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Character and Function of Anammox Bacteria under Environmental Stress

Ola Bäckman

Institutionen för kemi och molekylärbiologi
Naturvetenskapliga fakulteten

Akademisk avhandling för filosofie doktorsexamen i Naturvetenskap, inriktning kemi som med tillstånd från Naturvetenskapliga fakulteten kommer att offentligt försvaras fredagen den 4 oktober kl. 10:15 i KB, Institutionen för kemi och molekylärbiologi, Kemigården 4, Göteborg.

Fakultetsopponent: Professor Mark Trimmer, The School of Biological and Chemical Sciences, Queen Mary University of London

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Abstract

During the last few decades observations of novel processes involved in nitrogen transformations have fundamentally challenged the view of pathways and con-trolling mechanisms during local and global nitrogen cycling. Anaerobic ammonium oxidation (anammox) constitutes one of these new pathways where autotrophic bacteria oxidize ammonium by nitrite to dinitrogen gas under anaerobic conditions. Anammox provides a shunt during nitrogen transformations as it bypasses the classical pathway of aerobic nitrification coupled to anaerobic denitrification, a reaction scheme previously thought to be the sole source of dinitrogen gas in natural environments. Anammox is now acknowledged as a widespread and a globally important sink for nitrogen in water column and sediment systems.

The first part of this thesis emphasises factors that regulate anammox bacteria in natural environments. Particular focus relates to coastal marine sediments and the importance of anammox for nitrogen removal under environmental stress associated with the temporal availability of oxygen and nutrients. Measurements of anammox and denitrification were made by ^{15}N amendments including both shallow-water illuminated autotrophic (net oxygen producing) sediments and deeper heterotrophic (net oxygen consuming) sediments. While rates of anammox were insignificant in illuminated sediments with primary production by benthic microalgae, anammox was found almost as important as denitrification for total N_2 production in the dark heterotrophic sediments. Long term laboratory incubations under different oxygen conditions confirmed the importance of oxygen availability for the removal of bioavailable nitrogen by N_2 production in surface sediments.

In the second part of the thesis investigations focus on detailed mechanisms involved during anammox. Cutting edge analytical tools of membrane proteomics were utilized to identify and sub-cellularly localize key proteins involved in the anammox reaction. Two proteins, the hydrazine synthase (previously hydrazine hydrolase) and an F-ATPase, were identified by proteomics and LC-MS/MS analysis and subsequently targeted for antibody production. Through immunogold electron microscopy the hydrazine synthase was assigned to the interior of the anammoxosome, the unique “organelle” of anammox bacteria. The F-ATPase was associated with the anammoxosome membrane. These observations not only strengthen the important role of the anammoxosome during anammox metabolism, but also provide experimental support to the idea of the anammoxosome as an energized membrane.

Keywords: Anammox | N-cycle | environmental stress | redox oscillations | anammoxosome | key proteins

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