Self-control of vitamin K_2 production captured in the crystal

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Menaquinone (MK) or vitamin K_2 is an important metabolite that controls the redox/energy status of *Mycobacterium tuberculosis*. Although the major steps of MK biosynthesis have been delineated, the regulatory mechanisms of this pathway have not been adequately explored. Bashiri *et al.* now demonstrate that MenD, catalyzing the first committed step of MK production, is allosterically inhibited by a downstream cytosolic metabolite in the MK biosynthesis pathway.

Menaquinones (MKs),[2] also known collectively as vitamin K_2, are polyisoprenylated naphthoquinones synthesized by bacteria and defined by the number of their isoprene units. The etiologic agent of tuberculosis (TB), *Mycobacterium tuberculosis,* produces MK-9, carrying nine isoprene repeats. MK-9 is a central component of the respiratory chain, serving as an electron shuttle from dehydrogenases to the terminal electron oxidases (1). MK also plays a role in virulence of *M. tuberculosis.* This has been demonstrated by the reduced intramacrophage survival of a deletion mutant of *MenJ* (encoding an enzyme that reduces the β-isoprene unit of MK) (2). Thus, *M. tuberculosis* MK (*Mtb*-MK) represents an essential vulnerable point in the electron transport chain and a prime target for new drug development. The absence of MK biosynthesis in humans further strengthens the druggability of enzymes in this pathway. Whereas most MK enzymatic reactions, catalyzed by 10 enzymes (MenA–MenJ), have been described (2), regulatory aspects of *Mtb*-MK biosynthesis are still lacking. Given the importance of MK in the physiology and pathogenesis of *M. tuberculosis*, it is expected that biosynthesis of *Mtb*-MK is tightly regulated. This assumption has now been convincingly validated in the elegant study by Bashiri *et al.* (3).

MenD catalyzes the first committed step in *Mtb*-MK synthesis, converting isochorismate into 2-succinyl-5-enolpyruvyl-6-hydroxy-3-cyclohexadiene-1-carboxylate (SEPHCHC), using α-ketoglutarate as a co-substrate and thiamine diphosphate (ThDP) as co-factor. In a previous study, the authors solved the three-dimensional crystal structure of MenD (4), revealing it as a homotetrameric protein, where each monomer is composed of three domains. Whereas domains I and III form the active site, via interactions between domain I of one monomer with domain III of the neighbor monomer, the role of domain II remained elusive. To search for putative regulatory compounds, Bashiri *et al.* soaked the crystals of the holo-form of MenD (bound to ThDP) into solutions containing downstream products or metabolites from the MK synthesis pathway. After solving the three-dimensional structures, a clear extra electron density corresponding to 1,4-dihydroxy-2-napthoic acid (DHNA) was found in a cleft of domain II. DHNA is the substrate of MenA, which converts DHNA to demethylmenaquinone (5). In MenD, the DHNA binding site is distant by at least 20 Å from the active site and characterized by the presence of an "arginine cage" composed of three arginine residues, namely Arg-97, Arg-277, and Arg-303 (Fig. 1). Next, Bashiri *et al.* used ^{1}H NMR–based and UV-based spectroscopy assays to confirm that DHNA inhibits the conversion of isochorismate to SEPHCHC, supporting their structural analyses. In addition, assessment of the enzymatic activity of WT MenD and three MenD mutants, in which the three Arg residues forming the DHNA-binding pocket and required for MenD activity were substituted by Ala, confirmed that the three Arg residues play a crucial role for propagating the signal from the DHNA site to the active site.

To gain more insight into the role of DHNA binding, the authors compared the crystal structures of MenD in various states—in its apo-form, bound to DHNA, or bound to two different co-factor intermediates—with previously solved structures of MenD (4), unraveling subtle structural rearrangements occurring after binding of DHNA. The DHNA-occupied monomers adopt a similar conformation as the structure of the monomers bound to the ThDP cofactor. The changes observed in the structures containing DHNA, as compared with the apo-form structure, affect mainly domain I, particularly the flexible active site loop (residues 105–125), which appears more ordered in the DHNA-bound *versus* DHNA-free enzyme. The N terminus of domain I, containing one catalytic residue, also undergoes structural rearrangements upon DHNA binding. Of interest, binding of DHNA induces an asymmetry in which the active site in two of the four MenD monomers are not positioned in a catalytically favorable state, suggestive of intersubunit communication and allostery. Together with the fact that the MenD active sites are located at the interface of two monomers, the authors propose that DHNA may alter the propagation of signals between these active sites and, therefore, acts as an allosteric inhibitor perturbing the catalytic cycle.

From a fundamental perspective, the study by Bashiri *et al.* reports the discovery of a new feedback regulatory mechanism

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[2] The abbreviations used are: MK, menaquinone; TB, tuberculosis; SEPHCHC, 2-succinyl-5-enolpyruvyl-6-hydroxy-3-cyclohexadiene-1-carboxylate; ThDP, thiamine diphosphate; DHNA, 1,4-dihydroxy-2-napthoic acid.

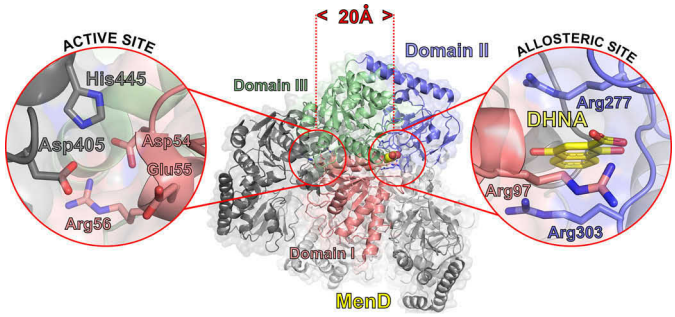

Figure 1. Allosteric inhibition of *Mtb*-MenD by DHNA. The three-dimensional structure (Protein Data Bank entry 6DOJ) of the MenD homotetramer is represented in a *cartoon* and *surface representation*. All monomers are shown in *gray*, except for one monomer that is colored as follows: domain I in *salmon pink*, domain II in *blue*, and domain III in *green*. The *right inset* displays the three arginine residues (*stick representation*) that form the allosteric site that accommodates DHNA (in *yellow stick representation*) and communicate with the active site of the enzyme (*left inset*).

that involves allosteric inhibition of MenD, which represents a major advance in our understanding of this essential and complex biosynthetic process. Whether other metabolites deriving from the MK pathway or any other pathway control MenD activity and whether they involve similar allosteric inhibition mechanisms remains to be investigated.

With 10 million new cases and 1.6 million deaths in 2017, TB remains a leading health problem worldwide (6). *M. tuberculosis* is a resilient microorganism that can persist silently through long chemotherapeutic courses and years of dormancy within the host. The standard chemotherapeutic treatments remain very challenging, substantiated by the slow growth of *M. tuberculosis* and the presence of a thick and drug-impermeable waxy cell envelope (7). In this context, new chemical entities that kill actively growing as well as persistent bacilli are needed. Exploiting MK biosynthetic enzymes as potential drug targets has already shown promise, and chemical inhibitors of MenA (8), MenB, MenE, and MenG (9) have proven efficacious in inhibiting actively growing and nonreplicating *M. tuberculosis*, validating the essentiality of this pathway. The discovery of an allosteric inhibitor of MenD with drug-like properties may thus pave the way for the design of new MK-specific inhibitors. The presence of hydroxyl and carboxylic acid groups in DHNA offers the possibility to perform chemical modifications that may guide for the rational design of inhibitors with improved biological and pharmacological properties. The absence of a strict conservation of the arginine cage forming the allosteric site of *Mtb*-MenD in other bacterial MenD homologues also provides a great advantage, as *Mtb*-MenD inhibitors are unlikely to affect the activity of MenD in human microbiota microorganisms. Finally, a recent study highlighted the synergistic activity of MenA inhibitors with other electron transport chain inhibitors, such as bedaquiline (10). Thus, testing whether *Mtb*-MenD inhibitors exert synergism with other drugs would be highly relevant, broadening the window to a number of drug combination opportunities against TB. It will be exciting to see how this DHNA-mediated self-control can be exploited in our efforts to control this devastating disease.

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References

1. Dhiman, R. K., Mahapatra, S., Slayden, R. A., Boyne, M. E., Lenaerts, A., Hinshaw, J. C., Angala, S. K., Chatterjee, D., Biswas, K., Narayanasamy, P., Kurosu, M., and Crick, D. C. (2009) Menaquinone synthesis is critical for maintaining mycobacterial viability during exponential growth and recovery from non-replicating persistence. *Mol. Microbiol.* **72,** 85–97 CrossRef Medline
2. Upadhyay, A., Fontes, F. L., Gonzalez-Juarrero, M., McNeil, M. R., Crans, D. C., Jackson, M., and Crick, D. C. (2015) Partial saturation of menaquinone in *Mycobacterium tuberculosis*: function and essentiality of a novel reductase, MenJ. *ACS Cent. Sci.* **1,** 292–302 CrossRef Medline
3. Bashiri, G., Nigon, L. V., Jirgis, E. N. M., Ho, N. A. T., Stanborough, T., Dawes, S. S., Baker, E. N., Bulloch, E. M. M., and Johnston, J. M. (2020) Allosteric regulation of menaquinone (vitamin K_2) biosynthesis in the human pathogen *Mycobacterium tuberculosis*. *J. Biol. Chem.* **295,** 3759–3770 CrossRef Medline
4. Jirgis, E. N. M., Bashiri, G., Bulloch, E. M. M., Johnston, J. M., and Baker, E. N. (2016) Structural views along the *Mycobacterium tuberculosis* MenD reaction pathway illuminate key aspects of thiamin diphosphate-dependent enzyme mechanisms. *Structure* **24,** 1167–1177 CrossRef Medline
5. Dhiman, R. K., Pujari, V., Kincaid, J. M., Ikeh, M. A., Parish, T., and Crick, D. C. (2019) Characterization of MenA (isoprenyl diphosphate:1,4-dihydroxy-2-naphthoate isoprenyltransferase) from *Mycobacterium tuberculosis*. *PLoS ONE* **14,** e0214958 CrossRef Medline
6. Dheda, K., Limberis, J. D., Pietersen, E., Phelan, J., Esmail, A., Lesosky, M., Fennelly, K. P., Te Riele, J., Mastrapa, B., Streicher, E. M., Dolby, T., Abdallah, A. M., Ben-Rached, F., Simpson, J., Smith, L., *et al.* (2017) Outcomes, infectiousness, and transmission dynamics of patients with extensively drug-resistant tuberculosis and home-discharged patients with programmatically incurable tuberculosis: a prospective cohort study. *Lancet Respir. Med.* **5,** 269–281 CrossRef Medline
7. Barry, C. E., 3rd, and Mdluli, K. (1996) Drug sensitivity and environmental adaptation of mycobacterial cell wall components. *Trends Microbiol.* **4,** 275–281 CrossRef Medline
8. Kurosu, M., and Crick, D. C. (2009) MenA is a promising drug target for developing novel lead molecules to combat *Mycobacterium tuberculosis*. *Med. Chem.* **5,** 197–207 CrossRef Medline
9. Sukheja, P., Kumar, P., Mittal, N., Li, S.-G., Singleton, E., Russo, R., Perryman, A. L., Shrestha, R., Awasthi, D., Husain, S., Soteropoulos, P., Brukh, R., Connell, N., Freundlich, J. S., and Alland, D. (2017) A novel small-molecule inhibitor of the *Mycobacterium tuberculosis* demethylmenaquinone methyltransferase MenG is bactericidal to both growing and nutritionally deprived persister Cells. *mBio* **8,** e02022-16 CrossRef Medline
10. Berube, B. J., Russell, D., Castro, L., Choi, S.-R., Narayanasamy, P., and Parish, T. (2019) Novel MenA inhibitors are bactericidal against *Mycobacterium tuberculosis* and synergize with electron transport chain inhibitors. *Antimicrob. Agents Chemother.* **63,** e02661-18 CrossRef Medline