Putting a Stop to L,D-Transpeptidases

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In this issue of *Structure*, Lecoq et al. investigated the structural and dynamic basis for the unexpected inhibition of peptidoglycan-crosslinking L,D-transpeptidases by carbapenem antibiotics. In addition to defining a neutral thiol-imidazole catalytic triad, their studies revealed extensive induced motions upon formation of a long-live covalent drug-enzyme complex.

The major pathway for bacterial peptidoglycan biosynthesis requires the formation of peptide-crosslinks catalyzed by penicillin-binding proteins (PBPs) with D,D-transpeptidase activity. Due to their resemblance to the terminal D-Ala-D-Ala motif present in the precursor stem pentapeptides, the β-lactam antibiotics are effective suicide inhibitors of D,D-transpeptidases. However, in some bacteria, L,D-transpeptidases bypass this classical pathway by catalyzing the formation of alternative peptidoglycan cross-links from variant stem peptides lacking a D-Ala-D-Ala motif (Figure 1A) (Mainardi et al., 2005). Most notably, L,D-transpeptidation is the predominant mode of cross-linking in *Mycobacterium tuberculosis* (Gupta et al., 2010). The D,D-transpeptidases and L,D-transpeptidases are structurally unrelated and exploit invariant serine or cysteine residues, respectively, for catalysis. As expected from their distinct substrate specificities, L,D-transpeptidases are insensitive to most β-lactams. However β-lactams of the carbapenem class, such as imipenem, are surprisingly active against these bypass enzymes (Mainardi et al., 2007). Understanding this activity may facilitate development of antibacterial strategies targeting L,D-transpeptidases, including those in extensively drug-resistant strains of *M. tuberculosis*.

Lecoq et al., 2012 (in this issue of *Structure*) have used a battery of NMR spectroscopic techniques to investigate the structural and dynamic properties of apo and imipenem-bound L,D-transpeptidase from *Bacillus subtilis* (Ldt_{BS}) and to characterize its Cys-His-Gly catalytic triad. It must be stressed that, while crucial for understanding kinetic mechanisms, it is very difficult to determine the ionization states and hydrogen bonding patterns of the active site residues in enzymes. For example, histidine, which has the highest propensity among all amino acids to be involved in catalysis, can exist in the cationic or neutral $N^{\delta1}$ or $N^{\varepsilon2}$ tautomeric states. Fortunately, each of these states has very diagnostic chemical shift patterns, allowing Lecoq et al. (2012) to unambiguously establish that His126 of Ldt_{BS} has an unusually depressed $pK_a < 3.1$ (over 3.5 log units lower than the average protein value [Grimsley et al., 2009]) and exists as the less common neutral $N^{\delta1}$ tautomer under physiological conditions. Furthermore, Cys142 does not titrate between pH 3 and 10, and thus has a pK_a outside these limits. Based on a careful structural analysis of apo Ldt_{BS}, Lecoq et al. (2012) convincingly demonstrated that Cys142 remains protonated under neutral pH conditions (hence its $pK_a > 10.5$ or over 3.5 log units above average) and hydrogen bonds to the $N^{\varepsilon2}$ of His126. The third component of the catalytic triad, Gly127, serves as a hydrogen bond acceptor for the histidine $N^{\delta1}H$ through its backbone carbonyl. Thus, in contrast to the generally accepted thiolate-imidazolium (S^-/NH^+) ion pair found in the active site of the prototypical cysteine protease papain (Lewis et al., 1981), Ldt_{BS} exploits a neutral, hydrogen bonded thiol-imidazole pair (SH/N) for catalysis (Figure 1B). Although mechanistic variations can be proposed, by analogy with serine proteases, His126 likely serves as a general base to activate the Cys142 S^{γ} for nucleophilic attack upon the donor peptide carbonyl and then subsequently as a general acid to protonate the nitrogen leaving group. Why are the pK_a values of His126 and Cys142 perturbed to favor their neutral states and why has nature evolved this configuration to catalyze peptidoglycan crosslinking? The answer may lie with the burial of the histidine, the electropositive nature of the Ldt_{BS} active site, or the need to protect the cysteine from oxidation in the periplasm. However, rationalizing the ionization states and hence precise catalytic functions of active site residues remains even more challenging than their experimental characterization.

Lecoq et al. (2012) also uncovered a striking example of induced dynamics in the long-lived imipenem-bound state of Ldt_{BS} (Figure 1B). The first clue to the altered mobility of residues near the active site of the complex came from the observed attenuation of their NMR signals. This hampered the measurement of NMR-derived restraints for many of these residues, as well as the bound imipenem, thus resulting in high root mean square deviations for the drug and for flanking active site regions in the calculated structural ensemble of the covalently-modified enzyme. However, this conformational disorder is not a trivial result of limited experimental data, but rather results from true dynamics. Using relaxation-dispersion experiments, which sensitively detect ms-μs timescale exchange between conformational states with different chemical shifts, Lecoq et al. (2012) demonstrated that the active site regions of imipenem-bound Ldt_{BS} (but not apo Ldt_{BS}) undergo significant motions with exchange rate constants of $\sim 2\ ms^{-1}$. This is not due to loss of the His126-Cys142 hydrogen bond because the C142A variant did not show such dynamic behavior. Rather, the induced motions result from the covalent modification of the enzyme since similar regions

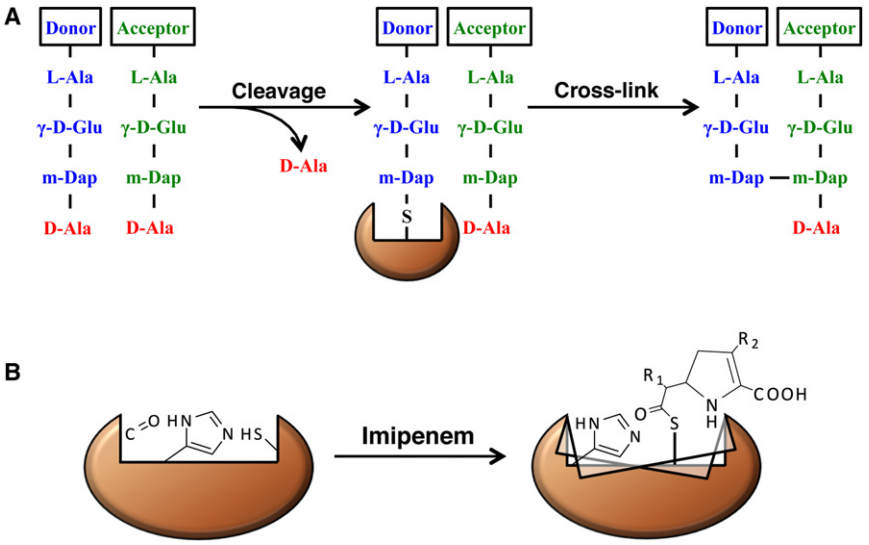

Figure 1. Inhibition of Bypass Peptidoglycan Biosynthesis by Carbapenems
(A) Mechanism of L,D-transpeptidase catalyzed peptidoglycan crosslinking (m-Dap, *meso*-diaminopimelic acid).
(B) The ionization states and hydrogen bonding configuration of the Ldt_{BS} Gly-His-Cys catalytic triad and induced dynamics upon Cys142 acylation by imipenem.

of Ldt_{BS} with a 2-nitro-5-thiobenzoate moiety attached to Cys142 also displayed ms timescale conformational exchange. The reasons for these induced motions remain unclear, as Cys142 is accessible in the apo enzyme and the bound imipenem does not appear to cause any steric conflicts in the calculated structural ensemble. However, this is somewhat complicated by the lack of experimental restraints involving the drug and highlights the need for additional studies directed toward understanding the structural properties of the "invisible" conformational states formed in the detected exchange equilibria (Baldwin and Kay, 2009). More importantly, are these motions catalytically-relevant, perhaps facilitating the second step of the transpeptidation reaction (Figure 1A), or are they somehow specific to the two long-lived complexes characterized in this study? Addressing this difficult question will certainly require detailed structural and dynamic characterization of the donor thioester enzyme intermediate (or a close stable analog) that reacts with the acceptor peptide to form a peptidoglycan crosslink.

The overarching goal of the Lecoq et al. (2012) study was to understand the surprising reactivity of carbapenem antibiotics toward L,D-transpeptidases. Through kinetic measurements and NMR-monitored titrations, the authors found that both imipenem and nonreactive ampicillin bind the active site region of wild-type or catalytically-inactive C142A Ldt_{BS} with similar, weak (>mM) affinities. Furthermore, the covalently-bonded imipenem does not adopt a well-defined conformation, but rather induces active site mobility. Thus, reactivity is not due to any specific, high affinity binding interactions in the Michaelis or product complexes. Indeed, it has long been known that β-lactams are generally effective inhibitors of PBPs not because they bind tightly, but because they are reactive acylating agents (Kluge and Petter, 2010). Therefore, the answer to this key question must lie with yet unknown complementary features of L,D-transpeptidases and carbapenems that stabilize the transition state for the chemical step of acylation. Do these features involve the induced motions detected in imipenem-bound Ldt_{BS}? The extensive results of the well-crafted Lecoq et al. (2012) study provide a much needed foundation for unraveling the structural and dynamic mechanisms underlying carbapenem inhibition of L,D-transpeptidases, as ultimately required for the development of improved antibiotics targetting bypass peptidoglycan biosynthesis.

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