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Light-induced aggregation of microbial exopolymeric substances

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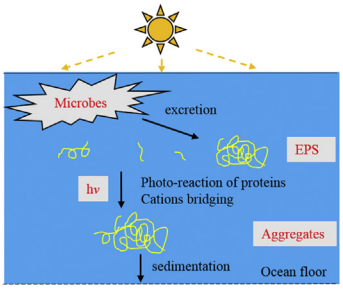
HIGHLIGHTS

- Sunlight can induce aggregation of bacterial exopolymeric substances.
- Reactive oxygen species played critical roles in the photo-oxidation process.
- First study that pinpoints proteins as likely components taking part in light-induced aggregation in the marine environment.
- New insights into polymer assembly, marine snow formation, and the fate of organic carbon and nitrogen in the ocean.

GRAPHICAL ABSTRACT



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ABSTRACT

Sunlight can inhibit or disrupt the aggregation process of marine colloids via cleavage of high molecular weight compounds into smaller, less stable fragments. In contrast, some biomolecules, such as proteins excreted from bacteria can form aggregates via cross-linking due to photo-oxidation. To examine whether light-induced aggregation can occur in the marine environment, we conducted irradiation experiments on a well-characterized protein-containing exopolymeric substance (EPS) from the marine bacterium *Sagitulla stellata*. Our results show that after 1 h sunlight irradiation, the turbidity level of soluble EPS was 60% higher than in the dark control. Flow cytometry also confirmed that more particles of larger sized were formed by sunlight. In addition, we determined a higher mass of aggregates collected on filter in the irradiated samples. This suggests light can induce aggregation of this bacterial EPS. Reactive oxygen species hydroxyl radical and peroxide played critical roles in the photo-oxidation process, and salts assisted the aggregation process. The observation that *Sagitulla stellata* EPS with relatively high protein content promoted aggregation, was in contrast to the case where no significant differences were found in the aggregation of a non-protein containing phytoplankton EPS between the dark and light conditions. This, together with the evidence that protein-to-carbohydrate ratio of aggregates formed under light condition is significantly higher than that formed under dark condition suggest that proteins are likely the important component for aggregate formation. Light-induced aggregation provides new insights into polymer assembly, marine snow formation, and the fate/transport of organic carbon and nitrogen in the ocean.

1. Introduction

Aggregation of marine colloids can play an important role in the

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transformation of dissolved organic matter (DOM) into particulate organic matter (Chin et al., 1998), and the formation of marine snow (Alldredge and Silver, 1988), especially after the inputs of pollutants (e.g. oil spill) into the ocean (Patton and Rigler, 1981; Passow et al., 2012; Quigg et al., 2016). Micron-sized marine aggregates can spontaneously assemble from free, nano-sized DOM polymers. Since the stability of tangled networks of microgels drastically decreases with shorter polymer length (Edwards, 1985), DOM polymers with shorter length chain generally assemble slower and form less stable gels. Ultraviolet (UV) light is thought to inhibit and disrupt the aggregation process by decreasing the DOM polymer length (Chin et al., 1998). The UV light can cleave high-molecular-weight DOM into low-molecular-weight DOM by random fragmentation (Mopper et al., 1991, 2001), resulting in smaller, less stable fragments that fail to assemble (Orellana and Verdugo, 2003). Several studies have shown that UVB radiation can cause significant photolysis of exopolymeric substances (EPS) and transparent exopolymer particles (Orellana and Verdugo, 2003; Ortega-Retuerta et al., 2010), and thus strongly disrupt the formation of microgels. However, there are few studies on the effects of less energetic sunlight (mainly in the UVA and the visible range). When UVB was reduced from natural sunlight (Ortega-Retuerta et al., 2010), the concentration of transparent exopolymer particles was increased by a factor of two after 1.5 day irradiation. In another study (Song et al., 2015), the EPS from a cyanobacterium *Chroococcus minutus* (*C. minutus*) formed stable flocculates under exposure to simulated solar irradiation.

From a biogeochemical perspective, many studies show that biomolecules such as proteins can cross-link to cause aggregation via photo-oxidation (Goosey et al., 1980; Shen et al., 2000). The cross-linking process may arise either from radical-radical termination reactions of two phenoxyl radicals (Shen et al., 2000; Parker et al., 2004), or from the oxidation of histidine residues to products (carbonyl compounds) followed by the reaction with lysine, cysteine or other histidine residues (Verweu and Steveninck, 1982; Balasubramanian et al., 1990; Dillon et al., 1993; Shen et al., 1996). During these processes, reactive oxygen species (ROS), such as singlet oxygen ($^1O_2$) (Goosey et al., 1980), hydroxyl radical (HO•) (Davies, 1987; Guptasarma et al., 1992), and peroxide (Davies et al., 1995) can play an important role. Since proteins are a major component of EPS released by microbes (Xu et al., 2009, 2011a), irradiation may facilitate cross-linked aggregation of marine colloids, which is the opposite to photolysis.

To examine the possibility of light-induced aggregation of EPS in the marine environment, we conducted irradiation experiments on well-characterized protein-containing EPS from *Sagitulla stellata* (*S. stellata*), a lignin-transforming marine gram-negative aerobic bacterium (Gonzalez et al., 1997; Ding et al., 2008). The turbidity level was monitored to follow the progress of flocculation during irradiation. After irradiation, the particle size change was measured using flow cytometry, and the aggregates were collected for mass measurements, protein and carbohydrate analysis, and attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) analysis. By comparing the protein contents of the bacterial *S. stellata* EPS to that of the diatom, *Amphora* sp., in conjunction with the tests of ROS effects, we reveal that proteins are likely the important component for aggregate formation.

2. Experimental

2.1. EPS extraction

S. stellata (ATCC 700073) was chosen as the representative of marine bacteria (e.g., *Roseobacter*) due to its ubiquity across various marine environments (Ding et al., 2008), while *Amphora* sp. (CCMP1389) was used as it is a common benthic diatom (Zhang and Santschi, 2009). EPS was obtained from these two cultures according to the procedures described in Zhang et al. (2008) and Xu et al. (2011a). In brief, *S. stellata* was incubated in Marine Broth 2216 (Difco Laboratories, 37.4 g/L) at 21 °C, and the growth was monitored by measuring the absorbance at 600 nm using Biotech-Epoch UV–Vis spectrometer. *Amphora* sp. was cultured in the artificial seawater (ASW) f/2 medium at 19 °C under a 14 h:10 h light: dark cycle and 130 μmol photons m^{-2} s^{-1}. Its growth was monitored by measuring the absorbance at 750 nm. At the end of the exponential phase when the absorbance was constant, the cultures were separated into dissolved (supernatant) and attached (pellet) fractions by centrifugation at 3000 g for 30 min. The dissolved phase was filtered through a 0.2 μm polycarbonate filter (Millipore) to remove any cells, and the EPS was collected after ultrafiltration and desalting using an Amicon Ultra-4 centrifugal filter unit with a 3 kDa cut-off membrane (Millipore). It was stored at 4 °C after being freeze dried.

Carbohydrates (CHO), proteins and uronic acids (URA) are the major components of EPS (Xu et al., 2009; Zhang et al., 2008). CHO and URA concentrations were determined by the anthrone method (Morris, 1948) and the phenyl phenol method (Blumenkrantz and Asboe-Hansen, 1973), respectively, while protein contents were determined by the Lowry Protein Assay Kit (Pierce, 23240, USA). The chemical composition of EPS from *S. stellata* and *Amphora* sp. is listed in Table 1.

2.2. Irradiation experiment

The EPS were dissolved in ASW to a final concentration of 10 mg/L for *S. stellata* and 5 mg/L for *Amphora* sp. Total organic carbon was 2.4 mg-C/L and 1.9 mg-C/L for *S. stellata* and *Amphora* sp. respectively. The samples were filtered through a 0.2 μm polycarbonate filter to remove aggregates before irradiation. The UV–Vis absorbance spectrum of *S. stellata* EPS was measured in the wavelength range of 250–600 nm in a well plate (Greiner). Samples were irradiated in 250 mL borosilicate glass or quartz flasks in a water bath by circulating water of temperature 24 ± 1 °C around the flasks. The samples were irradiated for 1–4 h under natural sunlight at noon time, with a mean light dose of 2.5 Wm^{-2} of UVB, 20 Wm^{-2} of UVA, and 900 Wm^{-2} of photosynthetically active radiation (PAR) measured by radiometers (Solarmeter Ltd). The borosilicate glass flasks were partially opaque to UVB light and transparent to UVA and PAR light (transparent 68% of UVB, 90% of UVA and 95% of PAR measured by radiometer), while quartz flasks

Table 1
Chemical composition and molecular weight of EPS from *S. stellata* and *Amphora* sp.

EPS	OC[a] (%)	Protein-C/OC (%)	CHO-C/OC (%)	URA-C/OC (%)	molecular weight[b]
S. stellata	24	7.7 ± 3.0	39.6 ± 3.6	1.3 ± 0.3	27 kDa
Amphora sp.	37.7	N.D.[c]	32.9 ± 0.2	46.6 ± 0.2	>1000 kDa

[a] OC, organic carbonin % of dry weight; CHO, total carbohydrates; URA, uronic acid; Protein-C, CHO-C, URA-C, protein, carbohydrate, uronic acid normalized to carbon, assuming that proteins contain 33% carbon, carbohydrates contain 40% carbon, and uronic acid contain 37% carbon.
[b] (Zhang et al., 2008; Xu et al., 2009).
[c] "N.D." denotes undetectable.