Ann. Ist. Super. Sanità, vol. 35, n. 3 (1999), pp. 397-400

The potential role of particulate diatom exudates in forming nuisance mucilaginous scums

Alice L. ALLDREDGE

Department of Ecology, Evolution and Marine Biology, University of California, Santa Barbara, California, USA

Summary. - Polysaccharide-specific staining techniques have revealed the existence and high abundance of gel-like mucilaginous particles formed from the polysaccharide exudates of diatoms. Evidence from both the field and laboratory indicates that these transparent exopolymer particles, known as TEP, are a major component of diatom aggregates and facilitate the flocculation of diatom blooms. TEP are composed primarily of sulfated polysaccharides, molecules known to be resistant to bacterial decomposition. Furthermore, the presence of TEP in diatom aggregates reduces the permeability of aggregates causing them to become neutrally buoyant and accumulate at picnoclines where they eventually develop gas bubbles and float to the surface. Diatom species, such as *Cylindrotheca closterium*, found abundantly in the Adriatic Sea, may produce particularly unique and copious quantities of TEP resistant to sinking and degradation. This material could accumulate as mucilaginous nuisance scums when environmental conditions favor strong stratification of the water column, high diatom biomass, and the dominance of these species.

Key words: marine algae, transparent exopolymer particles (TEP), mucilage.

Riassunto *(Il ruolo potenziale degli essudati prodotti dalle diatomee nella formazione delle mucillagini)*. - Tecniche specifiche di colorazione hanno messo in evidenza l'esistenza e la grande quantità di particelle mucillaginose prodotte dagli essudati di polisaccaridi delle diatomee. I risultati di studi condotti sia in laboratorio che in campo indicano che queste particelle trasparenti formate da esopolimeri, note con l'acronimo TEP *(transparent exopolymer particles)* sono componenti molto importanti degli aggregati ed evidenziano il loro ruolo nell'aumentare i processi di flocculazione conseguenti alle fioriture a diatomee. Il TEP è composto essenzialmente da polisaccaridi ricchi di solfati, molecole note per la loro resistenza alla degradazione batterica. La presenza di TEP negli aggregati prodotti dalle diatomee riduce inoltre la permeabilità degli aggregati stessi, determinandone la galleggiabilità. Ciò permette quindi il loro accumulo a livello del picnoclino da dove, grazie alla produzione di bolle gassose essi possono risalire alla superficie. Alcune specie di diatomee quali *Cylindrotheca closterium*, sempre abbondante in Adriatico, possono produrre grandi quantità di specifici TEP particolarmente resistenti alla sedimentazione e alla degradazione batterica. Questo materiale potrebbe accumularsi fino a formare mucillagini quando le condizioni ambientali favoriscono la formazione di un picnoclino particolarmente stabile, e l'abbondanza di diatomee con la dominanza di questa specie.

Parole chiave: alghe marine, transparent exopolymer particles (TEP), mucillagini.

Introduction

Carbohydrate-specific staining techniques have recently revealed the existence of a new class of large, discrete, transparent particles called TEP (transparent exopolymer particles) which are formed from dissolved extracellular polysaccharides exuded by phytoplankton, especially diatoms [1]. TEP form when long-chain polysaccharide exudates align via various mechanisms, including cation bridging, to form colloidal microfibrils [2, 3] which further aggregate to form larger, gel-like particles. Alternatively, TEP may form from spontaneous assembly of dissolved exudates into polymer gels [4]. Unstained TEP are invisible under the microscope and exist as spheres, films, webs, and strings up to hundreds of microns in size. They occur at concentrations up to thousands per ml in natural seawater [1, 5]. The existence of carbohydrate exudates as discrete particles in their own right rather than just as dissolved molecules or as coatings on other particles has far reaching implications for many processes including the formation of marine snow and mucilaginous scums.

TEP is essential for diatom bloom aggregation

Diatom blooms are an essential component of the mucilaginous phenomenon in the Adriatic Sea. These blooms aggregate to produce large flocs in the water column which are the precursors of scum formation.

Mucilaginous scums are rich in diatom remains. Considerable evidence now exists indicating that TEP is the agent responsible for the formation of large diatom aggregates and the mass flocculation of most diatom blooms. Abundant TEP has also been found in mucilage marine snow from the Adriatic Sea (Alldredge, unpublished). Evidence that TEP is necessary for formation of diatom bloom aggregation includes:

1) TEP has been found in all diatom aggregates and marine snow we have examined from the Pacific, Atlantic, and Southern Oceans and the Adriatic Sea;

2) the presence of TEP was essential for the flocculation of two diatom blooms investigated off California [5, 6]. Dissolved carbohydrates exuded throughout the blooms reached maximum concentrations at the bloom peak. TEP abundance and size continued to increase as these dissolved substances were converted to TEP. The percentage of diatom cells attached to TEP increased rapidly during the flocculation phase of the bloom from <10% to >95% indicating that TEP were aggregating rapidly with the abundant, largely unsticky [7] diatoms present, forming large TEP-diatom flocs heavy enough to sink;

3) diatom blooms where TEP is rare do not undergo mass aggregation to form large flocs [8];

4) TEP abundance, not phytoplankton cell abundance, predicted aggregation of blooms in three different systems [9]. This evidence is highly suggestive that the presence of TEP may be necessary for the aggregation and subsequent sedimentation of particles otherwise too small to sink rapidly to depth;

5) the relative concentration of TEP present during the flocculation of diatom blooms determines the composition of diatom flocs. At high concentrations of TEP all particles present appear to aggregate indiscriminately. If concentrations of TEP are low then cells which themselves have sticky cell coatings (like *Nitzschia* spp.) aggregate preferentially, whereas other particles remain unaggregated [10].

TEP appear to drive the aggregation of smaller particles to form large mucus flocs through 3 main avenues. First, as discrete particles, TEP increase the total concentration of particles available for aggregation [9]. Second, the gel-like structure of TEP allows these particles to become large (up to 100's μm). Particle size is highly significant in increasing aggregation rates [11-13]. Finally, TEP are very sticky. TEP consist primarily of deoxysugar-rich polysaccharides with sulfate half-ester groups [14]. The acidic and anionic properties of these groups make these polysaccharides highly surface-active due to their tendencies to form metal ion bridges and hydrogen bonds. Accordingly, the stickiness of TEP is very high. Almost all small marine particles, including detritus, sediment, and phytoplankton, are quite unsticky, with attachment probabilities (called α) upon collision below 1% ($\alpha = 10^{-2}$ to 10^{-4}) [15-17]. TEP, however, have α's of 0.1 to near 1, one to three orders of magnitude higher [5, 9]. This suggests that TEP, or exopolymeric material like it, must be present in order for most types of natural particles in the water column to aggregate at any appreciable rate. TEP collide with non-sticky particles, coating or embedding them, and serve as the "glue" sticking detritus, phytoplankton, and fecal pellets together to form aggregates of marine snow.

Properties of TEP are significant for mucilage formation

TEP has several properties particularly relevant to the formation of mucilage in the Adriatic Sea. First, recent evidence indicates that sulfated polysaccharides, such as those composing TEP, are much more resistant to decomposition than other types of polysaccharides. Arnosti [18] found that sulfated polysaccharides required many weeks to decompose, considerably longer than other types of polysaccharides she investigated. This suggests that once produced, TEP in the water column can persist for long periods of time against the activities of decomposing microorganisms. This is consistent with the long residence time of mucus aggregates and scums in the Adriatic Sea.

Second, gels are resistant to diffusion by salts. Alldredge and Crocker [19] presented a model and evidence suggesting that gels formed from diatoms in surface waters of the Adriatic Sea where salinity is lowest would become neutrally buoyant as they sank into higher salinity water at the picnocline. The TEP within these aggregates would tend to maintain its integrity and specific gravity against diffusion processes. Alldredge and Crocker [19] demonstrated that an aggregate of marine snow would need to be only about 0.5-2% mucilage by volume to become neutrally buoyant and accumulate at the picnocline. This is consistent with observations that scum formation can be proceeded by high abundances of mucilage at the picnocline resembling a false bottom [20].

Finally, the quantity and characteristics of TEP formed from diatom exudates is closely related to the species composition and abundance of the phytoplankton excreting these substances. The chemical composition of TEP is likely to vary between species and as a function of physiological stage because the composition of diatom exudates varies with these parameters [21-23]. It is possible that *Cylindrotheca closterium*, the most often reported species associated with the scum phenomenon, as well as other important scum forming species, produce TEP which is particularly resistant to both decay and to sinking. This results in extensive accumulation of mucilage in the water column when these species are abundant.

Hypothesis regarding mucilaginous scum formation

Based on our present knowledge of diatom blooms, TEP, and the mucilage phenomenon in the Adriatic Sea, the following hypothesis is proposed regarding production of nuisance scums. It must be stressed that this is only a hypothesis and further research will be necessary to determine its validity.

Large flocs of marine snow, many centimeters long, formed from diatoms are always seasonally abundant in the Adriatic Sea [20, 24-26]. When environmental conditions particularly favor the abundant growth of *Cylindrotheca closterium* and other taxa, such as *Chaetoceros* and *Nitzschia*, copious TEP is produced. This material aggregates with diatom cells and sinks to the picnocline where it becomes neutrally buoyant. The mucilage produced by these taxa is particularly resistant to decomposition and accumulates over many weeks. Abundant microbial communities eventually colonize the mucilage and produce gas (possibly nitrogen gas under anaerobic conditions as hypothesized by Azam, this volume). The mucilage then floats to the surface forming nuisance scums.

Why does mucilage scum form only in some years and not others? Conditions must exist which especially favor the seasonal dominance of those particular diatom species whose TEP is unusually copious, gelatinous, and resistant to decomposition. Conditions leading to the dominance of these species probably involve nutrient conditions and resuspension events regulated by flow rates from the Po river and circulation patterns in the northern Adriatic Sea. Once the causal diatom species are abundant, mucilaginous scum formation is further enhanced by periods of very calm, very sunny weather, which increases diatom biomass and facilitates stratification leading to the accumulation of mucilage at the picnocline. Much further research will be necessary to determine the importance of these and other factors in generating the fascinating mucilage phenomenon in the Adriatic Sea.

Submitted on invitation.
Accepted on 9 December 1998.

REFERENCES

1. ALLDREDGE, A.L., PASSOW, U. & LOGAN, B. 1993. The abundance and significance of a class of large, transparent organic particles in the ocean. *Deep-Sea Res. Part I* **40**: 1131-1140.

2. LEPPARD G.G., MASSALSKI, A. & LEAN, D.R.S. 1977. Electron-opaque microscopic fibrils in lakes. Their demonstration, their biological derivation and their potential significance in the redistribution of cations. *Protoplasma* **92**: 289-309.

3. LEPPARD, G.G. 1995. The characterization of algal and microbial mucilages and their aggregates in aquatic ecosystems. *Sci. Total Environ.* **165**: 103-131.

4. CHIN, W-C., ORELLANA, M.V. & VERDUGO, P. 1998. Spontaneous assembly of marine dissolved organic matter in polymer gels. *Nature* **391**: 568-572.

5. PASSOW, U., ALLDREDGE, A.L. & LOGAN, B.E. 1994. The role of particulate carbohydrate exudates in the flocculation of diatom blooms. *Deep-Sea Res. Part I* **41**: 335-357.

6. PASSOW, U. & ALLDREDGE, A.L. 1995. Mass aggregation of a diatom bloom in a mesocosm. The role of TEP. *Deep-Sea Res. Part II* **42**: 99-110.

7. DAM, H.G. & DRAPEAU, D.T. 1995. Coagulation efficiency, organic matter glues and the dynamics of particles during a phytoplankton bloom in a mesocosm study. *Deep-Sea Res. Part II* **42**: 111-124.

8. KIØRBOE, T.J., HANSEN, L.S, ALLDREDGE, A.L., JACKSON, G.A., PASSOW, U., DAM, H.G., DRAPEAU, D.T., WAITE, A. & GARCIA, C. 1996. Sedimentation of phytoplankton during a spring bloom. Rates and mechanisms. *J. Mar. Res.* **54**: 1123-1148.

9. LOGAN, B.E., PASSOW, U. , ALLDREDGE, A.L. , GROSSART, H-P. & SIMON, M. 1995. Rapid formation and sedimentation of large aggregates is predictable from coagulation rates (half lives) of transparent exopolymer particles (TEP). *Deep-Sea Res. Part II* **42**: 203-214.

10. CROCKER, K. & PASSOW, U. 1995. Differential aggregation of diatoms. *Mar. Ecol. Prog. Ser.* **17**: 249-257.

11. McCAVE, I.N. 1984. Size spectra and aggregation of suspended particles in the deep ocean. *Deep-Sea Res.* **31**: 329-352.

12. JACKSON, G.A. 1990. A model of the formation of marine algal flocs by physical coagulation processes. *Deep-Sea Res. Part I* **37**: 1197-1211.

13. JACKSON, G.A. & LOHMAN, S.E. 1992. Effect of coagulation on nutrient and light limitation of an algal bloom. *Limnol. Oceanogr.* **37**: 77-89.

14. MOPPER, K., ZHOU, J., RAMANA, K.S., PASSOW, U., DAM, H.G. & DRAPEAU, D.T. 1995. The role of surface-active carbohydrates in the flocculation of a diatom bloom in a mesocosm. *Deep-Sea Res. Part II* **42**: 47-74.

15. GIBBS, R.J. 1983. Effect of natural organic coatings on the coagulation of particles. *Environ. Sci. Technol.* **17**: 237-240.

16. KIØRBOE T., ANDERSON, K.P. & DAM, H.G. 1990. Coagulation efficiency and aggregate formation in marine phytoplankton. *Mar. Biol.* **107**: 235-245.

17. ALLDREDGE, A.L. & McGILLIVARY, P. 1991. The attachment probability of marine snow and their implications for particle coagulation in the ocean. *Deep-Sea Res. Part I* **38**: 431-443.

18. ARNOSTI, C. 1996. Evidence of a structural effect on enzyme hydrolysis of polysaccharides in marine sediments. *EOS, Transactions, American Geophysical Union* **76**: 41.

19. ALLDREDGE, A.L. & CROCKER, K.M. 1995. Why do sinking mucilage aggregates accumulate in the water column? In: Marine mucilages (special issue). *Sci. Total Environ.* **165**: 15-22.

20. STACHOWITSCH, M., FANUKO, N. & RICHTER, M. 1990. Mucus aggregates in the Adriatic Sea. An overview of stages and occurrences. *PSZNI Marine Ecology* **11**: 327-350.

21. PAINTER, T.J. 1983. Algal polysaccharides. In: *The polysaccharides*. G.O. Aspinall (Ed.). Academic Press, New York. p. 196-275.

22. MYKLESTAD, S., HAUG, A. & LARSEN, B. 1972. Production of carbohydrates by the marine diatom *Chaetoceros affinis* var. *willei* (Gran) Husted II. Preliminary investigation of the extracellular polysaccharide. *J. Exp. Mar. Biol. Ecol.* **9**: 137-144.

23. MYKLESTAD, S., HOLM-HANSEN, O., VARUM, K.M. & VOLCANI, B.E. 1989. Rate of release of extracellular amino acid and carbohydrates from the marine diatom *Chaetoceros affinis*. *J. Plank. Res.* **11**: 763-773.

24. HERNDL, G. J. &. PEDUZZI, P. 1988. The ecology of amorphous aggregations (marine snow) in the North Adriatic Sea: I. General considerations. *PSZNI Marine Ecology* **9**: 79-90.

25. BOCHDANSKY, A.B. & HERNDL, H.J. 1992. Ecology of amorphous aggregations (marine snow) in the Northern Adriatic Sea. III. Zooplankton interactions with marine snow. *Mar. Ecol. Prog. Ser.* **87**:135-146.

26. Marine mucilages. 1995. R.A. Vollenweider & R. Rinaldi (Eds). *Sci. Total Environ.* (Special issue) **165**: 1-235.