Abundant Oxygenates in the Atmosphere: A Biochemical Perspective

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Contents

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1. Introduction

There is now compelling evidence that volatile organic compounds (VOCs) released from the biosphere profoundly affect the chemical composition, oxidative processes, and aerosol content of the atmosphere (recently reviewed in refs 1−3). Although much of the past work on biogenic VOCs has focused on the hydrocarbon components, such as methane released from anaerobic bacteria, and isoprene and monoterpenes released from green plants, there is growing interest in a small group of oxygenated VOCs (oVOCs) that have been measured in surprising abundance throughout the remote troposphere and stratosphere.[4−6] These oVOCs include three compounds, methanol, acetaldehyde, and acetone, referred to here as C1−C3 oVOCs. Recent estimates suggest that biogenic sources of C1−C3 oVOCs are relatively large: 38−105 Tg of C yr^{-1} (methanol), 44−88 Tg C yr^{-1} (acetaldehyde), and >25−59 Tg C yr^{-1} (acetone).[4,5,7−9] While these quantities are less than annual emissions of other biogenic VOCs, such as methane and isoprene (each in the range of 175−500 Tg C yr^{-1}),[10,11] Singh et al.[5] have concluded that "Sources of acetaldehyde, acetone and methanol alone (∼200 Tg yr^{-1}) are estimated to be more than double the anthropogenic emissions of NMHCs (∼100 Tg yr^{-1}." NMHCs refer to non-methane hydrocarbons. Larsen et al.[12] have analyzed airborne acetaldehyde, acetone, and other carbonyl oVOCs in semiremote to semiurban sites in Europe and concluded that the background levels of these compounds are predominantly of biogenic origin. In addition, these three C1−C3 oxygenated VOCs are considered to be important in atmospheric chemistry. Their impacts on tropospheric and stratospheric processes are reviewed elsewhere,[4,7,8,13] including other articles in this issue.

Given this background, atmospheric scientists are interested in the biological processes that give rise

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to the C1−C3 oVOCs. This is more than just an academic interest, as atmospheric modelers are engaged in attempts to predict the role of the biosphere in the future behavior of the atmospheric chemistry system.[2,14−16] In addition to concerns about direct impacts of biogenic volatiles on the atmosphere, there is considerable effort underway to understand and predict indirect anthropogenic effects on biogenic VOC formation. For example, the potential impacts of atmospheric CO_2 increase on the biosphere include changes in biosphere−atmosphere exchange of biogenic VOCs.[14] Recently, somewhat surprising results were obtained on the effect of increased CO_2 on the release of the reactive VOC isoprene from a model forest ecosystem by Rosenstiel et al.[17] In this work, isoprene formation was significantly decreased by doubling or tripling ambient CO_2 concentration, and this effect was traced to an alteration of the carbon flow in leaf metabolism (away from isoprene formation in this case). Kreuzwieser et al.[18] have also described the effects of elevated CO_2 on emissions of acetaldehyde and acetone from Mediterranean oak (*Quercus*) species, where in one species (*Q. ilex*) emissions of both of these oVOCs were enhanced in the autumn but not in the summer. It will be of interest to determine if changes in atmospheric [CO_2] will increase or decrease the background emissions of other biogenic VOCs, including the C1−C3 oVOCs. Information of this type will help drive future modeling efforts on the biosphere−atmosphere exchange of important VOCs.

This paper attempts to provide a biochemical perspective on the processes that give rise to large emissions of C1−C3 oVOCs to the atmosphere. It draws upon several previous reviews on biological sources of oVOCs,[10,19−24] including two recent reviews on atmospheric sources of methanol[7,8] that attempt to reconcile the global atmospheric budget of this simple alcohol. These latter reviews also contain detailed information on potential sources of methanol that are not considered here, such biomass burning and processes in the oceans that may be sources or sinks of methanol. A review describing the biogenesis of acetaldehyde in plant roots was also especially useful.[24]

It should also be noted that there are indications that decaying vegetation could be a considerable source of C1−C3 oVOCs. For example, Lindinger and co-workers examined the volatiles released by decaying and dried leaves[25] and determined that there were substantial releases of the C1−C3 oVOCs discussed here. Global sources of 6−8 Tg (3.7−5 Tg C) of acetone and 18−40 Tg (6.8−15 Tg C) of methanol from decaying leaves were estimated; acetaldehyde was also released in substantial amounts. It is proposed that such volatiles could arise by Maillard-type reactions, which occur in natural materials by the reaction of reducing sugars with amino acids and other amines.[26−29] Methanol can also arise from these complex reactions in plant materials that are heated.[30] These decomposition reactions are not further discussed here.

This paper aims to update the information on biogenic methanol and acetaldehyde, to provide an overview of the formation of acetone in the biosphere, and to indicate the gaps in our knowledge concerning the biochemical and physiological processes that control C1−C3 oVOCs release to the atmosphere.

2. Methanol

Methanol has been described as the simplest natural product derived from plants,[20] and it is likely that pectin demethylation in plant cell walls is the major source of most of the methanol in the atmosphere.[7] However, as recent reviews on the atmospheric methanol budget have emphasized,[7,8] there are likely to be other biological processes that give rise to this alcohol. For example, field experiments on oVOC fluxes from western United States pine forests have detected substantial emissions of methanol attributed to processes occurring in soil and/or forest litter;[31,32] these processes remain to be characterized. The sections below review aspects of methanol formation that are more certain—biogenesis in plant cell walls—and consider how and why this alcohol escapes plant metabolism and enters the atmosphere.

2.1. Pectin Biosynthesis as a Source of Atmospheric Methanol

The primary cell walls of most land plants contain abundant amounts of the polysaccharide pectin. As reviewed by Willats and co-workers,[33,34] the pectin matrix contributes to the physical strength and various physiological aspects of the plant cell wall. Pectins are relatively complex polysaccharide structures, including three domains enriched in galacturonic acid (GalA). One of the domains, termed homogalacturonic acid (HGalA), is a linear homopolymer of 100−200 GalA residues linked by α(1−4) glycosidic bonds. Of interest here is that during its biosynthesis the HGalA region is largely methyl esterified, is exported to the growing cells walls in this form, and is then processed by pectin methylesterases (PMEs).[35] This process and the PME reaction are diagrammed in Figure 1.

As cell wall expansion occurs—necessary for cell division—the primary cell wall is formed in a very complex process,[36,37] including the secretion of the highly methylated pectins into the cell wall (Figure 1). Subsequently, as the cellulose and hemicellulose polymers of the new cell wall are assembled, there are important noncovalent associations that hold them together. One of these associations results from the PME reaction on methylated pectin, which can produce blocks of free carboxylic acid groups in the HGalA regions. The pK_as of these carboxylic acids have been measured and are in the range of 2.9,[38] which means that at the physiological pH of the cell wall (ranging around pH 6[39]) they will be ionized and capable of Ca^{2+} binding. Thus, blocks of carboxylate side chains can chelate calcium ions (Ca^{2+}), forming calcium−pectate gels that are thought to rigidify and strengthen the cell wall (reviewed in ref 40). It is estimated that the ability to form calcium−pectate gels occurs when the degree of methylation (DM) falls below about 40%, and then blocks of 14−20 consecu-

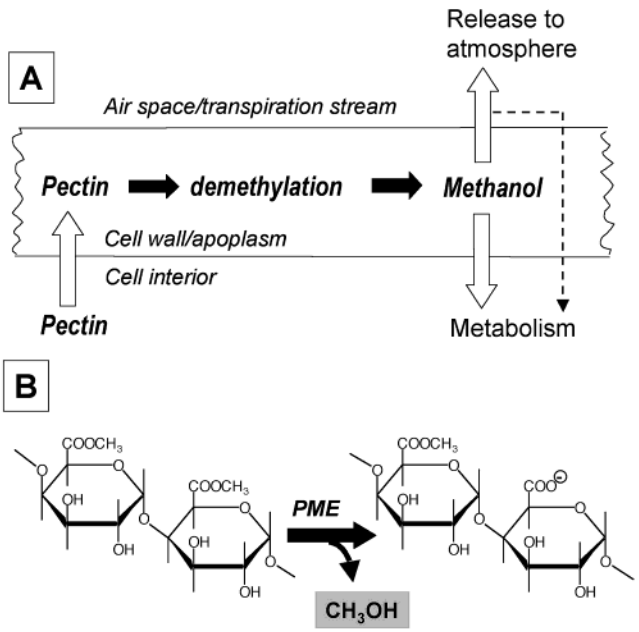

Figure 1. Scheme for the formation of methanol in the cell walls of plants (A), and the pectin methyl esterase reaction (B). As indicated in A, following its formation in the cell wall, as a part of the apoplasm in contact with the air space and transpiration stream, methanol can be released from the plant; an unknown fraction of methanol produced in the cell wall can partition back into the cell to be metabolized.

tive free carboxylate groups plus Ca^{2+} ions can assemble into structures resembling "egg boxes," as reviewed by Ralet et al.[38] It is assumed that during its biosynthesis the DM of pectin deposited in the cell wall is >70%, so it is likely that a large fraction of the methyl esterified groups are hydrolyzed during cell wall formation and refashioning. This is relevant to the biogenesis of methanol.

The PME reaction, Figure 1B, is a simple esterase reaction that forms a galacturonic acid side chain on the HGalA backbone and methanol as the other product. From the recently described crystal structure of a PME isoenzyme from carrot roots,[41] and in comparison to the crystal structure of a bacterial PME,[42] PMEs appear to contain a novel esterase active site. While many known esterases adopt an α/β hydrolase fold and have a Ser-His-Asp triad at the catalytic center, both the plant and microbial PMEs belong to the family of parallel β-helix proteins and have a pair of Asp residues at the active site. Ester hydrolysis in this case would occur by a general acid−base mechanism like that seen in aspartic proteases.[43] The pectin binding site in these enzymes appears as a shallow cleft lined with several aromatic residues, a pattern seen in other proteins that bind carbohydrate polymers. The structural features of this cleft region could explain the processivity seen with many (but not all) PMEs. Processivity allows the formation of the blocks of demethylated GalA side chains described above.

An interesting feature of plant PMEs is that they occur as multigene isoenzymes. As an example, Micheli[40] reports that the genome project for the plant *Arabidopsis* has identified 67 different PME-related genes, suggesting important roles for this family of enzymes. So many PME genes may reflect expression of particular PMEs in certain tissues and the fact that PMEs have roles in both (i) cell wall stiffening during growth and (ii) loosening during fruit ripening or leaf senescence and abscission.[33] For pectin demethylation that occurs during fruit ripening, a different type of PME enzyme, acting randomly rather than in blocks, assists in loosening of the cell wall pectin structure; Rose and Bennett[37] review the complex processes that occur in the primary cell wall during fruit ripening.

What is the evidence that pectin demethylation is the major source of plant (and atmospheric) methanol? This issue has recently been reviewed by Galbally and Kirstine[7] and includes circumstantial evidence from a variety of laboratory studies that relate the demethylation of pectin to liberation of methanol from plants. A pertinent example is the work of Nemecek-Marshall et al.,[44] who measured emission fluxes of methanol from developing leaves of bean, soybean, and cottonwood leaves. They showed that methanol emissions are high in young, rapidly expanding leaves and decrease in mature leaves. In leaves of cottonwood trees (*Populus deltoides*), this decrease was almost 2 orders of magnitude. In recent work from field experiments,[45] eddy correlation measurements of methanol fluxes from an aspen−oak forest canopy revealed very large fluxes during spring bud break and then a decline as leaves expanded. Each of these laboratory and field observations suggest that methanol release from leaves is correlated with the pectin demethylation that occurs during rapid cell wall growth during bud break and expansion of young leaves.

Direct evidence for the link between PME activity and methanol formation, albeit in tomato fruit, was recently obtained. As mentioned above, during fruit ripening the loosening of the cell wall occurs, and PMEs and other enzymes play an essential role.[37] Frenkel et al.[46] examined the role of PME activity in ripening tomato fruit in transgenic plants that expressed an antisense PME gene. In these fruits the levels of PME activity were reduced substantially throughout fruit ripening, and the levels of methanol were 5−7 times lower in immature fruit and did not rise as seen in wild-type fruit.

2.2. How and Why Does Methanol Escape from Plants?

2.2.1. Gas Exchange

In plant cell walls, PMEs are secreted into and function within the extracellular apoplast, which is usually defined as all compartments outside of the cellular plasma membrane. These compartments include the interfibrillar and intermicellar space of the cell walls, the xylem, and the intercellular gas- and water-filled spaces.[47] Thus PMEs are examples of extracellular enzymes that operate under the conditions of the cell wall apoplasm (e.g. pH, cations, and substrate availability; ref 40), and the methanol released by PME action is in direct contact with the interface between the aqueous apoplasm and the air space. As illustrated in Figure 1A, this can lead to

the partitioning of a volatile like methanol into the air space of the leaf. Such partitioning is controlled by methanol's Henry's law constant (0.461 Pa m^3 mol^{-1} at 25 °C).[48] In addition, some fraction of methanol produced also can diffuse into the cell for metabolism (Figure 1A); metabolism of methanol is discussed below. Given methanol's volatility and miscibility with water, this alcohol can thus exit plants with the transpiration stream through stomatal pores in leaves. In the work of Nemecek-Marshall et al.,[44] there was a significant correlation between stomatal conductance rate and methanol emission, except when leaves were first sampled in the morning, where a transient methanol "burst" was seen. This burst could be a result of build up of free methanol during the night when stomata are primarily closed and then its release when stomata are induced to open in the light. More recently, Harley et al.[49] have repeated this work and suggested that such "morning bursts" are consistent with the above mechanism as well as low stomatal conductance in the dark. They suggest, on the basis of the work of Niinemets and Reichstein,[48] that the extent to which stomata control methanol emissions over the course of a day is more complicated, being controlled by both light and temperature effects, but is fundamentally dependent on the rate of change of stomatal conductance versus the rate of gas−liquid phase equilibration of methanol within the leaf. A similar early morning release of methanol has been observed in a field experiment with alfalfa, with maximal fluxes of methanol from undisturbed plants occurring at 0800 local time.[50] In this case, however, methanol emission was attributed to the evaporation of dew, which is formed on the plants at night. These observations attest to the need to assign emission factors for methanol releases due to physical effects of leaf stomata on gas exchange, as well as surface evaporation of an oVOC. Such considerations are very important for those attempting to model methanol emissions from regional and global vegetation (Harley et al.[49]).

The controls on the emission of plant VOCs through stomata have recently been modeled by Niinemets and Reichstein.[48] They conclude that for more water-soluble VOCs, such as alcohols with H constants in the range from 10^{-2} to 10^{1} Pa m^3 mol^{-1}, that release from the leaf is controlled by stomatal conductance. Furthermore, their model showed (i) an excellent fit to the data for methanol emissions from bean leaves when stomata were induced to close with the hormome abscicic acid (Nemecek-Marshall et al.[44]) and (ii) could explain the bursts of methanol mentioned above.

2.2.2. Wounding

Methanol is also released from leaves and stems following wounding events. This has been observed in numerous laboratory and field experiments.[50−56] The simplest explanation for this release is that the aqueous phase methanol contained in the plant's transpiration stream is exposed to air at the wound surfaces, and this would promote evaporation and release of methanol. Longer term release of methanol from excised vegetation, such as with forage crops that are cut and allowed to dry[53−56] might be the result of activation of PME activity as the vegetation dries and decays, although this has not been directly demonstrated. The first seasonal study of methanol release from a forest canopy in the autumn during leaf senescence showed that there are small but significant releases of methanol all the way to leaf drop.[45] It is possible that during senescence, when many of the leaf's components are mobilized for transport to roots, that additional pectin demethylation occurs.

2.2.3. Metabolism

The metabolism of methanol in plants is not well characterized. As reviewed elsewhere,[20,57] it is known that ^{14}C-labeled methanol is readily assimilated by plant cells with formation of $^{14}CO_2$, and it is likely that this oxidation occurs in the following sequence:

$$\text{methanol} \rightarrow \text{formaldehyde} \rightarrow \text{formate} \rightarrow CO_2$$

However, the enzymatic machinery and subcellular location of these steps are somewhat uncertain. For example, as reviewed by Kreuzwieser et al.,[24] it is not known whether methanol oxidation occurs via a methanol oxidase, as occurs in methanol-assimilating yeasts, or by a catalase-mediated mechanism. Both of these mechanisms, in a subcellular context, would mean that methanol oxidation would be initiated in peroxisomes, oxidative organelles in higher organisms, and the subsequent oxidation of the formaldehyde and formic acid products would occur in other compartments. The control of the metabolic flow of methanol-derived carbon between these compartments is unknown.[57] However, the enzymes that mediate formaldehyde and formate oxidation in plants, formaldehyde dehydrogenase[58,59] and formate dehydrogenase,[60,61] are well characterized.

3. Acetaldehyde

Acetaldehyde is a simple volatile aldehyde that can be emitted from leaves as a result of a fermentative metabolism in anaerobic roots[24,62−65] and from other metabolic processes. The root pathway is very important in plants that are adapted to periodic flooding or to submergence of their roots in water.[66] It has also recently been discovered that acetaldehyde can arise in leaves directly from metabolism that occurs during light−dark transitions.[67,68] These two pathways are discussed separately below, along with a brief consideration of the origins of acetaldehyde in vegetation during senescence and following leaf wounding.

It should also be mentioned that recent field experiments have shown that forest soil and/or litter can be a significant acetaldehyde source[32] and that in a remote Amazonian forest during different seasons deposition of acetaldehyde often dominates emissions.[69] These findings add to the complexity of our understanding of biosphere−atmosphere exchange of acetaldehyde.

3.1. Root Metabolism as a Source of Atmospheric Acetaldehyde

On a biochemical level, it has been known for decades that many plants respond to root flooding by switching to a fermentative metabolism.[70] During root flooding episodes the soil air spaces fill with water, leading to an anoxic environment. Roots of many plants respond to this stress by utilization of the classic ethanolic fermentation pathway that is well known in yeasts:

$$\text{glucose} \longrightarrow \text{pyruvate} \xrightarrow{\text{PDC}} \text{acetaldehyde} \xrightarrow{\text{ADH}} \text{ethanol (plant roots)}$$

This pathway serves to provide energy (via glycolysis) by the conversion of glucose to pyruvate and utilizes the formation of ethanol to regenerate the essential glycolytic cofactor NAD^+ from NADH.[71] Pyruvate is converted first to acetaldehyde by reaction with pyruvate decarboxylase (PDC), and acetaldehyde is reduced to ethanol by action of alcohol dehydrogenase (ADH), forming the required NADH. In plants adapted to life with their roots in water (e.g. rice grown in paddies), the enzymatic machinery for fermentative metabolism and ethanol formation is highly induced.[72,73]

In flood-tolerant plants, the ethanol produced in this way can be delivered from roots to leaves via the xylem sap, and in leaves a large fraction can be reutilized by oxidation to provide energy.[24,63,65] This oxidative pathway appears to involve a leaf ADH isoenzyme that operates in the oxidative direction, producing acetaldehyde, which can be converted to acetate by an aldehyde dehydrogenase (AlDH):

$$\text{ethanol} \xrightarrow{\text{ADH}} \text{acetaldehyde} \xrightarrow{\text{AlDH}} \text{acetate} \rightarrow \text{oxidation (plant leaves)}$$

This sequence was established in poplar leaves by using ^{14}C-labeling experiments and metabolic inhibitors of leaf enzymes involved in ethanol oxidation.[63] A fraction of the ethanol delivered in this way, in the range of 0.08−0.16%, was found to be emitted from leaves. An even larger fraction of ethanol delivered, 0.31−0.42%, was emitted as acetaldehyde. The explanation for acetaldehyde emission is simply that it represents a leak of a highly volatile intermediate; acetaldehyde has a very low boiling point (bp 21°C) and a relatively high Henry's law constant of 7.0 Pa m^3 mol^{-1}.[48]

In further experiments confirming this model, Kreuzwieser et al.[64,65] observed that flooding poplar roots led to large increases (as much as 40-fold) in acetaldehyde emission from leaves and that inhibition of leaf AlDH by feeding the compound disulfiram also enhanced acetaldehyde emissions. These experiments also indicated that while stomatal conductance does not directly influence acetaldehyde emission rate, it does affect transpiration rates and thus controls delivery of ethanol from roots to leaves.

It is not known how widespread this mechanism for ethanol and acetaldehyde formation is in different green plants. It has been shown that leaves of several woody plants, but not herbaceous species, have constitutive levels of alcohol dehydrogenase (ADH) in leaves and thus are poised to oxidize ethanol arising in roots by the mechanism shown above.[74,75] Kelsey[76] surveyed a large number of conifers and found that the ability to form ethanol in stems was widespread, especially in the smallest and slowest growing stems; these observations suggest that the vascular cambium of conifers might be subject to periods of hypoxia when transpiration rates are low, leading to the metabolic formation of ethanol. More experimental work is needed to assess the significance of this type of mechanism in diverse types of plants that are subject to periodic root flooding (i.e. rainforest species) or hypoxia in internal tissues during periods of drought stress.[77] In this regard it is notable that Kesselmeier and co-workers did not see substantial fluxes of ethanol or acetaldehyde from rainforest canopies in Amazonia at the onset of the rainy season,[78] but this may have only indicated that the subsoil did not become saturated with water. In subsequent laboratory studies with several Amazonian tree species, the trees showed flooding-induced formation of ethanol as in the plants mentioned above (J. Kesselmeier, personal communication).

3.2. Sunflecks and Leaf Pyruvic Acid as a Source of Atmospheric Acetaldehyde

A second mechanism for transient acetaldehyde release from leaves has been recently discovered. Using tree leaves during light−dark transitions, Holzinger et al.[67] and Karl[79] both observed transient releases of a VOC at m/z 45 by proton-transfer-reaction mass spectrometry (PTR-MS); they attributed this VOC to be acetaldehyde. Subsequently, Karl et al.[68] investigated these transient releases in more detail and used a DNPH-cartridge method to verify that the VOC detected by PTR-MS at m/z 45 was indeed acetaldehyde. By using metabolic inhibitors and $^{13}CO_2$ labeling, they proposed that acetaldehyde arises during these light−dark transitions by a pyruvic acid overflow mechanism that is illustrated in Figure 2A.

The essential features of this overflow mechanism are the following: (i) During a light−dark transition, cytosolic pyruvic acid levels in leaves rise rapidly. (ii) The cytosolic enzyme pyruvate decarboxylase (PDC) acts as a sensor or safety valve, converting excess pyruvate to acetaldehyde. (iii) Acetaldehyde thus produced can either be oxidized to acetate for oxidative assimilation or be partitioned to the leaf air space and released to the atmosphere via stomatal pores. This model is based in part upon the "acidosis model" in which Harry and Kimmerer[62] proposed that cytosolic pyruvic acid can be converted to the pH neutral species acetaldehyde and CO_2. Such a conversion would help to prevent acidification of the cytosol when the glycolytic production of pyruvic acid exceeds its oxidation in mitochondria. A similar scheme for the aerobic formation of acetaldehyde (and ethanol) in plant pollen has been proposed to function in the control of pyruvic acid levels.[80] In this type of mechanism, as with the root-derived acetaldehyde, we can assume that the release of acetaldehyde would be a result of a leak of a volatile intermediate.

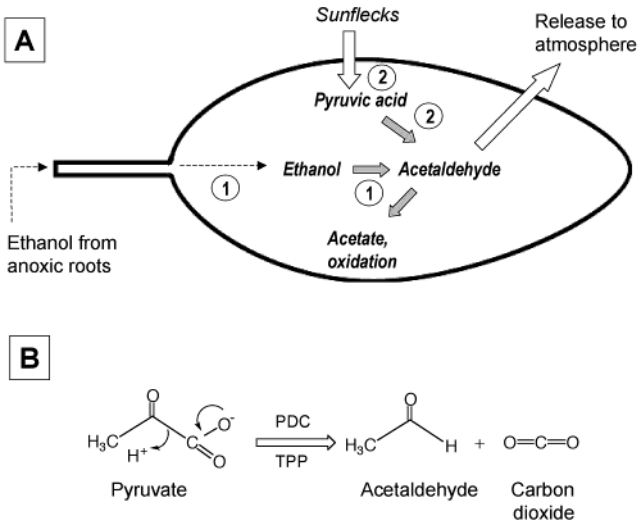

Figure 2. Scheme for the formation of acetaldehyde in leaves and its release to the atmosphere. As described in the text, acetaldehyde can be derived from both oxidation of ethanol derived in roots (path 1) and as a result of sunflecks that increase cytosolic pyruvic acid (path 2). In each case, acetaldehyde is metabolized to acetate for further oxidation, but some of it is emitted from leaves due to its high volatility.

In the pyruvate overflow model, the enzyme PDC is directly responsible for acetaldehyde formation in leaves. The PDC reaction mechanism is shown in Figure 2B. The structure and mechanism of PDC are best known from studies of the yeast enzyme,[81,82] and it appears that the plant PDCs are very similar.[83] The reaction is a simple decarboxylation of an α-keto acid (pyruvic acid), but it is notable that the enzyme requires a complex cofactor, thiamine diphosphate (TPP). TPP is unique in that its thiazolium ring can form an ylide (a dipolar carbanion), which can attack the α-carbonyl carbon of pyruvate to faciliate decarboxylation and acetaldehyde formation.[84] Studies of PDCs from a variety of yeasts indicate that it is a regulatory enzyme, subject to substrate activation.[81,82] A similar substrate activation phenomenon occurs in plant PDCs.[85,86] Substrate activation is seen in the sigmoidal dependence of the initial rate on pyruvate concentration and has been modeled as the binding of pyruvate to a regulatory site of the inactive enzyme, triggering a conversion to the active conformation.[87] This type of behavior is also seen in vitro by a lag in the initial rate of reaction. The enzyme from rice also shows a very steep response to pH, with the activity and apparent K_m for pyruvate greatly enhanced between pH 7 and 6.[85] This is consistent with a model where PDC acts as a sensor or pH stat, as discussed above, serving to convert excess pyruvic acid to acetaldehyde.

These transient releases of acetaldehyde emission from leaves mentioned above begin within a few minutes of darkening, peak rapidly and then subside.[67,68] The simplest interpretation—not yet proven—is that such bursts are a direct reflection of leaf cytosolic pools of pyruvic acid that rise and then fall due to PDC activity. Such a rapid response might have physiological relevance in relation to sunflecks.[88] Sunflecks are the variations in photosynthetically active radiation that illuminate leaves in plant canopies. Shade-adapted leaves, in the lower part of plant canopies, typically acquire 30−70% of daily carbon gain during sunflecks. It is possible that rapid changes (on the time scale of minutes) in illumination could trigger small, but frequent, releases of acetaldehyde by the pyruvate overflow mechanism, but this remains to be tested in field experiments.

As described above for methanol, releases of acetaldehyde have been noted in studies of VOC emissions from wounded vegetation and forage crops that have been cut and allowed to dry.[50−56] The mechanism(s) of these releases in unknown but may involve a combination of (i) release of small amounts of acetaldehyde present in the transpiration stream or, more likely, (ii) formation of acetaldehyde as part of a switch to a glycolytic type of metabolism in the wounded tissue. The latter mechanism would use the pyruvate overflow enzymatic machinery present in leaves and stems, and the acetaldehyde formed may again represent a leak of a volatile intermediate. Further research will be needed to investigate these or other possibilities.

4. Acetone

Acetone is the simplest ketone and can arise in biological systems by numerous pathways. Well-characterized mechanisms include those in cyanogenic plants, anaerobic bacteria, and higher animals. It is uncertain which of these pathways, or some as yet uncharacterized pathway, is the most important source of atmospheric acetone. Each of these pathways will be briefly discussed here as a guide to the types of biochemical processes that can produce acetone.

4.1. Cyanogenic Formation of Acetone

The atmospheric science community was slow to learn of the biological literature on cyanogenic plants, including those cyanogenic plants that produce acetone (Fall et al.[89]). Cyanogenesis, that is, the formation and release of hydrogen cyanide (HCN) to deter herbivores, has been known for decades and occurs in thousands of plant species.[90−92] Vetter[92] estimates that 11% of plant species could be cyanogenic. Figure 3A illustrates how acetone and HCN arise in crops such as cassava, an important food source in tropical Africa, and in common white clover. Cyanogenic varieties of these plants convert the amino acid valine into the cyanogenic glycoside linamarin, which accumulates in the cell vacuole. Then, if the cell is ruptured by feeding herbivores, linamarin can come into contact with a degradative β-glucosidase that is present in the cell wall. Reaction leads to the formation of acetone cyanohydrin, which is a substrate for another cell wall enzyme, hydroxynitrile lyase (HNL). The action of HNL results in formation of acetone and HCN. Volatile HCN acts as a deterrent to feeding animals and insects. Acetone is simply a volatile byproduct. It is noteworthy that by similar pathways a variety of other cyanogenic glycosides, all derived from amino acids, give rise to a host of carbonyl

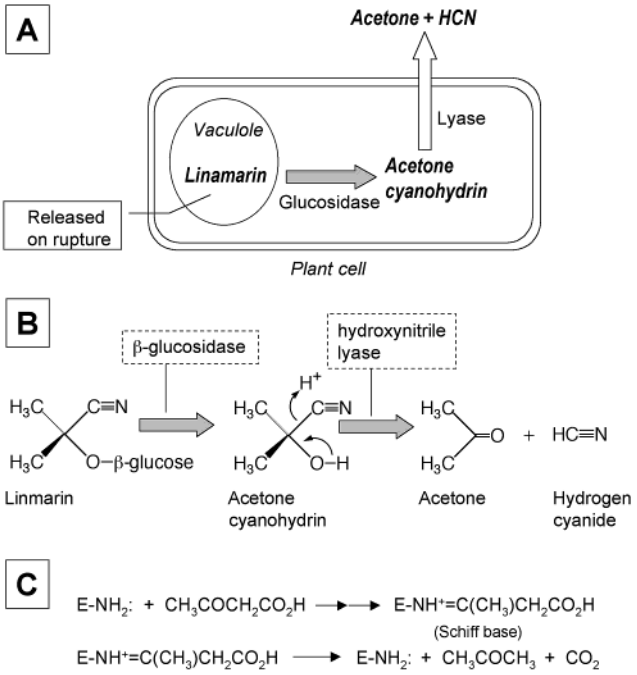

Figure 3. Known enzymatic pathways for the formation of acetone in biological systems. Scheme A illustrates the cyanogenic pathway in a damaged plant cell, which leads to the formation of acetone and HCN; scheme B shows the enzymatic reactions involved in cyanogenesis from linamarin, a cyanogenic glycoside. Scheme C shows the enzymatic mechanism of acetoacetate decarboxylase, an enzyme present in certain soil bacteria; in this reaction E-NH_2: represents the basic form of a lysine side chain in the enzyme active site that results in Schiff base formation upon binding of acetoacetic acid. The Schiff base facilitates decarboxylation of the β-keto acid.

products, such as butanone, methacrolein, and benzaldehyde. Some plants such as common white clover give rise to both acetone and butanone by this type of mechanism.[93]

The mechanisms of the HNLs that cleave acetone cyanohydrin and related cyanohydrins have been of interest, since these enzymes have potential uses in the bioorganic synthesis of cyanohydrins. Gregory[94] has reviewed the properties of the HNLs, which appear to have evolved into four different classes by both convergent and divergent evolution. The detailed mechanisms for HNLs are still under investigation, but recent crystal structures of several of these enzymes are now determined, including the structure of the cassava acetone cyanohydrin HNL.[95] In the latter case, general acid−base catalysis is used, as indicated in Figure 3B.

Could linamarin breakdown be a significant source of atmospheric acetone? Many major plant families contain linamarin-producing species, including Compositae (sunflower family, >25 000 species), Euphorbiaceae (spurge family, >8000 species), Linaceae (flax family, >150 species), Papaveraceae (poppy family, >240 species), and Leguminosae (pea family, >18 000 species) (reviewed by Vetter[92]). Notably, because the standard method of chemotaxonomic screening for cyanogenic species only utilizes detection of HCN, and not the carbonyl product, undoubtedly more linamarin-producing plants may exist. The use of a new GC method for the sensitive, simultaneous analysis of HCN and acetone[96] may assist in identification of additional cyanogenic, acetone-emitting plants.

The levels of cyanogenic glycosides in plant tissues vary over a large range, under control by genetic, developmental, and nutritional factors. In general, the youngest leaves have the highest level of cyanogenic glycosides (reviewed in Gleadow and Woodrow[97]), consistent with the role for these compounds in protecting the most tender parts of plants. Several investigations with cyanogenic varieties of clover have shown that the leaves and stems of these plants can contain large amounts of linamarin and lotaustralin (the precursor of butanone and HCN); for example, Stochmal and Oleszek[93] report linamarin and lotaustralin levels that yield 4−12 mg of HCN (g of dry matter)$^{-1}$ in a range of clover varieties. It is likely that this mechanism explains why Kirstine et al.[52] and de Gouw et al.[53] detected such substantial emissions of acetone and butanone from cut and drying clover plants—i.e. they were using cyanogenic varieties of clover for these experiments.

4.2. Enzymatic and Nonenzymatic Acetoacetate Decarboxylation

The biogenesis of acetone in bacteria and humans has been known for a long time.[98,99] In each case the immediate precursor of acetone is acetoacetic acid or its ionized form, acetoacetate, and as shown in Figure 3, acetone arises as a result of decarboxylation of this β-keto acid. In bacteria the reaction is enzymatic, and in humans and other animals it is nonenzymatic.

The enzyme acetoacetate decarboxylase (AD) was characterized as part of investigations of the industrial production of acetone and butanol by the anaerobic bacterium *Clostridium acetobutylicum*.[99] When this microbe is grown on glucose as carbon source, it utilizes a fermentation sequence of reactions that generates acetoacetate. The presence of AD is thought to pull the fermentative reaction sequence toward completion, and as a result acetone is one of the volatiles produced. A similar AD, but with higher affinity for acetoacetate, has been isolated and characterized from the soil bacterium *Bacillus polymyxa*;[100] in this case the metabolic role for the enzyme is less certain, as it is most highly induced by aerobic growth on starch with ammonium as nitrogen source. It is likely that this type of carbohydrate metabolism is responsible for the production of acetone in soils and sediments. It is possible that a substantial fraction of acetone seen in forest canopies[31,32] actually arises from the soil by such microbial mechanisms. The finding that acetone can also serve as a carbon source for the growth of a very diverse groups of aerobic and anaerobic bacteria in soils, sediments, and waters[101] also suggests that microbial processes could be very substantial sources of atmospheric acetone.

The mechanism of the *Clostridium* AD has been studied in detail and involves the decarboxylation of the β-keto acid via a Schiff base intermediate to an active site Lys115 residue (E-NH_2:),[102,103] as shown in Figure 3C. The pK_a of the ϵ-amino group of this Lys115 is very unusual; it is estimated to be about

6, which is 4.5 pK_a units lower than free lysine in solution. This pK_a shift is attributed to suppression of its protonation by an adjacent Lys116 side chain.[103]

The nonenzymatic decarboxylation of acetoacetic acid can also be a very significant source of acetone formation. It is this reaction that is responsible for the acetone that is present in human breath. This decarboxylation reaction occurs at physiological pH, assisted by enolization of the β-keto acid.[102] During periods of increased fatty acid oxidation, as occurs during fasting or in diabetic patients, serum levels of acetoacetate rise and there is a parallel increase in acetone levels.[98,104] Acetone then partitions to the air spaces in lungs and it released with exhaled breath. The levels of breath acetone are generally small (usually in the low end of the range of 1.2−1880 ppbv[105]), so it is unlikely that acetone from animal metabolism is a significant source of atmospheric acetone.

Is there a direct link between acetoacetate and acetone formation in plants? MacDonald and Fall[106] surveyed a few coniferous species and noted that (i) acetone was formed in buds but much less so in needles and (ii) the formation was seasonal. Preliminary analysis of bud extracts suggested the presence of acetoacetate, providing a possible link to acetone foramtion by the enzymatic or nonenzymatic pathways discussed above. However, more recent attempts to repeat these findings were unsuccessful.[107] Perhaps of greater interest are the recent findings that acetone formation is light dependent in various conifers[108,109] and that in the light acetone released from Gray pine needles is rapidly labeled, in all three carbons with $^{13}CO_2$, with up to 50% labeling.[107] Similar, but less extensive, labeling was observed in Scots pine needles.[109,110] These latter results suggest that acetone formation can occur from recently fixed photosynthetic carbon, and it will be of interest to determine if the mechanism of acetone formation in pines is related to the pathways described above. Curtis[107] demonstrated in various conifers that acetone formation is not related to the cyanogenesis pathway described above.

5. Conclusions

In recent years there has been considerable progress in understanding the biogenesis of three major C1−C3 oVOCs that are present in the troposphere, but many uncertainties remain. Here, we have outlined biochemical machinery that certainly play important roles in the formation of methanol, acetaldehyde, and acetone in plants and certain microbes. However, at present it is unlikely that all the significant sources and mechanisms of formation of these C1−C3 oVOCs have been discovered. What conclusions can currently be drawn about the likely biogenic sources of C1−C3 oVOCs, and what are the gaps in our knowledge concerning the processes giving rise to them?

5.1. Methanol

There is now a large body of evidence to link the major source of atmospheric methanol to the Earth's vegetation. It seems likely that a major fraction of methanol emissions arise in the cell walls of plants as a byproduct of the pectin methylesterase reaction. Since this enzymatic process occurs in the apoplast, methanol is in direct contact with leaf air spaces and is released from plants primarily by transport in the transpiration stream with exit from leaf surfaces via stomatal pores. It remains to be established if methanol emissions from wounded plants (including cut crops), as well as forest soils and litter are important sources of the alcohol and whether most of these sources derive from the large residual methylated pectin in mature, senescing, and decaying vegetation.

5.2. Acetaldehyde

Acetaldehyde is a primary product of a safety valve reaction—catalyzed by pyruvate decarboxylases—both in roots when they become anoxic due to flooding and in leaves following light to dark transitions. The latter may be very important in forest canopies as a result of sunflecks. Acetaldehyde release is likely to represent a leak of a highly volatile metabolic intermediate before it can be assimilated in the leaf aldehyde dehydrogenase reaction. Since acetaldehyde is a common metabolite of microbial fermentation (see ref 80), its formation in soils rich in organic material might explain observations of acetaldehyde emissions from forest floors (e.g. ref 32).

5.3. Acetone

Acetone can arise by several pathways in biological systems. In plants it is a product of a wounding reaction in cyanogenic species of plants, being formed as a product of a cyanohydrin lyase reaction. In addition, in noncyanogenic plants, such as pines, acetone arises in both light-dependent and -independent processes; these processes may be related to the well-known decarboxylation of acetoacetate that occurs in soil microorganisms and animals, or represent uncharacterized biochemical reactions.

5.4. Modeling the Leaky Biosphere

At our present level of understanding, the atmospheric C1−C3 oVOCs are all leaky byproducts of metabolism (methanol and acetone) or volatile intermediates (acetaldehyde), rather than end products that have a specific biological function. This conclusion does not greatly assist those who wish to model regional and global C1−C3 oVOC emissions. Consider, for example, the uncertainties in modeling methanol emissions from the Earth's forests and grasslands. (i) What is the pectin content of the major vegetation groups? Our knowledge of the leaf contents of methylated versus demethylated pectins in conifers and grasses is surprisingly sparse, especially considering that these plant groups are the major vegetation in boreal forests and grasslands. (ii) What fraction of methanol produced by pectin demethylation in cell walls is metabolized or released? This is apparently unknown for any plant. (iii) Methanol is produced in roots and growing stems, as well as in growing leaves; what fraction is exuded by roots or metabolized by other tissues during its transit from

roots to leaves? This is also unknown. (iv) Methanol emissions are driven by seasonal leaf development, daily variation in light and temperature, and diurnal influences such as accumulation at night and possible evaporation from plant surfaces. How can these diverse processes be accurately modeled? (v) Methanol is also a primary carbon source for abundant methylotrophic organisms on leaves.[111] How does this biological sink affect the net ecosystem release of methanol? When one adds the uncertainties of methanol-producing processes during lignin degradation (Galbally and Kirstine[7]) and in marine phytoplankton (Heikes et al.[8]), it is currently a daunting task to accurately predict global methanol source strengths. Even greater uncertainties in the biogenesis of acetaldehyde and acetone could be listed, testifying again to the challenges lying ahead for C1−C3 oVOV modeling efforts.

5.5. Future Efforts

Despite the challenges just listed, there are reasons for optimism about future efforts to understand and model biogenic C1−C3 oVOC emissions. First, there have been many recent analytical developments that will facilitate laboratory and field measurements of these VOCs. Past efforts to measure fluxes of methanol and acetaldehyde have been hindered by analytical challenges, such as the loss of methanol in sampling devices and the difficulties in measuring atmospheric aldehydes with derivatization methods. Now, on-line chemical ionization mass spectrometry (CIMS) methods, such as PTR-MS and ion trap-CIMS technologies, are maturing, and being applied to both ground-based and aircraft measurements.[6,112,113] In addition, these instruments can be used as fast sensors for eddy correlation measurements of biogenic VOC fluxes in various ecosystems.[45,50] With the development new CIMS reagent ions, such as hydroxide ion or hydrazine,[114,115] it may be possible to improve the selectivity of detection of biogenic carbonyls such as acetaldehyde and acetone. With such tools surely there will advances in assessing C1−C3 oVOC fluxes from different vegetation and from different levels of plant canopies in response to both environmental and seasonal influences. Coupled with the ability of these devices to measure biogenic VOC fluxes in the laboratory and with use of isotopic labeling,[68,116] we can expect to make progress in further understanding the underlying biochemical processes controlling oVOC formation and emission.

Second, the increased capabilities for on-line field measurement of C1−C3 oVOCs will also be important for examining linkages between biological processes in vegetation (and perhaps soil microbes) in response to anthropogenic influences. The ability to make measurements of biogenic VOCs over periods of weeks or months has recently revealed the importance of atmospheric CO_2 levels on the emission of isoprene from poplar canopies.[17] It is likely that by studying the influence of changing climate and atmosphere on the biogenesis of oVOCs over longer time scales, we will learn much more about the physiological controls on these processes. The availability of such robust datasets, including continuous diurnal VOC emission information, may allow more detailed understanding of biochemical events in both day and night, linking oVOC formation to both plant photosynthetic and respiratory metabolism.

Third, there is now international recognition that a clear understanding of biosphere−atmosphere exchange of VOCs will involve the collaborative efforts of atmospheric scientists with biologists and biochemists, as demonstrated at a recent international conference.[117] To make meaningful field and laboratory measurements of biogenic VOC fluxes to the atmosphere, it will probably be essential to make simultaneous measurements of physiological processes, such as photosynthesis, respiration, and nitrogen cycling, and possibly biochemical factors. The information gained this way can be incorporated into models that not only consider the physical and chemical aspects of VOC exchanges from biological surfaces but also link these exchanges to the underlying physiological and biochemical processes. In the future, we should be able to construct more sophisticated biogenic VOC emission models that can be successfully applied at a broad range of scales: cellular, canopy, regional, and global, for example. It is hoped that this paper is a step in the direction of raising the awareness of the biochemical complexity of C1−C3 oVOC formation in cells, a complexity that should be considered as these new biogenic VOC emission models evolve.

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