Oxidative C-H activation for C-C bond formation: Characterization of EasC/EasE and comparison to CnsA/CnsD

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INTRODUCTION

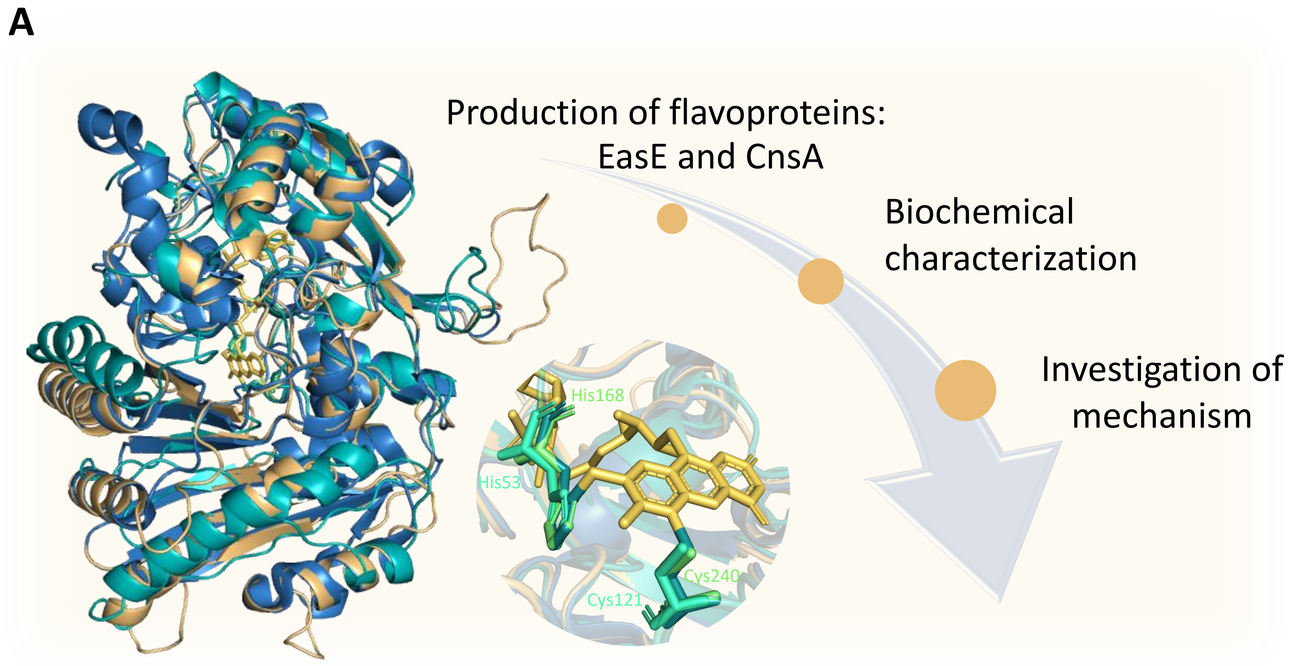

Figure 1: Homology models of EasE/CnsA and EasC/CnsD and the initial steps in alkaloid biosynthesis where the proteins are involved: **(A)** Overlay of the homology models of EasE (orange) and CnsA (teal) in comparison to *Ec*BBE (blue). The bicovalent linkage of the FAD cofactor is shown in the close-up view of the active site. **(B)** Overview of the initial and conserved part in ergot alkaloid bionsynthesis (above dashed line) and one branch of the initial biosynthetic pathway of communesins (below dashed line) starting from L-tryptophan. The interaction of EasE and EasC has been solved recently[1]. While EasE seems to be crucial for the formation of the intermediate "diene moiety" compound, EasC turned out to be the key enzyme, which leads to the ring closure and results in chanoclavine I formation. In contrast, the flavoprotein CnsA is responsible for the ring closure reaction to form *trans*-methyl-claviciptic acid and CnsD functions as a decarboxylase to form aurantioclavine. **(C)** Overlay of the homology models of EasC (orange) and CnsD (teal) in comparison to Peroxisomal Catalse from *Hansenula polymorpha* (blue). Amino acids in the range of 3.5 Å near the heme cofactor are shown in the close-up view - all amino acids that are identical are shown in grey, the amino acids that are different in EasC and CnsD are shown in the respective color of the model.

A crucial step in alkaloid synthesis is the carbon-carbon (C-C) bond formation. In synthetic organic chemistry, this step remains still challenging and therefore, time consuming and expensive. Hence, special emphasis has been placed on the field of enzyme catalyzed C-C bond formation in the last two decades.

To investigate this ring closure mechanism, **EasE -** a flavin adenine dinucleotide (FAD) dependent homologue of the berberine bridge enzyme-like (BBE) enzyme **-** and **EasC -** a heme dependent catalase **-** originating from ***Aspergillus japonicus*** should be produced and investigated. Further, **CnsA** (BBE-like) and **CnsD** (catalase), which represent a similar protein pair in ***Penicillium expansum***, will be investigated.

For a long time, the interactions of EasC and/or EasE to form chanoclavine I from *N*-Me-DMAT as well as CnsA and/or CnsD to form aurantioclavine from DMAT, was unclear. Just recently, this mystery has been solved[1,2]. Interestingly, the respective homologues from the different organisms catalyze different reactions[2], which makes further investigations all the more important.

FLAVOPROTEINS

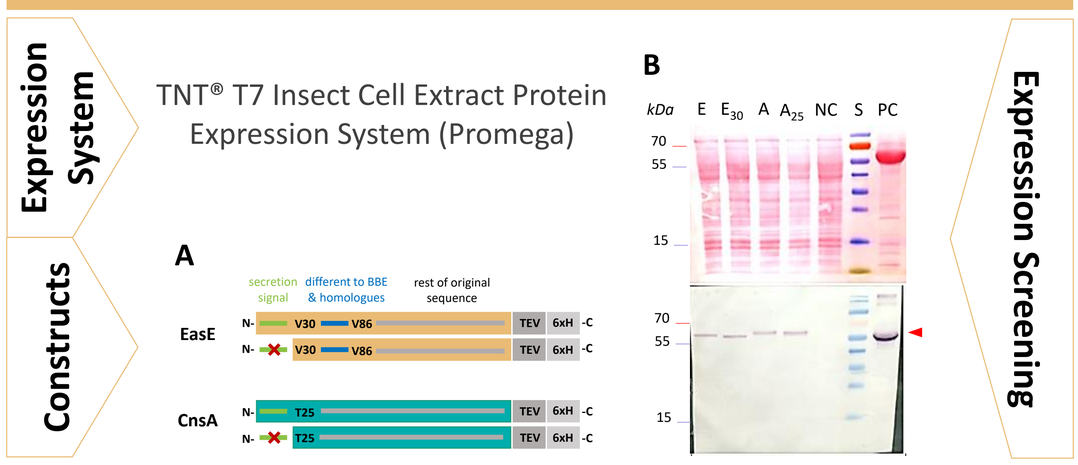

Figure 2: Expression of cnsA and easE: **(A)** Schematic representation of the coding sequences and the resulting constructs that were inserted into pF25K ICE T7 Flexi® Vector, **(B)** PonceauS staining and Western blot showing the flavoproteins at ≈64-68 kDa (red arrow): E=EasE, E_{30}=$EasE_{30}$, A=CnsA, A_{25}=$CnsA_{25}$, NC=extract w/o plasmid, S=PageRuler™ Prestained Protein Ladder, PC=purified heme protein (His-tag control)

HEME-PROTEINS

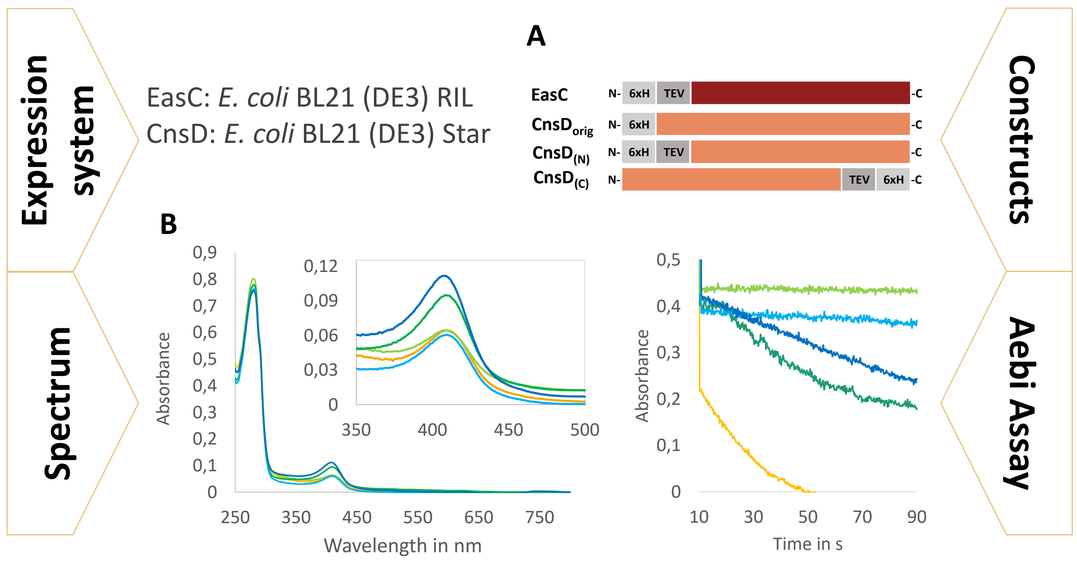

Figure 3: Expression of cnsD and easC: **(A)** Schematic representation of the coding sequences and the resulting constructs that were inserted into pET-30Xa/LIC ($CnsD_{orig}$) and pET-M11 ($CnsD_{(N)}$, $CnsD_{(C)}$, EasC) **(B)** Spectra of the native proteins showing the characteristic protein peak at 280 nm and the heme cofactor at 410 nm (left) and the corresponding H_2O_2 depletion assay (right) – expression conditions: with and w/o addition of 0.5 mM Δ-Ala: $CnsD_{orig}$+ Δ-Ala (orange), $CnsD_{(N)}$ (light green), $CnsD_{(N)}$+ Δ-Ala (dark green), $CnsD_{(C)}$ (light blue), $CnsD_{(N)}$+ Δ-Ala (dark blue)

OUTLOOK

- Switch from cell-free environment into insect cells for expression of *cnsA* and *easE*
- Optimization of expression conditions for *easC* and *cnsD*
- Crystallization and solving of crystal structure

1 Yongpeng Yao, Chunyan An, Declan Evans, Weiwei Liu, Wei Wang, Guangzheng Wei, Ning Ding, K. N. Houk, and Shu-Shan Gao, *Journal of the American Chemical Society* **2019** *141* (44), 17517-17521

2 Kuan-Lin Chen, Chen-Yu Lai, Mai-Truc Pham, Rong-Jie Chein, Yi Tang, and Hsiao-Ching Lin, *Organic Letters* **2020** 22 (8), 3302-3306