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Transfer of singlet oxygen from senescent irradiated phytoplankton cells to attached heterotrophic bacteria: Effect of silica and carbonaceous matrices

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ABSTRACT

The effect of silica and carbonaceous matrices (charged mineral surfaces) in phytoplankton cells on the transfer of singlet oxygen from irradiated phytodetritus to their attached bacteria was investigated under controlled laboratory conditions. Our results indicate that a silica matrix (i.e. as in diatom frustules) inhibits the transfer of singlet oxygen and limits the induced photodegradation of *cis*-vaccenic acid (a fatty acid generally considered as specific to bacteria). In contrast, a carbonaceous matrix (i.e. as in coccoliths) does not seem to inhibit the transfer probably due to the release of coccoliths upon cell death. As a consequence, bacteria associated with phytodetritus from diatoms should be in a healthy state and biodegradation of organic matter associated with these particles should be favoured. These results should contribute to a better understanding of photosensitized degradation processes and to a better estimation of the balance between degradation and preservation of organic material during sedimentation in seawater.

1. Introduction

Particulate organic matter (POM) consisting mainly of phytodetritus in the euphotic layer of oceans is recycled in surface waters within the food web and the microbial loop. Only a small part is exported by particles which fall to the deep ocean and thus serve as the primary vehicle by which carbon is exported to the deep ocean and seafloor (Boyd and Trull, 2006; Thunell et al., 2007). It is thus very important to understand the mechanisms by which this organic matter (OM) is degraded. Although the importance of photooxidative and autoxidative processes (abiotic processes) of POM degradation in the marine environment has been experimentally demonstrated (Rontani et al., 2011), there is a paucity of data concerning these processes. Indeed, most assessments of POM export from the euphotic layer to the deep ocean only consider biotic degradation processes including zooplankton grazing and enzymatic degradation by attached and free living heterotrophic bacteria (Goutx et al., 2007; Sempéré et al., 2000; Tamburini et al., 2009; Turley and Mackie, 1994). Ghiglione et al. (2007) previously showed that the contribution of attached bacteria to total bacterial activity can reach up to 83% under mesotrophic conditions. We could recently confirm these results in a culture of the haptophyte *Emiliania huxleyi* in late stationary phase (Petit et al., 2015). Indeed, quantitative PCR analyses allowed us to demonstrate that more than 90% of bacteria were attached to *E. huxleyi* cells.

During the senescence of phototrophic organisms, visible light and UV-induced photosensitized degradation processes act intensively due to the presence of a very efficient photosensitizer: chlorophyll (Foote, 1976; Knox and Dodge, 1985). These processes mainly involve singlet oxygen ($^1O_2$) and act on most of the unsaturated lipid components (including sterols, unsaturated fatty acids, and the chlorophyll phytyl side chain) of these organisms, producing allylic hydroperoxides (Christodoulou et al., 2010; Rontani, 2001).

The effects of photooxidation, however, are not limited to chloroplasts; indeed, during the natural senescence of higher plants, $^1O_2$ can migrate outside the chloroplasts and chemically react with unsaturated components of cuticular waxes (Rontani et al., 2005). In the case of senescent phytoplanktonic cells, $^1O_2$ can induce the degradation of heterotrophic bacteria attached to particles. Indeed, recent results demonstrated that singlet oxygen has a much larger intracellular sphere of activity than previously thought (Ogilby, 2010). Most of the calculated values of the radius of singlet oxygen's sphere of activity from its point of production (ranging from 155 to

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340 nm; Baier et al., 2005; Ogilby, 2010; Skovsen et al., 2005; Wilkinson et al., 1995) are sufficiently large to allow singlet oxygen, if produced near the cell membrane, to be able to cross over into the extracellular environment (Ogilby, 2010) and thus reach any attached bacteria. Cellular damage resulting from the transfer of substantial amounts of $^1O_2$ from phytoplanktonic cells to their attached heterotrophic bacteria may be substantial due to the lack of efficient photoprotective and antioxidant systems in these microorganisms (Garcia-Pichel, 1994) and this can affect their capability to degrade POM. Such a transfer of $^1O_2$ to bacteria has also been observed in situ in several POM samples (Christodoulou et al., 2009; Marchand and Rontani, 2001; Marchand et al., 2005; Rontani et al., 2011) and may help to explain the relative recalcitrance of already strongly abiotically degraded suspended POM towards further biotic degradation (Rontani et al., 2011).

The lifetime of $^1O_2$ in hydrophobic environments is longer, and its potential diffusive distance greater, than under hydrophilic conditions (Suwa et al., 1977). Charged mineral surfaces including silica matrices (e.g. diatom frustules) or carbonate matrices (e.g. coccoliths) could reduce the lifetime of $^1O_2$ and inhibit its migration to bacterial biomass. Indeed, if the transfer of $^1O_2$ is strongly favoured between two lipophilic membranes (such as those of phytoplankton and associated bacteria; Petit et al., 2013), this excited form of oxygen might be quickly deactivated if the two membranes are separated by a polar structure. Thus charged mineral surfaces, such as frustules or coccoliths, may allow for enhanced bacterial growth and biodegradation of phytodetritus by inhibition of $^1O_2$ transfer. It may be noted that transparent exopolymer particles (TEP), which are mainly composed of polysaccharides (Smith et al., 1995) and act as the glue for particle aggregation (Gärdes et al., 2011), may also (due to their polar character) inhibit $^1O_2$ transfer to bacteria.

Diatoms and coccolithophorids are two major groups in present-day marine phytoplankton. Diatoms are ubiquitous photosynthetic eukaryotes that are responsible for about 35% of the primary production of the oligotrophic oceans and 75% in the coastal zone and other nutrient-rich systems (Nelson et al., 1995). These cells build a rigid cell wall made of amorphous silica (frustules) that cause them to sink rapidly when the cells die or during senescence, carrying fixed organic carbon to the deep ocean. *Thalassiosira weissflogii* is a centric diatom occurring in coastal waters in the Atlantic and Pacific Oceans, as well as brackish and freshwater environments. Coccolithophorids, belonging to the algal class Prymnesiophyceae, are able to produce scales made of calcium carbonate called coccoliths. *E. huxleyi* is the most ubiquitous living coccolithophorid species in the ocean (Westbroek et al., 1984) and occurs in most of the oceanic subsurface (Honjo and Okada, 1974; Okada and Honjo, 1973) from polar to equatorial areas. This species is often present in high density (5×10^3 cell L^{-1}) and can form large blooms particularly in temperate and subarctic latitudes (Brown and Yoder, 1994). Consequently *E. huxleyi* plays an important part in the oceanic carbon cycle as a primary producer, contributing to the organic matter stock in marine sediments (Marlowe et al., 1990). *Dunaliella tertiolecta* (Chlorophyceae) is a naked cell (lack of a rigid cell wall) ranked among the most photophilic algae (Richardson et al., 1983). This species thrives in hypersaline, marine and freshwater habitats (Ben-Amotz and Avron, 1992).

In this study, we (i) compared the $^1O_2$ transfer from senescent diatom cells, coccolithophorid cells and free rigid wall chlorophyte cells to their attached bacteria, and (ii) monitored the effect of the biogenic silica concentration of the diatom frustules on $^1O_2$ transfer. For this purpose, the photooxidation of *cis*-vaccenic acid ($C_{18:1\Delta11}$; a fatty acid typical of Gram-negative bacteria; Sicre et al., 1988; Keweloh and Heipieper, 1996) in non-axenic senescent cultures of the haptophyte *E. huxleyi*, diatoms *T. weissflogii*, *Navicula* sp. cf. *jeffreyi* and *Skeletonema* sp. cf. *costatum* and the green alga *D. tertiolecta* was investigated under controlled laboratory conditions.

2. Methods

2.1. Photodegradation experiments

Two kinds of photodegradation experiments were conducted. The aim of the first one was to compare the effect of the matrices of *Navicula* cf. *jeffreyi* type, *E. huxleyi*, *D. tertiolecta* and *Skeletonema* cf. *costatum* on photodegradation where samples (one replicate for each point) were directly irradiated by artificial light with an Atlas Suntest solar simulator under an irradiance of 500 W m^{-2} in the 280–700 nm wavelength range. The second experiment was conducted to investigate the effect of biogenic silica (bSi) concentration of *T. weissflogii* where 5 triplicate samples taken during 3 weeks of growth (at days 0, 6, 13, 15 and 20) were stored at −20 °C (freezing/thawing cycles do not significantly affect the frustule of *T. weissflogii*; Bidle and Azam, 2001) until irradiated (for 4 h). In both experiments, Pyrex flasks (that limited irradiations to UVA + PAR, i.e. $\lambda > 300$ nm) containing culture samples were irradiated by artificial light with an Atlas Suntest solar simulator under an irradiance of 500 W m^{-2} in the 280–700 nm wavelength range. The flasks were maintained at 17 °C by submersion in a water bath connected to a cryothermostat. Flasks were also kept under darkness at 17 °C as controls over the time course experiment (one replicate per point).

2.2. Production of algal material

E. huxleyi strain RCC1215 (with coccoliths, carbonaceous matrix) from Roscoff marine station culture collection, *Skeletonema* cf. *costatum* (silica matrix) strain RCC70 (Roscoff), *D. tertiolecta* (no matrix) strain RCC6 (Roscoff) and *Navicula* cf. *jeffreyi* (silica matrix) strain CS513 from the CSIRO Algal Culture Collection were grown in 500 mL of f/2 medium under non-axenic conditions at 17 °C, in a constant environmentally controlled cabinet under an irradiance of 36 W m^{-2} (Osram, Fluora, 12:12 hour light:dark cycle), until stationary phase. Senescence was induced by transfer of the cells (after centrifugation at 3500 rpm for 5 min) to 500 mL of old natural seawater (collected several months previously) followed by incubation for 4 days. The cells employed during the experiments were senescent, except in the case of *D. tertiolecta* which were dead. Indeed, it was not possible to induce senescence in this alga by transfer in seawater, incubation under darkness or freezing–thawing cycles so it was killed with mercuric chloride (final concentration 2.5×10^{-4} M).

In parallel, synchronous growth of *T. weissflogii* (silica matrix) strain AC813 from Caen Culture Collection was obtained by removing silicate from the medium which is well known to stop the cell cycle (Hildebrand et al., 2007). First 2 L of starter culture was grown in f/2 medium (with silicate) under the conditions above described, until stationary phase. Then 200 mL of this starter culture was centrifuged (3500 rpm for 5 min), cells were washed with 10 mL of filtered sea water and transferred in 2 L of silica-free f/2 medium in a Nalgene flask. After 24 h under these growth conditions, 0.5 mL of $NaSiO_3$ solution was added (final concentration 200 μmol L^{-1}) and diatom growth followed over time for 3 weeks.

It may be noted that during the different experiments the production of TEP was not favoured by the presence of EDTA in the f/2 medium (Decho, 1990), the lack of nutrient limitation (Malejl and Harris, 1993) and the shaking (Thornton, 2002). Moreover, the strain employed during the study of the effect of biogenic silica concentration on the transfer of singlet oxygen (*T. weissflogii*) does not aggregate (Kiørboe and Hansen, 1993).

2.3. Biogenic silica and dissolved silica concentration

The particulate biogenic silica (bSi) concentration for the *T. weissflogii* photodegradation experiment was determined using a variation of the method of Ragueneau and Tréguer (1994). As no