Relationship between ectoenzymatic activity and availability of organic substrates (Ross Sea, Antarctica): an experimental approach

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Organic matter consumption and decomposition were studied in four experimental systems, having collected different organic substrates in the Ross Sea in December 1994. For the experimental approach selected, processes normally acting on a mixed pool of substances could be separated and the main features of each phenomenon could be focused on. Through the strict relationship between each experimental system and natural conditions shown by organic matter assessment, ectoenzymatic activity trends and their relation with Antarctic water substrates could be described. Through ice melting the water column becomes rich in large pools of substrates, as well as enzyme-producing micro-organisms, capable of quick development. The quantitative predominance of leucine-aminopeptidase throughout the year is well known, but its relative importance seems to decrease when, owing to production events, the environment is enriched with autotrophic- and heterotrophic-derived substances, leading to glycolytic enzymes expression. Thus, ectoenzymatic activity is supposed to be one of the factors responsible for organic matter variations, showing quantitative and qualitative changes depending on substrate availability.

Introduction

Particulate organic matter in seawater is derived from a number of sources and shows a wide range of biochemical kinds (Amy et al. 1987). Despite environmental variability, consumers are a common step in organic matter bulk transformations (Nelson and Smith 1986; Knox 1994; Vetter and Deming 1994). The role of heterotrophic microbial organisms in organic matter changes is quite a complex problem when an ecological approach is followed. Bacteria, for example, are metabolically capable of exhibiting plasticity to face environmental changes and, owing to their consumption and production abilities, are able to modify sharply the organic matter pool (Azam et al. 1992). Beside uptake and production evaluation, the assessment of hydrolytic activity of microbial enzymes is a cornerstone and a popular approach to describe the main patterns and rates of organic matter exploitation (Vives-Rego et al. 1985; Meyer-Reil 1987; Chröst 1989; Chröst and Velimirov 1991; Hoppe 1991; Smith et al. 1992; Vrba et al. 1992; Unanue et al. 1993; Christian and Karl 1995). This approach may give clues on both quantitative expression and on the efficiency of organic matter consumption (Christian and Karl 1995).

Autotrophic-derived material, which is the main organic matter fraction in the Antarctic environment (Cho and Azam 1990; Fabiano et al. 1993; Fabiano et al. 1996), causes a very patchy distribution of production-consumption systems, characterised by quick development and small influence areas (El Sayed and Taguchi 1981; Smith and Nelson 1985). These areas may be found in pack-ice marginal zones, which are the main production sites, especially in spring, when ice-associated algae embedded during previous year freezing (Smith and Nelson 1985; Nelson and Smith 1986; Treguer and Jacques 1992) are released by ice melting in the surface water column. In the water column phytoplankton and zooplankton are a typical feature of mature systems, capable of supporting a high tertiary production (Jacques 1989; Fabiano et al. 1993). Furthermore, they lead to water column and sediment enrichment with available and labile substances (Bodungen et al. 1986; Fabiano et al. 1996).

This paper reports the investigations on ectoenzymatic activity development in relation to short-term...
changes in the biochemical composition of three different organic matter pools (ice-associated organic matter, phytoplankton, zooplankton). These substrates are usually found in the Antarctic marine environment during its seasonal development. They show a different mixture, depending on the season and on the functional state of the system itself. In order to characterise the different experimental systems and link them to natural conditions, the following parameters were measured: ectoenzymatic activity ($\beta$-glucosidase, $\beta$-N-acetylglucosaminidase, leucine aminopeptidase), bacterial abundance, autotrophic fraction (chlorophyll-a and phaeopigments) and particulate organic matter (nitrogen, carbon, proteins and carbohydrates).

### Materials and methods

#### Substrate choice and collection

The substrates utilised for the experiments were collected between 14 and 15 December 1994 next to the marginal ice zone of Terra Nova Bay polynya (75°08'.74'S, 166°07'.59'E), where drifting pack ice was observed. The substrates were chosen in order to obtain a wide scenery of natural conditions. The first experimental system focused on the development of particulate organic matter deriving from pack-ice melting. The sampled ice showed evident ice-associated algal communities at the bottom and, as suggested by DeLille et al. (1995) and Archer et al. (1996), probably also high abundances of heterotrophic micro-organisms. The second experiment dealt with water column organic matter, mainly of phytoplankton origin but with some heterotrophic contribution. This substrate was supplied by means of vertical phytoplankton sampling (0–50 m) performed with a 40-µm net. A great abundance of diatoms (over 10⁶ cells l⁻¹) and the presence of Prymnesiophyceae (about 10⁵ cells l⁻¹), usually assembled in large colonies (Knox 1994), were recorded in the sampling area (Marino and Cabrini 1997). The third experiment was performed with a detrital substrate of zooplanktonic origin. The zooplankton-derived organic matter was collected with a 500-µm net. The community mainly consisted of copepods (especially Calanoides, female and C5; P. Licandro, personal communication).

#### Experimental arrangement

Each experimental system was made up of a plastic tank, filled with 12 l of sub-surface (5 m depth) seawater, collected in the substrate sampling area. To avoid particulate matter contamination, this water was prefiltred through Whatman GF/F filters, while keeping the free bacterial cells passing through the filter pore (actually about 1 µm). Gentle vacuum (<100 mm Hg) was employed to avoid cell damage during prefiltration and the related, unpredictable increases of the Dissolved Organic Matter (DOM) fraction.

To replace the particle-attached and large bacteria lost during prefiltration, and thus enhance organic matter transformation processes by micro-organisms, the prefiltred seawater was added with an in situ bacterial culture, reaching natural bacterial abundances ($1.3\times10^6$ cells l⁻¹), according to the results of Bruni et al. 1997 for the Ross Sea. Aerobic bacteria were cultured on Marine Agar 2216 (Difco) by means of the spread plate method (0.1 ml inoculum), incubating plates at in situ temperature. According to the general features of the area (Bruni et al. 1997), the bacteria were mainly non-fermentative and gram-negative rods (*Pseudomonas, Flavobacterium* and *Vibrio* genera).

Addiction of substrates to the experimental systems

#### Ice-added experimental system (ICE)

An ice block (approximately 1 kg weight), showing evident algae assemblages, was melted and the water placed in the tank with the prefiltred seawater. Thus, the system had a starting organic matter concentration of 879.0 µgC l⁻¹.

#### Phytoplankton-added experimental system (PHYTO)

The sample collected with the 40-µm net was placed in the experimental system immediately after collection, to avoid physiological alterations of the living fraction. The system starting-concentration amounted to 2.65 mgC l⁻¹.

#### Zooplankton-added experimental system (ZOO)

The sample was dried for a short time (about 30 min at room temperature) in order to kill the organisms and to provide an actual detrital substrate. The starting organic matter concentration amounted to 2.73 mgC l⁻¹.

#### Control experimental system (control)

One of the tanks received no organic substrate to evaluate any contamination and the processes acting in an organic matter-poor system (214.4 µgC l⁻¹ at the starting point).

#### Sampling

The experiments were carried out under natural illumination placing the tanks outside the laboratory, in a continuous seawater flowing system to maintain the temperature within a range of approximately −1 ±0.5°C. Salinity values ranged from 34.4 to 34.5 PSU.

Water sampling for chemical analyses started immediately after substrate introduction in the systems (hour 0) and after 13, 25, 37, 61, 85 and 130 h, collecting about 1 l of water for each sampling. The sampled water was then pre-filtered through a 200-µm-mesh net to avoid the mesopelagic fraction.

Ectoenzymatic analyses were carried out on the particulate matter retained by Nuclepore polycarbonate filters (0.2-µm pore diameter). About 10–30 ml of sample water was filtered for each sub-sample. For particulate organic matter sampling, Whatman GF/F filters were employed. About 50 ml of water was filtered for each sample.

#### Biochemical analyses

**Ectoenzymatic activity**

Ectoenzymatic activity determination was performed following Christ and Velimirov (1991) and Vrb and Macháček (1994). The artificial substrates, purchased by Sigma, were 4-methylumbelliferone (4-D-glucoside, 4-methylumbelliferone) N-acetyl-$\beta$-D-glucosaminide and L-leucine 7-amido-4-methylcoumarin hydrochloride respectively for $\beta$-glucosidase (BG), $\beta$-N-acetylglucosaminidase (GA) and leucine aminopeptidase (LA).

The five sub-samples employed for each ectoenzymatic activity determination [one filter in 5 ml of prefiltred (0.2 µm) and autoclaved seawater] and five blanks [5 ml of prefiltred (0.2 µm) and autoclaved seawater each] were supplemented with 0.5 ml of substrate (1–100 µmol l⁻¹ final concentration) and then incubated for