

## ETHYLENE

1864 Girardin

Trees near gas lines that had leaks became defoliated

1908 Crocker and Knight

Greenhouse blossoms near gas lamps closed

1901-13 Neljubov

Pea seedling in his laboratory germinated horizontally instead of vertically. If the lab air was filtered by passage over hot copper oxide, germination was normal. (this results in combustion:  $\text{CuO} + \text{CH}_2\text{CH}_2 \rightarrow \text{Cu} + \text{CO}_2 + \text{H}_2\text{O}$ ; identify  $\text{H}_2\text{O}$ ,  $\text{CO}_2$  chemically and quantitatively).

He examined individual components of the lighting gas to the lab, and found that ethylene and acetylene were the components that caused horizontal germination

### Ethylene had been used in agriculture before it was understood

In Puerto Rico, pineapple farmers knew to light fires near the pineapples to synchronize flowering, as did Philipino mangrove growers

Lemon growers in California stored lemons in a humid, warm environment to ripen them. When the source of heat for these storage areas changes, and the outmoded kerosene heaters were replaced, the lemons stopped ripening.

### Ethylene as an endogenous regulator

1910 Cousins

Oranges produced gases that caused bananas to ripen

1932-6 Elmer

Citrus inhibited potato sprouting. Ethylene mimics this effect

1934 Gane

Olefinic volatiles from 27 Kg of apples trapped by bromination ( $\text{Br}_2$ ) at  $-65$  degrees C for 4 weeks ...identified ethylene dibromide ( $\text{Br}_2$  is more electronegative than ethylene;  $\text{Br}^+$  adds to the double bond electron of ethylene; this leaves  $\text{Br}^-$  which adds to the same side therefore get cis only). Fractional distillation of the resultant oil yielded 0.65 mg of a compound which boiled at less than  $140$  C (ethylene dibromide boils at  $131$  C) and this product was treated aniline. Crystals formed that were identified as N,N'-diphenyldiethylenediamine.

Denny et al 1930s

Many plant species and organs produce ethylene

Because of the difficulty in assaying ethylene, and because it was unfashionable to consider ethylene a hormone or any kind of endogenous regulator, as it was a gas, little attention was paid to ethylene until the 1960s, when GC identification became available.

Ethylene is synthesized by all species of higher lower plants examined, as well as by algae, and prokaryotes including *E. coli*.

## ETHYLENE IMPLICATED IN

Breaking of potato dormancy

Geotropism

Fruit ripening (climacteric fruits, includes apple, banana, avocado, mango)

Flower induction (Bromeliads, mango) and opening

Shoot, root, ovule development (root hairs, adventitious roots)

Senescence (flower petal abscission, leaf abscission)

Wounding responses (gum production, subset of pathogen resistance genes)

Stress (leaf abscission, anaerobic stress, glucose starvation)

### Sites of synthesis

made in most tissues, in response to stress, during senescence, ripening

### Transport

Diffusion

Precursor ACC (1-aminocyclopropane 1-carboxylic acid) transported and then converted to ethylene

### Biosynthesis

From methionine, via S-adenosyl methionine and ACC

### Endogenous derivatives and related compounds

Ethylene is active form

(malonated derivative of ACC)

### Ethylene is an unusual hormone

only one active form

gaseous

not compartmentalized (diffuses freely)

only one active form

only one major conjugate (of precursor!)(malonyl-ACC)

short, defined biosynthetic pathway

inhibitors to each enzyme available

precursors available

non-destructively measured

induced by development (senescence, ripening, germination) and environment

(wounding, drought, infection, auxin)

## BIOSYNTHETIC PATHWAY

General pathway of synthesis, malonation

Some history of the elucidation of the pathway:

Most done in apple fruit

Labeled L-methionine at the 3,4 positions, converted to labeled ethylene

S-adenosylmethionine (abbreviated AdoMet or SAM) postulated as intermediate, because inhibitors of oxidative phosphorylation also inhibited ethylene synthesis from MET (ie ATP was required)

(Adams and Yang, 1977, Plant Physiol, 60, 892-896)

- $^{35}\text{S}$  methionine,  $^3\text{H}$  MET converted to MTA (5'methylthio-adenosine) and its catabolic product MTR (5'methylthioribose).
- (U- $^{14}\text{C}$ ) MET fed to apple in anaerobic conditions that block ethylene synthesis...converted to ACC, MTA (Methylthio-adenosine), MTR (methylthio-ribose)
- Return same apple slices to air, radiolabeled aminocyclopropane-1-carboxylic acid (ACC) (derived from MET) converted to  $^{14}\text{C}$  ethylene

Exogenous ACC stimulates ethylene production (Lurssen et al, 1979, Z Pflanzenphysiol. 92, 285) (ACC had been identified in 1957 from ripe cider apples, pears, and thought to be stimulator of ripening, but this wasn't followed up further until it was found to be an ethylene precursor.)

The Pathway:

Methionine  $\rightarrow$  S-adenosylmethionine (AdoMet)  $\rightarrow$  ACC  $\rightarrow$  C<sub>2</sub>H<sub>4</sub>

Malonyl-ACC formed from ACC

Characterization and cloning of enzymes involved in ethylene synthesis

### ACC synthase

Activity dramatically affected by environment

Correlation between ACC activity, ethylene biosynthesis

First step in ethylene biosynthesis

Requires pyridoxyl phosphate for activity (covalently bound to lysine at SLSK motif)

Sensitive to pyridoxyl phosphate inhibitors

AVG (K<sub>i</sub>=0.2 uM) aminoethoxyvinylglycine

AOA (K<sub>i</sub> 0.8 uM) aminoxyacetic acid

Inhibitors can be used to distinguish between the regulation of ethylene biosynthesis by ACC synthase vs ACC oxidase (formerly called "ethylene forming enzyme" EFE)

AVG, AOA inhibit ethylene synthesis and physiological responses thought to be caused by ethylene (germination, ripening, senescence, and stress responses, -including wounding, drought, waterlogging, chilling, toxic agents, infection, insect infestation). Consistent with ACC synthase as regulatory enzyme.

Pyridoxal phosphate interacts with a particular lysine on ACC synthase. Partially purified ACC synthase was reacted with radiolabeled PLP, digested with trypsin, the fragment bound to the PLP was sequenced.

Very short half life (about 30 min)

Inactivated by product "**suicide inactivation**". 1/30,000 catalytic reactions results in vinylglycine formation (by beta, gamma elimination) (ACC is formed by alpha, gamma elimination). The product binds to ACC synthase and deactivates it (may contribute to short half life of this enzyme, and consequently precise regulation of ethylene synthesis) Inactivation of ACC synthase by AdoMet. (Sato and Yang :1989 Plant Physiol 91, 1036-1039).

Suicide inactivation (using radiolabeled AdoMet) exploited to radiolabel and identify enzyme in semipurified mix.

ACC synthases present as multigene families, expression of individual genes differentially regulated by hormonal and environmental stimuli.

### **ACC synthase activity is altered by application of hormones that alter ethylene formation**

**auxin**- stimulates ACC synthase activity, ethylene evolution. Treat with AVG, block ethylene synthesis.

**ABA**- decreases ethylene evolution and ACC synthase activity increase associated with wilting of leaves (ie inhibits wilting-induced ACC activity increase), but doesn't alter ACC synthase in turgid leaves.

**cytokinins**- Stimulates water-stress-induced and auxin-induced ethylene formation and ACC synthase activity. *Doesnt* effect ethylene formation or ACC synthase activity in uninduced conditions

**ethylene**-can cause increased ethylene formation (autocatalysis) or decreased ethylene formation (autoinhibition) (response varies with tissue and developmental stage). Due in part to changes in ACC synthase activity

ACC synthase appear to typically be the regulatory step in ethylene biosynthesis:

ACC synthase **RNA accumulation** is induced by wounding and by a variety of other signals that induce ACC synthase activity.

Many ACC synthase genes cloned (typically members of 4-5 gene families). Gene-specific probes and promoter-GUS fusions used to analyze patterns of expression of each gene. ACC synthase genes are differentially regulated: induced in response to various stimuli.

e.g., Tomato: Four different homologues of ACC synthase identified; have different but overlapping patterns of accumulation in response to different stimuli.

### ACC N-malonyltransferase

studies quantifying endogenous levels of ACC and ethylene showed that ACC disappearance was not completely accounted for by ethylene synthesis. Partially purified from mungbean hypocotyls, also studied in tomato (eg Su, L-Y, Liu, Y, S-F Yang. 1985. *Phytochemistry* 24: 1141-1145)

reduces ACC that can be converted to ethylene

ACC converted to its malonyl derivative by addition of malonyl CoA

( $K_m$  0.25mM)...over 0.75mM malonyl ACC is inhibitory

ACC ( $K_m$  = 0.15mM)

AEC, non-polar amino acids (DMET, Dphenylala, Dala) and alpha-aminoisobutyric acid can also be malonated by this enzyme

### ACC oxidase

**(formerly: Ethylene Forming Enzyme)**

Ethylene formation via ACC oxidase is stereospecific

make ethyl derivatives at carbons 2 and 3 of ACC\*\*\*\*\*

ACC oxidase preferentially converts one particular stereoisomer of ACC to ethylene.

Other enzymatic and non-enzymatic reactions in cell free extracts also cause ethylene formation; but these are not stereospecific.

Studies of ACC oxidase must confirm the reaction being assayed is stereospecific.

Studies of the enzyme were very difficult because of its perceived instability. The enzyme couldn't be purified.

### Subcellular site of synthesis:

ACC oxidase is **cytosolic**, however some sticks to vacuolar membranes; in the past, the conditions in which the soluble enzyme was isolated deactivated it.

### Cloning of ACC oxidase

The gene for ACC oxidase could not be cloned using an antibody to the purified protein, because the protein could not be purified. Genetic approach resulted in cloning.

Davies and Grierson. 1989. Identified mRNAs that are preferentially expressed both in response to wounding and during ripening. One of these was ACC oxidase.

Identification of cDNA clones for tomato (*Lycopersicon esculentum* Mill.) mRNAs that accumulate during fruit ripening and leaf senescence. *Planta* 179:73-80. seven cDNAs

appeared in both processes. 1992. The cloned cDNAs were transformed into tomato so that they expressed the antisense RNA under control of the 35S CaMV constitutive promoter; one such transgene inhibited ethylene formation.

This putative ACC oxidase cDNA was introduced in a sense orientation into yeast; the yeast had ACC oxidase with correct stereospecificity.  
(cDNA sequence has homology to a hydroxylase, supporting the biochemical mechanism originally proposed by Yang and Hoffman in which n-hydroxyl-ACC was an intermediate)

Enzyme can be purified much more efficiently now, using the procedures developed for flavonone 3-hydroxylase (under N<sub>2</sub>, in presence of Fe<sup>++</sup> and ascorbate).

Enzyme is 40,000 D, inhibited by Co<sup>++</sup>, Z<sup>++</sup>, CO<sub>2</sub>, n-propylgallate.

Gene has been cloned from many species (zucchini, carnation, Arabidopsis, avocado).

ACC oxidase may sometimes be rate limiting in ethylene formation.:

1. GA decreases ACC oxidase and inhibits cell division in cultured cells
2. Tomato cells + elicitor at log phase of growth: ACC plentiful, ACC oxidase level regulates ethylene synthesis. Stationary phase cells: ACC level low, ACC synthetase regulates ethylene synthesis.

Tomatoes have at least 3 ACC oxidase genes, which are differentially regulated.

### **C≡N metabolism**

HCN is made when ethylene is formed, does this cause respiratory problems in vivo?

The level of CN generated during ethylene formation was calculated to be sufficient to inhibit the cytochrome **a/a3** respiration.

Beta-cyanoalanine synthase detoxifies cyanide. The CN released on ethylene formation is converted into beta cyanoalanine, or asparagine. Present in all species of plants examined, present in most tissues has a role as a protective mechanism in plants that accumulated cyanogenic glycosides.

### **Recycling**

The sulfur from the 5'methylthioadenosine generated when ACC is made from SAM is recycled back to form SAM. ("Salvage pathway")