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 cyanuric 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), 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-carboxyamidomamide, 1-nitrobiuret, and succinamic acid (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 Gln-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):
Phylogenetic and additional structural studies are needed to unravel the mode of action of atrazine, particularly when other nitrogen sources are limiting. AtzGE for a different, unknown reason, is speculated that AtzH is also important for the delivery of 1-carboxybiuret from cyanuric acid and AtzGE, as 1-carboxybiuret is an unstable intermediate that can spontaneously decarboxylate.

Figure 1. AtzGE and GatCA catalyze similar reactions. A, it has been proposed that AtzE would catalyze the hydrolysis of biuret to allophanate as a central step in the degradation of atrazine and other S-triazines; but, this activity had not been demonstrated. B, top: AtzGE actually deaminates 1-carboxybiuret to produce ammonium and 1,3-dicarbonylurea. The ammonium is released into the medium. Co-expression of AtzG is required for stable overexpression and purification of AtzE. Bottom: the GatA subunit of the GatCAB complex deaminates L-glutamine, producing ammonium; this ammonium is used by GatB (in the same enzyme complex) to convert two misacylated tRNAs, Asp-tRNAAsn and Glu-tRNAGln, into Asn-tRNAAsn and Gln-tRNAGln, respectively. The amino acid side chains in Asp-tRNAAsn and Glu-tRNAGln are phosphorylated in GatB prior to transmission (shown as a P). Co-expression of GatC is required for stable overexpression and purification of GatA.

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 gatCA 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 (ammonia) to convert Asp-tRNAAsn and Glu-tRNAGln into Asn-tRNAAsn and Gln-tRNAGln (Fig. 1) (5). GatCAB contains a long ammonia tunnel that delivers ammonium from GatA to GatB where it is used for transmission (9). It is unlikely, but not impossible, that another component of the atrazine biodegradation pathway forms a GatB-like complex with AtzGE. However, it is more likely that stable or transient interactions occur between AtzD (the enzyme that produces 1-carboxybiuret from cyanuric acid) and AtzGE, as 1-carboxybiuret is an unstable intermediate that can spontaneously decarboxylate. Such interactions would allow for direct delivery of 1-carboxybiuret from AtzD to the active site of AtzE. In fact, 1-carboxybiuret was generated in situ by AtzD in the evolution of AtzE. Additionally, the atzGE genes are part of a larger operon (atrDGEHF). Prior to this work, the atrH gene, like atzG, had remained unannotated. This atzH gene putatively encodes a 129-amino acid protein whose function remains unknown. It is possible that AtzH is also important for the delivery of 1-carboxybiuret to AtzE or that it assembles into a complex with AtzGE for a different, unknown reason.

Many environmental bacteria have the capability to degrade atrazine, particularly when other nitrogen sources are limiting. Phylogenetic and additional structural studies are needed to better understand why some organisms use biuret hydrolyase (e.g. BiuH from Rhizobium leguminosarum bv. viciae 3841 (4)) versus 1-carboxybiuret hydrolyase (AtzE) during the breakdown of cyanuric acid to carbon dioxide and ammonium. Pseudomonas sp. strain ADP lacks AsnRS and has retained a functional copy of GatCAB for the biosynthesis of Asn-tRNAAsn. Phylogenetic analyses could also help pinpoint the organism or clade that chose to co-opt GatCAB for atrazine degradation. The utilization of GatCAB as the precursor to AtzGE was obviously opportunistic, but it was also clever, in that it capitalized on the structural similarities between L-glutamine (the GatA substrate) and 1-carboxybiuret (the AtzE substrate). The amidase Lys-Ser-Ser catalytic triad in GatA could be conserved in AtzE with active site mutations only required to adapt to 1-carboxybiuret. By ignoring GatB, the third component of the GatCAB enzyme, any requirement for tRNA as a substrate was instantly eliminated. These intriguing evolutionary and functional connections between GatCA and AtzGE highlight the remarkable creativity used by bacteria to respond to new evolutionary pressures, the introduction of atrazine into the environment in this case. Fragments of aminoacyl-tRNA synthetases are found throughout evolution (10). This work demonstrates that indirect tRNA aminoacylation can also be a source of alternative functions.

References