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Article / Book Information

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論 文 要 旨

THESIS SUMMARY

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学籍番号： Student ID Number			指導教員（主）： Academic Advisor(main)	Professor Yuki YAMAGUCHI	
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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

Salicylic acid is a classic non-steroidal anti-inflammatory drug that was originally discovered as an active ingredient in willow bark. Although the drug has been in use for at least 3500 years, it is now replaced by acetyl salicylic acid (aspirin), which is rapidly hydrolyzed to salicylic acid *in vivo*. In addition to their analgesic actions, aspirin and salicylic acid have various adverse pharmacological effects, including gastrointestinal toxicity and mitochondrial injury. Although several proteins, such as cyclooxygenase (COX), IκB kinase (IKK-β), and adenosine monophosphate-activated protein kinase (AMPK), are now recognized as therapeutic targets of aspirin and/or salicylic acid, a comprehensive understanding of the pharmacological mechanism of action of these drugs has not yet been achieved. In this study, I present ferrochelatase (FECH), a terminal enzyme in the heme synthesis pathway, as a new molecular target of salicylic acid that mediates its anti-mitochondrial activity. Several lines of evidence support the idea that salicylic acid decreases heme biosynthesis by inhibiting FECH: (i) FECH binds to salicylic acid -immobilized beads (Chapter 2), (ii) salicylic acid inhibits the enzymatic activity of recombinant FECH *in vitro* (Chapter 2), (iii) salicylic acid binds to FECH at the dimer interface, which is thought to be critical for its activity (Chapter 3), (iv) salicylic acid decreases heme content in cultured human cells, and its inhibitory effect is abrogated by the knockdown of FECH (Chapter 4), (v) salicylic acid inhibits heme synthesis in zebrafish, which can be partially rescued by the overexpression of zFECH (Chapter 4), and (vi) salicylic acid isomers, *meta*-hydroxybenzoic acid and *para*-hydroxybenzoic acid, are less effective than salicylic acid in all the assays examined.

To identify new targets of salicylic acid, a one-step affinity purification scheme was carried out by using salicylic acid-immobilized beads. FECH was purified by these beads. Also, salicylic acid was found to specifically inhibit recombinant FECH activity. To understand the mechanism of salicylic acid inhibition, co-crystal structure of FECH-salicylic acid complex was determined by X-ray crystallography at a resolution of 2.0 Å. In the complex structure, FECH was found to exist as dimer with salicylic acid bound at the dimer interphase. *In vitro* site-directed mutagenesis study validated the salicylic acid binding site and it was also found that residues Trp301 and Leu311 are important for salicylic acid interaction. Further, gel filtration experiments showed that salicylic acid inhibits FECH enzymatic activity by causing conformational changes in FECH. Thus, in Chapter 2 and Chapter 3, I clearly showed that FECH is a novel target of salicylic acid.

To determine the significance of this interaction, salicylic acid induced effects on FECH activity were studied *in vivo*. In agreement with the *in vitro* results, salicylic acid decreases heme synthesis in K562 human erythroleukemia cells and zebrafish. Knockdown of FECH in 293T cells abrogated the effect of salicylic acid; while overexpression of FECH in zebrafish rescued the salicylic acid induce heme decrease in zebrafish. At pharmacologically relevant concentrations salicylic acid was also found to induce mitochondrial injury in K562 cells. Salicylic acid-treated zebrafish embryos also showed a substantial increase in the FECH substrate protoporphyrin-IX detected by its auto fluorescence. Thus, results in Chapter 4 suggest that inhibition of FECH activity by salicylic acid is involved, at least in part, in salicylic acid -induced mitochondrial injury.

Since, heme serves as a prosthetic group of many hemoproteins, such as cytochromes in the electron transport chain and COXs in the prostaglandin synthesis pathway, a reduction of heme synthesis by salicylic acid may contribute to the other pharmacological effects of salicylic acid. Possibly, salicylic acid-mediated inhibition of mitochondrial ATP synthesis may result in the release of adenosine, an endogenous anti-inflammatory agent, and therefore may be partially responsible for its anti-inflammatory activity. Clearly, further investigation is required to determine the possible role of FECH in the many pharmacological effects of salicylic acid.

備考 ： 論文要旨は、和文 2000 字と英文 300 語を 1 部ずつ提出するか、もしくは英文 800 語を 2 部提出してください。
Note : Thesis Summary should be submitted in either a copy of 2000 Japanese Characters and 300 Words (English) or 2 copies of 800 Words (English).