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An unexpected role for ergothioneine

Zedu Huang and Wilfred A. van der Donk*

Ever since the appearance of atmospheric oxygen, organisms have needed ways to protect themselves from damage caused by oxidative stress. Glutathione (GSH, Fig. 1A) is the dominant organothiol in eukarya to detoxify products formed from oxidative stress. In bacteria, several different small molecule thiols have evolved including GSH, mycothiol (MSH, Fig. 1A), a cysteinyl pseudo-disaccharide found in actinomycetes, bacillithiol discovered first in bacilli and since then found in specific bacterial genera, and ergothioneine (EGT, Fig. 1A) produced by fungi and actinobacteria and widely taken up by plants and vertebrates [1]. These compounds have in common that they are more stable to oxidation than cysteine, and hence serve as an improved reservoir of reducing agents. Of these, EGT is the only compound that is not a direct cysteine derivative, although its sulfur atom still derives from cysteine [2]. EGT's thiol–thione equilibrium lies in favor of the thione, but the thione has thiolate resonance character that may account for its reported antioxidant activity, although its specific physiological role has yet to be elucidated [3].

In addition to their role as an intracellular redox buffer, GSH and MSH detoxify many endogenous electrophilic compounds and xenobiotics through the action of their nucleophilic sulfhydryl group [4,5]. Both compounds also serve as cofactors in some isomerization reactions, and GSH plays a role in the biosynthesis of some important natural products. For example, the sulfur atoms in gliotoxin originate from GSH [6]. However, EGT had not been observed to engage in any enzymatic reactions until the recent work by Professor Wen Liu and coworkers

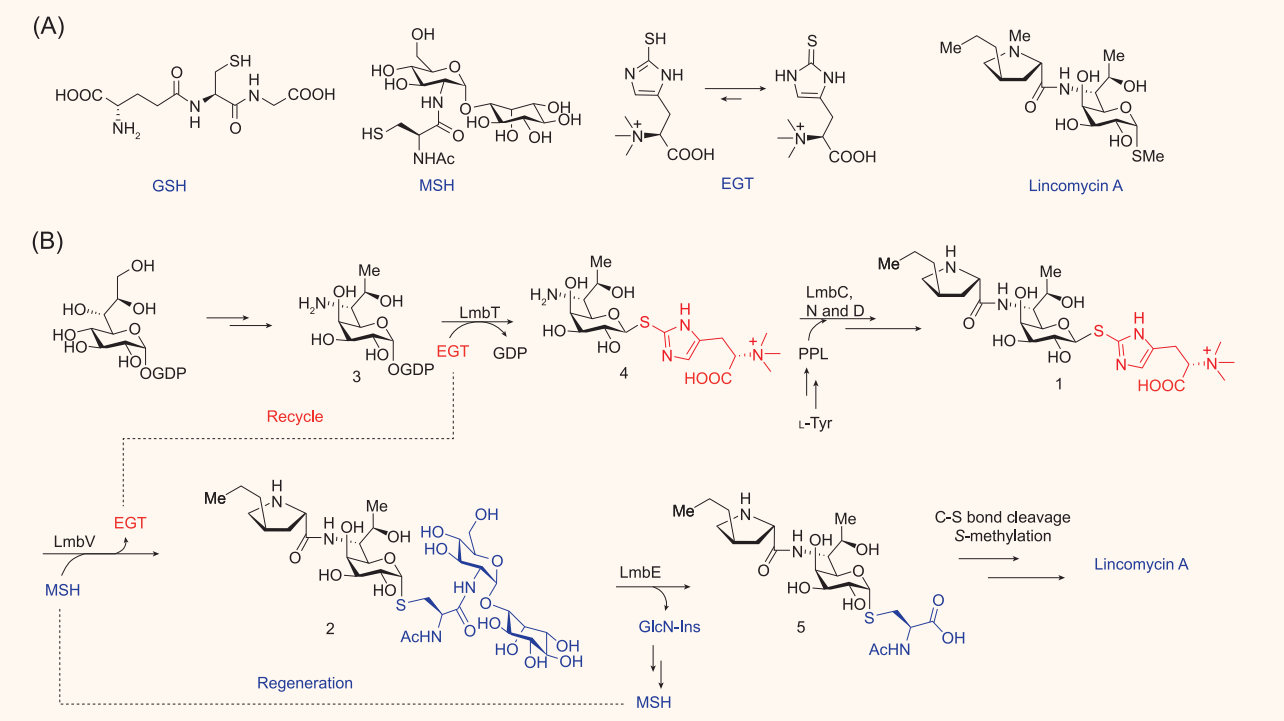

Figure 1. (A) Structures of GSH, MSH, EGT, and lincomycin A. (B) Involvement of EGT (red) and MSH (blue) in the biosynthesis of lincomycin A. Panel B is adapted from [7].

on the biosynthesis of lincomycin A (Fig. 1A) [7].

The Liu group at the Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences holds a long-standing interest in the biosynthesis of natural products. Their recent work regarding the biosynthesis of the clinically used antibiotic lincomycin A (Fig. 1A) has revealed that MSH is the ultimate donor of sulfur in the natural product, but that unexpectedly, EGT is conjugated to several biosynthetic intermediates. Their findings not only provide key insights into the biosynthesis of an antibiotic that is also the precursor of the semisynthetic drug clindamycin, and they also present the first biochemical evidence for EGT-associated enzymatic reactions [7]. This report also sets the stage for a search of other examples in which EGT may be a temporary sulfur carrier during sulfur metabolism.

Professor Liu and coworkers used a state-of-the-art approach combining genome sequencing, synthetic chemistry, genetics, and *in vitro* biochemistry to unravel the biosynthesis of lincomycin A. A key piece of their detective work came from inactivation of one of the genes in the lincomycin biosynthetic gene cluster (*lmbV*), which resulted in the unexpected accumulation of compound **1** that contained EGT via a β-S glycosidic linkage (Fig. 1B). Then, using an ortholog of LmbV (the enzyme CcbV involved in the biosynthesis of lincomycin cousin celesticetin), they demonstrated enzymatic exchange of the β-linked EGT in **1** for α-linked MSH in **2**. This result then raised the question of how EGT is incorporated into **1**. As summarized in Fig. 1B, the authors were able to demonstrate that the glycosyltransferase (GTase) LmbT catalyzes the displacement of guanosine diphosphate (GDP) from GDP-D-α-D-lincosamide (**3**) by EGT to generate an EGT *S*-conjugated lincosamine (**4**). Thus, the EGT released by LmbV can be recycled to be used in the LmbT reaction. The pathway is then driven forward by the coupling of compound **4** with 4-propyl-L-proline (PPL) by the combined action of LmbC, LmbN, and LmbD to afford **1**. LmbE, a member of the amidase family that is involved in MSH-mediated detoxification, then selectively cleaves the amide bond to furnish compound **5** and GlcN-Ins, which can be used to regenerate MSH. Finally, compound **5** is expected to be transformed to lincomycin A by several downstream enzymes [7].

The demonstration that EGT acts as a carrier in lincomycin A biosynthesis is the first demonstration of the involvement of EGT in enzymatic reactions. Why EGT is used as a temporary scaffold for the LmbCDN reactions rather than installing MSH from the start is not clear, but the answer might be related to the evolutionary history of these enzymes that may have had an EGT binding site. In addition to the first use of EGT in an enzymatic reaction, the establishment of MSH as the sulfur donor of lincomycin A indicates that MSH can be a functional surrogate of GSH in actinomycetes not only for protection against oxidative stress and electrophilic toxins, but also as an important sulfur donor in natural product biosynthesis. As homologs of LmbE are present in the biosynthetic pathways of other sulfur-containing natural products, it will be interesting to see if the use of MSH as a sulfur donor is common. In this regard, the current groundbreaking work lays the foundation for future study of MSH and EGT-dependent proteins and associated biochemical processes.

Zedu Huang and Wilfred A. van der Donk*
Institute for Genomic Biology, Department of Chemistry, Howard Hughes Medical Institute, University of Illinois at Urbana-Champaign, USA
*E-mail: vddonk@illinois.edu

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