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Antimetabolite Poisoning of Cofactor Biosynthesis

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In this issue of *Chemistry & Biology*, van der Westhuyzen et al. describe the synthesis and characterization of the natural product CJ-15,801, which acts as an antimetabolite of the coenzyme A biosynthetic pathway.

The lack of new antibiotics coupled with the increased prevalence of multidrug-resistant bacterial pathogens is cause for great concern (Wright, 2012). The genomics revolution predicted a new era in antibiotics discovery with the delivery of an abundance of conserved and essential targets (Payne et al., 2007). However, target-based approaches for antibacterial discovery have proven quite challenging due to many problems including conversion of a biochemical inhibitor into a cell-permeable analog, rapid evolution of resistance to single targets, and potential lack of vulnerability of a given target to inhibition (i.e., may require >99% inhibition in order to impact growth and/or survival). Given the historical success of natural products in the field of antibiotics (74% of all approved antibiotics are derived from natural products), revisiting abandoned and neglected natural product scaffolds represents a viable strategy to overcome the shortage of new antibiotics (Newman and Cragg, 2012). In fact, three of the four new antibiotic scaffolds introduced in the last decade were all from natural products initially discovered more than 30 years ago (e.g., fidaxomicin, a polyketide; retepamulin, a diterpene; and daptomycin, a lipodepsipeptide).

In this issue of *Chemistry & Biology*, van der Westhuyzen et al. (2012) describe their investigation of a natural product antibiotic named CJ-15,801, which was originally discovered by scientists at Pfizer (Sugie et al., 2001). CJ-15,801 possesses promising activity against multidrug-resistant strains of *Staphylococcus aureus*. This microorganism is a Gram-positive bacterium associated with a wide range of infections, from minor skin infections to severe cases of endocarditis and sepsis. Methicillin-resistant strains of *S. aureus*, termed MRSA, are particularly troublesome because of their resistance to the classic β-lactam antibiotics (Gilmore et al., 2008).

CJ-15,801 (hereafter referred to as CJ) is a dehydro analog of pantothenate (vitamin B5), and this close structural similarity suggests that it interferes with coenzyme A (CoA) biosynthesis. CoA is an essential cofactor in the central pathways of respiration and lipid metabolism and is synthesized in five steps from pantothenate (**1**) as shown in Figure 1 (Begley et al., 2001). The first step is carried out by PanK (pantothenate kinase, encoded by the gene *coaA*) that phosphorylates pantothenate to afford 4′-phosphopantothenate (**2**) (Hong et al., 2006). Three PanK isoforms exist, and *S. aureus* is unique among bacteria, as it possesses a type-II PanK. PPCS (4′-phosphopantothenoylcysteine synthase, encoded by the gene *coaB*) then catalyzes the CTP-dependent ligation of **2** with cysteine to afford **4** (Yao et al., 2009). Notably, this reaction proceeds through an acyl-cytidylate intermediate (**3**). Subsequent decarboxylation, adenylation, and phosphorylation of **4** provides CoA (**5**) through the actions of CoaC, CoaD, and CoaE, respectively.

The authors show that CJ (**6**) is an excellent substrate for PanK from *S. aureus*, but not other bacterial PanKs, and is converted into phosphorylated-CJ (P-CJ, **7**) as shown in Figure 1. P-CJ is a substrate for PPCS, the next enzyme in the pathway, and is efficiently cytidylated to form an acyl-cytidylate intermediate termed P-CJ-CMP (**8**). However, rather than reacting with cysteine, this intermediate acts as a dead-end inhibitor of PPCS. Despite the presence of the Michael acceptor moiety, irreversible inhibition is not observed, since enzyme activity is restored after removing the compound by gel filtration. Thus, CJ innocently enters the CoA biosynthetic pathway through PanK, then inhibits the second enzyme (PPCS) in the pathway, but only after enzymatic activation to form P-CJ-CMP. P-CJ exhibits time-dependent inhibition of PPCS from *S. aureus* with a K_i of 13 nM. The potent K_i value is consistent with the bisubstrate nature of P-CJ-CMP, which effectively interacts with both the pantothenate and CTP binding pockets. The authors provide further overall support for inhibition of the CoA biosynthetic pathway by showing that supplementation of the growth media with pantothenate causes a shift to a higher minimum inhibitory concentration (MIC). Additionally, synergy is observed with other CoA biosynthesis inhibitors. Collectively, these results reveal that CJ poisons the CoA biosynthetic pathway and acts as a classic antimetabolite. The intrinsic specificity of CJ for *S. aureus* results from the selective phosphorylation by PanK in this organism.

The basis for PPCS inhibition by P-CJ-CMP is intriguing, since it contains a high energy acyl-phosphate linkage that appears capable of reacting with cysteine. To assess the impact of P-CJ-CMP on protein stability, circular dichroism (CD) was used to measure the melting temperature (T_m) of PPCS unfolding. P-CJ-CMP leads to enhanced protein stability relative to the native phosphopantothenate-CMP intermediate **3**, as a result of the reduced conformational entropy caused by the more rigid alkene. The authors speculate this increased stabilization favors a closed conformation of the active site, which prevents the subsequent ligation with cysteine from occurring. Furthermore, the carbonyl carbon of the P-CJ-CMP is proposed to be less reactive than in **3** due to the electron-donating vinylogous amino group. Consistent with this observation, a simple methyl ester of CJ was resistant to base-mediated hydrolysis. While further

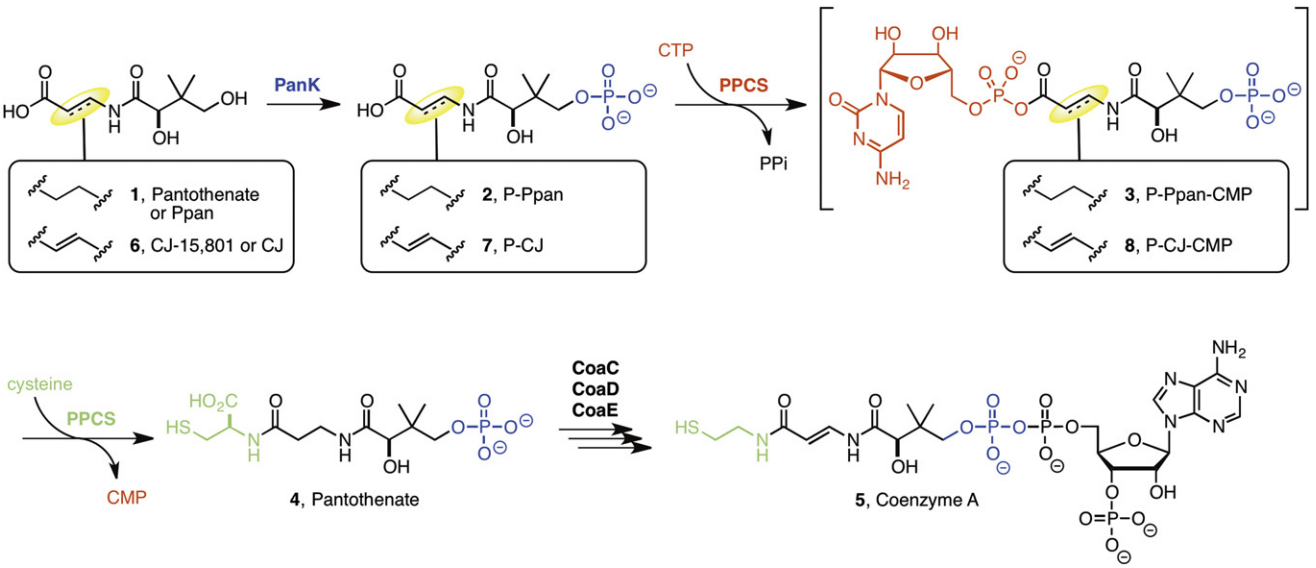

Figure 1. Biosynthesis of CoA and Mechanism of CJ Bioactivation

detailed analysis will be required to delineate the precise mechanism of inhibition by CJ, it seems plausible that both factors contribute to CJ's unique reactivity.

In this article, the authors have succeeded in synthesizing CJ, elucidating its mode of action, and uncovering the origin of its remarkable specificity. This work highlights the importance of enzymology in antibiotics discovery and reveals a conceptually elegant mechanism of action. CJ acts as an antimetabolite poisoning the CoA pathway by exploiting the promiscuity of PanK from *S. aureus* to enter and then inhibit the downstream enzyme PPCS through formation of a catalytically incompetent dead-end inhibitor. The sulfonamides, the first class of antibiotics used in human medicine, are also antimetabolites that interfere with folate biosynthesis. Nucleotidylating enzymes are ubiquitous (Duckworth et al., 2012), and this research potentially offers a general strategy for inhibiting these enzymes by incorporation of a vinylogous carbamic acid in the respective enzyme substrate.

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