COMMENTARY

Anaerobic bacteria need their vitamin B_{12} to digest estrogen

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Often described as nature's most beautiful cofactor (1), vitamin B_{12} (cobalamin) is a complex and fascinating organometallic molecule that, although made only by some prokaryotes, has key functional roles in microbes, animals, and humans (2). Its two major biological forms, methylcobalamin (MeCbl) and adenosylcobalamin (AdoCbl), have a central cobalt atom in a corrin ring that is coordinated via a cobalt–carbon bond to an upper axial methyl or 5′-deoxyadenosyl group, respectively; in both forms the lower axial ligand is the 5,6-dimethylbenzimidazole (DMB) base of a nucleotide tail attached to the corrin ring (2–5). Finely controlled enzymatic or photolytic cleavage of the upper axial cobalt–carbon bond underlies the use of B_{12} as an enzyme cofactor or, in a new twist, as the light-sensing molecule in a photoreceptor protein (2–5). As an enzyme cofactor, MeCbl is used for methyl transfer reactions by methyltransferases and AdoCbl for radical-based transformations by mutases, dehydratases, deaminases, and ribonucleotide reductases (2–4). In a broad range of organisms and biological processes, B_{12}-dependent methyltransferases catalyze the transfer of a methyl group from a donor to a final acceptor, utilizing MeCbl or its methylcobamide analogs (with a base other than DMB) as methyl carriers. In these enzymes the nucleotide base in B_{12} is always displaced, often by a histidine ligand of a conserved DxHxxG protein motif (6–8). Methionine synthase, a well-studied MeCbl-dependent methyltransferase present in many bacteria and mammals, is a single polypeptide with four modules that uses methyltetrahydrofolate as donor and homocysteine as acceptor to catalyze the terminal step in methionine biosynthesis (6, 7). In many microbes that thrive in anaerobic habitats, such as methanogens and acetogens, growth and energy production rely on B_{12}-dependent methyltransferase systems that employ a variety of methyl donors (e.g., methanol, methylamines, and methyltetrahydromethanopterin) and acceptors (coenzyme M and tetrahydrofolate) (6, 7). Rather than a single polypeptide, these B_{12}-dependent methyltransferases are often multiprotein enzyme complexes with one subunit each for binding the methyl donor, the B_{12} cofactor, and the methyl acceptor (6, 7). In PNAS, Wang et al. (9) report on a B_{12}-dependent methyltransferase system that targets estrogen, a previously unsuspected methyl acceptor, in an anaerobic denitrifying bacterium. As a result, the estrogen gets transformed into androgen for subsequent degradation and use, thus linking B_{12} with anaerobic bacterial steroid catabolism.

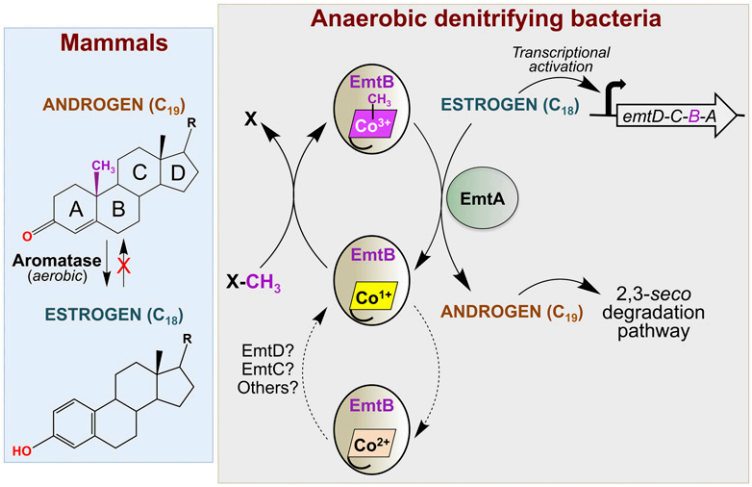

Fig. 1. Proposed B_{12}-dependent methylation of estrogen to androgen in anaerobic denitrifying bacteria. The putative catalytic and B_{12}-binding units are EmtA and EmtB, respectively, which are encoded by the estrogen–up-regulated *emtABCD* operon. As in well-characterized B_{12}-dependent methyltransferases, the cobalt in B_{12} is expected to cycle between the +3 and +1 oxidation states. The latter is a potent nucleophile that suffers occasional oxidative inactivation to the +2 state and requires reductive activation to reenter the catalytic cycle (dotted arrows). This may be mediated by EmtD (and EmtC) and/or other candidate proteins, whose genes are up-regulated in estrogen-fed cells. "X" is a methyl donor, whose identity is as-yet unknown. For comparison, a simplified schematic of the aerobic, irreversible, aromatase-catalyzed conversion of androgen to estrogen in mammals is shown on the left.

The sex steroids, androgens and estrogens (Fig. 1), are cholesterol-derived hormones with critical roles in

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mammalian physiology, development, reproduction, and behavior (10). Steroid biosynthesis typically occurs in eukaryotes but is rare in bacteria, where it is observed in *Gemmata obscuriglobus* and in some methanotrophs and myxobacteria (11–13). Because steroids are generally recalcitrant to degradation and can disrupt endocrine functions even at low concentrations in a variety of organisms including humans, the constant release of natural and synthetic steroids into the environment due to agriculture, industry, and sewage is of ever-increasing concern. Hence, bacteria capable of degrading steroids are valuable bioremediation agents (14, 15). How bacteria deal with steroids is also important in the context of host–microbe metabolic interdependencies, including in pathogenesis (16). Thus, the findings by Wang et al. (9) not only add estrogen methylation to the repertoire of B_{12}-dependent methyltransferase activities but are also relevant in bioremediation/wastewater treatment and in host–microbe metabolic interactions.

Bacterial steroid degradation mechanisms, pathways, and intermediates are well characterized for androgens but less so for estrogens, and aerobic pathways are better charted than anaerobic ones, with most insights emerging from studies of some Actinobacteria and a few α-, β-, and γ-Proteobacteria (14, 15). Thus, androgens are degraded aerobically via the 9,10-*seco* pathway (bond cleavage between ring B carbon atoms C9 and C10 to generate the corresponding secosteroid) or anaerobically in denitrifying bacteria via the 2,3-*seco* pathway (bond cleavage between ring A atoms C2 and C3) (14, 15). Estrogens are more refractory to degradation due to their stable aromatic A-ring and are degraded in some aerobic bacteria by oxygenase-catalyzed cleavage of the bond between ring A atoms C4 and C5 (the 4,5-*seco* pathway) (14, 15). Anaerobic estrogen degradation had been reported only in two bacterial species, and the genes and mechanisms involved have remained unknown. The study in PNAS (9) contributes toward filling this gap by identifying a degradation strategy that is based on "retroconversion" of estrogens to androgens for subsequent degradation via the anaerobic 2,3-*seco* pathway (Fig. 1). Such an estrogen-to-androgen conversion represents a challenging reaction that has not been reported previously. Indeed, in eukaryotes an oxygen/NADPH-dependent cytochrome P450 aromatase (or estrogen synthase) specifically targets the androgen "A" ring for the biosynthesis of estrogens with one less carbon, in a complex three-step hydroxylation–demethylation–aromatization reaction that is considered irreversible (refs. 17 and 18 and Fig. 1).

In the study in PNAS (9), an anaerobic β-proteobacterium, *Denitratisoma* sp. strain DHT3, which exhibits efficient B_{12}-dependent estrogen degradation under denitrifying conditions, was isolated from a municipal wastewater treatment plant. Its genome revealed a complete set of genes for the 2,3-*seco* androgen degradation pathway and for B_{12} transport and utilization but a lack of most genes for B_{12} biosynthesis. Transcriptomic analysis indicated that while 2,3-*seco* pathway genes were similarly expressed under both estrogen- and androgen-fed conditions, genes for B_{12} transport and utilization, and those in a cluster denoted *emtABCD* (*emt* for estradiol methylation), were upregulated under estrogen-fed conditions. Consistent with a role in estrogen metabolism, *emtABCD*, which is transcribed as a polycistronic RNA, also occurs in the other two known denitrifying, estrogen-degrading microbes: the β-proteobacterium *Denitratisoma oestradiolicum* and the γ-proteobacterium *Steroidobacter denitrificans*. Protein sequence analysis suggested that EmtA may be the catalytic subunit of a putative B_{12}-dependent methyltransferase complex, whose B_{12}-binding subunit would be EmtB, based on the presence of the $DxHxxGx_{41/42}SxLx_{24–28}GG$ motif found in MeCbl-dependent methione synthase and many B_{12}-binding proteins (3–8). EmtC is a hypothetical protein, and EmtD resembles the F420/FMN-dependent oxidoreductase involved in the reductive activation of B_{12}-dependent methyltransferases, required to regenerate the active cofactor +1 state upon its sporadic oxidation to the +2 state (Fig. 1 and refs. 6 and 7).

In PNAS, Wang et al. report on a B_{12}-dependent methyltransferase system that targets estrogen, a previously unsuspected methyl acceptor, in an anaerobic denitrifying bacterium.

Evidence that EmtA mediates B_{12}-dependent methylation of estrogen to androgen, which is then channeled into its established anaerobic degradation pathway, came from data obtained using genetic, biochemical, and ultraperformance liquid chromatography–high resolution mass spectrometry methods (9). Thus, disrupting the *emtA* gene abrogated the ability to utilize estrogen, and metabolite profiling for ^{13}C-labeled estrogen-fed bacteria revealed characteristic intermediates of the 2,3-*seco* androgen degradation pathway. Moreover, a decrease in estrogen levels with a concomitant appearance of androgens was observed when estrogens were incubated anaerobically with cell extracts from the wild-type strain (but not from the *emtA*-disrupted one) in the presence of MeCbl and of cofactors for the reductive activation of B_{12}-dependent methyltransferases. The effect of propyl iodide, an inhibitor of B_{12}-dependent enzymes in the dark but not in the light, provided further support for the requirement of B_{12} in the estrogen-to-androgen conversion reaction.

Although the evidence presented for B_{12}-dependent methylation of estrogen and its conversion to androgen is persuasive, the study also raises a slew of exciting new questions that remain to be addressed. Functions of the Emt proteins and the proposed catalytic mechanisms were hypothesized based on sequence comparisons and phylogenetic analysis and need to be demonstrated. Whether the proteins are subunits of larger complexes, as with various B_{12}-dependent methyltransferase systems, must be assessed. The molecular basis for the specific recognition and methylation of estrogens, ascribed to EmtA, has to be determined, as does also the identity of the initial methyl donor involved in the catalytic cycle. These will require purification, biochemical characterization, and in vitro reconstitution studies, as well as structure elucidation of these proteins, besides genetic analyses. From the standpoint of gene regulation, it will be interesting to decipher how estrogens up-regulate expression of the *emt* cluster and of the genes for B_{12} transport, salvage, and reductive activation. Nonetheless, the discovery of estrogen as the methyl acceptor of a putative B_{12}-dependent methyltransferase and its conversion to androgen, a reaction thought to be formidably difficult, is noteworthy and fills an important niche in bacterial steroid metabolism.

The B_{12}–steroid degradation link identified in the study has other ramifications. Because the anaerobic bacteria that degrade estrogen require B_{12} but cannot synthesize it de novo they must necessarily depend on exogenous B_{12} to "digest" estrogen. Thus, they may have to rely on other microbes in their natural milieu that do produce B_{12}, a frequently shared (and precious) resource

in microbial consortia (19). Such intermicrobe nutritional interactions will be relevant not only in optimizing any bioremediation strategy against environmental estrogen but also in the host–microbe metabolic interactions that are subject to modulation by sex steroids (16) and by B_{12} (19).

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