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How plants sense ethylene gas — The ethylene receptors

Randy F. Lacey, Brad M. Binder *

Department of Biochemistry, Cellular and Molecular Biology, M407 Walters Life Sciences, University of Tennessee, Knoxville, TN 37996, United States

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ABSTRACT

Ethylene is a hormone that affects many processes important for plant growth, development, and responses to stresses. The first step in ethylene signal transduction is when ethylene binds to its receptors. Numerous studies have examined how these receptors function. In this review we summarize many of these studies and present our current understanding about how ethylene binds to the receptors. The biochemical output of the receptors is not known but current models predict that when ethylene binds to the receptors, the activity of the associated protein kinase, CTR1 (constitutive triple response1), is reduced. This results in downstream transcriptional changes leading to ethylene responses. We present a model where a copper cofactor is required and the binding of ethylene causes the receptor to pass through a transition state to become non-signaling leading to lower CTR1 activity.

1. Introduction

Ethylene was the first gaseous biological signaling molecule discovered. Initial evidence for this was provided in 1901 when the Russian scientist Neljubov reported that ethylene was the active compound in illuminating gas that caused altered growth of pea seedlings [1]. Three decades later Gane measured ethylene biosynthesis by apple fruits [2]. Since these early observations, ethylene biosynthesis has been observed in a large number of plants, the pathway for biosynthesis has been elucidated, and ethylene has been shown to affect many important processes in plants including seed germination, growth, flowering, senescence, fruit ripening, and responses to a variety of stresses [3,4].

Despite these early advances, the mechanism for ethylene signal transduction remained unknown for decades. This began to change in the late 1970s and early 1980s when ethylene-binding sites in plants and plant cell extracts were characterized [5–10]. The development of *Arabidopsis thaliana* as a model plant system led to significant progress in identifying components of the ethylene signaling pathway, including the receptors. Dark-grown *Arabidopsis* seedlings show a characteristic response when exposed to ethylene that includes inhibition of growth, increased diameter of the hypocotyl, and an exaggerated apical hook. This easy and quantifiable assay was used to screen for and identify ethylene response mutants, which led to the identification of genes involved in ethylene signaling [11–14]. From these and other experiments, a model of ethylene signal transduction has emerged where the receptors signal to and stimulate CTR1 (constitutive triple response1), a serine/threonine kinase, which acts to inhibit downstream signaling. In this model, ethylene inhibits the receptors leading to diminished CTR1 activity. Lower CTR1 activity leads to release of inhibition of downstream signaling resulting in transcriptional changes required for responses to ethylene [Reviewed by: 15–17]. In this review we summarize what is known about the binding of ethylene to these receptors and propose a model for receptor output.

2. Overview of the ethylene receptors

The ETR1 (ethylene receptor1) ethylene receptor from *Arabidopsis* was the first hormone receptor identified and cloned from plants [11,18]. Evidence that ETR1 is a receptor for ethylene came from two main observations. First, specific missense mutations in the transmembrane domains led to ethylene-insensitivity throughout the plant [11,19]. Second, ethylene-binding activity was observed when ETR1 was expressed in yeast [20]. Later studies showed that there are four other receptor isoforms called ETR2, ERS1 (ethylene response sensor1), ERS2, and EIN4 (ethylene insensitive4) in *Arabidopsis* [21–23] that bind ethylene with high affinity [24–26].

The different isoforms share several common structural features as shown in Fig. 1. All are predicted to contain three transmembrane α-helices at the N-terminus that form the ethylene-binding domain. This is followed by a GAF (cGMP-specific phosphodiesterases, adenylyl cyclases, and FhlA) domain, and a protein kinase domain. Three of the five *Arabidopsis* receptors also contain a receiver domain at their C-termini that is similar to domains found in bacterial two-component receptors [27]. Based on sequence comparisons of the ethylene-binding domains, the ethylene receptors in plants fall into two subfamilies [28]. In *Arabidopsis*, subfamily 1 includes ETR1 and ERS1 that contain all amino acid residues needed for histidine kinase activity [18,21] and show histidine kinase activity *in vitro* [29,30]. Subfamily 2 includes ETR2, EIN4, and ERS2 that contain degenerate histidine kinase domains [22,23] and show serine/threonine kinase activity *in vitro* [30]. ERS1 is unusual in that it has both histidine and serine/threonine kinase

* Corresponding author. Fax: +1 865 974 6306.
E-mail address: bbinder@utk.edu (B.M. Binder).

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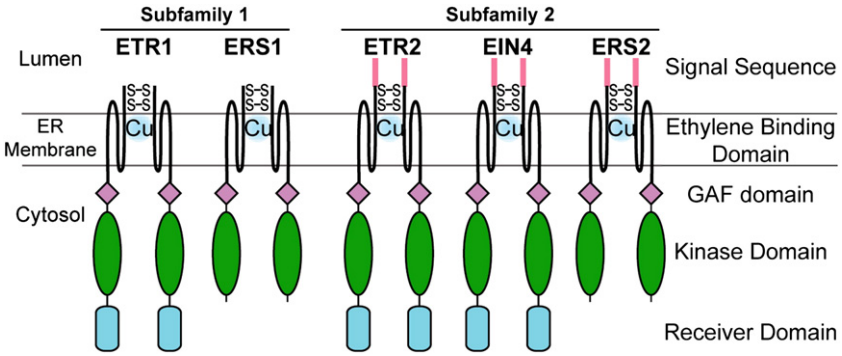

Fig. 1. Ethylene receptor structure and subfamilies in *Arabidopsis*. The plant ethylene receptors fall into two subfamilies as shown. The receptors are homodimers stabilized by two disulfide bonds. Each monomer is composed of three transmembrane α-helices followed by a GAF and kinase domain. A subset also contains a receiver domain and the subfamily 2 members contain an extra N-terminal signal sequence. The α-helices of the dimer form the ethylene binding domain that coordinates Cu(I).

activities *in vitro* [30]. These studies analyzed autophosphorylation activity making it unclear whether or not there are additional substrates for these receptor kinases. The subfamily 2 receptors contain additional amino acids at the N-terminus that may function as a signal sequence.

The receptors form homodimers that are stabilized at their N-termini by two disulfide bonds [25,31,32]. These disulfide bonds are not necessary for binding of ethylene since ETR1 mutants lacking them still bind ethylene [32]. Additionally, a homolog of ethylene receptors from the cyanobacterium, *Synechocystis* sp. *PCC6803*, also binds ethylene even though it lacks the cysteines required for these disulfide bonds [33]. These disulfide bonds are not required for a functional ETR1 receptor *in planta* [34].

Using [^{14}C]-ethylene, ethylene-binding sites were localized to the membranes of the ER (endoplasmic reticulum) in pea plants [7,8]. Subsequent studies on specific receptor isoforms of *Arabidopsis*, melon, and tobacco confirm that these receptors are predominantly localized to the ER, although, it is possible that they are also localized to other membranes of the cell [35–40]. Since ethylene is lipophilic and can diffuse in both aqueous and lipid environments, the endomembrane localization of the receptors does not prevent perception.

The GAF, kinase, and receiver domains likely represent the output domains of the receptors. However, the biochemical mechanism for receptor signaling and how binding of ethylene affects this is not clear. The ethylene receptors are homologous to bacterial two-component receptors that function *via* a histidine to aspartate phosphorelay mechanism [18,41]. This observation led to a model invoking this activity for ethylene signaling. In support of this, a biochemical study showed that the binding of ethylene to ETR1 lowered autophosphorylation [42]. Additionally, the level of ethylene receptor phosphorylation in tomato was reduced upon exposure to ethylene [43]. However, the sites of phosphorylation were not determined in these studies. In contrast to the biochemical studies, genetic experiments suggest that the binding of ethylene to ETR1 activates the histidine kinase [44]. Thus, there is uncertainty as to how ethylene affects this kinase activity. Despite this uncertainty, it is clear that modulation of histidine kinase activity has only a minor role in ethylene signaling since mutational studies show that this activity is not required for ethylene responses in plants [34,45,46]. Rather, this activity may subtly modulate receptor signaling to downstream components [44,45,47]. The role of serine/threonine kinase activity of subfamily 2 members has also been explored. As with the above studies, this activity does not appear to be required for ethylene responses, but may have a modulatory role in ethylene receptor signal transduction [48].

Since kinase activity of the receptors is likely to not be required for ethylene responses, an alternative model has developed that posits ethylene modulates receptor-protein interactions to cause ethylene responses. Interactions between the receptors and various proteins have been noted in numerous studies using diverse techniques [32,38,49–57]. CTR1 is a major component of ethylene signaling [13] and the receptors directly interact with CTR1 [38,50–52]. Since CTR1 has a central role in ethylene signaling, interactions between the receptors and CTR1 are likely to be critical for this process. This is supported by the observation that mutations in CTR1 that abolish these interactions cause CTR1 to be nonfunctional [52,58]. Also, the application of ethylene causes CTR1 to associate with the ER membranes in a receptor-dependent manner [52]. Consistent with results discussed above, this modulation of interaction occurs independently of ETR1 histidine kinase activity [52]. Together, these studies suggest a model where ethylene-induced conformational changes in the receptors cause inhibition of CTR1 (Fig. 2). Other receptor–protein interactions are occurring that probably modulate signaling from the receptor [Reviewed by: 59], but the interactions with CTR1 are likely to be of primary importance in mediating responses to ethylene.

3. Ethylene binding to the receptors

The first step in ethylene perception is when ethylene binds to the receptors. By expressing truncated versions of the receptors in yeast, researchers found that ethylene binding occurs in the three N-terminal transmembrane domains of the receptors from both *Arabidopsis* and tomato [20,24,33]. Ethylene binds to the *Arabidopsis* receptors with a K_d in the nM range [20,26,33]. Even though ethylene detectors have been synthesized, none bind ethylene with this high affinity [60–65]. Therefore, a better understanding of how the plant receptors bind ethylene also has implications for agricultural, horticultural, chemical, and industrial applications. Here we summarize research on both the metal cofactor and the protein environment that are important for this high-affinity, ethylene-binding activity found in ethylene receptors from plants.

3.1. A metal cofactor is required for ethylene binding

One of the central issues in ethylene signaling has been to determine how a protein is capable of binding ethylene with high affinity and specificity. Initially, based upon olefin chemistry, several transition metal cofactors were suggested including cobalt, copper, iron, nickel, and zinc [66–69]. This issue was answered using ETR1 exogenously expressed in yeast where it was found that copper ions act as the cofactor for the binding of ethylene [33]. A later study showed that copper ions are also used by the other four receptor isoforms [26]. Additional support that copper is the cofactor for binding comes from the observation that the etr1-1 receptor mutant cannot coordinate copper and fails to bind ethylene [33]. Also, several studies have determined that the copper transporter, RESPONSE TO ANGAGONIST1, acts upstream of the receptors and is needed for the biogenesis of the ethylene receptors [70–73]. The copper ion is coordinated in the transmembrane portion of the receptors [33]. Studies on metal–olefin complexes indicate that it is Cu(I), rather than Cu(II), that is involved and the interactions between ethylene and Cu(I) in the receptor could be complex involving σ-bonds and π-orbital back-bonding [66,74–76]. Since Cu(I) is found