# Copper-Ethylene Interactions in Biology: Synthesis and Signaling

James Lansing Literature Seminar 10/12/2010

Ethylene is a valuable chemical feed stock, whose manufacture reached $1.2\times10^8$ tons in 2008.[1] Ethylene is utilized extensively in the production of polymers, both as a precursor to monomers and as a monomer itself. Additionally, ethylene finds use as a raw material for the production of other feed stock chemicals such as ethylene oxide, acetaldehyde, and synthetic ethanol.[1] It 1901 it was first reported that plants near natural gas leaks died much faster than their counterparts who had not been exposed.[2] Since then much research has demonstrated that ethylene plays an important role in biological systems, acting as a plant hormone. Ethylene modulates many changes in flowers and fruits, with the most important being ripening and eventual spoiling and death of the flowers or fruit. Extensive research has been performed to determine the biosynthetic mechanism of ethylene and its role in plants as a hormone.

The biosynthesis of ethylene begins with the naturally occurring amino acid, methionine. Methionine is converted into 1-aminocyclopropane-1-carboxcyclic acid (ACC) via the Yang cycle.[3] Subsequent transformation of ACC into ethylene also produces an equivalent of cyanide, carbon dioxide and two equivalents of water. The mechanism of ethylene production has been proposed to involve chelation of ACC to an iron center of ACC oxidase followed by conversion to ethylene. This process was found to be dependent on the presence of bicarbonate and ascorbic acid, the former to stabilize reactive oxygen species and the latter to provide electrons.[4, 5] Ethylene then diffuses through the fruit and binds to a receptor protein known as ETR1, thought to contain a copper cofactor.[6]

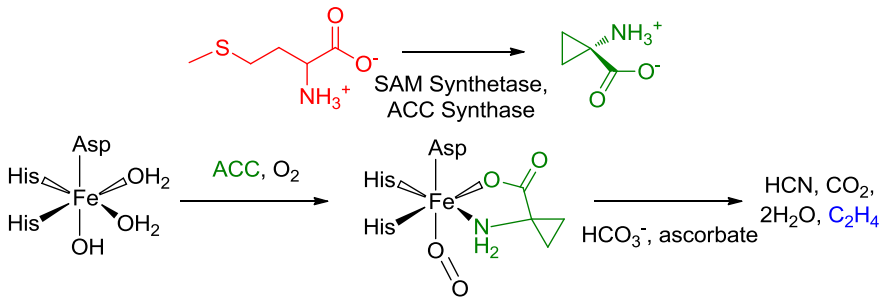


**Figure 1**: Biological synthesis of ethylene (blue) following conversion of methionine (red) to ACC (green)

The crystal structure of ETR1 has been determined. However the active site has not been sufficiently well-resolved to verify the identity of the proposed copper cofactor. The identity of the metal cofactor was determined by reconstituting the ethylene binding portion of ETR1 and introducing a wide array of metal ions. The receptor demonstrates negligible ethylene binding on its own. Introduction of non-coinage metal ions demonstrated no ethylene binding. Binding of ethylene was found to occur in the presence of silver ions, but further responses leading to ripening processes within the

protein did not occur. Gold displayed similar responses to the natural receptor with respect to ethylene action; however some toxicity was observed.[7] The ethylene binding portion of the protein only displays high activity without toxic effects in the presence of copper ions. Site-directed mutagenesis studies have deduced that copper is most likely bound in the active site via the $Cys^{65}$ and $His^{69}$ residues.

Ethylene is known not only to ripen fruits, but also to cause them to spoil. In order to prolong fruit shelf life, considerable research has been preformed with the goal of controlling ethylene biosynthesis or preventing ethylene binding to ETR1. It is proposed that the binding of ethylene modulates the electron density at copper, promoting a response that leads to downstream signaling. While silver will block ethylene binding, toxicity issues arise for use in food products. The use of olefins to block ethylene binding has been investigated. Due to strong competitive binding for copper receptor sites, 1-methylcyclopropene (1-MCP) has emerged as a viable inhibitor and has been implemented commercially. Solutions of 1-MCP will extend the shelf life of bananas for an additional 12 days.[8]

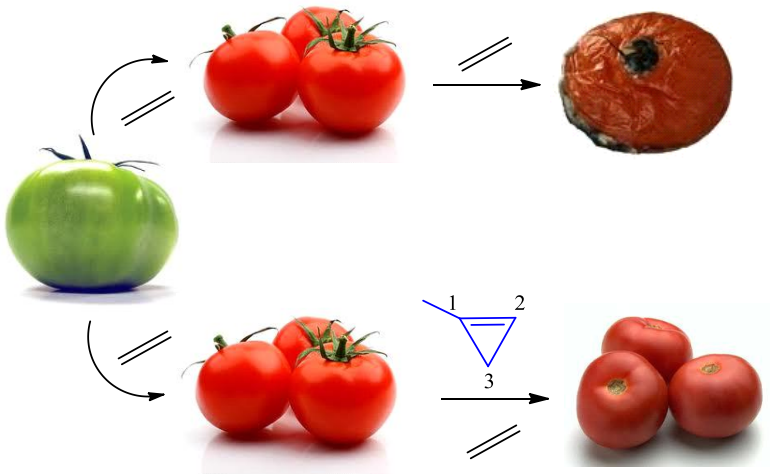


**Figure 2**: Ethylene effects on tomatoes, with (bottom) and without (top) protection by 1-MCP (blue, with carbons labeled)

Structural activity studies of substituted cyclopropenes have been undertaken. The alkyl group at the 1-position is necessary for efficient ethylene inhibition. Substituents at the 3-position or at both the 1- and 2-positions displayed less affinity for the active site, possibly due to steric or electronic effects. Lengthening the chain at the 1-position increased ethylene inhibition. For example, the use of 1-decylcyclopropene extended shelf life an additional 24 days over 1-MCP.[9] Amine complexes containing at least one cyclopropene also increased shelf life an additional 20 days over 1-MCP.[10] These alkyl-substituted and amine substituted 1-MCP compounds are reasoned to bind to the copper site more efficiently than 1-MCP due to interactions with either the hydrophobic pocket found near the active site or by hydrogen bonding interactions respectively.

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