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GUEST COMMENTARY

New Insights into Acetone Metabolism▽

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In this issue of the *Journal of Bacteriology*, Kotani et al. (11) elucidate the latter half of a new pathway for propane metabolism in *Gordonia* sp. strain TY-5, thus complementing their work on the earlier steps of the pathway from 3 years ago (10). Utilization of this gaseous three-carbon substrate, depicted in the boxed region of Fig. 1, provides new insights into the microbial metabolism of acetone—a central intermediate of the overall scheme. The initial step of propane utilization involves a four-component putative di-iron monooxygenase (encoded by *prnABCD*) that catalyzes the NADH-dependent subterminal hydroxylation of the alkane to form 2-propanol. Three distinct NAD^+-dependent secondary alcohol dehydrogenases (encoded by *adh1*, *adh2*, and *adh3*) convert the hydroxylated intermediate to acetone. A flavin adenine dinucleotide (FAD)-dependent monooxygenase (encoded by *acmA*) catalyzes an NADPH-dependent Baeyer-Villiger reaction that inserts an oxygen atom into a carbon-carbon bond of acetone to produce methyl acetate. Finally, a hydrolase (encoded by *acmB*) splits the ester into acetic acid and methanol. Consistent with their proposed functions, these genes are induced by propane, 2-propanol, and acetone.

The central position of acetone in the propane oxidation pathway is placed into a broader context by comparison to the other acetone-related reactions illustrated in Fig. 1. Many anaerobic bacteria, such as those in the genus *Clostridium* (4), produce acetone during fermentation by decarboxylation of acetoacetate (reaction a). Selected plants decompose cyanogenic glucosides to generate acetone cyanohydrin, which is metabolized by a lyase (reaction b) to form hydrogen cyanide and acetone (7). Epoxypropane, formed in certain bacteria by monooxygenation of propene, can undergo isomerization (reaction c) to acetone (18). Various members of the vibrio family possess a multistep pathway of leucine catabolism that produces 3-hydroxy-3-methylglutaryl-coenzyme A (15), which decomposes to yield this ketone (reaction d). Other microbes oxidize 2-nitropropane (reaction e), atrazine (reaction f), or other compounds to produce this molecule (13, 14). In the same manner, alternative pathways for acetone decomposition have been described. One well-characterized pathway involves the ATP-dependent, manganese-containing enzyme acetone carboxylase (reaction g) that forms acetoacetate, which is subsequently converted through multiple steps to form two molecules of acetyl-coenzyme A (1, 17). The conversion of acetone to acetol by cytochrome P450 has long been known (9), and other types of monooxygenases also may be capable of catalyzing such terminal hydroxylation (scheme h). Acetol cleavage to form acetaldehyde and formaldehyde (reaction i) has been proposed (5); however, no characterization of this enzyme (presumably containing thiamine pyrophosphate to facilitate this chemistry) has been reported. Acetol dehydrogenase (reaction j) and methylglyoxal dehydrogenase (reaction k) reactions have been characterized (3) and would convert acetol to pyruvate. Finally, evidence was presented 20 years ago for an NADPH-dependent acetol monooxygenase that catalyzes Baeyer-Villiger chemistry (reaction l) in *Mycobacterium* sp. strain P1 (8); the resulting hydroxymethylene acetate would spontaneously decompose (reaction m) to yield acetic acid and formaldehyde. In sum, the reactions shown in Fig. 1 highlight the position of acetone at a central metabolic crossroads.

Although Baeyer-Villiger-type monooxygenases have been studied for decades, the *Gordonia* enzyme encoded by *acmA* appears to be unique in using the three-carbon compound acetone as an effective substrate. Indeed, a review of the literature reveals only slight activity with butanone as the smallest alternate substrate in one enzyme representative (2). The Baeyer-Villiger monooxygenases are flavoenzymes, and this is certainly the situation for AcmA. On the basis of its weak yellow color, this protein probably loses a portion of its FAD cofactor during purification—a situation known to occur during isolation of other family members (6, 12). Figure 2 depicts a reasonable FAD-dependent catalytic reaction for AcmA, using as a precedent the results of kinetic, mechanistic, and structural studies carried out with related enzymes. The figure is not meant to imply the order of substrate binding. For example, AcmA may exhibit a ter-ter kinetic mechanism, as described for cyclohexanone monooxygenase (16). Alternatively, the *Gordonia* enzyme might reduce its flavin by using NADPH, undergo a conformational change to react with oxygen, and then bind the substrate, as suggested by structural studies carried out with *Thermobifida fusca* phenylacetone monooxygenase (12). This structurally characterized family member is 43% identical in sequence to AcmA, raising questions concerning the structural basis of substrate specificity. Unfortunately, no structure is available for a substrate-bound form of any Baeyer-Villiger monooxygenase. Figure 3 depicts a surface representation of the *T. fusca* protein with its flavin illustrated in yellow. The residues lining its substrate-binding pocket are compared to the *Gordonia* enzyme and shown in

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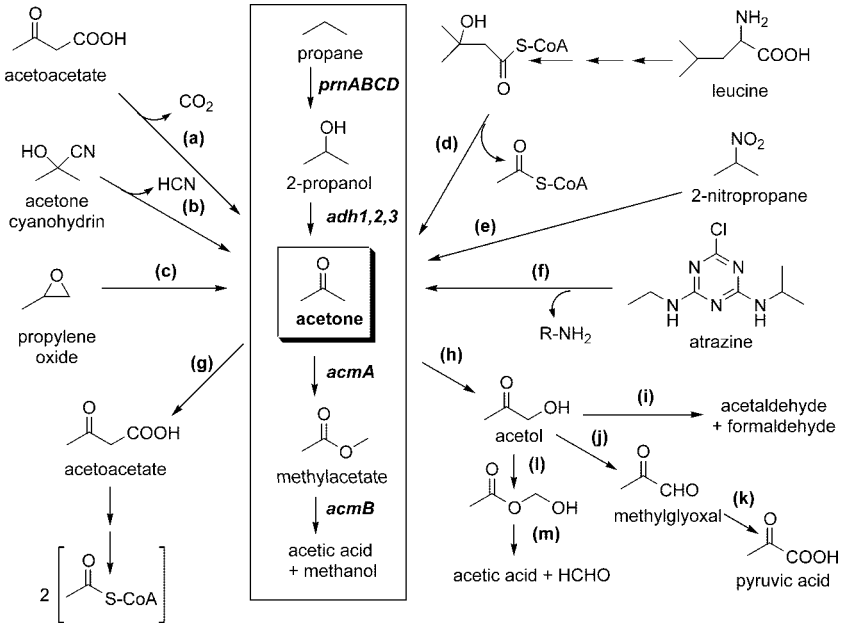

FIG. 1. Overview of acetone metabolism. The boxed region depicts the novel pathway of propane metabolism uncovered in *Gordonia* sp. strain TY-5 (10, 11). Additional reactions that generate or degrade acetone are illustrated outside of the box.

green if conserved (Asp66, Leu153, Arg217, Thr218, Tyr331, Arg337, Gly388, Phe389, Ala391, and Ser500) or in other coloring when substituted (C65F, blue; S196A, teal; H220Q, magenta; K336H, cyan; I339P, red). Further comparisons of the sequences indicate a single amino acid deletion in AcmA, corresponding to Thr256 of the *T. fusca* enzyme, and three single amino acid insertions in the *Gordonia* protein (between Ile288 and Leu289, Glu375 and Arg376, and Ser441 and Ala442 of the phenylacetone monooxygenase). The first three of these changes are distant from the active site and unlikely to be important to substrate specificity or catalysis, whereas the latter insertion is predicted to occur near the FAD. Additional

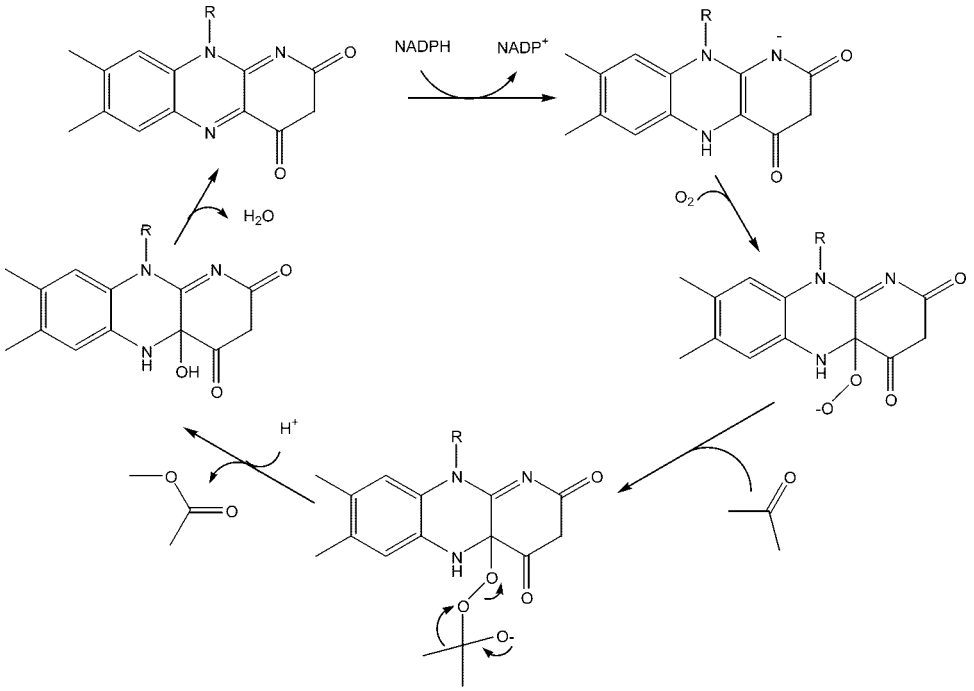

FIG. 2. Mechanism of acetone monooxygenase AcmA, a Baeyer-Villiger-type monooxygenase. The order of substrate binding and product release is unknown; however, the reduced nicotinamide must be used to first reduce the flavin, the reduced flavin then reacts with oxygen, the peroxyflavin intermediate reacts with acetone, and a Criegee rearrangement leads to product formation.

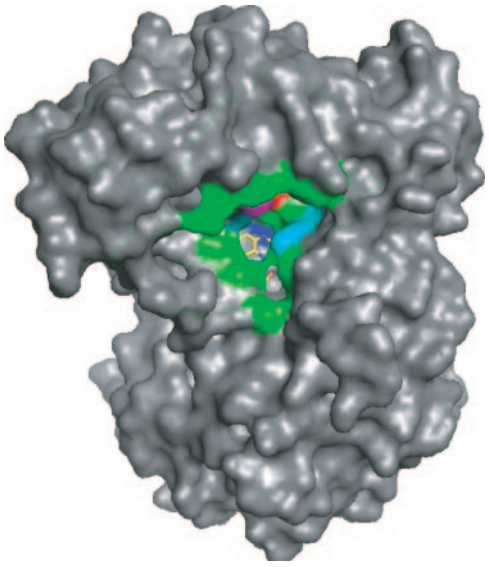

FIG. 3. Surface representation of phenylacetone monooxygenase and comparison of residues lining its substrate-binding pocket with those of AcmA. The structure of *T. fusca* phenylacetone monooxygenase (Protein Data Bank access code 1w4x) is shown in a surface representation (with the FAD depicted in yellow). Residues likely to be important for access to the active site or catalysis are compared to the corresponding residues of AcmA (green if conserved or other colors if they differ; see text).

kinetic studies are needed to determine the catalytic efficiencies of the various substrates of AcmA, and mutagenic or structural investigations are required to discern the structural basis of the substrate specificity of these enzymes.

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