Biosynthesis of Gliotoxin: Studies on a Fungal Virulence Factor

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Infections with the human pathogenic fungus *Aspergillus fumigatus* are often lethal to immunocompromised patients. One of the virulence factors of *A. fumigatus* is the secondary metabolite gliotoxin. Belonging to the class of epipolythiodioxopiperazine compounds, gliotoxin features a reactive transannular disulfide bridge that is crucial for its toxicity. The biosynthesis of gliotoxin requires numerous proteins, 13 of which are organized in the *gli* gene cluster. Although most enzymes have been assigned a specific function and their overall order of action has been elucidated, some steps are mechanistically still poorly understood or catalyzed by uncharacterized enzymes.

The talk will present the X-ray structures of six enzymes engaged in gliotoxin biosynthesis along with biochemical, mutagenesis, molecular modeling and activity data. The first two enzymes act on the toxic, reduced version of gliotoxin. The oxidoreductase GliT oxidizes in a flavin-adenin-dinucleotide dependent manner the free dithiols to a disulfide bridge. Being structurally and mechanistically related to thioredoxin reductases, GliT protects *A. fumigatus* from damage by reduced gliotoxin[1]. Alternatively, the enzyme TmtA, which has been spotted outside of the *gli* gene cluster, methylates the thiol groups of reduced gliotoxin[2] and the resulting bis-methyl derivative shuts down gliotoxin biosynthesis.

Besides, two additional methyltransferases, GliN and GliM, are encoded in the *gli* gene cluster. While *N*-methylation by GliN is pivotal for stability and toxicity of gliotoxin, GliM has not been functionally characterized so far. The X-ray structures of the three methyltransferases associated with gliotoxin biosynthesis, TmtA, GliN, and GliM, are compared with each other and functional implications are derived[3].

The sulfur atoms of gliotoxin originate from two glutathione molecules that are attached to the diketopiperazine core by the enzyme GliG. Our X-ray data on GliG in complex with its reaction product, illustrates the bis-glutathione-adduct and allows for a mechanistic proposal of C-S bond formation[3].

Finally, the structure of GliJ is discussed. GliJ is a dipeptidase that together with two other enzymes breaks down the glutathione moieties attached by GliG. The active site of GliJ contains two catalytically relevant metal ions that can be of surprisingly diverse nature[4].

Altogether, the biosynthesis of gliotoxin involves numerous types of chemical reactions. Because of their pseudosymmetry, most gliotoxin intermediates require symmetric step-wise processing and the respective enzymes need to deal with two different but closely related substrates. Biochemical and structural studies thus provide insights into the enzymatic reaction mechanisms and may enable *in vitro* reconstitution of the pathway in the future.

[1] D. H. Scharf, M. Groll, A. Habel, T. Heinekamp, C. Hertweck, A. A. Brakhage, E. M. Huber, *Angew. Chem. Int. Ed. Engl.* **2014**, *53*, 2221-2224.

[2] E. R. Duell, M. Glaser, C. Le Chapelain, I. Antes, M. Groll, E. M. Huber, *ACS Chem Biol* **2016**, *11*, 1082-1089.

[3] unpublished data.

[4] A. Marion, M. Groll, D. H. Scharf, K. Scherlach, M. Glaser, H. Sievers, M. Schuster, C. Hertweck, A. A. Brakhage, I. Antes, E. M. Huber, *ACS Chem Biol* **2017**, *12*, 1874-1882.