Review

Salicylic acid signaling in disease resistance

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ABSTRACT

Salicylic acid (SA) is a key plant hormone that mediates host responses against microbial pathogens. Identification and characterization of SA-interacting/binding proteins is a topic which has always excited scientists studying microbial defense response in plants. It is likely that discovery of a true receptor for SA may greatly advance understanding of this important signaling pathway. SABP2 with its high affinity for SA was previously considered to be a SA receptor. Despite a great deal work we may still not have true a receptor for SA. It is also entirely possible that there may be more than one receptor for SA. This scenario is more likely given the diverse role of SA in various physiological processes in plants including, modulation of opening and closing of stomatal aperture, flowering, seedling germination, thermotolerance, photosynthesis, and drought tolerance. Recent identification of NPR3, NPR4 and NPR1 as potential SA receptors and α-ketoglutarate dehydrogenase (KGDHE2), several glutathione S transferases (GSTF) such as SA binding proteins have generated more interest in this field. Some of these SA binding proteins may have direct/indirect role in plant processes other than pathogen defense signaling. Development and use of new techniques with higher specificity to identify SA-interacting proteins have shown great promise and have resulted in the identification of several new SA interactors. This review focuses on SA interaction/binding proteins identified so far and their likely role in mediating plant defenses.

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1. Introduction

Salicylic acid (SA), a simple phenolic compound is well studied for its role in activating plant defenses especially systemic acquired resistance (SAR) [1,2]. SA and its derivative (aspirin: acetyl SA) have been widely used for years as an anti-inflammatory drug. Initially SA was discovered as a major component in bark extract of willow (*Salix*) tree. Aspirin became the first synthetic drug to be used for anti-inflammatory agent [3]. The role of SA in plants was recorded for the first time in 1987 [4]. Tobacco plants treated with

aspirin, exhibited increased resistance against tobacco mosaic virus (TMV) [4,5]. Treatment with SA and its derivative induced expression of pathogenesis-related proteins [6–8]. SA is required for the activation of robust SAR and is marked by the increased expression of many defense proteins including pathogenesis-related (PR) proteins. Plants defective in SA synthesis/accumulation exhibit enhanced susceptibility to pathogens [9,10]. Besides SA, other plant hormones known for their direct/indirect role in plant signaling are jasmonic acid (JA), ethylene (ET), abscisic acid (ABA), auxins, gibberellins (GA), brassinosteroids, and cytokinins (CKs) [11]. Many of these hormone mediated signaling pathways are also known to crosstalk resulting in an antagonistic or synergistic interaction [12]. JA pathway when activated in response to herbivory or wounding triggers a systemic response similar to SAR.

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Treatment of plants with SA is known to suppress JA induced wounding response [13,14]. Some pathogen like *Pseudomonas syringe* induces activation of both SA and JA pathways [13]. Additional studies have shown that SA-induced defense mostly acts against biotrophs while JA activated defense is targeted toward wounding and necrotropic pathogens [15,16]. Activation of an immune pathway against biotrophic pathogens suppress defense against necrotropic pathogens [17,18]. *Arabidopsis* plants treated with low concentrations of JA and SA exhibited a synergistic effect on the expression of *PR1* and *PDF1.2* genes while treatment with higher concentrations resulted in an antagonistic effect [19]. Mutation in a fatty acid desaturase (*ssi2*) resulted in the upregulation of the SA pathway and suppression of the JA mediated pathway [20,21]. ET also shows extensive crosstalk with SA–JA signaling pathways. ET potentiates expression of SA dependent *PR1* gene expression in *Arabidopsis* and in tobacco plants it is required for activation of the SAR response [22,23]. ABA, an important hormone in signaling abiotic stress has recently emerged as a key component of plant immune signaling [24]. ABA antagonizes the SA mediated plant defense responses at multiple steps [11,25]. Overall, molecular details of ET, JA and SA interactions are still poorly understood and require further investigations.

2. SA biosynthesis and metabolism

In plants, SA is synthesized in plastids via two routes from chorismate, a product of the shikimate pathway. One route is through isochorismate synthase (ICS), which is believed to be responsible for >90% of SA synthesized during activation of stress response [10]. The other route uses the phenylalanine ammonia-lyase (PAL) mediated pathway [26]. SA is readily modified to its many derivatives (via glucosylation, methylations, amino acid conjugation, sulphonation, hydroxylation, etc.) but most are not active compounds [27]. Most of the SA produced in plants is glucosylated (SAG) and believed to be the main storage form with the potential to be converted back to SA through enzymatic reactions catalyzed by a SA β-glucosidase [28,29]. A methylated derivative (MeSA; methyl salicylate) is also inactive but is volatile and could readily diffuse through membranes. Volatilization of SA through MeSA synthesis could help plants excrete SA outside of the cell in which it is synthesized for eventual diffusion out of the plant [30,31]. This mechanism may help plants to reduce the accumulation of SA and its resulting toxic effects, which has the potential to cause cell death [32]. Besides plant immune signaling, SA also serves as an important signaling molecule in various physiological responses such as drought [33], thermogenesis [34–36], stomatal closure [37], seed germination [38], flowering [39–42], salt stress [43], ozone [44], and chilling [45]. A recent study suggests a role for SA in clathrin-mediated endocytic protein trafficking [46]. The main focus of this review is to discuss major SA interacting/binding proteins identified to date and their role in understanding of SA signaling pathway in disease resistance. There are a number of excellent reviews describing other aspects of SA signaling [2,27,47].

3. SA-binding proteins

To identify cellular proteins which physically interact and bind to SA, a combination of biochemical and traditional column chromatography was used. Proteins from tobacco plants which bound to SA labeled with ^{14}C or ^3H were identified, purified and characterized for their role in SA-mediated plant defense response. Several tobacco proteins were identified as SA-binding proteins. Meanwhile a genetic approach using *Arabidopsis* mutants identified a number of key components of the SA signaling pathway but did not directly identify any SA-binding proteins. The presence of redundant proteins with overlapping functions is one reason why T-DNA insertion may not be suitable for the identification of SA interacting/binding proteins.

The SABP, catalase was the first soluble plant protein found to physically bind SA [48]. SABP was identified and purified using ^{14}C-SA [49,50]. In plants, catalases are known to detoxify H_2O_2 produced during various metabolic processes. Binding of SA to the catalase resulted in inhibition of its H_2O_2 hydrolyzing activity [49]. It was hypothesized that inhibition of catalases by SA could potentially lead to accumulation of toxic H_2O_2 which then activates expression of defense genes and systemic acquired resistance (SAR). Supporting this hypothesis, another SAR inducer, 2,6-dichloroisonicotinic acid (INA), has been shown to inhibit catalase activity in tobacco [51]. Transgenic tobacco plants expressing a yeast catalase gene (*CAT1*) accumulated less H_2O_2 around Tobacco mosaic virus (TMV) induced necrotic lesions compared to control plants. TMV induced necrotic lesions were larger compared to control plants both in primary inoculated and secondary inoculated upper leaves, suggesting that catalase has a role in inducing disease resistance. Increased levels of catalase in these transgenic plants more likely detoxified H_2O_2 resulting in a decrease in its availability for activating resistance [52]. The transgenic tobacco with reduced catalase activity developed necrotic lesions and induced expression of *PR* genes only under high light conditions, suggesting that SA inhibition of catalase may not be required for the induction of the defense response. Later studies showed that SA binds to many iron containing enzymes, e.g. aconitase, catalase, lipoxidase and peroxidase suggesting that SA binding to catalase was not specific [53]. SA bound to SABP with a moderate affinity ($K_d = 14\ \mu M$) [48]. To search for high affinity SA-binding proteins, a ligand with higher affinity (^3H-SA) was synthesized and used for identification of additional SA-binding proteins [54].

SA-binding protein 3 (SABP3) was identified as a stroma localized carbonic anhydrase. It has moderate affinity $K_d = 3.7\ \mu M$ for SA compared to SABP2 ($K_d = 90$ nM) [55] (Table 1). But unlike SABP, SA binding has no effect on the carbonic anhydrase activity of SABP3 [55]. Carbonic anhydrase in animals helps to transport CO_2 out of muscle cells and provide bicarbonate to mitochondria for gluconeogenesis [56]. In C4 plants, cytosol localized carbonic anhydrase catalyzes the conversion of CO_2 into bicarbonate, which is used during carbon fixation by the C4 enzyme, phosphoenolpyruvate carboxylase [57]. In contrast, antisense carbonic anhydrase tobacco plants with 99% reduction in activity had little or no effect on photosynthesis or general fitness of the plant [58]. Recent studies using a T-DNA insertion mutant of a plastid localized carbonic anhydrase in *Arabidopsis* showed a reduction in seedling establishment compared to wild type plants at ambient CO_2 levels [59]. Overexpression of carbonic anhydrase in chloroplast led to an increase in Rubisco activity [58]. Virus-induced gene silencing of a *SABP3* homolog in *Nicotiana benthamina* led to the suppression of the Pto:avrPto mediated hypersensitive response [55]. In yet another study, carbonic anhydrase transcripts were shown to be upregulated in compatible reactions while down regulated in incompatible reactions at the 12 h time point [60]. By 24–28 h carbonic anhydrase transcripts were completely downregulated. Only by 72 h, where carbonic anhydrase transcripts upregulated again [60]. Silencing of carbonic anhydrase in *N. benthamiana* allowed increased growth of *Phytophthora infestans* [60]. These results suggest that SABP3/carbonic anhydrase is needed for positive regulation of defense responses in plants. SABP3 is a target for modification via S-nitrosylation during later stages of *R*-gene mediated protection against avirulent plant pathogens [61]. S-nitrosylation is the covalent attachment of nitric oxide moiety to a cysteine thiol of a protein to form S-nitrosothiol [62]. Modification by S-nitrosylation at Cys280 renders SABP3 unable to bind to SA and lose its carbonic anhydrase activity [63]. SABP3 is a positive