

1 Interaction of Ethylene and Other Compounds with the Ethylene Receptor: Agonists and Antagonists

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1.1 Introduction

Although ethylene has long been recognized as a plant hormone, it is only recently that the ethylene receptor has been subjected to detailed study. Most reviews on ethylene signal transduction do not discuss much about ethylene interaction with the ethylene receptor except to mention that ethylene does bind to the receptor. This review will concentrate on the interaction of ethylene, ethylene agonists, and antagonists with the receptor. It is important that we identify the factors that determine compound binding and activity whether the compound is an ethylene agonist or an ethylene antagonist. It is important that findings from past work be noted in concordance with new-found results that contribute to our knowledge of the many compounds known to bind to the receptor. In recent years, the number and type of compounds that interact with the receptor has been expanded considerably. Some of these compounds appear to be useful both for basic research and for practical purposes. Many more may be discovered. It is the intent here to present some of what is known about both ethylene antagonists and agonists that have been found with the hope that the information will help lead to other compounds.

1.2 Ethylene and Agonists

1.2.1 Discovery of Ethylene Action and Some Important Lessons from the Past

Ethylene is one of the five original basic plant hormones. Many of the responses caused by ethylene were observed before it was known that it was the cause of the response (Abeles et al. 1992). In 1901, Neljubov reported that

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ethylene caused a triple response in etiolated pea seedlings: i.e., epicotyl thickening, growth retardation, and horizontal growth of the epicotyl. It was soon recognized that ethylene was not alone in causing a triple response in plants. Soon it was known that propylene, acetylene, and carbon monoxide were ethylene agonists also giving a triple response in pea. In 1967, Burg and Burg identified several other alkenes and alkene-related compounds that were active. Isocyanides were added to the list of ethylene agonists 10 years later (Sisler 1977). These were important clues as to how ethylene may act. Now a great number of plant responses have been shown to be regulated by ethylene. Ethylene, which is produced by almost all plants, mediates a wide range of different plant responses and developmental steps. Ethylene plays an active role in seed germination, tissue differentiation, formation of root and shoot primordia, root elongation, lateral bud development, flowering initiation, anthocyanin synthesis, flower opening and senescence, pollination, fruit degreening and ripening, the production of volatile organic compounds responsible for aroma formation in fruits, leaf and fruit abscission, the response of plants to both biotic and abiotic stress, and plant-microbial interactions that are important for plant's growth and survival (Abeles et al. 1992; Grichko and Glick 2001a). Agricultural and horticultural loss is high due to ethylene-accelerated post-harvest ripening and deterioration of perishable commodities.

1.2.2 Molecular Requirements

In 1967, Burg and Burg published a paper on the molecular requirements for ethylene action in plants. Applying techniques used in enzyme kinetics, they compared a number of active compounds for their ability to give an ethylene agonistic response in peas. Using a gas chromatographic technique, they also compared the ability of the same compounds to bind to silver ions. They reported the binding of the compounds to silver ions to be in the same order as their ability to inhibit pea seedling growth. Burg and Burg (1967) then proposed that there was a metal in the supposed ethylene receptor. This was an important step toward understanding the way ethylene acts to bring about a response in plants. That ethylene binds to certain metals was not new. It had been known since 1827 that ethylene formed a complex with platinum and there was much chemical literature available on metal complexes of ethylene and other olefins, but the report by Burg and Burg (1967) was the first report extending this concept to plant responses. Consequently, there were several early attempts and suggestions to explain the mechanism of ethylene activity. Did ethylene act by being oxidized? Did ethylene act by producing some essential component as in an enzymatic reaction, or did ethylene serve to turn on a signal transduction pathway? Experimental evidence has favored a signal transduction pathway and this has been the focus of much recent work.

Some early work focused on the putative metal involved in ethylene action. Based on some deficiency experiments, Burg and Burg (1967) found that only zinc deficiency seemed to alter ethylene sensitivity in plants. For ethylene oxidation, copper seemed more likely as the metal. The reversible binding of ethylene to Cu(I) was well known (Cotton and Wilkinson 1980) and it seemed a likely prospect for being the metal involved (Sisler 1976, 1977). To support the proposed role of monovalent copper in the ethylene binding in plants, complexes of Cu(I) with imidazole-like ligands were synthesized (Thompson et al. 1983; Thompson and Whitney 1984; Thompson and Swiatek 1985). The complexes were the rather stable Cu(I) adducts with ethylene and its agonists and exhibited either a trigonal-planar geometry or a distorted tetrahedral structure. In a membrane environment, ligands bound to a metal ion may considerably alter its properties and the properties of its complex with ethylene, and there is a possibility that other metals might be involved in ethylene binding *in situ*. Rodriguez et al. (1999) did include other metals in an *in vitro* study where ethylene receptor gene *ETR1* from *Arabidopsis* was cloned in yeast. Only Cu(II) and Ag(I) significantly increased ethylene binding. Supplying ions such as Fe(II), Co(II), Ni(II), or Zn(II) did not increase ethylene binding. In the 2-D model of an ethylene receptor, which was developed based on these experiments, the transmembrane, hydrophobic ethylene-binding domain contained one Cu(I) ion per protein dimer, and coordinating amino acids were thought to be Cys65 and His69 (Rodriguez et al. 1999). The ethylene receptor has been suggested to contain either one or two Cu(I) ions per dimer (Hirayama et al. 1999; Pirrung 1999; Klee 2002; Taiz and Zeiger 2002; Weiler 2003). The stoichiometry gives little clue as to the structure. The coordination number of Cu(I) ions can be anywhere from two to six, and it is possible that Cu(I) forms a tetrahedral complex with both Cys65 and His69 (Pirrung 1999). A sulfur-ligated Cu(I)-ethylene complex exhibits very weak metal-ligand bonding interactions (Hirsh et al. 2001), and it is also possible that each cysteine residue is not a coordinating ligand. Cysteine residues instead may form disulfide bond *in situ*, and histidine residues and water may serve as ligands. Ethylene is likely to displace a weak ligand, and water is one of the most suitable candidates for this role. Displacement of water by ethylene followed by expelling of water molecule(s) from the hydrophobic domain is likely to result in the formation of a stable complex. An experiment in which a specific metal is shown to function *in situ* in ethylene perception has not yet been reported and is needed, but much recent evidence has favored copper as the metal involved in the receptor. Hirayama et al. (1999) restored antagonistic activity to an *Arabidopsis ran1* mutant, which gave an agonistic response with *trans*-cyclooctene (TCO), by either cloning a Cu(I) transporter into it or by supplying Cu(II) ions. This essentially confirms that copper can function in the receptor. The fact that *ran1* loss-of-function mutants were responsive to both ethylene and *trans*-cyclooctene is rather fascinating. Because 1-methylcyclopropene (1-MCP), a potent ethylene antagonist, also appeared to function normally, a metal

must have been present in the receptor. Was that metal copper? *trans*-Cyclooctene was not included in the *in vitro* study of Rodriguez et al. (1999), and it is not known if the ethylene receptor associated with a different metal binds alkenes other than ethylene. Unusual behavior of the *ran1* mutant might be a result of either alteration of ethylene receptor conformation, decrease in ligand specificity, or the stability of receptors (Hirayama et al. 1999; Woeste and Kieber 2000). It can also be a result of irreversible disruption of altered ethylene receptors by *trans*-cyclooctene, enhanced sensitivity or insertion of different metal into some ethylene receptors under the conditions of a severe Cu(I) ion deficiency.

Some data suggest that binding of ethylene to the receptor may result in a structural rearrangement of the receptor, which can serve as an initial event in a signal transduction pathway. The role of histidine kinase activity of the ethylene receptor subfamily I is proven to be rather complex (Wang et al. 2003; Qu and Schaller 2004). It was shown that the ethylene receptor directly interacts with the downstream negative regulator CTR1 (Clark et al. 1998), and kinase activity of ETR1 is not required for its interaction with CTR1 (Gao et al. 2003).

There are still many questions about how ethylene acts. The exact structure of the ethylene-binding domain of the ethylene-receptor family is still unknown. The 3-D structure may be determined soon following a high-level expression of ETR1 in *E. coli* (Voet-van-Vormizeele and Groth 2003) and this may greatly facilitate the process of selection of the best candidates from the pool of synthetic compounds and phytochemicals and make it easier to predict anti-ethylene potency of their derivatives.

1.2.3 Ethylene Binding

Another important step in understanding the action of ethylene was the development of methods of measuring ethylene binding in plants. Using ^{14}C -ethylene, the rate of ethylene binding and the rate of ethylene release could be measured in plant tissue (Jerie et al. 1979; Sisler 1979). Using this technique, it could be shown that in vegetative tissue, there appeared to be a major component that bound and released ethylene rapidly. The time-radiolabeled ethylene remained bound to the major component varied in different plants. In most plants, the $t_{1/2}$ was about 10 min. However, in tomato leaflets it was only 2 min. The rapid component correlates well with the data of Warner and Leopold (1971) for response times by pea plants to ethylene. The value for K_d as determined by a Scatchard plot correlated well with the value for K_m as determined by a Lineweaver-Burk plot (Sisler 1979). There usually also was a small amount that was released with a much longer half-time. In some seeds, there were large amounts of ethylene, which remained bound for long periods of time. Because there is no known

function for ethylene in these seeds, this probably represents binding to a storage component.

In measuring ethylene action, pea plants responded to ethylene in just 10 min and recovered with $t_{1/2}$ of about 18 min after its withdrawal (Warner and Leopold 1971). In *Arabidopsis* hypocotyls of etiolated seedlings, there were two phases of growth inhibition by ethylene, a rapid phase followed by a prolonged slower phase. Full recovery occurs about 90 min after ethylene removal (Binder et al. 2004a). The recovery time was significantly smaller than the time of ethylene dissociation from ETR1 receptors expressed in yeast (Schaller and Bleecker 1995; Binder et al. 2004a). The inhibition appears to be a complex process (Binder et al. 2004b). In ethylene binding studies, the shortest value of $t_{1/2}$ for ^{14}C ethylene diffusion from the binding site measured *in vivo* was 2 min (Sisler 1982).

In vitro, the short-lived component is absent; in extracts of mung bean sprouts, $t_{1/2}$ of 1 h and $t_{1/2}$ of 50 h were measured (Sisler 1990). In a cell-free system from cotyledons of *Phaseolus vulgaris*, $t_{1/2}$ was about 10.5 h (Bengoechea et al. 1980). In yeast expressing ETR1 at a level of about 4.0×10^{-8} M, $t_{1/2}$ was 12.5 h (Schaller and Bleecker 1995). Based on K_d and $t_{1/2}$ (Sisler 1991), one can estimate that the rate constant of ethylene binding to the receptor is about $5 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ for the short-lived component and $2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ for the long-lived component, indicating that rate of ethylene binding is likely to be determined by the rate of its interaction with the active center of the receptor.

1.3 Ethylene Antagonists

1.3.1 Chemical Adjuvants Counteracting Ethylene

Ethylene responses in plants can be prevented to some extent by a number of chemical adjuvants. High concentrations of sucrose, carbon dioxide, and cycloheximide delay senescence in flowers (Dilley and Carpenter 1975). Carbon dioxide is used in controlled atmosphere storage of fruits and vegetables and it has been known for many years that it is a natural inhibitor of ethylene responses. Early studies of the carbon dioxide effect suggested that it competes with ethylene in ethylene action (Burg and Burg 1967); however, direct measurement with ^{14}C -labeled ethylene did not indicate that carbon dioxide competes with ethylene for the receptor sites (Sisler 1979). Recently it was shown that carbon dioxide acts by suppressing ethylene biosynthesis (John 1997). Indoleacetic acid can prevent ethylene action under some circumstances. Application of indoleacetic or 2,4-dichlorophenoxyacetic acid to plant tissue will retard some ethylene-induced processes, but there is no evidence that they act by preventing ethylene binding, and their action also seems to be indirect (Sisler et al. 1985).



<http://www.springer.com/978-3-540-32716-5>

Ethylene Action in Plants

Khan, N.A. (Ed.)

2006, XVII, 206 p., Hardcover

ISBN: 978-3-540-32716-5