Bacterial Mortality: A Pathway for the Formation of Refractory DOM?

T. Nagata[1], D. L. Kirchman[2]

[1] Ocean Research Institute, University of Tokyo, Minami-dai, Nakano, Tokyo 164-8639 Japan
[2] College of Marine Studies, University of Delaware, Lewes, DE 19958 U.S.A.

ABSTRACT

Dissolved organic matter (DOM) represents a major part (>90%) of the organic carbon pool in seawater, and plays important roles in oceanic biogeochemistry. Recent studies have suggested that bacterial membrane proteins (porins) and a cell wall component (peptidoglycan) account for a large fraction of high-molecular-weight DOM in a variety of oceanic environments including deep waters. These new findings have stimulated interest among investigators in the mechanisms underlying the formation, degradation and preservation of detrital biopolymers derived from bacteria. The purpose of this paper is to discuss the following two hypotheses: (i) liposome-like colloidal particles (phospholipid vesicles with an aqueous phase interior) are released to seawater during bacterial death and; (ii) these small particles play a role in the formation of semi-labile and refractory DOM. Phagotrophic flagellates grazing on bacteria release liposome-like particles (picopellets) consisting of bacterial membranes, proteins and other prey components. Also, liposomes are likely formed during lysis of bacteria by viruses. Because of the structural feature of these particles, biopolymers associated with liposomes are sterically protected from rapid ectoenzymatic attack in seawater. Owing to the protection and close association of macromolecules at high concentrations, liposome-like particles probably provide "micro-reaction chambers" that facilitate diagenetic reactions that may create refractory DOM. Our review suggests that bacterial death processes are central among controls of DOM dynamics in oceanic waters.

Introduction

In oceanic environments, large amounts of dissolved organic matter (DOM) are produced by several biological mechanisms including phytoplankton excretion, sloppy feeding, and egestion by grazers. Although there is a great variety of compounds in DOM, they can be ordered, as a first approximation, along a continuum of decreasing biological reactivity. At one end, highly labile components, such as dissolved free amino acids and free sugars, are rapidly consumed by bacteria (on a time scale < days), providing a basis for a tightly coupled flow of carbon through the microbial loop [1]. At the other extreme, selected components of DOM strongly resist microbial degradation and accumulate in seawater as a highly refractory DOM pool (average age > 1,000 years [24]). The gap between the labile and refractory components is filled by semi-labile DOM [3], which is degraded by microorganisms but only at very slow rates (average turnover times of months to hundreds of years). The DOM pool consists of roughly 50% refractory and 50% semi-labile DOM in the upper oceans [24] and plays important roles in global and regional biogeochemistry

Microbial Biosystems: New Frontiers
Proceedings of the 8th International Symposium on Microbial Ecology
Bell CR, Brylinsky M, Johnson-Green P (eds)
Atlantic Canada Society for Microbial Ecology, Halifax, Canada, 1999.

[3, 24]. Concentrations of labile DOM are very small and represent a trivial fraction of total DOM (< 1%). However, the chemical identity of semi-labile and refractory DOM, and the mechanisms by which these DOM compounds escape rapid microbial degradation in seawater, is not well understood.

Recent studies have suggested that biopolymers derived from bacteria can represent dominant components of high molecular weight DOM (HMW-DOM) in the oceans. Tanoue and colleagues [21, 22, 23] found that a porin, a type of bacterial outer membrane protein, dominates the dissolved protein pool in both surface and deep oceans. Based on the enantiometric ratio of amino acids in HMW-DOM, McCarthy et al. [13] suggested that peptidoglycan remnants originating from bacterial cell walls constitute a major fraction of the dissolved organic nitrogen in the oceans. These new observations suggest that bacteria are a major proximate source of DOM in oceanic waters, but production and preservation mechanisms of bacterial polymers in seawater remain unknown.

This paper discusses recently emerging ideas on microbial controls of the cycling of HMW-DOM in seawater. We specifically focus on the following two processes: i) production of liposome-like small particles during protist grazing and viral infection and; ii) protection by liposomes of labile protein from rapid microbial degradation in seawater. Our aim is to examine if labile DOM can become more refractory because of steric protection by liposome-like structures produced during bacterial death.

Production of Liposome-like Particles in Seawater

One major cause of bacterial death in seawater is grazing by protists such as phagotrophic flagellates [6]. Digestion of prey bacteria by protists is a dynamic process in which prey cell material is vigorously attacked by digestive enzymes within acidic food vacuoles. Observations by electron microscopy have shown that membraneous vesicles, possibly of prey bacterial origin, are abundant in food vacuoles during the later stage of digestion, which suggests that bacterial membrane components are relatively resistant to digestive enzymes [5]. Owing to high concentrations inside the vacuoles, these membraneous materials spontaneously form liposome-like vesicles, i.e., phospholipid particles with an aqueous center. Along with egestion, incompletely digested prey materials including liposomes are released to seawater as “picopellets” [14, 18].

Biochemical measurements have provided data in support of the above model that bacterivorous flagellates release liposome-like picopellets. Nagata and Kirchman [16] have shown that macromolecules account for 10 - 30% of the total DOM that is released by heterotrophic flagellates grazing on bacteria, and that these macromolecules are rich in lipids. They also found that one component of released macromolecules is acid phosphatase, a digestive enzyme of the flagellates. Notably, the released acid phosphatase is highly resistant to proteases but is susceptible to proteases after treatment with polymyxin B, which disrupts bacterial membranes. This observation suggests that labile proteins released by flagellates may be protected by liposome-like structures composed of prey bacterial membranes.

Liposomes are likely formed by the burst of bacteriophages, another major causative factor of bacterial death [7, 8]. In support of this, it has been observed that detrital colloids (size 0.4 - 1 μm) are abundantly produced along with the burst of viruses infecting marine bacteria [20]. An important implication of this observation is that large amounts of bacterial fragments are introduced into seawater due to viral-induced mortality. Membranes and walls probably represent the major constituents of these fragments, which

may spontaneously form liposome-like structures, although biochemical characterization of released material has yet to be done.

Preservation by Liposomes of Labile Protein in Seawater

Associations of protein with liposome-like structures greatly affect degradability of protein in seawater. These associations include adsorption to the outer surface of liposome-like particles, entrapment inside the aqueous center, and integration within the membrane component [2, 18]. Experiments have been conducted to examine how different types of associations affect bacteria - protein interactions.

In a study on the degradation of protein adsorbed to surfaces of model colloidal particles (polystyrene beads, diameter 0.1 - 1 μm), Nagata and Kirchman [17] found that adsorbed protein is degraded by marine bacteria at much slower rates (1/10 - < 1/100) than the same protein freely dissolved in seawater. These results are explained partly by collision frequency between particles and bacteria, but the data also suggest that hydrolysis is impeded by adsorption. This observation is consistent with other data showing that labile protein can be quickly (< day) transformed into more refractory protein in seawater, possibly due to adsorption to other organic matter [10]. Thus adsorption appears to be a mechanism by which protein temporally escapes ectoenzymatic attack in seawater.

Borch and Kirchman [2] suggested that protein entrapped inside liposomes is physically protected from degradation by bacteria. This hypothesis was supported by experimental data showing that protein entrapped inside synthetic liposomes are more slowly degraded than free protein in seawater. The data also showed that bacteria degrade liposomes (phospholipid) more slowly than protein. At the end of their experiment, which was conducted for up to 22 days, about half of the entrapped protein initially added was preserved, while free protein was completely degraded during the same period.

The hypothesis that bacterial membrane proteins resist degradation was tested by Nagata et al. [19] who determined degradation rates of soluble and membrane protein isolated from a bacterium. The membrane protein was prepared by destruction of bacterial cells by sonication followed by ultracentrifugation. Radiolabeled proteins were either inside the lipid bilayer, adsorbed to vesicles, or trapped inside the liposomes. The results showed that proteins in this crude membrane extract are degraded at significantly slower rates (1/2 - 1/6) than the soluble proteins. The authors also found that proteins intimately associated with membrane (i.e., integrated into the membrane matrix or trapped inside liposomes) are even harder to degrade. These results are consistent with a model in which membrane and wall components severely restrict access of bacterial proteases to proteins. Alternatively, some proteins in the membrane fraction could be resistant to protease hydrolysis because of inherent structural properties of the protein molecules. However, membrane proteins isolated from membrane and wall components appear to be degraded as easily as soluble protein (R. Fukuda, unpublished), suggesting that association with liposomes plays a major role in resistance of membrane proteins to degradation.

The Liposome Hypothesis

Lipid concentrations in the DOM fraction are typically 10 - 100 μg $liter^{-1}$ in upper waters, representing < 10% of total DOM [2]. Even though lipids are a relatively minor component of DOM, they can be important because they can form liposome-like structures. Note that, even in living cells, the content of lipids is generally low (ca. 10% of total mass), but this amount is high enough to maintain cell (i.e., liposome) structures. We

hypothesize that liposomes protect labile proteins and other DOM from rapid enzymatic degradation by bacteria. This hypothesis may help explain substantial accumulation of membrane protein in oceanic waters [22, 23]. Also, the presence of large amounts of peptidoglycan remnants in the oceanic DOM pool [13] is consistent with a model in which bacterial wall components (peptidoglycan) are tightly complexed with membrane components (phospholipids, lipopolysaccharide) to become a part of liposome-like structures. Data from the laboratory experiments we described support the liposome hypothesis; different types of associations of proteins with liposomes all impede degradation. The liposome hypothesis is consistent with the double layer hypothesis of Lee and Wakeham [12], which suggests that the presence of one organic layer can impede the degradation of other material not exposed directly to ectoenzymes. Similarly, Chin et al. [4] hypothesized that labile material can be protected from degradation due to entrapment within polymer networks consisting of lipids, proteins and carbohydrates.

It is intriguing to speculate that preservation of organic matter by liposomes contributes to the formation of refractory DOM. Membrane proteins [22] and peptidoglycan remnants [13] are abundantly detected even in intermediate and deep waters, suggesting that these molecules are preserved longer than time scales of the circulation of these water masses (> 100 years). Nagata and Kirchman [16] hypothesized that high concentrations of diverse organic molecules within liposomes facilitate diagenetic reactions that may create refractory compounds [25]. In surface waters, ultraviolet irradiation may enhance these reactions [10]. Thus, structural properties of liposomes could provide “micro-reaction chambers” in which geochemical modifications of sterically protected organic matter are substantially enhanced.

Community and Ecosystem Implications

The release of labile DOM induced by bacterivory and viral infection has been regarded as a short-circuit of the microbial loop [7, 15], where organic carbon is intensively recycled via a cyclic flow of matter within a DOM - bacteria loop. At a community level, bacterial recycling of organic matter may significantly increase the ratio of secondary production relative to primary production [7] by a mechanism called network virtual amplification [9]. The production of semi-labile and refractory DOM along with bacterial mortality substantially adds to this concept; bacteria are now among major inputs to the organic carbon reservoir in seawater. Storage of DOM in upper oceans may result in large-scale transport of carbon mediated by mixing and advection [3]. The storage also allows temporal and spatial uncoupling between production and consumption of organic matter [11]. Importantly, only a small portion of total DOM production needs to be preserved in order to contribute greatly to the formation of large DOM storage. Furthermore, small differences in degradability of DOM may result in substantial accumulation. Thus, controls and dynamics of semi-labile and refractory DOM are inherently subtle and complex. Traditionally, the only source term seriously considered in DOM modeling has been phytoplankton. However, mechanistic considerations presented in this paper, and geochemical observations [13, 23], emphasize a need to formulate a revised model in which bacterial death processes are among the central regulatory elements of DOM dynamics in oceanic waters. Along this research path, the liposome hypothesis should provide a useful framework for further investigating controls of DOM-bacteria systems in the oceans.

Acknowledgments

We thank Kazuhiro Kogure for providing us with an opportunity to present this paper at ISME-8. This work was supported by grants from MESSC and NSF.

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