Characterization of a Novel Oxylipin Defense Signal in Maize

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Introduction

Plant oxylipins constitute a family of oxygenated fatty acid-derived small molecules formed by enzymatic processes or chemical (auto)oxidation. The predominant mode of synthesis is initiated enzymatically by lipase cleavage of linoleic acid (LA) or α-linolenic acid (LeA) from membrane lipids and subsequent processing by the 9- or 13-lipoxygenase (LOX) pathways. Accumulating evidence demonstrates the potent signaling activity of plant oxylipins in development, reproduction, resistance against abiotic stress, and modulation of innate immune responses against pathogens and pests. Recently, we discovered a series of maize cyclopente(a)none oxylipins that are produced in response to pathogen infection [1]. Abundant in dying necrotic tissue, we termed these metabolites “death acids” (DAs). One of the DAs predominantly induced by pathogens is DA$^{0-4:0}$ (4-[(1, 5)-2-oxo-5-pentylcyclopent-3-ene-1-yl] butanoic acid), a small molecule derived from the 18-carbon cyclopentenone 10-oxo-11-phytoenoic acid (10-OPEA). Two β-oxidation steps shorten the octanoate side chain to butanoate, followed by enzymatic reduction of the cyclopentenone ring via oxo-phytodienoic acid reductase (OPR) to yield the saturated product DA$^{0-4:0}$. Interestingly, DA$^{0-4:0}$ is strongly induced by the common maize pathogen *Cochliobolus heterostrophus* and demonstrates potent signaling activity with regulation of maize defense genes such as Pathogenesis-Related 4b, OPR2, and Glutathione-S-transferase 2. The focus of this project was to use NMR to elucidate the chemical structure of a novel oxylipin defense signal and gain a better understanding of plant responses to pathogen attack.

Experimental

Infected maize tissue was ground in liq. N$_2$ and extracted with ethyl acetate. The resulting organic extract was concentrated and purified by silica flash chromatography using a Teledyne silica system. Fractions were screened for the presence of oxylipins using GC/MS on a DB-35 column and EI mass detector. Compounds of interest were purified by multiple HPLC steps including both normal and reverse phase conditions followed by GC/MS analysis. Purified oxylipines were acquired in CDCl$_3$ (Cambridge Isotope Laboratories, Inc.), 2.5-mm NMR tubes (Norell) at 22 °C using a 5-mm TXI cryprobe (Bruker Corporation) and a Bruker Avance II 600 console (600 MHz for 1H and 151 MHz for 13C). 1D and 2D NMR spectroscopy were performed using Bruker Topspin 2.0 and MestReNova (Mestrelab Research) software packages.

Results and Discussion

An oxylipin with a molecular weight of 240 was isolated by extraction of infected corn tissue and subsequent chromatography. Careful NMR analysis of the purified metabolite confirmed a novel 14-carbon cyclopentanone derivative of 10-OPEA, termed DA$^{0-4:0}$. The structure was determined using correlations from 1D and 2D experiments.

Conclusions

We have identified DA$^{0-4:0}$ and four additional related maize oxylipins. Initial investigations into biological activities demonstrated potent transcriptional regulation of genes associated with pathogen defense.

Acknowledgements

This project is supported by AMRIS grant ML-Schmelz-001 and NSF grant 1139329.

References


Figure 1. Structure and predicted biosynthesis of DA$^{0-4:0}$. Red dashed arrows indicate predicted enzyme activity requiring future confirmation.