Project Ute Krämer

Biosynthesis of the natural high-affinity metal chelator nicotianamine with applications in biofortification and medicine

Background and preliminary work: Nicotianamine (NA), a high-affinity metal chelator and low-molecular-mass non-proteinogenic amino acid, is a well-known natural product of flowering plants. NICOTIANAMINE SYNTHASE (NAS) proteins, first discovered in tomato and barley, catalyse the biosynthesis of NA from three molecules of S-adenosylmethionine (SAM).

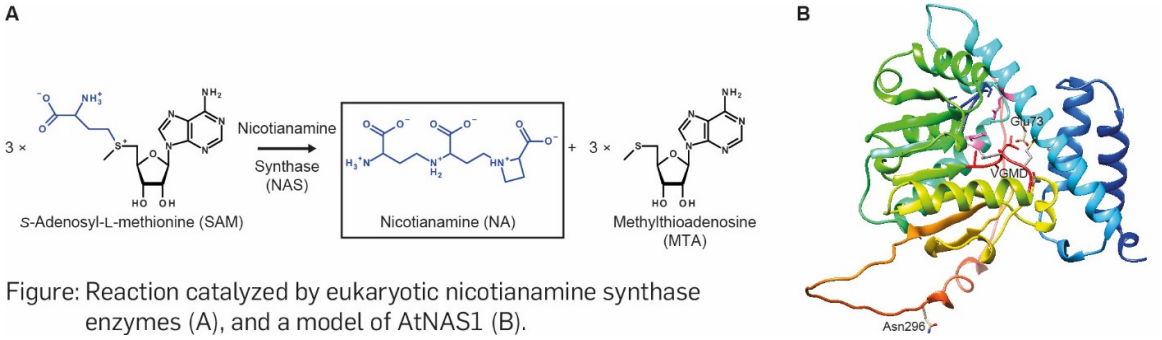

Figure: Reaction catalyzed by eukaryotic nicotianamine synthase enzymes (A), and a model of AtNAS1 (B).

Long after the identification of NAS, similar proteins were discovered in other organisms: NA is also synthesized by some filamentous fungi, and NA-related chelators are produced by some archaea and bacteria. Indispensable in flowering plants, NA maintains the inter-cellular mobility of the nutrient metals, for example iron and zinc (Zn), and NA-derived molecules named phytosiderophores are required for root iron uptake by YELLOW STRIPE-LIKE transporters in cereals. NA has become a prime target compound in crop biofortification towards combating human malnutrition, and it also possesses anti-hypertensive pharmacological activity.
NAS and NAS-related proteins catalyse several consecutive reactions in a single active site, as was concluded based on crystal structures of an NAS-like archaeal thermoNAS. Its reaction product thermoNA contains a glutamate instead of the terminal SAM-derived azetidine-2-carboxylate moiety characteristic of NA. After establishing a quantitative NAS enzyme assay, the Krämer group discovered that NAS proteins of flowering plants consist of a core-NAS domain and a C-terminal extension that is autoinhibitory *in vitro*. They also pinpointed a number of essential residues at the active site of *Arabidopsis thaliana* NAS1. Earlier, the group showed that the Arabidopsis membrane transport protein ZINC-INDUCED FACILITATOR 1 (ZIF1) mediates the vacuolar sequestration of NA, which functions to control sub-cellular and whole-plant Zn partitioning. Interestingly, no proteins acting in the catabolism of NA are known. A possible NA breakdown product – the toxic proline analogue and allelopathic chemical azetidine-2-carboxylate – was detected in only a few plants to date.

Work planned: The proposed project will address **structure-function relationships of fungal and plant NAS proteins** and **explore avenues for enhancing cellular NA production**.
(1) Our first goal is to identify residues of *Neurospora crassa* NAS required for intermediate and final product formation during catalysis, including the formation of the azetidine ring that is exceedingly rare in biological chemistry. To this end, we will combine site-directed mutagenesis, biochemical *in vitro* and *in vivo* activity assays, and LC-mass spectrometry-based identification of products (cooperation with Frank Schulz), based on protein structural modelling using alphafold2 and phyre2 (cooperation with Eckhard Hofmann; see Figure above).
(2) Our second goal is to understand the mechanisms underlying NAS autoinhibition and its release. Following up on our previous observations, we will use site-directed mutagenesis to comprehensively map all amino acids required for autoinhibition of AtNAS1 and AtNAS4 in the C-terminal extensions as well as on the surface of the core-NAS domains. Furthermore, we will employ sequence comparisons and amino acid/domain swaps with NcNAS. Biochemical activities and properties of the C-terminal peptides will be analysed using microscale thermophoresis, and Inductively-Coupled Plasma Optical Emission and Mass Spectrometry (ICP-OES, ICP-MS) for the quantification of bound metal cations, for instance. Both project parts

will involve the use of heterologous yeast (*Saccharomyces cerevisiae*) and homologous plant expression systems.

Selected references:

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