Bioorganic & Medicinal Chemistry 24 (2016) 493–500

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Chemical manipulation of plant water use

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ARTICLE INFO

Article history:
Received 2 September 2015
Revised 5 November 2015
Accepted 10 November 2015
Available online 11 November 2015

Keywords:
Drought
Abscisic acid
Receptor
Agonist

ABSTRACT

Agricultural productivity is dictated by water availability and consequently drought is the major source of crop losses worldwide. The phytohormone abscisic acid (ABA) is elevated in response to water deficit and modulates drought tolerance by reducing water consumption and inducing other drought-protective responses. The recent identification of ABA receptors, elucidation of their structures and understanding of the core ABA signaling network has created new opportunities for agrochemical development. An unusually large gene family encodes ABA receptors and, until recently, it was unclear if selective or pan-agonists would be necessary for modulating water use. The recent identification of the selective agonist quinabactin has resolved this issue and defined *Pyrabactin Resistance 1* (PYR1) and its close relatives as key targets for water use control. This review provides an overview of the structure and function of ABA receptors, progress in the development of synthetic agonists, and the use of orthogonal receptors to enable agrochemical control in transgenic plants.

1. Introduction

Drought is the major source of crop losses worldwide and major improvements to agricultural productivity may be realized by improving water use and drought tolerance.[1] There are many strategies for mitigating the effects of drought on yield, including the development of drought tolerant crops through breeding or transgenic approaches;[2–4] here we focus on abscisic acid (ABA **1**, Fig. 1) receptor agonists, which afford direct control of plant transpiration by targeting a highly conserved family of receptors that control a negative regulatory response pathway (Fig. 1). ABA controls plant water use primarily through modulating ion transport in guard cells, pairs of specialized epidermal cells that form a stomatal pore that opens and closes in response to environmental signals (Fig. 1). The accumulation of new biomass through photosynthesis depends on entry of atmospheric CO_2 to inner leaf mesophyll cells through stomata, but this comes at the cost of H_2O escape driven by the large difference in water vapor pressure between the inner leaf and atmosphere. Plants therefore face an intrinsic tradeoff between water conservation and growth, and consequently perturbations that reduce water consumption typically come at the cost of reduced growth. Conversely, selection for high yielding crop varieties has been associated with increased stomatal conductance in some crops.[5,6]

Although the water/growth tradeoff may appear to create an insurmountable dilemma from the perspective of increasing yields during drought, the effects of drought vary throughout a plant's life cycle. In maize, for example, drought during the early juvenile growth phases or late growth phases is less detrimental to final grain yield than during flowering, where drought can cause reproductive failure.[7] Monsanto's recently introduced DroughtGard™ trait achieves ~6% yield increases under conditions of moderate drought by overexpression of a *Bacillus subtilis* cold-stress induced RNA chaperone protein.[4] This trait reduces the water consumption of juvenile plants during water deficit, which in turn increases soil water content at flowering relative to non-GMO controls.[4] The molecular mechanism of the trait's physiological action is unclear, but it nonetheless illustrates the potential of 'water banking' to improve yield during drought. Synthetic ABA agonists, such as quinabactin (**2**) and pyrabactin (**3**),[8,9] are attractive because agonists can, in principle, enable an agrochemical strategy for water banking in any crop of interest.

2. Molecular aspects of ABA perception and action

S-(+)-ABA is a chiral sesquiterpenoid, with a decorated cyclohexenone ring appended to a dienoic acid sidechain. ABA is derived from β-carotene and was discovered in the 1960s by activity guided identification of plant growth regulators.[10–12] In addition to its role in guard cell physiology, ABA mediates other abiotic stress responses (for example freezing tolerance) and plays a central role in inducing seed dormancy, controlling root architecture,

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http://dx.doi.org/10.1016/j.bmc.2015.11.010
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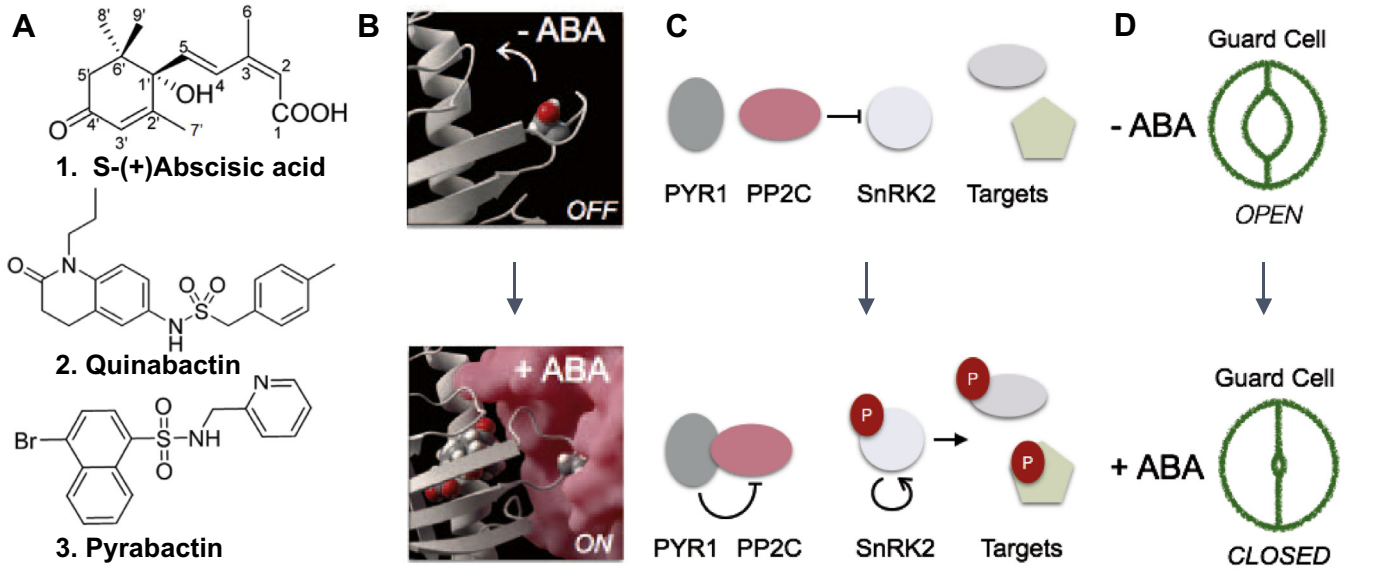

Figure 1. (A) Structures of *S*-(+) abscisic acid (**1**), quinabactin (**2**) and pyrabactin (**3**). (B) The gate-latch-lock structural mechanism for ABA recognition and biochemical activation. The receptor is depicted as a gray cartoon, ABA is depicted in a gray CPK model, and the PP2C is depicted as a pink surface. (C) Biochemical pathway downstream of activation of ABA receptors. (D) The physiological response of guard cell closure in the presence of ABA or other receptor agonists.

and influences several biotic interactions.[10] ABA biosynthesis is tied to water status and cellular osmotic pressure; reductions in osmotic pressure lead to rapid transcriptional induction of ABA biosynthetic enzymes, in particular nine-*cis* epoxycarotenoid dioxygenases (NCEDs), which act at the first committed step in ABA biosynthesis.[12] ABA levels rise greater than 25-fold under mild drought conditions due to de novo ABA biosynthesis and hydrolysis of inactive glucose-esters.[12,13] Mutants deficient in NCEDs or other biosynthetic enzymes lose leaf turgor (i.e., wilt) more rapidly than wild type plants.[12,14] Conversely, treatment of plants with exogenous ABA or synthetic agonists causes guard cell closure, reduces transpiration, and prolongs the time before wilting occurs relative to untreated plants.[8,9,13,15] ABA also induces the transcription of genes encoding enzymes that increase cellular osmolytes levels, and has other drought-protective effects.[16]

ABA responses are mediated by a negative regulatory signaling module that involves soluble *Pyrabactin Resistance 1/PYR1-Like/Regulatory Component of ABA Receptor* (PYR/PYL/RCAR) ABA receptors, clade A type 2C protein phosphatases (PP2Cs) and subfamily 3 Snf1-related kinases (SnRK2s; Fig. 1). The SnRK2s directly phosphorylate and control the activity of several downstream effectors such as transcription factors, and anion channels that are required for guard cell closure.[17] The SnRK2s autoactivate by *cis*- and *trans*-autophosphorylation, but their activity is suppressed by the PP2Cs, which dephosphorylate and inactivate the kinases.[18,19] When ABA binds to soluble PYR/PYL/RCAR ABA receptors, the receptors bind stably within PP2C active sites and inhibit PP2C activity, this in turn enables accumulation of activated SnRK2 kinases, which regulate downstream factors by direct phosphorylation.[20–22]

ABA receptors are members of the START/Bet v 1 superfamily,[5] an ancient family characterized by an α-β-α_2-β_6-α topology that forms a helix-grip fold in which 7 anti-parallel beta-sheets (and intervening short loops and helices) enclose a long C-terminal helix to form a central ligand binding pocket.[23–25] The structures of several ligand–receptor complexes have been elucidated by X-ray crystallography and depict the conformation of receptor-bound ABA as a half-chair with a pseudoaxial sidechain[26–32] (Fig. 2). ABA binding induces a conformational change that enables the receptors to dock into and inhibit PP2C activity. The largest conformational change occurs in a 'gate'-loop that flanks the ligand

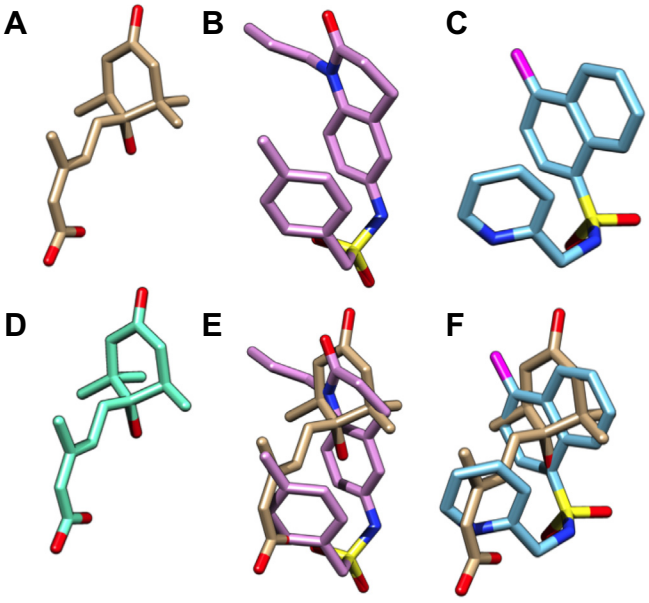

Figure 2. 3-D aligned structures of receptor-bound ABA receptor agonists. (A) ABA (3QN1), (B) quinabactin (4LA7), (C) pyrabactin (3NMN), (D) R-(−)-ABA, (E) overlay of ABA and quinabactin, and (F) overlay of ABA and pyrabactin. The PDB accessions used for the structures are listed in parentheses.

binding pocket, which adopts a closed conformation via direct hydrophobic contacts to ABA.[26,27,29,30,33] A second 'latch'-loop also changes conformation and encloses the bound ligand (Fig. 1). The sidechain of an invariant serine in the –SGLPA– gate-loop points in towards the ligand binding pocket in apo-receptor structures but becomes solvent exposed after agonist binding/gate closure, which enables the closed conformer to bind and competitively inhibit PP2C enzymatic activity. The majority of ABA recognition occurs inside the receptors and involves 25 highly conserved residues that make direct or water-mediated contacts to ABA. Additionally, a critical PP2C tryptophan located in a recognition loop, which is, specific to ABA regulated PP2Cs, called the 'lock', inserts into a small pore directly above ABA's 4′-carbon and makes a water