Physiological role of novel sterol-amino acid derivatives in fungi.

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Research objective

Sterols are ubiquitous in eukaryotic cell membranes. Fungi contain ergosterol as their principal sterol, which is functionally equivalent to cholesterol in animals and plays diverse roles, including the regulation of membrane fluidity, osmotic balance, and membrane protein activity. Since ergosterol is specific to fungi, it has long served as a target for antifungal drug development. Several ergosterol derivatives, including fatty acid esters, acetylated forms, and glycosylated derivatives, have been reported; however, the physiological roles of most of these derivatives remain largely unknown. We recently identified a novel class of ergosterol derivatives in filamentous fungi termed ergosterol–amino acid derivatives (Erg-aa), in which an amino acid is conjugated to a hydroxyl group at the C3 position[1]. To-date, Erg-aa has not been identified in any other organisms. Erg-aa was discovered through functional characterization of a domain of unknown function (DUF2156) fused to a tRNA synthetase (Fig. 1). Fungal aspartyl-tRNA synthetase (AspRS) containing a C-terminal DUF2156 catalyzes the formation of 1-ergosteryl-L-aspartate (Erg-Asp) in a tRNA-dependent manner; this enzyme is designated ErdS[1]. In addition, the stand-alone DUF2156 enzyme catalyzes the formation of 1-ergosteryl glycine (Erg-Gly) and was named ErgS[2]. ErdS is highly conserved in the Dikarya subkingdom, which includes Ascomycota and Basidiomycota, and represents approximately 70% of all fungi. These observations suggested that Erg-aa plays a physiologically important role in filamentous fungi. Functional studies using *erdS* and *ergS* knockout mutants of *Aspergillus oryzae* revealed that conidiation was reduced to 20–50% of wild-type levels. Furthermore, these mutants produced numerous sclerotia when cultured on agar plates. A similar phenotype was observed in the *erdS* knockout mutant of *Fusarium graminearum*. Collectively, these results indicate that Erg-aa plays a critical role in the regulation of both conidiation and sclerotium formation in filamentous fungi. In this study, we aimed to clarify the relationship between Erg-aa and physiological phenomena, including conidiation and sclerotium formation, as well as to elucidate the molecular pathways through which Erg-aa acts.

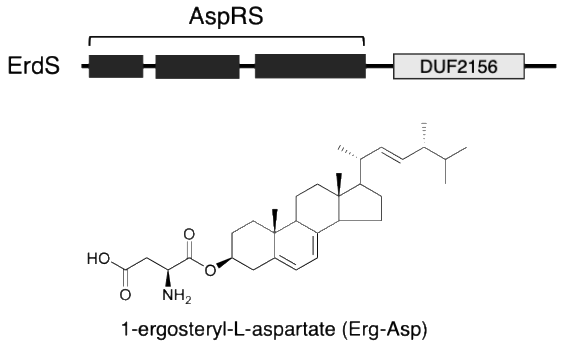

Fig. 1 Domain structure of ErdS and its product Erg-Asp

Methods

Wild-type *A. oryzae* (HiMe10 strain), the *erdS* deletion mutant, and the *ergS* deletion mutant were cultured on minimal agar medium (Czapek-Dox (CD) medium) for 3–5 days, and the expression levels of genes related to conidiation and sclerotium formation were quantitatively analyzed by RT-qPCR. To investigate the relationship with reactive oxygen species (ROS), the strains were also cultured for 5 days on CD medium supplemented with 1.6 μM copper(II) sulfate, or with one of the following antioxidants: glutathione (15 mM), DMSO (5%), tiron (100 μM), or butylated hydroxyanisole (BHA) (100 μM).

To clarify whether Erg-Asp is involved in pathogenicity, wild-type *F. graminearum* and the *erdS* deletion mutant were inoculated onto the tips of wheat coleoptiles at 3 d post-germination, and the lesion size was measured after 7 d of cultivation.

To examine the relationship between Erg-Asp and sexual reproduction, an *AnerdS* deletion mutant of *A. nidulans* was generated. A strain in which the upstream region of *AnerdS* was replaced with the xylose-inducible promoter (P*xylP*), thereby allowing repression or induction of *AnerdS* expression, was also constructed (P*xylP-AnerdS* strain). Analysis of sterigmatocystin was performed by extracting 5-day-old cultured mycelia with chloroform, followed by HPLC (column: ODS-80T_M, solvent: acetonitrile/water = 45/55).

Results

Wild-type *A. oryzae*, *erdS* deletion mutant, and *ergS* deletion mutant were cultured on minimal agar medium for 5 days, and expression analyses of genes related to conidiation and sclerotium formation were performed by RT-qPCR. Expression levels of *brlA*, *abaA*, and *wetA*, the central transcription factors for conidiation, were significantly reduced in the *erdS* and *ergS* deletion mutants compared to the wild type[3)]. In contrast, no significant differences were observed in the expression levels of *sclR*, which positively regulates sclerotium formation, or *ecdR*, which negatively regulates this process. Similarly, no differences were observed in *veA*, *velB*, or *vosA*, which constitute the velvet complex that balances conidiation and sclerotium formation. To investigate the relationship between Erg-Asp and reactive oxygen species (ROS), wild-type, *erdS*, and *ergS* deletion mutants were grown on media supplemented with copper(II) ions, which are thought to suppress sclerotium formation by enhancing antioxidant enzyme activity, or with antioxidants. Under these ROS-inhibiting conditions, sclerotia formation was abolished. These findings suggest that loss of Erg-Asp or Erg-Gly promotes sclerotium formation by enhancing the intracellular accumulation of ROS.

To examine how Erg-Asp affects the pathogenicity of plant fungi, wild-type and *erdS* deletion mutants of *F. graminearum* were inoculated into wheat coleoptiles. The *erdS* deletion mutant showed a significant reduction in lesion size, indicating that Erg-Asp positively affects

the pathogenicity of *F. graminearum*.

To explore the relationship between Erg-Asp and sexual reproduction, an *AnerdS* mutant of *A. nidulans* was constructed by replacing the upstream region of the *AnerdS* gene with the xylose-inducible P*xylP* promoter, yielding the P*xylP-AnerdS* strain. Under xylose-supplemented conditions, induction of *AnerdS* expression led to excessive accumulation of Erg-Asp, whereas under glucose-supplemented conditions, *AnerdS* expression was repressed and Erg-Asp was not produced, mimicking the *AnerdS* deletion mutant. When the P*xylP-AnerdS* strain was cultured under these conditions, an increase in cleistothecia was observed compared to the wild type, regardless of whether *AnerdS* expression was induced or repressed. This suggests that Erg-Asp is involved in sexual reproduction and that its endogenous level may influence the balance between sexual and asexual reproduction. Furthermore, when the accumulation of sterigmatocystin (ST), a representative secondary metabolite of *A. nidulans*, was examined, ST levels increased significantly when *AnerdS* expression was repressed. These results indicated that Erg-Asp also affects the production of secondary metabolites.

Conclusions

The present study revealed that Erg-aa promoted conidiation by altering the expression of genes involved in conidiation, but had no effect on the velvet complex. Analyses using *A. nidulans* further demonstrated that Erg-aa influenced cleistothecium formation, indicating its involvement in sexual reproduction. In addition, Erg-aa was suggested to be involved in sclerotium formation via ROS signaling. As ROS signaling in filamentous fungi regulates asexual and sexual reproduction, sclerotium formation, pathogenicity, and secondary metabolism, it was inferred that Erg-aa is closely associated with ROS signaling. Future studies will aim to elucidate how Erg-aa modulates ROS signaling. Additionally, the observation that Erg-aa influences the pathogenicity of phytopathogenic fungi highlights its potential as a promising target for the development of novel antifungal agents.

References

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