Old enzymes versus new herbicides

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The introduction of manmade chemicals, including the herbicide atrazine, into the environment has led to the emergence of microorganisms with new biodegradation pathways. Esquirol et al. demonstrate that the AtzE enzyme catalyzes a central step in atrazine degradation and that expression of AtzE requires coexpression of the small protein AtzG. Remarkably, AtzG and AtzE appear to have evolved from GatC and GatA, components of an ancient enzyme involved in indirect tRNA aminoacylation, providing an elegant demonstration of metabolic repurposing.

When introduced into the environment, synthetic chemicals can have unexpected consequences on bacterial metabolism. For example, the introduction of synthetic s-triazines into the environment as herbicides and in plastics has provided selective pressure for the evolutionary emergence of new metabolic degradation pathways against them. These ad hoc pathways can result from the development of completely new enzyme activities or enhancement of existing activities (often amplifying moonlighting roles of known proteins). They can include multiple steps co-opted from distinct biochemical processes or a single permissive enzyme to allow flux through known pathways. Elucidation of these strategies can therefore lead to the discovery of unexpected enzymatic transformations and offers promise for the development of bioremediation processes. A remediation pathway for the herbicide atrazine would be especially welcome, as this controversial compound has been banned in Europe due to its accumulation in groundwater (1), but one key enzymatic step in this pathway has remained unknown. Esquirol and colleagues (2) now fill this gap through their characterization of two proteins that work together to remove ammonia from an atrazine degradation intermediate. This protein complex and its catalytic activity are derived from an unexpected evolutionary source, once again demonstrating nature’s resourcefulness.

Pseudomonas sp. strain ADP carries a plasmid (pADP1) that encodes the machinery necessary to degrade atrazine to carbon dioxide and ammonium, via cyanoacetic acid, a common intermediate in the enzymatic degradation of s-triazines (3). Most of the atrazine degradation enzymes encoded in pADP1 have been characterized, with the notable exception of AtzE, which proved recalcitrant to heterologous expression and purification for in vitro studies. The predominating hypothesis was that AtzE would be a biuret amidohydrolase (BiuH),2 catalyzing the conversion of biuret to allophanate during atrazine degradation (Fig. 1A). However, direct evidence of this activity remained elusive (2), and AtzE does not share sequence homology with known BiuH enzymes (4).

Esquirol and colleagues (2) successfully purified AtzE directly from Pseudomonas sp. strain ADP by following ammonia production. No evidence of biuret hydrolysis, the anticipated reaction, was detected. Instead, they discovered that AtzE deaminates 1-carboxybiuret to produce 1,3-dicarboxyurea and ammonia (Fig. 1B, top panel) (2). Unexpectedly, AtzE co-purified with a 7.4-kDa protein, the product of a previously neglected gene in the atrazine catabolism operon that they named AtzG. Heterologous co-expression in Escherichia coli of AtzG and AtzE from an atzGE operon led to the successful isolation of these two proteins in a complex. Subsequent enzyme assays demonstrated that AtzE is indeed a 1-carboxybiuret hydrolase (Fig. 1B). AtzE also shows substrate promiscuity, as it is capable of hydrolyzing 1-carboxyammonamide, 1-nitrobiuret, and succinimide (2).

The authors solved the crystal structure of the AtzE-AtzG complex (2). Interestingly, AtzE and AtzG share sequence and structure homology with the ancient, bacterial proteins GatA and GatC, respectively, suggesting an origin for their evolution. GatA and GatC are part of a heterotrimeric GatCAB complex found in many bacteria (5). GatCAB is the final enzyme in a process called indirect tRNA aminoacylation, which is essential in organisms that lack genes for asparaginyl-tRNA synthetase (AsnRS) and/or glutaminyl-tRNA synthetase and therefore cannot produce Asn-tRNAAsn or Gln-tRNAGln directly (Fig. 1, bottom panel). GatCAB hydrolyzes glutamine and uses the resulting ammonia to convert the misacylated tRNAs Asp-tRNAAsn and Glu-tRNAGln (produced by misacylating aspartyl- and glutamyl-tRNA synthetases, respectively) into Asn-tRNAAsn and/or Gln-tRNAGln. GatCAB represents the ancestral pathway for the production of these two aminoacyl tRNAs, and it appears to have existed prior to the last universal common ancestor (LUCA). In contrast, glutaminyl-tRNA synthetase and AsnRS are believed to be the last two aminoacyl-tRNA synthetases to have evolved, probably emerging post-LUCA (6).

The gatCA and atzGE systems share other similarities in addition to structure. For example, heterologous protein expression of GatA in E. coli requires a gatCA operon (7, 8), analogous to the atzGE operon that was required for successful heterologous expression of AtzE. The reason(s) behind these coexpression requirements remain unclear for both systems. GatA and AtzE also catalyze similar reactions (Fig. 1, boxes):

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2 The abbreviations used are: BiuH, biuret amidohydrolase; AsnRS, asparaginyl-tRNA synthetase; LUCA, last universal common ancestor.
AtzE hydrolyzes 1-carboxybiuret (2) and GatA hydrolyzes glutamine (5); both produce ammonium as a product. Given these similarities, it is reasonable to conclude that a copy of the atrzABC operon was used as a starting point for the evolution of atzGE.

The active sites of both AtzE and GatA contain a general amidase signature sequence (KSS). One notable difference is that AtzE, like other amidases, releases ammonium into the medium (2), whereas GatA uses its ammonium product (as ammonia) to convert Asp-tRNA^Asn and Glu-tRNA^Gln into Asn-tRNA^Asn and Gln-tRNA^Gln, respectively. The amino acid side chains in Asp-tRNA^Asn and Glu-tRNA^Gln are phosphorylated in GatB prior to transamination (shown as a P). Co-expression of GatC is required for stable overexpression and purification of GatA.

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