Deciphering and reprogramming the cyclization regioselectivity in bifurcation of indole alkaloid biosynthesis

Zhuo Wang,[1,2#] Yiren Xiao,[1,2#] Evangelos C. Tatsis[1, 3*]

[1]CAS Center for Excellence in Molecular Plant Sciences, Shanghai Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, Shanghai, 200032, China
[2]University of Chinese Academy of Sciences, Beijing, China
[3]CEPAMS - CAS-JIC Centre of Excellence for Plant and Microbial Sciences
E-mail: etatsis@cemps.ac.cn; evangelos.tatsis@jic.ac.uk

Background *Akuammilan* alkaloids are a structurally diverse class of monoterpene indole alkaloids (MIAs) isolated mainly from plants of Apocynaceae family across different parts of the world. *Akuammilan* alkaloids exhibit a broad range of biological activities, like rhazimol (**1**) and akuammiline (**2**) are considered to be the bioactive ingredients of Traditional Chinese Medicine application Deng-tai-ye which is based on extracts from leaves and bark of *Alstonia scholaris* tree to treat diseases. Here we report the discovery of novel enzymes from *Alstonia scholaris* tree that together synthesize the indole alkaloid akuammiline with the unique methanoquinolizidine cage structure and investigated the catalytic mechanism of the two P450 enzymes by homology modelling and reciprocal mutations.

Results and Disccusion

1. *Akuammilan* alkaloids

C. roseus GO catalyses the oxidative cyclization and rearrangement including bond formations between C2-C16, C3-C7 to synthesis of *strychnos* alkaloid akuammicine. The SBE (CYP71AY4) from *R. serpentina* catalyses the oxidative cyclization between C5-C16 to generate polyneuridine aldehyde marking the distinctive transition into *sarpagan* alkaloids metabolism (Figure 1).

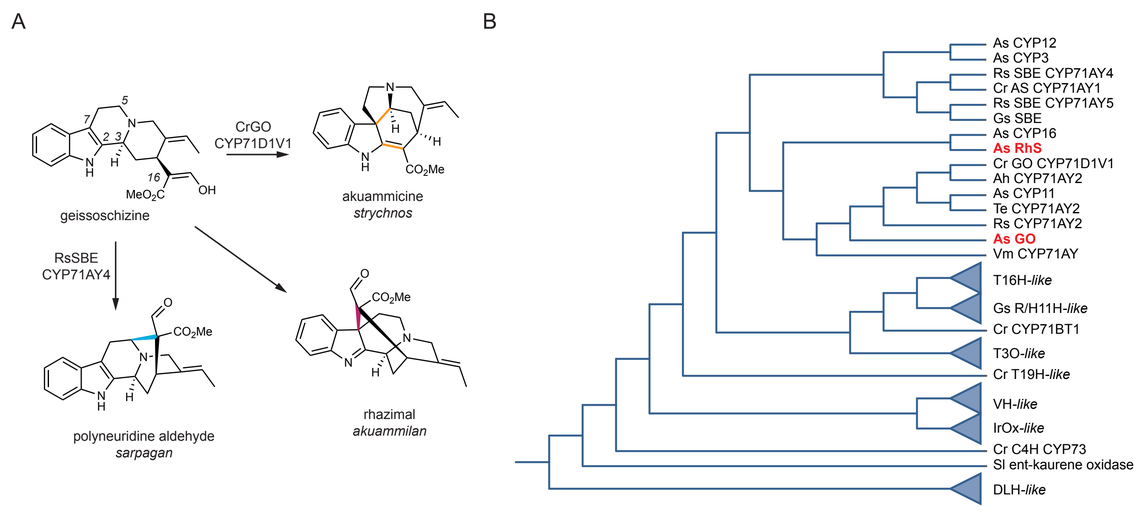

Figure 1. *Akuammilan* alkaloids and their close biogenetic relationships with *strychnos* and *sarpagan* alkaloids.

2. Pathway characterization of *Akuammilan* alkaloids

The entry point to akuammilan biosynthesis is the transformation of geissoschizine through intramolecular cyclization and the bond formation between C7 and C16 of geissoschizine for the synthesis of aldehyde rhazimal. Here we characterized five new enzymes with novel RHS, RHR and AKS activities that together synthesize akuammiline.

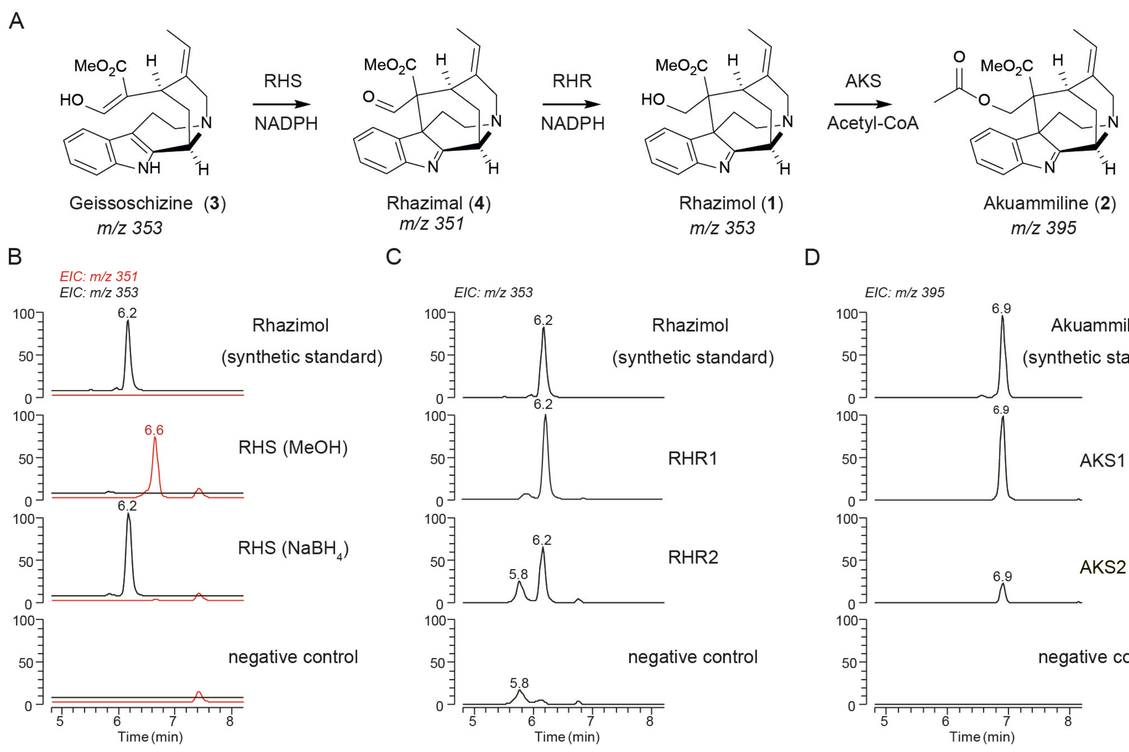

Figure 2. Discovery of akuammiline biosynthetic pathway genes.

3. Mutagenesis analysis on active site

The key amino acid AsRHSF372V displayed reduced RHS activity while its GO activity seemed to be favoured. The mutant enzyme AsGOV372F
was the only mutant AsGO enzyme that observed RHS activity. It imply the key role of F372/V372 amino acids in regioselectivity of cyclization reactions on geissoschizine. The model structures (Figure 3) demonstrate that the side chains of the two amino acids at position 372 occupy the space between the heme and the substrate (geissoschizine).

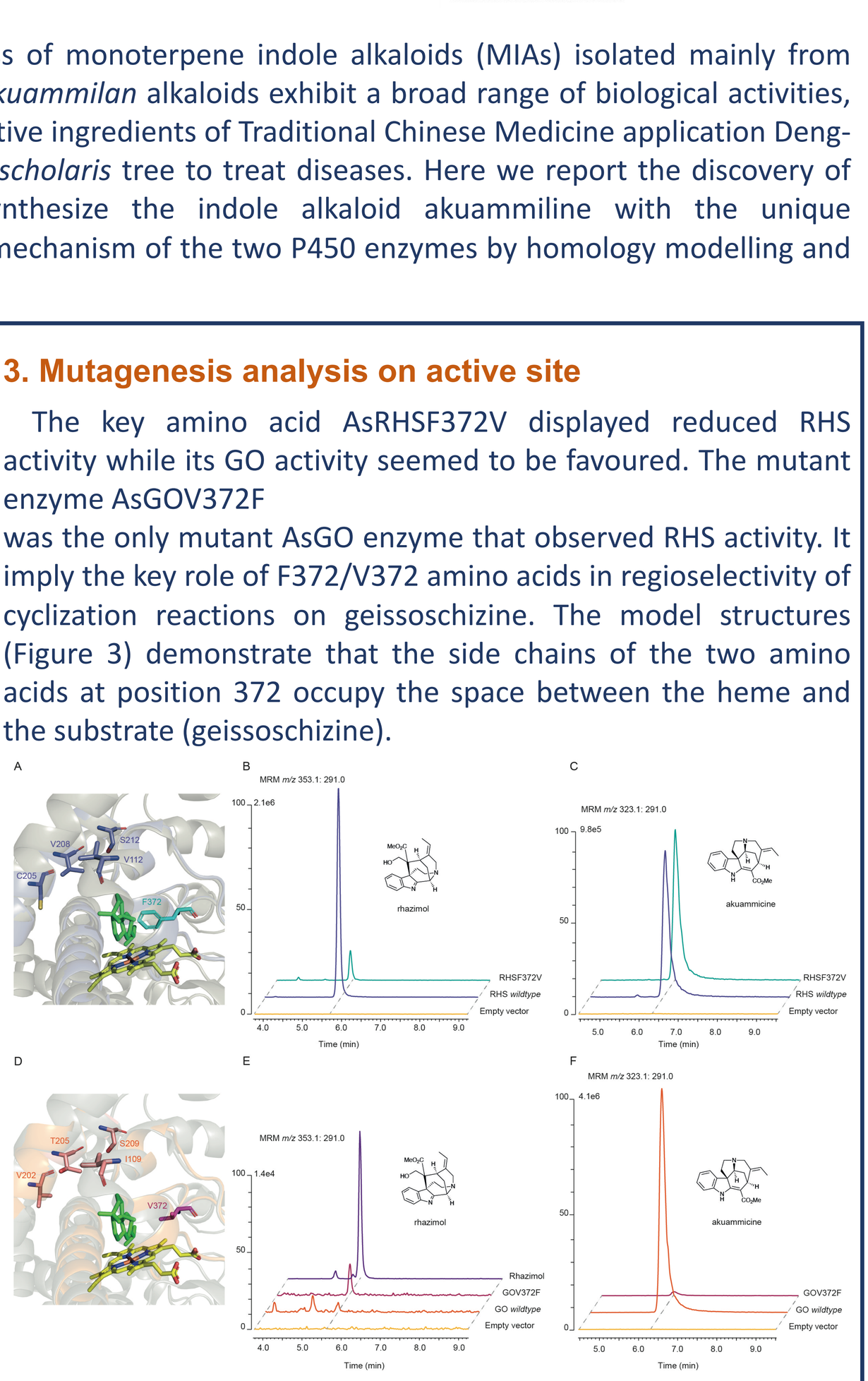

Figure 3. Molecular modelling, and mutagenesis on active site of AsRHS and AsGO.

4. Proposed mechanism of rhazimal formation

The AsRHS first catalyse the oxidation of geissoschizine and then is followed by a nucleophilic attack from the keto-enol function of geissoschizine which serves as an excellent nucleophile may proceed the formation of rhazimal.

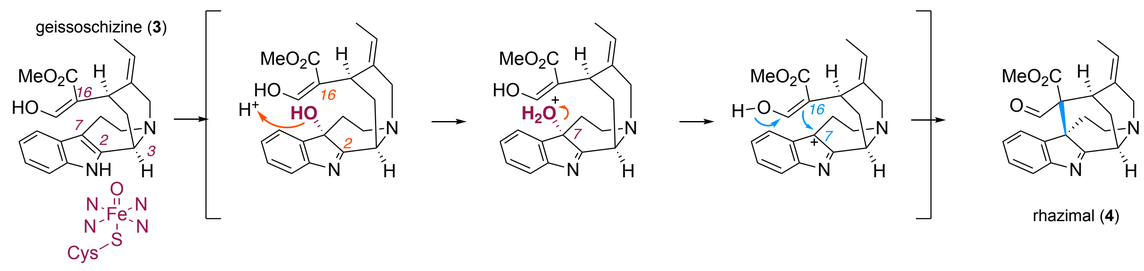

Figure 4. Proposed mechanism for catalytic activity of RHS

In conclusion, the discovery of these enzymes demonstrates the astonishing biochemistry that occurs in plants, and the current work paving the way for discovery downstream genes in *akuammilan* alkaloids biosynthesis and facilitate future synthetic biology applications.

Reference

1. E. C. Tatsis, I. Carqueijeiro, T. Dugé de Bernonville, J. Franke, T. T. Dang, A. Oudin, A. Lanoue, F. Lafontaine, A. K. Stavrinides, M. Clastre, V. Courdavault and S. E. O'Connor, Nat Commun, 2017, 8, 316.
2. T. T. Dang, J. Franke, I. S. T. Carqueijeiro, C. Langley, V. Courdavault and S. E. O'Connor, Nat Chem Biol, 2018, 14, 760-763.
3. K. Yamamoto, D. Grzech, K. Koudounas, E. A. Stander, L. Caputi, T. Mimura, V. Courdavault and S. E. O'Connor, Plant Physiol, 2021, 187, 846-857.