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Ethylene Oxide in Plant Biological Systems: A Review

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ABSTRACT

This study reviews the role of ethylene oxide in biological systems and the progress made within this study area since the earliest identification of this gas. Current study and review articles provide vast information on the toxicity of ethylene oxide and the potential health risks that are associated with its use. While a few sections in this study are dedicated to the effects of ethylene oxide, a majority of the material will be confined to the gas and its role in plant biological systems.

Key words: Ethylene oxide, sterilization, ethylene oxidase, fruit ripening

INTRODUCTION

What the world knows of Ethylene Oxide (EO) is that it is an industrial gas that is widely used to sterilize medical equipment, cosmetics, pharmaceuticals and food stuffs. Generally, the higher the EO levels, the more effective the sterilization process and the shorter the time required. Unfortunately, it is highly toxic, even a sniff can be fatal. In addition, EO is explosive in concentrations ranging from 3-100% by volume and because it carries its own oxygen supply, it can explode in anaerobic atmospheres. However, for over 4 decades now, plant biologists have encountered minute quantities of this gas in plant biological systems and believe that it plays an important role in plant tissues where it is often detected as one of the products of ethylene metabolism. Surprisingly, the levels of EO detected in plant tissues have been at low levels that almost always escape detection and require radiolabelling to be detected and adequately measured. For these reasons, widespread research into the role of EO in biological systems has been hindered. In addition the purification and characterization of the enzyme catalyzing the conversion of ethylene to EO, ethylene oxidase (ethylene monooxygenase), has been met with tremendous difficulty and lack of success because the enzyme appears to be rather delicate and is often lost before any number of purification steps can be completed.

ETHYLENE OXIDE THE INDUSTRIAL GAS

Ethylene oxide is a major industrial chemical used primarily as an intermediate in the manufacture of other chemicals such as ethylene glycol, a major component of automotive and other antifreeze products. It is also useful in the synthesis of polyesters, laundry detergent and as an industrial sterilant. The EO is predominantly synthesized from the catalytic oxidation of ethylene in air or oxygen. It is very reactive because its highly strained ring can be opened easily and as a result, it is not persistent in the environment because it may be dispersed via a combination of mechanisms of dilution, biodegradation, volatilization and hydrolysis.

The sterilization of medical equipment with EO has for decades been the method of choice for the production of sterile thermolabile materials, owing to the microbicidal and virucidal properties of this agent as well as its penetration capacity (Hucker and Kramer, 2001; Mendes *et al.*, 2007). Despite the increasing use of novel sterilization methods such as radiation sterilization and research into the potential risks of EO exposure, more than 60% of all medical devices are sterilized in this manner (Lambert *et al.*, 2011; Buchalla *et al.*, 1995). Identification of the risks associated with the widespread use of EO has sparked varied research worldwide. Ongoing study involves the creation and/or improvement of sterilizing equipment (Hucker and Kramer, 2001; Smith *et al.*, 2007; Mendes *et al.*, 2007). Occupational safety bodies such as National Institute for Occupational Safety and Health (NIOSH) and the National Healthcare Association (NHA) in the USA have set Permissible Exposure Limits (PEL) at 0.5 ppm, however the degree to which these standards are followed on EO production plants as well as in hospital and other areas of use is difficult to ascertain with any degree of certainty.

Based on studies in animals, cancer is considered the critical endpoint for the effect of EO on human health (Liteplo *et al.*, 2001). In humans, EO has been implicated in DNA damage, reduction in reproduction potential and spontaneous abortion, skin irritation and sensitization, neurological disorders and various types of cancers (Shore *et al.*, 1993; Axelson, 2004; Valdez-Flores *et al.*, 2010). These issues have been complicated by the detection of EO residues in medical equipment and skin products, however the effects of these residues has not been measured.

BIOSYNTHESIS OF EO IN PLANT TISSUES

Ethylene is a natural plant growth regulator often produced in sufficient quantities to alter cellular and developmental processes in a characteristic hormonal manner (Abeles, 1973). Almost all phases of plant development are affected including germination, growth, flowering, dormancy, abscission, senescence and sex expression (Blomstrom and Beyer, 1980). It is well established that ethylene is derived from methionine via the ethylene pathway in a complex cycle. In the presence of ATP, methionine joins with adenosine to form S- adenosyl methionine, a process catalyzed by S-adenosyl methionine transferase. S-adenosyl methionine then undergoes a γ-elimination process cleaving into methylthioadenosine and 1-aminocyclopropane-1-carboxylic acid (ACC). This process is catalyzed by ACC synthase. In the final step ACC is acted upon by ACC oxidase producing ethylene (Yang and Hoffman, 1984).

The earlier view that ethylene is metabolically inert (Abeles, 1972; Varner and Ho, 1976) is untenable. Low but readily detectable rates of ethylene metabolism have been observed in several plant tissues (Beyer, 1975). Studies indicate that the metabolism of ethylene does not represent a system for the removal of ethylene from the plant but instead represents the initial biochemical events in the action of ethylene in the plant (Beyer, 1977; Beyer and Sundin, 1978). Evidence of ethylene metabolism is reflected in studies which show that a portion of the ethylene is oxidized to EO (Jerie and Hall, 1978; Dodds *et al.*, 1979; Smith *et al.*, 1985), ethylene glycol (Blomstrom and Beyer, 1980) and/or carbon dioxide (Beyer, 1975; Beyer and Sundin, 1978).

Surprisingly, in spite of all the research done on various aspects of ethylene and its metabolism, little is known about its metabolic fate (Beyer, 1975). It is still not clear whether or not ethylene is incorporated into plant tissues, despite the fact that several studies (Hall *et al.*, 1961; Shimokawa and Kasai, 1968) have reported that a small but significant amount of radioactive label was incorporated by fruit and vegetable tissues. Work on the fate of $^{14}C_2H_4$ (radioactive ethylene) in pea seedlings (Beyer, 1975; Giaquinta and Beyer, 1977) and in cut carnation flowers

(Beyer, 1977), revealed a previously unrecognized ethylene metabolic system in these tissues. This system oxides ethylene to carbon dioxide and incorporates ethylene into water-soluble tissue metabolites (Beyer and Sundin, 1978). Efforts to determine the fate of radioactive ethylene in plant tissues have shown that little (1% maximum) or no incorporation occurs. Where incorporation does occur, radioactivity has been detected in a wide variety of compounds including benzene (Jerie and Hall, 1978), the presence of which in higher plants is unexpected. Flowers of *Ipomoea tricolor* Cav. (cv. heavenly blue) demonstrated the ability to metabolize ethylene to CO_2 (Beyer and Sundin, 1978). Like ethylene sensitivity and biosynthesis, the ability of plant tissues to metabolize ethylene changes during development. This complex metabolic pathway does not seem to serve as a detoxification or degradative system since based solely on the amount of ethylene removed, it would appear totally ineffective (Beyer, 1975).

ENZYMATIC CONVERSION OF ETHYLENE TO ETHYLENE OXIDE BY ETHYLENE OXIDASE

Although progress on the isolation of cell, free preparations of the ethylene oxidizing enzyme (ethylene oxidase) has been slow, important data has been provided using partially purified enzyme preparations (Dodds *et al.*, 1979; Smith *et al.*, 1985). Progress in the precise localization of the enzyme has been hampered by the fact that sucrose and other polyhydroxy compounds commonly used in the fractionation of subcellular components drastically inhibits enzyme activity (Smith *et al.*, 1985). Dodds *et al.* (1979), found that cell-free preparations of *Vicia faba* L. metabolized ethylene to EO with a high degree of efficiency. This system had a high affinity for ethylene and showed a molecular requirement for oxygen (Blomstrom and Beyer, 1980; Smith *et al.*, 1985) with a K_m of 3.63×10^{-3} M (Dodds *et al.*, 1979). The conversion of ethylene to ethylene oxide is not confined to plants. *Mycobacterium paraffinicum* has been shown to convert ethylene to ethylene oxide (Abeles, 1984; Hartmans *et al.*, 1991).

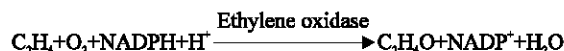
The incorporation of ^{18}O (radioactive oxygen) into EO was demonstrated in *Vicia faba* L. cotyledons using *in vivo* studies (Beyer, 1975). The evidence to date then suggests that ethylene oxidase is a mono-oxygenase (Hartmans *et al.*, 1991) and this is substantiated by the fact that the apparent K_m for NADPH is within the range for K_m values reported for other plant mono- oxygenases (Beyer, 1975). The enzyme's requirement for oxygen was also demonstrated by Beyer (1975), who showed that enzyme activity was reduced by over 60% when extracts were incubated in vials that had been flushed with N_2. The K_m of this system corresponds to a concentration of 0.096 $\mu L\ L^{-1}$ in the gaseous phase which corresponds well with values for endogenous ethylene concentrations in the air spaces of plant tissues (Beyer and Sundin, 1978).

The first studies using partially purified enzyme preparation of ethylene oxidase was carried out by Dodds *et al.* (1979). The enzyme extract was prepared from *Vicia faba* L. cotyledons by centrifugation of cell, free extracts to generate 6 fractions. Each fraction was tested for its ability to metabolise ^{14}C ethylene into ^{14}C ethylene oxide. More than half the activity of the original homogenate was discovered in the pellet obtained from centrifuging at the lowest speeds (3000-10 000×g). The first reports of kinetic studies carried out on ethylene oxidase showed that ethylene oxidase had a K_m of 4.17×10^{-10} M and required an optimum pH of 8.5 (Dodds *et al.*, 1979).

Later, Blomstrom and Beyer (1980) reported the metabolism of ethylene to ethylene glycol in cotyledons of *Vicia faba* L. From the results, it was suggested that EO being a likely precursor of ethylene glycol, might be involved in the formation of ethylene glycol from ethylene, serving as an

intermediate. Inhibition studies carried out showed that propylene competitively inhibits ethylene oxidase where the metabolite formed is ethylene glycol. Although the conversion of ethylene glycol may be unique to this tissue, it is of interest because EO is a likely precursor of ethylene glycol. In conditions like those found in *Vicia* cotyledons, where the ethylene is rapidly metabolized, perhaps EO escapes before rapidly undergoing hydrolysis, or that this later step may be blocked in this tissue (Blomstrom and Beyer, 1980).

Some of the most intense work done on ethylene oxidase to date seems to be that from Smith *et al.* (1985) and this gave independent confirmation to the work carried out by Dodds *et al.* (1979). Smith *et al.* (1985), generated a partially pure enzyme preparation from homogenized cotyledons of *Vicia faba* L. From these experiments, Smith *et al.* (1985) showed that ethylene oxidase had a molecular requirement for oxygen and NADPH and a high affinity for ethylene. As a result of kinetic studies with partially purified enzyme preparations, a reaction path for the metabolism of ethylene to EO in the presence of ethylene oxidase (a mono-oxygenase) has been proposed (Smith *et al.*, 1985). The reaction is shown as:



$$C_2H_4+O_2+NADPH+H^+ \xrightarrow{\text{Ethylene oxidase}} C_2H_4O+NADP^++H_2O$$

Ethylene oxidase had a K_m of 4.2×10^{-10} M for the substrate ethylene (*in vivo*) and 2×10^{-8} M for desalted extracts (Dodds *et al.*, 1979), which agrees with earlier theories that only extremely small yet significant amounts of ethylene is fixed and metabolized in fruit and vegetable tissues (Beyer, 1975). The high affinity of the enzyme for ethylene makes it very likely that ethylene is the true substrate. Inhibition studies using propylene gave a K_i of the order of 5×10^{-6} M (Blomstrom and Beyer, 1980) and 1.8×10^{-6} M (Dodds *et al.*, 1979), which supports the likelihood that ethylene, is the true substrate, having a K_m of 4.17×10^{-10} M. A requirement for NADPH was also shown by ethylene oxidase (Smith *et al.*, 1985). The NADH (0.5 mM) and NADPH (0.5 mM) increased the activity of ethylene oxidase but the NADPH alone was twice as effective as NADH. At the concentration tested, there was no evidence of synergism when both co-factors were applied.

The actual role of EO in plant metabolism is unclear. It does not appear to serve as a shunt pathway for controlling endogenous ethylene concentrations because of the fact that only trace amounts of ethylene is fixed and metabolized to EO. Although the function of ethylene metabolism is unclear, the process does appear to be correlated with ontogeny since production of ethylene increases with stage of maturity (Jerie and Hall, 1978).

ETHYLENE OXIDE OPPOSES ETHYLENE ACTION

EO has been recognized in the volatiles emanating from tissue slices and homogenates of fruit. It has also been identified in very low concentrations in volatiles of intact fruit. The physiological significance of this finding was realized in the fact that EO was observed to inhibit ethylene production and ripening of intact fruit (Lieberman and Mapson, 1962). This has led to the idea that EO may play an important role in plant metabolism.

In one of the earliest experiments involving EO, green tomato fruits were exposed to an atmosphere containing 0.75% EO for 16-22 h (Lieberman and Mapson, 1962). The treated fruits ripened at 20°C after a delay of 5-21 days depending on the ripeness of the fruit at the start of the experiment. The tissues of fruits that were exposed to EO concentrations of over 2% or tissues of fruits that had 2-3 days extended exposure were irreparably damaged and did not ripen at all after

the atmosphere was removed. However, fruits that were already in the "Climacteric stage" and producing fairly large amounts of ethylene, when they were exposed to the EO, were not affected. Ethylene production and ripening are inhibited so long as EO remained in the tissues (Lieberman *et al.*, 1964). After dissipation of absorbed EO by expulsion or reaction in the tissues, the fruits resume ripening in a normal manner without any alteration in the palatability, colour or texture of the fruits. In this manner EO acts as an anti-ageing metabolite of ethylene metabolism. From this it was concluded that EO possessed an anti-ageing effect, in that it retarded the ripening process and stalled ethylene production in pre-climateric fruits. Studies carried out on roses and carnations revealed that the mechanism of action of EO in the retardation of the ripening process and inhibition of ethylene production involved water retention. This is in contrast to the effect of ethylene in the ripening process which is alteration of the permeability of cell membranes and increasing water loss (Lieberman *et al.*, 1964).

More recent studies involving EO treatment of preclimacteric banana fruits yielded similar results (Williams *et al.*, 2003). A variation of treatments resulted in a delay of banana ripening as long as 4-6 weeks depending on the concentrations used and the number of applications applied. When a proper concentration of EO was applied to some fruits during the preclimacteric period, ethylene production was suppressed and ripening was often retarded. While residual EO remained in the tissues of exposed fruits, ripening was retarded (Lieberman *et al.*, 1964; Williams *et al.*, 2003). After dissipation of the absorbed EO, either by expulsion or reaction within the tissues, the fruit resumed production of ethylene in a normal manner. Sensitivity to EO varies considerably among different fruit tissues. The higher the rate of ethylene production at the time of treatment, the higher the concentration of EO required suppressing ripening.

The antagonistic effect of ethylene oxide on ethylene action was also demonstrated with roses and carnations (Lieberman *et al.*, 1964). Senescence in these flowers was significantly delayed after treatment with appropriate concentrations of ethylene oxide. A significant indicator of the possible role of EO in plant metabolism lies in the partial prevention of the triple response assay for ethylene when both ethylene and ethylene oxide were simultaneously applied to etiolated seedlings (Lieberman *et al.*, 1964). Although the etiolated seedlings were stunted, they did not show epinasty or swollen growing tips.

CONCLUSION

Recognition of EO as an inhibitor of ethylene production in fruits (Lieberman and Mapson, 1962; Williams *et al.*, 2003) presents new possibilities for exploring the pathway of EO biosynthesis and its mode of action. These areas of research will undoubtedly require intense work to be able to adequately assay for ethylene oxide. Because of its reactive nature, it is possible that it is easily converted to other by products and thereby evades detection. Nonetheless it should be interesting to explore these areas of research if only to shed some light on the levels of EO that accumulate in plant tissues and the effects on the tissues themselves. Perhaps the results of such study help us to better understand the use of EO in industry.

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