

The Biosynthetic Pathways of Salicylic Acid in Plants

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Summary : Salicylic acid (SA) is a signaling molecule which plays an essential role in plant resistance to pathogens. Biosynthesis of SA is induced by many environmental stresses including pathogen attack. In bacteria, SA is synthesized from chorismate via isochorismate by the reactions of isochorismate synthase and isochorismate pyruvate lyase. In plants, biosynthetic pathways of SA are still not fully understood; however, two pathways have been proposed to date. The first is isochorismate pathway in which, similar to bacteria, SA is simply produced from chorismate via isochorismate. The second pathway is phenylpropanoid pathway which is initiated by phenylalanine ammonia lyase from chorismate-derived phenylalanine. In phenylpropanoid pathway, SA has been proposed to be synthesized from benzoic acid by the reaction of benzoic acid 2-hydroxylase. In this review, the advances in the understanding of SA biosynthetic pathways and their regulatory mechanisms will be summarized.

Key word : Disease resistance, salicylic acid, biosynthesis, ICS, PAL

Salicylic Acid Plays an Important Role in Plant Resistance

Plants are sessile organisms and thus are always exposed to a risk of infection by pathogenic microbes. To cope with the problem, plants have evolved sophisticated defense mechanisms which are rapidly induced by pathogen attack. In response to pathogen attack, plants produce many hormones and signaling molecules which mediate induction of defense reactions. One of such signaling molecules is salicylic acid (SA). SA is induced when plants are exposed to pathogen attack and then induces many defense responses including expression of defense genes such as pathogenesis-related proteins¹. The importance of SA in resistance to pathogens has been genetically confirmed. Reduction of SA levels in *Arabidopsis thaliana* and tobacco (*Nicotiana tabacum*) by the mutation in SA biosynthetic gene or expression of SA degrading enzyme compromised resistance to fungal, bacterial and viral pathogens^{2,3}.

Proposed Biosynthetic Pathways of SA

Classically, the biosynthetic pathways of SA have been studied by tracer experiments using radioisotope-labeled putative precursors of SA in many plant species such as potato (*Solanum tuberosum*), pea (*Pisum sativum*) and rice (*Oryza sativa*). These experiments have suggested that SA is produced from *trans*-cinnamic acid, the first product of the phenylpropanoid pathway which is initiated by phenylalanine ammonia lyase (PAL)^{4,5} (Fig. 1). Subsequent analyses in tobacco suggested that the final step of SA biosynthesis is 2-hydroxylation of benzoic acid (BA) catalyzed by BA 2-hydroxylase (BA2H) activity although *BA2H* gene is not identified to date⁶. The importance of PAL in SA biosynthesis has been confirmed by pharmacological and genetic experiments. In *Arabidopsis*, exogenous application of PAL inhibitor suppressed SA induction and compromised resistance to oomycetes pathogen⁷. Similarly, tobacco plants, in which *PAL* expression is suppressed by co-suppression, showed reduced level of SA induction after tobacco mosaic virus inoculation⁸. These results have strongly indicated that SA production is mediated

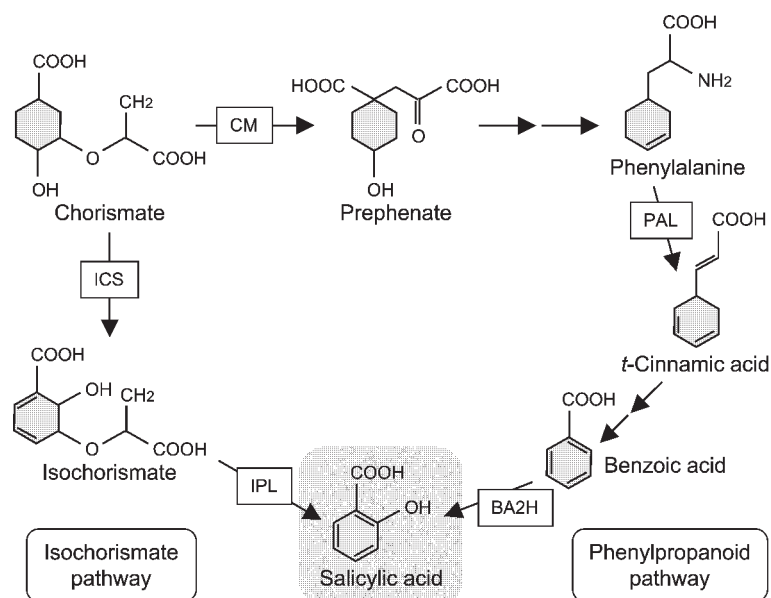


Fig. 1 Proposed pathways of SA biosynthesis in plants.

BA2H, benzoic acid 2-hydroxylase; CM, chorismate mutase; ICS, isochorismate synthase; IPL, isochorismate pyruvate lyase; PAL, phenylalanine ammonia lyase.

by PAL.

Wildermuth et al. (2001)⁹, however, found that *Arabidopsis* SA induction deficient 2 (*sid2*) mutants, which produce a lower level of SA than wild-type plants following pathogen attack, have a defect in isochorismate synthase (ICS) 1. In bacteria, SA is synthesized from chorismate via isochorismate by the reactions of ICS and isochorismate pyruvate lyase (IPL)¹⁰. Subsequently, it was reported that silencing of *Nicotiana benthamiana* ICS also reduces SA production induced by biotic and abiotic stresses¹¹. These lines of genetic evidence clearly showed the involvement of ICS in pathogen-induced SA production and raised the possibility that plants also produce SA by the reactions of ICS and IPL as do bacteria (Fig. 1). This hypothesis, however, contradicts the key role of PAL in SA biosynthesis which has been proposed for a long time⁷. Very recently, the importance of PAL in SA production was further supported by the report that quadruple knockout mutants of *Arabidopsis* *PAL1*, *PAL2*, *PAL3* and *PAL4* show substantially reduced levels of SA and display increased susceptibility to a bacterial pathogen¹². Therefore SA biosynthetic pathways in plants will be not as simple as those in bacteria. In fact, there are no genes which show significant

similarity to bacterial IPL in *Arabidopsis* genome.

Interestingly, SA levels in the healthy state of plants vary dramatically dependent on species. For example, healthy rice contains more than 50 folds SA than do *Arabidopsis* and tobacco⁵. Therefore rice and *Arabidopsis* might use different SA biosynthetic pathways. This hypothesis is supported by the fact that isochorismate is a precursor of not only SA, but also phyloquinone, which is required for photosynthesis¹³. If a large amount of SA is constitutively produced from isochorismate, plant will suffer from a decrease in the production of phyloquinone. The mechanisms that rice maintains such a high level of SA will be a subject of future analysis.

Regulation of SA Biosynthetic Pathways

Regulatory mechanisms of SA biosynthetic pathways have been poorly understood except for the transcriptional regulation of *ICS1*. Very recently, Zhang et al. (2010)¹⁴ reported that two transactors, *SARD1* and *CBP60g*, are required for pathogen-induced SA synthesis in *Arabidopsis*. They are recruited to the promoter of *ICS1* in response to pathogen infections, and overexpression of *SARD1* resulted in the constitutive accu-

mulation of SA. These results clearly showed that one of key mechanisms of SA induction in *Arabidopsis* is the transcriptional induction of *ICS1*.

In addition to *sid2* (*ICS1*), genetic analysis in *Arabidopsis* identified *eds5/sid1* mutant which does not accumulate SA after pathogen inoculation^{3,15}. Map-based cloning of *EDS5* revealed that it is homologous with members of the MATE (multidrug and toxin extrusion) transporter family¹⁶. This result suggested that *EDS5* might be involved in the transport of SA or its precursors, but biochemical function of *EDS5* remains unclear.

Several lines of evidence have indicated the involvement of mitogen-activated protein kinases (MAPKs) in the negative regulation of SA biosynthesis. In *Arabidopsis*, loss-of-function mutant of *MPK4* constitutively accumulates a large amount of SA and induces defense reactions which are inducible by SA¹⁷. Recently, we found that SA is abnormally induced by wounding in the tobacco plants silenced with *WIPK* and *SIPK*, two pathogen- and wound-induced MAPKs¹⁸. Future identification of target proteins of these MAPKs will help our understanding in the negative regulation of SA biosynthesis.

Concluding remarks

As described above, the biosynthetic pathways of SA and its regulatory mechanisms remain still unclear despite its importance in plant resistance. Although two SA biosynthetic pathways have been proposed to date, enzymes which catalyze the final step of SA biosynthesis have not been identified. Future identification of *BA2H* and/or *IPL* will help us understand the SA biosynthetic pathways.

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植物におけるサリチル酸生合成経路

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要約

サリチル酸 (salicylic acid: SA) は植物の病害抵抗性に不可欠のシグナル物質である。SA の生合成は病原体ストレスをはじめとした様々な環境ストレスにより誘導される。細菌において、SA はイソコリスミン酸合成酵素とイソコリスミン酸ピルビン酸リアーゼの反応により、コリスミン酸からイソコリスミン酸を介して合成される。植物における SA 生合成経路はまだ完全には明らかになっていないが、二つの合成経路が提唱されている。一つ目はイソコリスミン酸経路であり、細菌と同様に SA はコリスミン酸からイソコリスミン酸を介して合成される。二つ目はコリスミン酸由来のフェニルアラニンよりフェニルアラニンアンモニアリアーゼの働きにより開始されるフェニルプロパノイド経路である。フェニルプロパノイド経路においては、SA は安息香酸より安息香酸 2-水酸化酵素の反応により合成されると考えられている。本総説では、SA 生合成経路とその制御機構について現在までに得られた知見を総括する。