Aminoglycoside Modification Exploiting FAD-Dependent and Ligand-Enzyme NOS Bridge Dual Chemistry

Orf1 is characterized as a noncanonical FAD-dependent N-formimidoyl/iminoacetyl synthase, employing a ligand-enzyme NOS bridge mechanism for transforming natural products, a handy valuable tool for designing next-generation antibiotics.

Aminoglycoside antibiotics are effective in treating bacterial infections by binding to bacterial ribosomes, thereby hindering protein synthesis. The amine groups within aminoglycosides play crucial roles in recognizing their binding sites on ribosomes. However, pathogenic bacteria employ various drug-resistance mechanisms, such as acetylating amine groups, resulting in the inactivation of aminoglycoside antibiotics. Darwin once stated, "It is not the strongest of the species that survives, nor the most intelligent, but the one most responsive to change." In evolutionary competition, some bacteria employ aminoglycoside antibiotics to suppress competitors, prompting a responsive counter-strategy of amine acetylation. To prevail, bacteria evolve new modifications, such as *N*-formimidoylation or *N*-iminoacetylation, in response to this disarmament. Hence, the transformed antibiotics are no longer susceptible to inactivation. An international team which comprised Tsung-Lin Li of Academia Sinica, Yoshimistu Hamano of Fukui Prefectural University (Japan), Toru Dairi of Hokkaido University (Japan), and Chin-Yuan Chang of National Yang Ming Chiao Tung University, identified the enzyme (Orf1) that dictates the *N*-formimidoylation or *N*-iminodiacetylation transformation while elucidating the biosynthesis of the aminoglycoside antibiotic BD-12. Orf1 is the committed enzyme, as established by *in vivo* gene knockout and *in vitro* biochemical experiments (**Fig. 1**).[1] To delve into the reaction mechanism of the enzyme at the molecular level, they determined the Orf1 apo and complex structures by leveraging **TLS 15A1**, **TPS 05A,** and **TPS 07A** beamlines at the NSRRC.

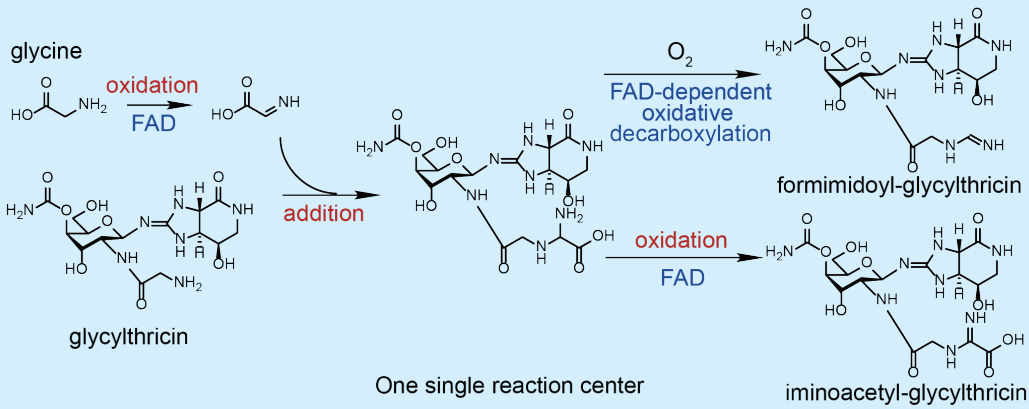

Fig. 1: *N*-formimidoylation or *N*-iminoacetylation was believed to follow multiple steps of reactions in one single reaction site, as does a typical FAD-dependent enzyme. [Reproduced from Ref. 1]

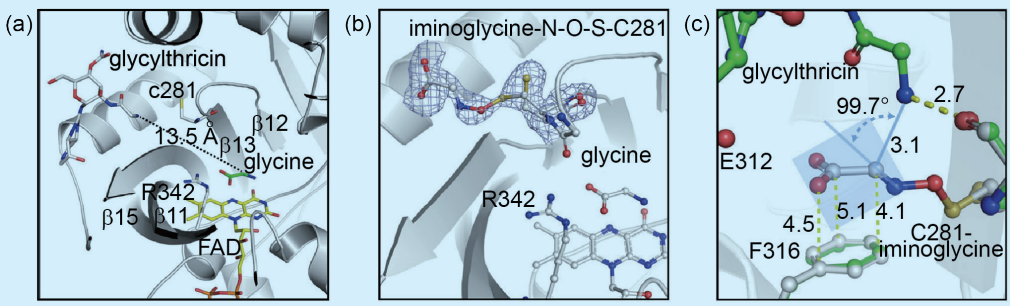

Fig. 2: (a) Two substrate-binding sites sit 13.5 Å apart. (b) The electron density region suggests a glycine imine adduct in a covalent linkage to C281 through an NOS bridge. (c) The arrangement between the amine group of glycylthricin and α-carbon of the iminoacetate adduct poses a trajectory appropriate for an addition reaction. [Reproduced from Ref. 1]

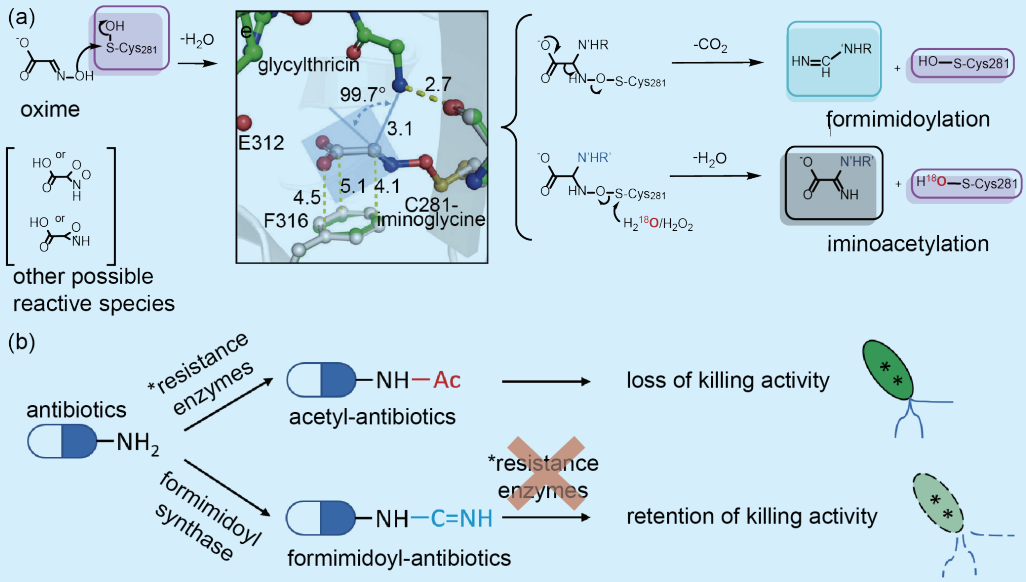

Fig. 3: (a) The proposed catalytic mechanism of Orf1 based on intrinsic experimental findings. (b) A strategy that antibiotic-producing species employ to counter drug resistance in competing species. [Reproduced from Ref.1]

The complex structures shed new light, revealing that the glycine and glycylthricin binding sites are separate and distant, ruling out the previously proposed single reaction chamber; the coupling reaction takes place at the latter site (**Fig. 2(a)**). Notably, a "nitrogen-oxygen-sulfur" covalent bond bridge between the glycine imine substrate and the sulfur atom of the enzyme cysteine was observed (**Fig. 2(b)**). This NOS bridge differs from others, occurring as a post-translational modification amid a reactive oxygen species, with a lysine residue and a cysteine residue likely involved in protein functional regulation.[2,3] In this case, the ligand-protein NOS bridge is brought about between an oxidized glycine formed at the first binding site (FAD) and an oxidized cysteine (sulfenic acid) residue at the second binding site (glycylthricin), with the oxidized glycine transported to the second binding site through a delicate intramolecular tunnel. The glycylthricin is positioned at a suitable distance and trajectory toward the glycine imine NOS bridge (**Fig. 2(c)**) for an addition reaction.

The team further revealed that two amino acid residues (E312 and F316) located near the NOS bridge are crucial, as the *N*-formimidoylated product converts to the *N*-iminodiacetyl one when mutated. These two amino acids coordinate to facilitate decarboxylation and elimination reactions, resulting in bond cleavage sites at either N–O or O–S, accounting for *N*-formimidoylation or *N*-iminoacetylation. When the bond breaks between oxygen and sulfur atoms in the context of *N*-iminoacetylation, the sulfenic group can be regenerated by water, in contrast to that of *N*-formimidoylation by molecular oxygen, ready for the following reaction cycle to proceed (**Fig. 3(a)**). Finally, the team concluded that the given modifications position themselves to prevent antibiotics from inactivation by the acetylation modification of strains carrying the resistance genes (**Fig. 3(b)**), emphasizing that the modification is a convenient approach to designing the next generations of aminoglycoside antibiotics. (Reported by Yung-Lin Wang, Academia Sinica).

This report features the work of Tsung-Lin Li and his collaborators published in Nat. Comum. ***14****, 2528 (2023).*

TPS 05A Protein Microcrystallography
TPS 07A Micro-focus Protein Crystallography
TLS 15A1 Biopharmaceuticals Protein Crystallography

- XPS, Protein Crystallography
- Biological Macromolecules, Protein Structures, Life Science

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