Retracing the Rapid Evolution of an Herbicide-Degrading Enzyme by Protein Engineering

Reinhard Sterner

Institute of Biophysics and Physical Biochemistry, Regensburg Center for Biochemistry, University of Regensburg, D-93040 Regensburg, Germany.

The mechanisms underlying the rapid evolution of novel enzymatic activities from promiscuous side activities are poorly understood. Recently emerged enzymes catalyzing the catabolic degradation of xenobiotic substances that have been spread into the environment only during the last decades provide an exquisite opportunity to study these mechanisms. A prominent example is the herbicide atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine), which is degraded by several consecutive enzymatic reactions, constituting the Atz pathway. We analyzed the evolution of the hydroxyatrazine ethylaminohydrolase AtzB, a Zn(II)-dependent metalloenzyme that adopts the popular amidohydrolase fold and catalyzes the second step of the Atz pathway. We started by searching for promiscuous side activities of AtzB, which might point to the identity of its progenitor. These investigations revealed that AtzB has a low promiscuous guanine deaminase activity. Furthermore, we found that the two closest AtzB homologues, which have not been functionally annotated up to now, are guanine deaminases with modest promiscuous hydroxyatrazine hydrolase activity. Based on sequence comparisons with the closest AtzB homologues, the guanine deaminase activity of AtzB could be increased by three orders of magnitude by only four active site mutations. Interestingly, the inverse four mutations introduced into AtzB homologues resulted in a hydroxyatrazine hydrolase activities that in one case even equaled that of wild-type AtzB. Molecular dynamics simulations elucidated the structural and molecular basis for the mutation-induced activity changes. The example of AtzB highlights that novel enzymes with high catalytic proficiency can evolve from low promiscuous side activities by only few mutational events within a short period of time.

Reference: Markus R. Busch, Lukas Drexler, Dhani Ram Mahato, Caroline Hiefinger, Sílvia Osuna & Reinhard Sterner (2023). *ACS Catalysis* **13**, 15558-15571.