by TLC (19) and thin-layer electrophoresis (18), confirmed that SAM was the only UV-absorbing compound within the SAM-containing fraction. The concentration of SAM was determined spectrophotometrically, assuming a molar extinction coefficient of 15,000 $cm^{-1} \cdot M$ at 260 nm (8). Recovery of SAM during the whole procedure was about 80%, as established by adding a known amount of SAM as an internal standard.

The rate of the *in vivo* conversion of methionine to SAM was estimated by labeling leaf discs with L-[U-^{14}C]methionine and determining the amount of radioactive SAM formed. The pH 4.5 extracts were passed through a Bio-Rex 70(H^+-form) column (20 × 5 mm) by elution with water to remove the labeled methionine. SAM was then eluted with 0.1 M HCl according to Schlenk (18) and its radioactivity determined by liquid scintillation counting.

Determination of ACC Concentration and *in Vivo* ACC Production. After grinding of the leaf material, 2 ml of 5% sulfosalicylic acid/g fresh weight were added. The mixture was stirred for 30 min at room temperature and centrifuged for 10 min at 30,000*g*. The concentration of ACC in the supernatant was determined by chemical conversion of the ACC to ethylene according to Lizada and Yang (15). ACC determinations were carried out in 40-ml sealed serum flasks; the amount of ACC was calculated from the concentration of ethylene reached after conversion.

To identify the ethylene-releasing compound as ACC, extracts were passed through a Dowex 50 (H^+-form) column (0.4 × 4 cm). After the column was washed with water, amino acids were eluted with 10 ml 2 M NH_4OH. This fraction was taken to dryness by rotary evaporation at 30°C. The small residue was dissolved in 96% ethanol and the amino acids were separated by TLC on silica gel plates using chloroform:methanol:(17%)ammonia (2:2:1, v/v/v) as the solvent system, according to Boller *et al.* (2). Different zones of the chromatogram were scraped off and subjected to ACC determination. More than 95% of the total amount of ethylene produced by these zones was derived from the one containing ACC, as established by co-chromatography with authentic ACC.

In all determinations, corrections were made for the efficiency of the conversion of ACC to ethylene by using authentic ACC as an internal standard. Ethylene recovery from ACC was generally about 80%.

Attempts to isolate ACC-synthase activity from either healthy or TMV-infected tobacco leaves by methods similar to those used by Boller *et al.* (2) and Yu *et al.* (23) for tomato fruits were unsuccessful. Probably ACC synthase is inactivated as a result of decompartmentation upon homogenization. Therefore, as a measure of ACC-synthase activity *in vivo*, ACC production was assayed by determining ACC accumulation during incubation of tobacco leaf discs under anaerobic conditions. Since the conversion of ACC into ethylene requires oxygen, anaerobiosis blocks the last step in ethylene biosynthesis (1). Discs from one leaf half were extracted immediately after detachment to determine the level of ACC at the start of incubation, whereas discs from the other half were placed in an atmosphere of N_2. To this end, a desiccator was 3 times evacuated and filled with oxygen-free nitrogen. This procedure reduced the O_2 concentration to less than 0.1%, resulting in an inhibition of ethylene production of more than 80%.

Preliminary experiments indicated that accumulation of ACC under these conditions was equal in light and darkness. The rate of ACC accumulation remained constant for at least 8 h. Thus, within this time period, any influence of anaerobic conditions on ACC-synthase activity, such as the induction found as a long-term effect of anaerobiosis in tomato roots (4) could be excluded.

Routinely, leaf discs were incubated for 4 h at 20°C in light. After opening of the desiccator, the leaf material was immediately frozen in liquid N_2 for extraction and determination of ACC. ACC production was expressed as the increase in ACC content in nmol/g fresh weight.

Measurement of Ethylene Production. Individual leaf discs (25 mm diameter) were incubated in 1 ml water in 40-ml sealed serum flasks at 20°C in fluorescent light (10 w/m^2). At specific times, 1-ml gas samples were withdrawn through the rubber seal. The concentration of ethylene was determined with a gas chromatograph, equipped with an alumina column, and a flame-ionization detector. Ethylene production was calculated as the mean of at least three independent incubations. Each experiment was repeated at least twice.

After labeling leaves with either L-[U-^{14}C]methionine or *S*-adenosyl-[3,4-^{14}C]methionine the ethylene produced was trapped with mercuric perchlorate and its specific radioactivity was determined as described earlier (6).

Determination of the *in Vivo* Capacity of the ACC-Converting Enzyme(s). This capacity should be assessed by exogenous application of a saturating amount of ACC. However, due to the low affinity of the ACC-converting enzyme system to ACC (13), saturation could not be reached (7). Nevertheless, it can be assumed that comparison of the ethylene production of leaf discs incubated in a high concentration of ACC will reflect their relative capacities for ACC conversion. To this end, discs were incubated in 1 ml 1 mM ACC solutions in 40-ml serum flasks. Ethylene production was measured during a 4-h incubation in light (6).

Chemicals. L-[U-^{14}C]methionine (285 mCi/mmol) was purchased from The Radiochemical Centre (Amersham, UK) and *S*-adenosyl-[3,4-^{14}C]methionine (about 40 mCi/mmol) from the "Commissariat à l'Energie Atomique" (CEA) (Gif sur Yvette, France). ACC was obtained from Bayer AG (Leverkusen, F.R.G.) and AVG from Dr. R. Maag AG (Dielsdorf, Switzerland).

RESULTS

Ethylene Production. When discs were punched out of freshly detached leaves at specific intervals after inoculation, ethylene production during the first 4 h showed a pattern identical to the one established earlier for whole detached leaves during continuous incubation (6). Discs from TMV-inoculated leaves showed a sharp peak in ethylene production with a maximum at 52 h after inoculation, 4 h after lesions became macroscopically visible (Fig. 1). The ethylene production by leaf discs from water-inoculated

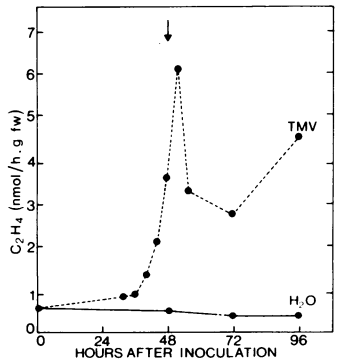

FIG. 1. Time course of ethylene production by leaf discs (25 mm diam), punched from water- (——) or TMV-inoculated (-----) leaves at the indicated times after inoculation. Ethylene production was measured during a 4-h incubation period at 20°C in light. The time that lesions became macroscopically visible is indicated by the arrow.

leaves remained at a steady low level.

Influence of Exogenously Applied Methionine, SAM, and ACC on Ethylene Production. Leaf discs, cut from freshly detached, noninoculated leaves, were vacuum infiltrated with, and further incubated in water or methionine solutions at concentrations varying from 10^{-5} to 10^{-2} M. At all concentrations tested, ethylene production was equal to that on water (about 0.3 nl/disc·h). Also, after preincubation of leaf discs in an humidified atmosphere for 24 h, application of methionine did not affect ethylene production.

Leaf discs were then cut 2 days after inoculation with either water or TMV, and infiltrated with and further incubated in solutions of methionine, SAM, or ACC, at a concentration of 1 mM. This is far higher than the endogenous concentrations of these ethylene precursors (see below). Methionine and SAM did not influence ethylene production in either the control, or in the virus-infected leaf discs. In contrast, the ethylene production of discs incubated in ACC was increased 5- and 2-fold, respectively (Table I). This indicates that ethylene production in tobacco is limited at the level of ACC.

After labeling leaves by vacuum infiltration with either L-[U-^{14}C]methionine or *S*-adenosyl-[3,4-^{14}C]methionine, radioactive ethylene was produced without any noticeable lag phase (Fig. 2), indicating that exogenously applied methionine or SAM are directly available to the ethylene-synthesizing enzyme system.

After labeling leaves with [^{14}C]methionine, the specific radioactivity of the methionine pool within the leaf decreased from 5,500 dpm/nmol at the beginning, to 250 dpm/nmol after 7 h of incubation, indicative of a high turnover rate. As a result, the specific radioactivity of the ethylene produced by these leaves decreased rapidly with time.

Although after vacuum infiltration, ethylene was produced from applied [^{14}C]SAM without a lag phase, the specific radioactivity of the ethylene was low and remained constant for at least 7 h. Apparently, SAM was taken up only gradually by the leaf cells. This prevented a reliable estimation of the specific radioactivity of SAM within the tissue proper. Due to the labeling with [^{14}C]SAM, the specific radioactivity of the methionine pool increased from 30 dpm/nmol at the beginning to 80 dpm/nmol at the end of the incubation period, indicating that some of the [^{14}C]SAM was hydrolyzed in the tissue. However, since the specific activity of the ethylene produced from [^{14}C]SAM was about 450 dpm/nmol, the ethylene was produced directly from SAM and not via SAM-derived methionine.

Table I. *Ethylene Production of Tobacco Leaf Discs, Cut Two Days after Inoculation with Either Water or TMV*

The discs were incubated in 1-ml solutions of 1 mM methionine, SAM, or ACC. Ethylene production was measured during a 5-h incubation period immediately after punching of the discs, and was expressed as the average (± SD) from four leaf discs.

Incubation	Ethylene Production	
	Water-inoculated	TMV-inoculated
	nl/disc·h	
Water	0.22 ± 0.03	3.13 ± 0.29
Methionine	0.18 ± 0.04	3.10 ± 0.24
SAM	0.21 ± 0.05	3.25 ± 0.38
ACC	1.05 ± 0.04	6.37 ± 0.40

Time Course of Methionine and SAM Concentration and the *in Vivo* Capacity to Convert Methionine to SAM During the Hypersensitive Reaction. In water-inoculated leaves, the methionine content remained stable at about 15 nmol/g fresh weight. No appreciable change occurred in TMV-infected leaves during the development of the lesions when ethylene production was rapidly rising (Fig. 3A).

Similarly, SAM content was unaffected during the hypersensitive reaction and remained at a level of about 7 nmol/g fresh weight during the first 3 days after inoculation, equal to the level of SAM in water-inoculated leaves (Fig. 3B). Only at 4 days after inoculation did the concentrations of both methionine and SAM increase significantly in the TMV-inoculated leaves, reflecting metabolic changes well after occurrence of the necrotic lesions.

To compare the conversion rates of methionine to SAM between water- and TMV-infected leaves, leaf discs were punched from leaves 48 h after inoculation, and labeled with L-[U-^{14}C]methionine. In a previous paper (6) we demonstrated that after labeling with L-[U-^{14}C]methionine by vacuum infiltration both healthy and virus-infected leaf discs produced ethylene with the same specific radioactivity, indicating uptake of the labeled methionine to be identical. This was confirmed by pulse-chase labeling: from 57,000 dpm L-[U-^{14}C]methionine introduced in the leaf discs by vacuum infiltration, after successive washings with 0.1 M KCl and 0.1 M unlabeled methionine 20 min after the pulse, 9,500 ± 1,100 dpm and 10,100 ± 1,500 dpm were retained in the water- and TMV-inoculated leaf discs, respectively.

Figure 4 shows that the amount of radioactivity recovered in SAM increased with time up to a maximum 30 min after labeling in both water- and TMV-inoculated leaf discs, apparently reflecting methionine uptake into the cells. At longer incubation times, the amount of radioactive SAM decreased, probably due to both the rapid decrease of the specific radioactivity of the methionine pool and the rapid turnover of SAM.

In each of three experiments, the peak in radioactive SAM formed was about 2 times higher in the virus-infected leaf discs than in the water-inoculated controls. Since the SAM-pool size was not increased after TMV-infection (Fig. 3B), this indicates that the turnover of SAM is increased about 2-fold upon TMV infection.

Time Course of *in Vivo* ACC Production and ACC Content During the Hypersensitive Reaction. Figure 5 shows the time course of the *in vivo* ACC production after water or TMV inoc-

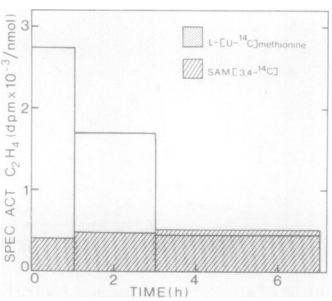

FIG. 2. Time course of the specific radioactivity of the ethylene produced by TMV-infected tobacco leaf discs 2 days after inoculation. Leaf discs were labeled with either L-[U-^{14}C]methionine (■) or SAM-[3,4-^{14}C]methionine (▨) at time 0.

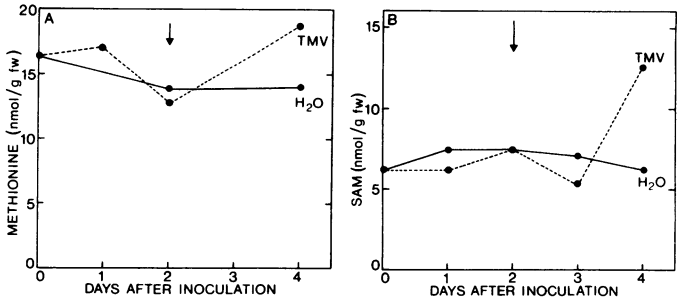

FIG. 3. Changes in endogenous concentrations of free methionine (A) or SAM (B) in extracts from tobacco leaves at specific times after inoculation with either water (——) or TMV (-----). The time of lesion appearance is indicated by the arrow.

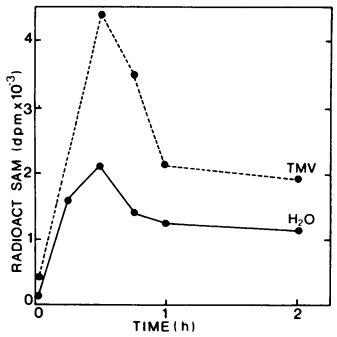

FIG. 4. Radioactive SAM formed after labeling leaf discs with L-[U-^{14}C]methionine. Discs were cut from leaves 2 days after inoculation with either water (——) or TMV (-----), and incubated at 20°C in light.

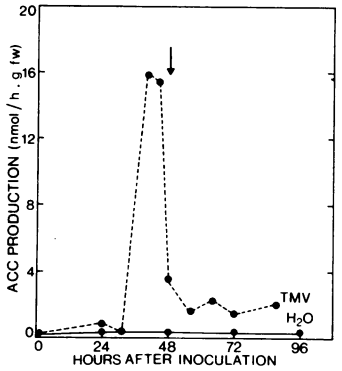

FIG. 5. ACC production at specific times after water (——) or TMV (-----) inoculation of leaves. ACC-synthase activity was calculated from the amount of ACC accumulated in leaf discs during a 4-h incubation in a nitrogen atmosphere. The time of lesion appearance is indicated by the arrow.

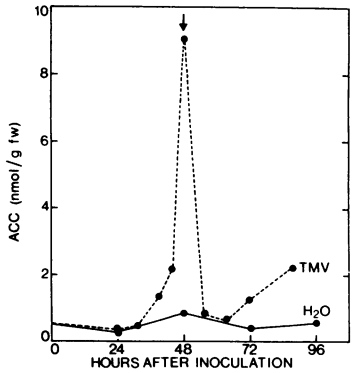

FIG. 6. Time course of ACC content in tobacco leaves after inoculation with water (——) or TMV (-----). The time of lesion appearance is indicated by the arrow.

ulation. After water inoculation, the activity of ACC production remained at the level of about 0.3 nmol/g fresh weight·h, whereas, in TMV-infected leaves, it increased more than 50-fold (up to 16 nmol/g fresh weight·h), as early as 40 h after inoculation. At 48 h, when local lesions had become macroscopically visible, ACC production had fallen to about 2 nmol/g fresh weight·h. It remained at this comparatively high level during subsequent days.

In both water- and TMV-inoculated discs, the endogenous ACC content fully reflected ACC synthesis. After water inoculation, it remained about 0.5 nmol/g fresh weight during the whole period, whereas in the TMV-infected leaves it increased from 40 h after inoculation onwards (Fig. 6), with a sharp peak at 48 h (about 9 nmol/g fresh weight). In four different experiments, the maximal increase was 15-, 9-, 24-, and 12-fold, respectively. By 60 h, the ACC content had decreased to the control level, but it slowly increased again to about 2 nmol/g fresh weight from 3 days after inoculation on.

As described before (6), AVG, at a concentration of 0.1 mM, very efficiently inhibited ethylene production of water- or TMV-inoculated leaves. ACC-synthase activity of AVG-treated, noninfected leaves was nearly abolished, whereas the rate of ethylene production of leaf discs incubated in 1 mM ACC was not influenced by AVG (Table II). Similar effects of AVG were found in TMV-infected leaves. These results confirm the conclusions of Boller *et al.* (2) and Yu and Yang (24) that AVG inhibits methionine-derived ethylene production by blocking ACC synthesis, rather than by inhibition of the conversion of ACC into ethylene.

Conversion of ACC into Ethylene. The sharp peak in ACC production during the hypersensitive reaction caused a temporary accumulation of ACC within the leaf tissue (Fig. 6). Since the increased ethylene production during the hypersensitive reaction is confined to the ring of tissue immediately adjacent to the necrotic lesions, it is tempting to assume that ACC accumulated only in that area. However, the amount of ACC had already declined when lesions became clearly visible. This indicates that, in the lesion area, at least from 36 to 48 h after infection, the final step in ethylene biosynthesis was rate-limiting.

The time course of the capacity of leaf discs to convert ACC

Table II. *Influence of (0.1 mM) AVG on Ethylene Production (Expt. 1) or ACC-Synthase Activity (Expt. 2)*

Ethylene production of noninfected leaf discs was measured between 2 and 7 h of incubation either in the presence or absence of 1 mM ACC. ACC-synthase activity was calculated from the amount of ACC accumulated during a 6-h anaerobic incubation following vacuum infiltration of the leaf discs with either water or 0.1 mM AVG.

Expt. No.	Incubation conditions	Ethylene Production	
		nl/disc·h	*% of control*
1	Water	0.115	100
	AVG	0.010	8.7
	ACC	0.813	707
	ACC + AVG	0.781	679
		ACC-Synthase Activity	
		nmol/g fresh wt·h	*% of control*
2	Water	0.285	100
	AVG	0.003	1.1

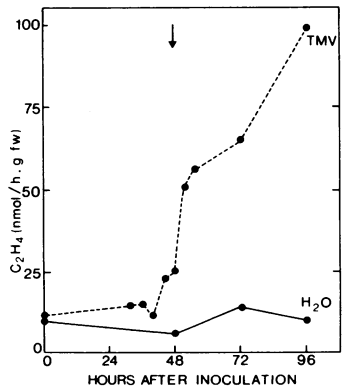

FIG. 7. Ethylene production of leaf discs, cut from water- (——) or TMV- (-----) inoculated leaves, upon incubation in 1 mM solutions of ACC at 20°C in light for a 4-h period. The time of lesion appearance is indicated by the arrow.

into ethylene was studied by incubating discs, cut from leaves at specific times after inoculation, in 1 mM solutions of ACC (Fig. 7). A comparison of Figure 1 with Figure 7 shows that the ethylene production of the water- and the TMV-inoculated leaf discs was increased manifold upon incubation in ACC. Starting around lesion appearance on day 2, the capacity of the TMV-inoculated leaf discs to convert exogenously applied ACC into ethylene increased 5- to 10-fold during the following days. Contrary to its production, ACC conversion kept increasing once local lesions had become visible.

DISCUSSION

After labeling leaves with L-[U-^{14}C]methionine, the specific radioactivity of the ethylene produced decreased rapidly with time, in accordance with the rapid decrease of the specific radioactivity of the methionine pool (*cf.* ref. 6). The specific radioactivity of the ethylene produced by *S*-adenosyl-[3,4-^{14}C]methionine-labeled leaves was low in comparison with that of the [^{14}C]methionine-labeled ones, but was far higher than that reached by the methionine pool within these leaves, indicating that the applied [^{14}C]SAM was not converted into ethylene via methionine, but that it was taken up as such and acted as an intermediate in methionine-derived ethylene production.

Since after labeling of the leaves with L-[U-^{14}C]methionine or *S*-adenosyl-[3,4-^{14}C]methionine, radioactive ethylene was produced without any lag time, exogenously applied methionine or SAM must have been directly available for the ethylene-synthesizing enzyme system. However, neither methionine nor SAM stimulated ethylene production of tobacco leaf discs when applied at 1 mM in the incubation solution. This indicates that the conversion rate of SAM to ACC is the limiting factor in ethylene biosynthesis as was shown to be the case in several other systems (3, 5, 11, 22, 24, 25). Furthermore, the increase in ethylene production 2 days after TMV infection was not accompanied by an increase in the pool size of either methionine or SAM. These results exclude the possibility suggested by Kende and Baumgarter (12) that ethylene production is regulated at the level of either the concentration or the availability of methionine or SAM.

Determination of the *in vivo* conversion of methionine to SAM in leaf discs, punched from leaves 2 days after inoculation with either water or TMV, revealed that the rate of [^{14}C]SAM production from [^{14}C]methionine was elevated in the TMV-infected leaves. This fits with our observations that, on the one hand, the SAM pool size remained constant during the hypersensitive reaction, whereas, on the other hand, SAM is converted to ACC at an increased rate.

As described earlier (6), ethylene production by tobacco leaf discs was increased severalfold upon incubation in ACC, in accordance with Cameron *et al.* (4), who demonstrated ACC to increase ethylene production in 16 plant species. This further demonstrates that ACC synthesis is the rate-limiting step in ethylene biosynthesis. As expected, the peak in ethylene production 52 h after inoculation with TMV was preceded by an increase in ACC production peaking at 40 h. Whether this stimulation of ACC production results from *de novo* synthesis of ACC synthase, as found for chemically- or mechanically-induced wound ethylene in tomatoes (25), or is caused by activation of a preexisting enzyme, was not investigated because inhibitors of protein synthesis inhibit virus multiplication and, hence, interfere with the virus-induced hypersensitive reaction and ethylene production.

In most experiments, the amount of ethylene evolved was less than expected on the basis of the ACC production. Inasmuch as accumulation of ACC during the 4-h incubation in the nitrogen atmosphere was linear, an effect of anaerobiosis as described by Bradford and Yang (4) for water-logged tomato plants is improbable. Perhaps the discrepancy between ACC and ethylene production results from an inhibition of other reactions in which ACC and/or ethylene are normally involved.

Apparently, as a result of the increase in ACC production, ACC accumulated within the leaf tissue. A maximal content (up to a 15-fold increase) was reached at 48 h after inoculation. Since ethylene production in TMV-infected tobacco leaves is increased only in the leaf cells surrounding the developing local lesions (6), the increase in ACC content is expected to be restricted to these cells only. Thus, the concentration of ACC within these cells must be excessively high. Under these conditions, the capacity of the ACC-converting enzyme system turned out to become limiting. Only around the time of lesion appearance did the capacity of the leaf discs to convert ACC into ethylene strongly increase, accompanied by a decrease in ACC-content and an increase in ethylene production during the first hours after lesions had become visible. Because the final step in methionine-derived ethylene production, the conversion of ACC into ethylene, is assumed to be mediated by a peroxidase-like enzyme (1), the increase in the ACC-converting enzyme activity is in good agreement with the increase in peroxidase activity after lesion appearance in TMV-infected tobacco leaves, observed by Van Loon and Geelen (21).

From the observations that ethylene production in noninfected leaves is increased severalfold upon incubation in ACC solutions and the increase in ACC synthesis precedes the stimulation of ethylene production in hypersensitively reacting leaves, we can conclude that virus-stimulated ethylene formation is regulated primarily at the level of ACC production. However, inasmuch as ACC accumulates near the time of lesion appearance, and the peak in ethylene evolution occurs only when the capacity for conversion of ACC into ethylene is increased, ethylene production is also limited temporarily at the level of ACC oxidation.

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