Salicylic acid (SA) has a central role in activating plant resistance to pathogens. SA levels increase in plant tissue following pathogen infection and exogenous SA enhances resistance to a broad range of pathogens. To study the relevance of the SA signaling in the flg22 response, we investigated the responses of SA-related mutants to flg22, a 22-amino acid peptide of the flagellin bacterial protein. We identified SA as an important component of the flg22-triggered oxidative burst, a very early event after flg22 detection, and gene induction, an early event. SA acted partially by enhancing accumulation of FLS2 mRNA. We also provide new evidence that NPR1 play a role in SA-induced priming event that enhances the flg22-triggered oxidative burst, which is correlated with enhancement of the flg22-induced callose deposition. Based on these observations, we conclude that SA signaling is required for early as well as late flg22 responses.

The plant immune system is broadly divided into 2: microbe-associated molecular pattern (MAMPs)-triggered immunity (MTI) and effector-triggered immunity (ETI).\cite{1-3} MTI is activated in response to the detection of MAMPs, such as flagellin. Direct recognition of the flg22 epitope of flagellin by the FLAGEL-LIN SENSING2 (FLS2) receptor protein is sufficient for inducing immune responses.\cite{4,5} ETI is mediated by resistance (R) proteins. In the host plant, recognition of an effector or its activity by the appropriate R protein triggers ETI, which leads to strong local defense responses that stop pathogen growth.\cite{2} Recent studies have shown considerable overlap in the events occurring during MTI and ETI. Both involve SA accumulation, pathogenesis-related (PR) gene expression, and systemic acquired resistance (SAR).\cite{6-9}

Flg22 recognition by FLS2 initiates a plethora of signaling responses in sequential order. Very early responses, within 1 to 5 minutes, triggered by flg22 include the production of reactive oxygen species (ROS), altered ion fluxes across the plasma membrane, and the activation of mitogen-activated protein kinases. Early responses follow within 2 to 30 minutes and include ethylene biosynthesis, receptor endocytosis, and gene activation. Late responses include callose deposition, seedling growth inhibition,\cite{10} and SA biosynthesis,\cite{6} and occur within hours to days.

In Arabidopsis, basal levels of cellular SA are approximately 15 μM. Following pathogen infection, SA is synthesized through the isochorismate pathway\cite{11-13} and can increase to approximately 200 μM. Flg22 treatments also enhance SA biosynthesis. This increased synthesis is dependent on SID2, which encodes an enzyme in the isochorismate pathway. Flg22-induced SA caused gene expression change and pathogen growth inhibition.\cite{6} These results led us to ask if SA signaling was involved in very early flg22 responses, specifically in the oxidative burst. To test this, we measured the flg22-triggered oxidative burst in the wild-type Arabidopsis (WT, ecotype Columbia) and the following mutants: sid2 and eds5 are deficient in SA biosynthesis and have low SA levels compared to the WT\cite{14,15}, cim6 has higher SA levels than the WT,\cite{16} and npr1 has a block in SA signaling.\cite{17} The flg22-triggered oxidative burst was suppressed in sid2 and eds5 compared to the WT, while it was greater in cim6. Because the oxidative burst occurred within a few minutes of flg22 exposure, it is possible that endogenous basal SA was responsible for the...
oxidative burst, acting at an early step in the signaling pathway. Another early event, gene activation, was also measured by qRT-PCR analysis. Flg22-dependent induction of WRKY29 and FRK1 genes was reduced by almost 50% in the sid2 mutant compared to WT. This results suggest that basal SA is required to establish sufficient flg22-triggered early responses such as oxidative burst and marker gene induction.

The levels of the FLS2 transcript and its protein affect the flg22-triggered oxidative burst. According to a recent report, ethylene signaling contributes to FLS2 expression. The ethylene insensitive protein 2 (EIN2)-dependent transcription factors, EIN3 and EIN3-like1 (EIL1), directly control FLS2 expression. In our system, basal FLS2 mRNA was strongly enhanced in ein6 and suppressed in sid2, compared to the WT. However, this suppression was less in sid2 than in ein2. A possible explanation is that ISOCHORISMATE SYNTHASE2 (ICS2) may have a partially redundant function, by mutation in isochorismate synthase 1 (ICS1) so that SA accumulation is not completely blocked in sid2. Basal expression of EIN2 mRNA was also suppressed in sid2 compared to WT. It may be that the basal levels of SID2 and SA in the WT plant are required for EIN2 transcript accumulation, a suggestion that implies that SA signaling components are accompanied by other factors, such as EIN2, on regulation of FLS2 gene. Based on our observations, we suggest that SA contributes to early flg22 responses through activating FLS2 mRNA accumulation. Signaling downstream from SA is primarily regulated by NPR1. NPR1 functions in SA-mediated induction of pathogenesis-related gene 1 (PR1) in the nucleus. In our study, NPR1 was required for SA-mediated priming of the enhanced flg22 responses. Pretreatment with low concentrations of SA enhanced not only the FLS2 transcript level but also the amplitude of FLS2-mediated flg22 responses, including the oxidative burst and callose deposition, mediated by NPR1. Other reports are consistent with our finding that NPR1 plays a role in SA-mediated priming for enhanced defense responses. Although the molecular basis of the SA-mediated priming effect in flg22 responses is unclear, we hypothesize that SA pretreatment act at the post-translational level through protein modification. SA has been shown to control the nuclear translocation of NPR1 through cellular redox changes. NPR1 homeostasis is controlled by SA binding to NPR3 and NPR4 in a concentration-dependent manner. In WT plants, basal SA may bind to the NPR4 complex, thereby allowing some NPR1 to accumulate, conferring basal resistance.

In our system, pre-incubation with SA alone promoted FLS2 transcript accumulation compared to the untreated plant. The enhanced level of FLS2 mRNA and free stable NPR1, possibly due to SA pretreatment, might contribute to accelerated FLS2-dependent flg22 responses.

Conclusions

Our study suggests an effect of SA signaling at a very early stage of the flg22 response, the oxidative burst. This very early effect may have consequences in the late flg22 responses, such as callose deposition. The molecular basis is currently unknown but warrants further study.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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