Significance of anaerobic ammonium oxidation in the ocean

Bess B. Ward

Department of Geosciences, Princeton University, Princeton, NJ 08544, USA

A missing link in the nitrogen cycle has recently been found: bacteria capable of oxidizing ammonium to nitrogen gas ($N_2$). This new source of $N_2$ has now been detected in both the water column and the sediments in marine environments. This discovery is important because it necessitates re-evaluation of (1) major fluxes in the global nitrogen budget and (2) regulation of the processes leading to fixed nitrogen loss.

Microbiologists often argue that some bacteria will figure out how to exploit any reaction with a favourable $\Delta G$. This claim is consistent with the impressive capabilities of bacteria to grow on substrates such as TNT and jet fuel, substances that are not only potentially toxic, but relatively recent additions to the substrate mixtures available to bacteria in nature. Only rarely can a novel metabolism be proven in the absence of a pure culture. This is the case, however, with the most recently discovered metabolism in bacteria, this time in the nitrogen (N) cycle. The anammox bacterium has yet to be isolated, but has nevertheless been described [1–3] and detected in the water column of two marine environments, both by its activity [4] and by its signature biochemistry [5].

The conventional N cycle

Denitrifying bacteria, which respire nitrate (NO$_3^-$) and nitrite (NO$_2^-$) to produce nitric (NO) and nitrous oxides (N$_2$O), and eventually nitrogen gas ($N_2$) (Fig. 1), are assumed to be responsible for the net sink of fixed nitrogen (i.e. the production of $N_2$) in the marine nitrogen budget. Some bacteria reduce NO$_3^-$ to ammonium (NH$_4^+$) instead of $N_2$, and denitrifiers (and other heterotrophic bacteria) release NH$_4^+$ from the mineralization of organic matter. This NH$_4^+$ was assumed to be either assimilated by microbes or oxidized back to nitrate by autotrophic aerobic nitrifying bacteria (Fig. 1). Inorganic nitrogen distributions in suboxic regions of the water column and sediments led oceanographers to suspect the existence of bacteria capable of coupling the oxidation of ammonium directly to the reduction of nitrate [6]. Denitrifiers and nitrifiers, however, are distinct and separate organisms, with quite different metabolisms and physiological requirements. The chemists argued that it must be possible; the microbiologists said there were no isolates that could do it.

The missing link

All of that changed with the discovery of the anaerobic ammonium oxidation (anammox) consortium [2,7], first described from a wastewater bioreactor in the Netherlands, and subsequently detected in sewage treatment plants all over Europe. Whether North American sewage treatment plants harbor anammox is as yet unknown, but it seems unlikely that bacteria involved in the process would observe geopolitical boundaries. The importance of the new pathway in the environmental nitrogen cycle must be evaluated to answer two questions: Does it change the overall magnitude of the flux from fixed nitrogen to $N_2$ (such that we must recalculate the global nitrogen budget); and does it change the regulation of the $N_2$ flux? If some of the organisms responsible for the $N_2$ production are obligately anaerobic autotrophs, rather than facultatively anaerobic heterotrophs, their activity is likely to be differentially regulated by environmental conditions and by interactions with other members of the microbial community.

The anammox bacteria (dominant members of the cultured consortium) are peculiar in several ways, including the use of hydrazine (a component of rocket fuel) as an...
intermediate [3]. Such a highly reactive intermediate has apparently necessitated the development of a subcellular compartment, the anammoxosome, which is bounded by unique ladderane lipids [8]. The anammox bacteria are members of the order Planctomycetales, characterized by unique cellular compartmentalization and unusual cell walls. Oxidation of NH$_4^+$ to N$_2$ is barely favourable energetically, and like conventional nitrifiers, the anammox bacteria compound their inefficiency by living as autotrophs, consequently squandering their hard-earned reducing power to fix their own carbon dioxide (CO$_2$). It is not surprising therefore that their shortest reported generation time is 10.6 days in a sequential batch reactor system [3]. Such slow steady growth might be selective in a bioreactor, but is it in the ocean?

**Anammox in the ocean**

Ammonium often accumulates in anoxic sediments owing to the lack of aerobic nitrification. On the basis of inorganic nitrogen distributions (e.g. [9]), ammonium oxidation coupled to nitrate reduction has, in the past, been implicated in sediments, but often the mass balances of ammonium and nitrate were not precise enough to suggest that any nitrogen was ‘missing’. Dalsgaard and Thamdrup [10,11] showed, by the unique labeling pattern in anoxic $^{15}$N-tracer experiments, that an anammox-like reaction was indeed occurring in sediments. Conventional denitrification could not account for the production of $^{29}$N$_2$ from $^{15}$NH$_4^+$ and $^{14}$NO$_3^-$, that Dalsgaard and Thamdrup [10,11] observed, but anammox should produce precisely that product. Although the identity of the bacteria responsible for the anaerobic oxidation of ammonia using nitrite (reduced from nitrate by denitrifiers) in marine sediments has not been verified, it seems likely that the tell-tale signature of the Planctomycetales will be discovered there.

The first occurrence of anammox in the water column of natural environments has now been reported. Several lines of evidence support the discovery of anammox in the Golfo Dulce and the Black Sea. Kuyper et al. [5] demonstrated the presence of the anammox type cells by detection of their signature ladderane lipids and retrieval of 16S rRNA sequences that cluster with known anammox rRNA genes. The similarity between the Black Sea sequences and those from bioreactor enrichments (87.6% to 98.1%) indicates high diversity within the group (a bacterial ‘species’ is often defined as encompassing strains with similarities of 97%). Kuyper et al. [5] also demonstrated the potential for anammox when they detected the accumulation of $^{29}$N$_2$ in incubations to which high concentrations of $^{15}$NH$_4^+$ and $^{14}$NO$_3^-$ were added.

Dalsgaard et al. [4] determined the rate of $N_2$ production from anammox using $^{15}$N-tracers, and concluded that anammox contributed 19% and 35% respectively of the total $N_2$ flux in the water column at their two stations in the Golfo Dulce. In both cases of water column anammox, the depth interval over which the process was detected was narrowly constrained to anoxic waters where nitrate or nitrite was present. The known anammox organisms are reversibly inhibited by oxygen concentrations higher than 2 $\mu$M [3]. At the other limit, where sulfate reduction occurs, the presence of sulfide inhibits conventional coupled nitrification/denitrification [12], thus removing the oxidant required for anammox. These limits constrain the depth distribution of anammox observed in the Black Sea and the Golfo Dulce, and imply that the extent of oceanic oxygen minimum zones in which anammox might occur is rather limited. This kind of interface environment, however, is common in marine sediments. Dalsgaard and Thamdrup [10,11] found that the contribution of anammox to the total $N_2$ flux increased (to a maximum of 67%) as the magnitude of the total $N_2$ flux decreased. Extrapolated to the entire ocean, this pattern would imply a low rate of anammox over a vast expanse of deep-sea sediments. Frietag and Prosser [13] did not detect the Planctomyces-like sequences in their search for anaerobic ammonia oxidizers in anoxic marine sediments from an organic rich sea loch, and this might be consistent with the predicted low anammox activity in rich environments.

**Impact on the N Cycle**

The higher rates of anammox activity detected in intense oxygen minimum zones, combined with the potential for low-level but widespread sediment activity, led Devol [14] to suggest that anammox could account for 30–50% of the $N_2$ production in oceans. Mineralization by denitrification of organic matter with an average elemental composition implies that the maximum potential contribution of anammox to $N_2$ production is 29%. A larger contribution is possible where N-rich organic matter is decomposed or where NH$_4^+$ is supplied from outside the anammox zone. This does not mean that the global rate of $N_2$ production has just been increased by 30–50%; at the least, it simply partitions some of the $N_2$ flux into a new pathway. The extent to which the loss of fixed N has been previously underestimated varies widely depending on the method used to make the estimates. If it has been underestimated, then re-evaluation of the N cycle must also focus on nitrogen fixation, to understand the state of balance or imbalance in the N budget. Equally as important however, are the implications for the regulation of a pathway that was previously ignored and is still poorly characterized in the environment. The sensitivity of nitrogen fluxes, including the trace gases NO and $N_2O$, to environmental changes such as nutrient loading, increased anoxia (such as in the Gulf of Mexico) and even temperature, must be evaluated in light of the bacterial metabolisms involved. In the laboratory, anammox can produce small amounts of $N_2O$, but this intermediate is not observed in wastewater bioreactors or in the sediment and water column examples of anammox. Conventional nitrification and denitrification both produce NO and $N_2O$. Therefore, the ratio of anammox to conventional coupled nitrification/denitrification could influence net oceanic fluxes of these important trace gases, with ramifications for greenhouse warming and the catalytic destruction of ozone in the stratosphere. A mystery in the marine nitrogen cycle has been resolved, then, but important research remains to be carried out on the molecular biology, physiology and ecology of anammox.
Gastric cell apoptosis and *H. pylori*: has the main function of VacA finally been identified?∗

Patrice Boquet1, Vittorio Ricci2, Antoine Galmiche3 and Nils C. Gauthier1

1INSERM U452, Faculty of Medicine, 28 Avenue de Valombrose, Nice, France
2Department of Experimental Medicine, Human Physiology Section, University of Pavia, Pavia, Italy
3Max Planck Institute for Infection Biology, Department of Cellular Microbiology, Campus Charité Mitte, Berlin, Germany

The vacuolating cytotoxin VacA is one of the most important virulence factors of *Helicobacter pylori*, a bacterium causing severe gastric diseases such as ulcers and cancer. VacA forms large cytoplasmic vacuoles in cultured cells, although its effects on host cells in vivo remain to be elucidated. Three independent groups have reported that VacA induces epithelial cell apoptosis. In particular, a recent study has demonstrated unambiguously the role of VacA in inducing epithelial gastric cell apoptosis.

The observation that large cytoplasmic vacuoles form in cells that are incubated with *Helicobacter pylori* broth-culture supernatants led, 15 years ago, to the discovery of the vaculating toxin VacA [1]. VacA-induced vacuoles are now recognized to originate from late endosomes [2,3] (see supplementary video). The vacA gene is present in all *H. pylori* strains examined, although these bacteria differ considerably in their production of vacuolating cytotoxins. This is mainly because of variations in cytotoxin structure, the regions of highest diversity being localized at the N-terminal part of the toxin (corresponding to two different toxin signal sequences s1/s2) and in the mid-region of VacA (m1/m2, corresponding to two different mid-regions of the toxin that are required to bind different cell types). VacA of genotype s1/m1, unlike that of genotype s2/m1 (a form of VacA associating the signal sequence type 2 with the mid-region type 1), can vaculate cells [3]. This difference is due to an additional sequence of 12 amino acids at the N-terminal end of s2 VacA, which inactivates the toxin [3]. The association of VacA-positive *H. pylori* strains with gastritis, gastroduodenal ulcers and gastric carcinoma favours the notion that the cytotoxin is an important pathogenic factor of this bacterium [3,4]. VacA is produced as a monomeric protein (88 kDa) that exhibits the structure of an A–B toxin (Fig. 1a). Oligomerization of VacA monomers in the plane of artificial lipid membrane [3] or in the HeLa cell plasma membrane [5] forms selective anionic transport channels [3,5]. Based on these results, it has been postulated that VacA channels formed at the cell surface could be endocytosed and, upon reaching late endosomes, might activate the electrogenic V-ATPase by allowing an influx of Cl− into this vesicle [6].

Other activities of VacA

Despite 15 years of research, it is not known whether cell vacuolation is the main function of the VacA cytotoxin in the overall virulence of *H. pylori*. It has been proposed that

References

0966-842X$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0966-842X(03)00181-1