

Liberate and Grab It, Ingest and Digest It: the GbdR Regulon of the Pathogen *Pseudomonas aeruginosa*

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The compatible solute glycine betaine is a powerful osmoprotectant, but many microorganisms can also use it as a nutrient. K. J. Hampel et al. (J. Bacteriol. 196:7–15, 2014) defined a regulon in the notorious pathogen *Pseudomonas aeruginosa* that comprises modules for the harvest and import of the glycine betaine biosynthetic precursor choline and its subsequent catabolism to pyruvate. The reported data link the GbdR activator with the metabolism of host-derived compounds (e.g., phosphocholine) and virulence traits of *P. aeruginosa*.

Glycine betaine (*N,N,N*-trimethylglycine) (Fig. 1) is without any doubt the most widely used compatible solute in nature, as members of all kingdoms of life use it to fend off the detrimental effects of high osmolarity on cell volume, turgor, cellular physiology, and growth (1–4). Compatible solutes are operationally defined (5) as organic osmolytes that are highly compliant with the proper functioning of proteins and biochemical processes, a physicochemical feature of these compounds that largely stems from their preferential exclusion from the immediate hydration shell of the protein backbone (6, 7). This uneven distribution of compatible solutes in the cell water generates a thermodynamic driving force for proteins and protein complexes to adopt a compact, well-folded native conformation under otherwise denaturing conditions. As a consequence, compatible solutes can be amassed to exceedingly high concentrations without negative side effects on cell physiology; they thereby counteract the osmotically instigated efflux of water from the cell and optimize at the same time the solvent properties of the cytoplasm (8, 9). Microorganisms can accumulate compatible solutes either through uptake or through synthesis when they are exposed to osmotic stress. This physiological trait is widely present both in *Bacteria* and in *Archaea* (1, 2, 5).

Transporters for glycine betaine allow microorganisms to make effective use of precious environmental resources (10), and their high affinity and osmotic control (5, 9, 11, 12) permit the amassing of their substrate(s) against steep concentration gradients and to pool sizes that are correlated with the severity of the imposed osmotic stress (13). Many microorganisms can also synthesize glycine betaine. This can be accomplished under aerobic conditions, either through the stepwise methylation of glycine (14) or through the oxidation of choline (15–18). Synthesis of glycine betaine from choline is widespread in bacteria and typically involves the acquisition of choline from external sources and its two-step oxidation, with glycine betaine aldehyde as the intermediate. Various combinations of enzymes can be used for this biosynthetic process (15–18).

There is rarely a compound synthesized by microorganisms that cannot be used by other bacteria as a nutrient, and this is also true for the osmoprotectants choline and glycine betaine (10, 19). The use of glycine betaine as osmoprotectants and nutrients requires very careful control over the cellular content of this solute to perform the task at hand (18, 20), since these two processes rely on very different pool sizes. One of the organisms that can both

catabolize glycine betaine and exploit it as a stress protectant is the notorious human pathogen *Pseudomonas aeruginosa* (21).

In the current issue of the *Journal of Bacteriology*, Hampel et al. (22) present a comprehensive analysis of the acquisition, import, and catabolism of choline and glycine betaine by *P. aeruginosa*, a process that is genetically controlled by GbdR, a glycine betaine-responsive activator protein and member of the AraC family of transcriptional regulators (23). Through a clever combination of chemical biology, genome-wide transcriptional profiling, bacterial genetics, DNA binding studies, and bioinformatics, Hampel et al. (22) were able to define the GbdR regulon and identify those members that allow *P. aeruginosa* to liberate choline from host cells through hydrolytic enzymes, mediate its import, and afford its subsequent catabolism, first to glycine betaine and then further to the central metabolite pyruvate. (For details of the catabolic pathway, see Fig. 1 in reference 22.) Since some members of the GbdR regulon have previously been identified as virulence factors (24–26), the data reported by Hampel et al. (22) provide a link between metabolism and the ability of *P. aeruginosa* to colonize its hosts and persist at infection sites. The harvest, transport, and catabolic genes in the GbdR regulon are evolutionarily conserved in a number of microorganisms that can metabolize choline under aerobic conditions, in particular in several plant-associated bacteria (e.g., *Pseudomonas syringae* [20, 27]), thereby extending the physiological relevance of the reported findings well beyond the particular microorganism studied by Hampel et al. (22).

The GbdR activator protein responds to the inducer glycine betaine and its degradation product, dimethylglycine (DMG), and M. J. Wargo and coworkers have previously identified several genes and gene clusters that are under GbdR control (23, 24). To define the GbdR regulon of *P. aeruginosa* more comprehensively, Hampel et al. (22) now used genome-wide transcriptional profiling experiments, but they used a very clever approach to avoid

Published ahead of print 25 October 2013

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doi:10.1128/JB.01243-13

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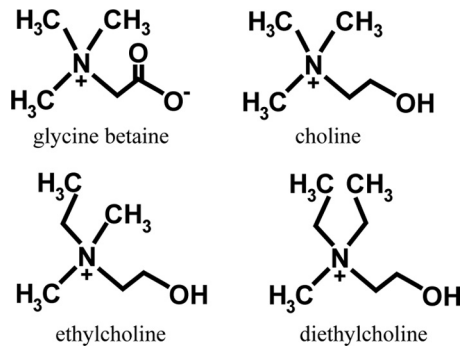


FIG 1 Chemical formulas of the quaternary amines glycine betaine and choline and of the synthetic choline derivatives diethylcholine and ethylcholine.

possible pitfalls that potentially could result from comparing the transcriptional profiles of a wild-type *P. aeruginosa* strain and its isogenic *gbdR* mutant. Since GbdR was already known to regulate genes for the import of choline and the inducers glycine betaine and dimethylglycine, *gbdR* mutant strains could potentially exhibit undesirable downstream metabolic and regulatory effects. Therefore, instead of simply comparing the transcriptome of a *gbdR*⁺ and *gbdR* mutant pair of strains, Hampel et al. (22) resorted to chemical biology methodology by exploiting the properties of synthetic choline derivatives (28). As shown by the authors, ethylcholine (Fig. 1) is a nonmetabolizable GbdR inducer; its inducing properties rely, however, on its BetBA-dependent *in vivo* conversion into a glycine betaine derivative. On the other hand, the chemically related diethylcholine (Fig. 1) is a GbdR-noninducing compound that, like ethylcholine, cannot be used as a nutrient by *P. aeruginosa*.

Using a set of very carefully chosen growth conditions with low concentrations of choline and the aforementioned synthetic choline derivatives as supplements, Hampel et al. (22) identified 11 GbdR-responsive transcriptional units in *P. aeruginosa* that comprise 25 genes. Inspection of the functional annotation of these genes revealed the presence of both previously discerned and newly identified members of the GbdR regulon and suggested their involvement in the acquisition, import, and metabolism of choline and glycine betaine.

P. aeruginosa can be found in various soil, estuarine, and marine habitats, and it will encounter in these ecosystems low concentrations of both choline and glycine betaine. It possesses several high-affinity import systems for these compounds (29) that allow the direct uptake of these compounds for the purpose of both osmoprotection and catabolism. Free glycine betaine and choline can also be found in organisms and host organs that can be infected by *P. aeruginosa* (e.g., the lung in cystic fibrosis patients) (18, 25, 30). However, in host cells and at infection sites, most of the available choline is bound in macromolecules, in particular in the head group of phosphatidylcholine, a major component of the plasma membrane of eukaryotic cells. Pulmonary surfactants are rich in phosphatidylcholine and they also comprise the choline-containing lipid sphingomyelin (25, 30); they are thus good sources for choline. Interestingly, the GbdR regulon (22) comprises members that can liberate the choline moiety from host-derived compounds: the hemolytic phospholipase PlcH (24), the phosphorylcholine phosphatase PchP (24), and the acetylcholine esterase ChoE (31). The PlcH enzyme is an excreted hydrolase and

is highly active in liberating the head group (phosphocholine) from phosphatidylcholine, a major constituent of the eukaryotic cell membrane and host lung surfactants. The subsequent enzymatic action of the PchP phosphatase then generates free choline.

Once harvested from host components, choline needs to be imported into *P. aeruginosa*, and the GbdR regulon members CbcXWV and BetX can accomplish this task. CbcXWV is a remarkable ABC transport system since its membrane-associated core components (CbcWV) can interact with several high-affinity periplasmic ligand-binding proteins (e.g., CbcX and BetX) that have distinct substrate profiles (27, 29, 32). As shown by a comprehensive analysis by Chen et al. of the plant pathogen *Pseudomonas syringae*, CbcX captures choline and glycine betaine, and BetX recognizes glycine betaine (27, 32). Once inside the cell, choline cannot be catabolized directly; instead, it is first converted through a two-step oxidation process by the BetBA enzymes into glycine betaine, which is then subsequently stepwise demethylated to dimethylglycine, monomethylglycine (sarcosine), and finally to glycine. Glycine is then further converted into serine, which in turn is catabolized to the central metabolite pyruvate. (For details of the catabolic pathway, see Fig. 1 in reference 22.) The expressed genes encoding the enzymes for the demethylation of glycine betaine (GbcAB, DgcAB, and SoxBDAG) and its conversion into serine (GlyA1) and further to pyruvate (SdaB) are all under GbdR control. This provides for the genetic integration of the harvest, import, and catabolic functions within the GbdR regulon of *P. aeruginosa* and their common induction in response to the choline metabolite glycine betaine and its degradation product, dimethylglycine (23). Additional regulatory input into the catabolism of glycine betaine is provided by a so-far-unidentified transcription factor that responds to monomethylglycine (sarcosine) and which controls those genes whose encoded proteins mediate the catabolism of sarcosine to pyruvate (23).

A significant finding of the study by Hampel et al. (22) is the observation that the genes encoding the enzymes for glycine betaine from choline (BetBA) are not under GbdR control. The expression of these genes, along with that of choline-specific transporter proteins BetT1 and BetT3, is osmotically and choline inducible and responds to the TetR family transcriptional regulator BetI (29), whose function as a regulator for glycine betaine synthesis was first elucidated in *Escherichia coli* (15, 33). The separate regulation of the choline to glycine betaine synthesis pathway from that of the catabolism of glycine betaine makes physiological sense, since *P. aeruginosa* uses glycine betaine not only as a nutrient but also as an osmoprotectant. The latter process requires the maintenance of very significant pool sizes of glycine betaine in osmotically challenged cells (18). Hence, a finely tuned balance between synthesis of glycine betaine and its catabolism needs to be achieved (18, 20, 34). Hampel et al. (22) suggest that the separation in the genetic regulation of the choline to glycine betaine synthesis pathways (via BetI) and of the route for the catabolism of glycine betaine to pyruvate (via GbdR) are key intersections for keeping situation-specific control over the cellular glycine betaine pools under osmotic stress and infection conditions. The two metabolites of phosphatidylcholine, choline and glycine betaine, also have stimulating effects through an unknown mechanism on the oxygen-sensitive regulator Anr of *P. aeruginosa* (25). Evidence suggests that Anr is important for the colonization of abiotic and biotic surfaces: increased Anr activity results in enhanced biofilm formation, and its absence impairs virulence of

P. aeruginosa in animal models (25). Hence, the quaternary amines choline and glycine betaine connect, via their influence on the GbdR and Anr regulators, metabolism and virulence traits of *P. aeruginosa*.

After having identified the core components of the GbsR regulon, Hampel et al. (22) did not rest their case and instead went on to validate the leads generated through the transcriptome analysis by DNA binding and footprinting studies with the purified GbdR activator protein. This allowed them to define the DNA sequence and salient features of the GbdR operator site, and through a bioinformatics approach, the authors exploited this information further by searching for possible GbdR-binding sites within the entire *P. aeruginosa* genome sequence. These combined approaches allowed Hampel et al. (22) to expand the knowledge on the *gbdR* regulon beyond the core set of genes highlighted by the transcriptome experiments and permitted the authors to recognize and experimentally verify *choE* as a GbdR-controlled gene. *choE* encodes a previously identified acetylcholine esterase (31), a protein that can liberate choline from a host-derived compound; it is thus part of the “harvest” subgroup of the GbdR regulon.

There are many genome-wide transcriptome studies that faithfully record the ups and downs in gene expression in response to environmental or cellular cues or in response to a particular set of regulatory proteins. However, the information gleaned from such studies frequently remains wanting, since the observed changes in the transcriptional profiles are not linked with solid physiological, biochemical, or genetic data, and their interpretation often rests solely on genome annotations. The study by Hampel et al. (22) stands out because it links a global approach with chemical biology and then uses the derived data set for in-depth studies to define, verify, and extend the knowledge on a complex regulon.

In addition to studying a process that not only is of substantial interest from an environmental point of view but also is of considerable significance for understanding the activities of pathogens at their infection sites, the properties of the GbdR protein itself are of great interest. The gene-activating function of the GbdR regulatory protein is triggered *in vivo* by the presence of glycine betaine in the *P. aeruginosa* cell (23, 24), thereby implying that GbdR binds glycine betaine directly. However, *in vitro* GbdR can also bind its operator sites at the *plcH* and *pchP* promoters in the absence of glycine betaine (24).

Why is the presumed direct binding of the inducer glycine betaine by GbdR of note? Glycine betaine is normally preferentially excluded from protein surfaces (6, 7); therefore, the following question arises: how can GbdR bind this compound with high specificity to activate gene expression? Aromatic cages of different architectures that can accommodate the positively charged head groups of choline and glycine betaine (Fig. 1) via cation- π interactions have been found in soluble ligand-binding proteins that operate in conjunction with ABC transport systems from *Bacteria* and *Archaea* and in the membrane-embedded glycine betaine carrier protein BetP from *Corynebacterium glutamicum*. Hence, nature has used common design principles to allow the specific and high-affinity binding of glycine betaine by evolutionarily not closely related transporter proteins (35). Will these design principles also extend to the GbdR regulatory protein? Time and experiments will tell!

ACKNOWLEDGMENTS

I am grateful to Chiliang Chen for thoughtful suggestions on the initial version of the manuscript and Vickie Koogle for help with language editing.

Work in my laboratory on compatible solutes and microbial responses to osmotic stress is funded by the Deutsche Forschungsgemeinschaft (DFG) through SFB-987 and by the LOEWE Program of the State of Hessen via the Center for Synthetic Microbiology (SynMicro, Marburg, Germany).

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