Bacterial porin in the ocean and its ecological implications

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ABSTRACT

Dissolved protein is an important component of the flow of organic substances in the sea. This study attempted to elucidate the mechanisms responsible for the formation and stabilization of specific dissolved protein molecules. The dissolved proteins studied were the porin homologues of *Pseudomonas* and *Vibrio*. A survey using antisera specific for bacterial cell (anti-*P. aeruginosa*) and porin P that most likely originated from *P. aeruginosa* (anti-48DP N-14) revealed that cell numbers detected with anti-48DP N-14 were higher than that detected with anti-*P. aeruginosa*. This suggests that porin homologues of *P. aeruginosa* are present in bacteria other than *P. aeruginosa*. Furthermore, antiserums against porin of *V. anguillarum* (anti-Omp35La), which can detect major porin of *Vibrio* genera, reacted similar to proteins of non-*Vibrio* group bacteria. These results strongly suggest that the porins originated from a diversity of bacterial groups rather than solely from the two genera above, and that they are able to survive and form dissolved proteins in the ocean.

Introduction

Dissolved organic matter (DOM) in the ocean is one of the largest pools of organic matter on the planet. DOM includes a diversity of molecules including proteins, nucleic acids, polysaccharides and numerous low molecular weight compounds. These biological molecules are believed to be largely decomposed into inorganic matter, although some become geochemicaly transformed into polymerized organic compounds that are relatively resistant to degradation.

In recent years, however, it was found that 48 kDa specific dissolved protein is present as DOM and does not appear to be degraded [1]. Fig. 1 represents a hypothetical illustration of the fate of these specific proteins in relation to the overall degradation of biological materials. N-terminal amino acid analysis of the 48 kDa protein revealed that the dissolved protein is a homologue of a bacterial porin OprP of *Pseudomonas aeruginosa* [2]. The OprP was induced when *P. aeruginosa* was exposed to phosphate deficient environments. However, *P. aeruginosa* is a human pathogen and is not found in the ocean. These findings lead to the questions of what the 48 kDa OprP homologue is and why is it present in the ocean. In an attempt to answer these questions we carried out studies using antibody probes against two porins to determine whether there are dissolved proteins homologous to bacterial porins in seawater samples collected in subarctic, subtropical, tropical and antarctic areas. We also carried out trials to directly count and isolate probe-reactive bacteria. We here summarize the discovery of the porin homologues in seawater and some more recent findings.

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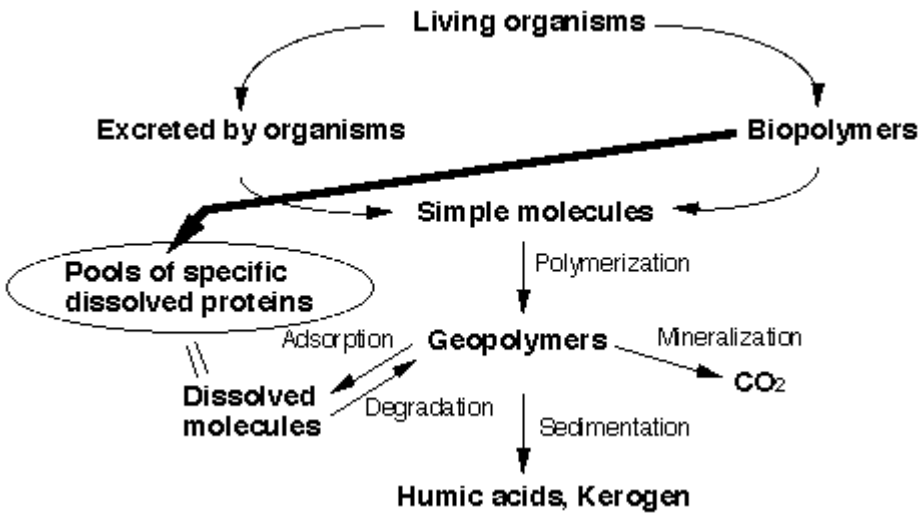

Fig. 1 Metabolic pathway of biomolecules in the ocean. The specific dissolved proteins escape from degradation. A new pool is added to the pathway proposed by Cauwet [1].

Dissolved Proteins in the Ocean

Tanoue et al. [5] discovered that approximately 30 polypeptides could be detected in SDS-PAGE in concentrated seawater samples that were collected from the arctic to the antarctic. Among the polypeptides, the 48 kDa-band was found in most samples suggesting that this protein is widely distributed in the ocean. The N-terminal amino acid sequence revealed that the 48 kDa protein has a 100% identical sequence in the N-terminus to that of *Pseudomonas aeruginosa* porin OprP. Since *P. aeruginosa* has not been recognized as being present in the ocean, it was questioned as to what the origin of the 48 kDa protein is. In order to examine the distribution of the 48 kDa protein molecule in many samples, an antibody against the primary sequence of the N-terminal amino acids of the protein was prepared [4]. Using this immunological probe (anti-48DP N-14), it was found that the dissolved proteins have similar antigenicity to the N-terminal 14mer of the 48 kDa protein [4]. We also used another antibody which recognizes the Omp35La porin of *Vibrio anguillarum* and other species belonging to the genus *Vibrio* [3]. This antibody reacted to 34 to 48 kDa proteins of seawater samples, indicating that the porin of the *Vibrio* genus is also a candidate for the origin of the dissolved protein [4]. The above suggests that bacterial porins may be one of the sources of the dissolved protein.

Bacteria Producing Dissolved Proteins

Studies were carried out to determine what kinds and how many bacteria produce the porin homologues reacting to the antisera. The first experiment was conducted by the University of Tokyo group. Using immunofluorescent microscopy, they counted the bacteria reactive to anti-*P. aeruginosa* whole cell antibody (commercially available anti-*P. aeruginosa*) and to anti-48DP N-14 in a sample collected during the Tnasei-maru R/V (KT-97-6) research cruise. They found the number of cells reactive to anti-48DP N-14 to be much greater than the number reactive to anti-*P. aeruginosa* (Kimata, unpublished data). This suggests that bacteria possessing antigen similar to *P. aeruginosa* are present in the ocean. Furthermore, it was found that bacteria other than the anti-*P. aeruginosa* reactive cells possess similar

antigenic sites to the N-terminal 14mer of the 48 kDa dissolved protein and *P. aeruginosa* OprP. These results indicate that the origin of the OprP homologue is not from one species.

The second experiment was carried out by the Kochi University group using anti-Omp35La. Culturable bacteria were isolated from the sample obtained on the KT-97-6 cruise. Anti-Omp35La reactive bacteria were screened by colony-Western blotting. After screening two times, 37 isolates were recognized as real positives and these were used for further Western blotting using their outer membrane proteins (OMP). The bacteria having reactive proteins sizing 30 to 40 kDa composed 16 strains. Other strains were negative in Western blotting suggesting that their positive reaction in colony-Western blotting was due to other antigenic substances, such as lipopolysaccharides. The strains with anti-Omp35La reactive OMP were classified by general biochemical properties and restriction fragment length polymorphism (RFLP) analysis. The antibody reactive isolates included species of the *Vibrio* genus and other genera. RFLP analysis showed that all Omp35La producing-isolates were not *V. anguillarum* alone, although strains close to *V. orientralis*, *V. pelagium*, and *V. tubiasii* were included. These results indicate that the Omp35La homologue was produced by other organisms beside *V. anguillarum*. Taken together, this strongly suggests that the organisms responsible for producing the homologue are not restricted to the genus *Pseudomonas* and *Vibrio*.

Stability of Omp35La Against Protease

In order to examine the stability of bacterial porin in the ocean, we designed a preliminary *in vitro* experiment to test whether Omp35La is hydrolyzed by protease. Since it was hypothesized that stability is dependent on the physical form of the protein, the outer membrane containing Omp35La and isolated-Omp35La were prepared from *V. anguillarum*, and then digested with trypsin. The results showed that Omp35La embedded in the membrane envelope was resistant to trypsin. When analyzed by native SDS-PAGE without using a reducing agent or heating, Omp35La was detected as trimer, indicating that this protein is present as native trimer after treatment with trypsin. Other minor proteins associated with the envelope were hydrolyzed under this condition. On the other hand, isolated Omp35La was hydrolyzed with trypsin, although Omp35La was more stable than other proteins. This suggests that if in the natural environment the porins are present embedded in membrane, the proteins may be protected from protelytic degradation. A schematic drawing of the possible stabilizing mechanism of the porins and related membrane proteins is presented in Fig. 2. We plan to carry out further experiments to clarify the mechanism of the stability of the dissolved protein molecules. Bacterial cells are decomposed by many kinds of processes, such as infection by phage and by grazing by heterotrophic nanoflagellates. The different "cell death" processes are thought to be important in determining the nature of the specific protein pool.

Conclusions and Unanswered Questions

1. The source of dissolved proteins in sea water is partly from bacterial porins and possibly from related membrane proteins.
2. Porin embedded in membrane is resistant to proteolytic degradation.
3. The native form of the dissolved protein is not known.
4. The quantity and dynamics of dissolved protein in the ocean is not well understood.

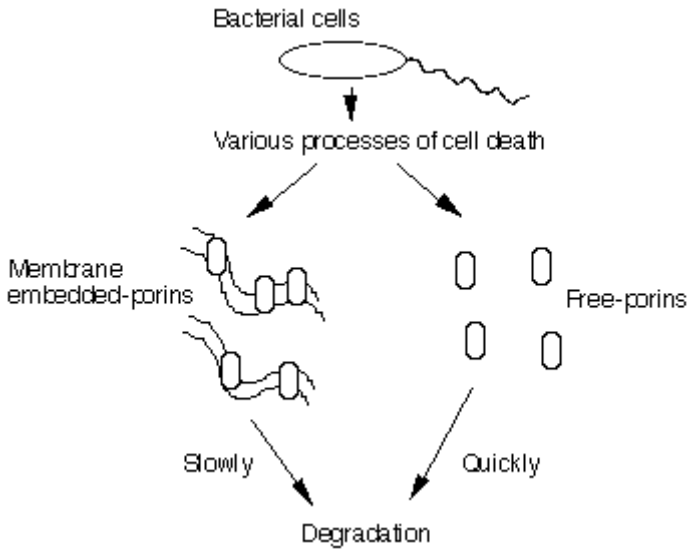

Fig. 2 Postulated destiny of bacterial outer membrane proteins. Various cell death processes could be important in determining the degradation kinetics of the proteins.

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