Structure/function/activity relationships in marine snow. Current understanding and suggested research thrusts

Gary G. Leppard

Aquatic Ecosystem Protection Branch, National Water Research Institute, Burlington, Ontario, Canada

Summary. - Marine snow and marine snow components contribute to the mucilage phenomenon in the northern Adriatic Sea. Of special relevance is the matrix material, composed of extracellular polymeric substances, which are packaged into fibrils of colloidal dimensions. These 0.005 μm diameter fibrils are physical units of mucilage which can be visualized by transmission electron microscopy (TEM). They form polymer bridges between the various biotic and mineral components of marine snow, creating three-dimensional networks which affect floc porosity, density and settling behaviour. Recent observations of the matrix by TEM reveal complex fibril-delimited channels and capillary systems which partially traverse marine snow flocs and which are postulated to play roles in anomalous settling. Considering the marine snow floc as a microecosystem, the relationships between ultrastructure, chemistry and environmental properties are being explored. On the assumption that colloidal matrix materials, including those released into the bulk water, might provide advance information on anomalous floc behaviour, two new methods are recommended for monitoring the northern Adriatic Sea. One is a technique for chemical quantification of colloidal organic carbon, the other uses TEM, applied to water fractions derived from cascade ultracentrifugation, to estimate fibril quantities as a proportion of colloidal organic carbon.

Key words: mucilage, marine snow, fibrils, colloidal organic carbon, transmission electron microscopy.

Riassunto (Relazioni tra struttura, funzione e attività nella neve marina: risultati attuali e possibili metodi di ricerca). - La neve marina ed i suoi componenti contribuiscono al fenomeno mucillagino nell’Adriatico settentrionale. Particolarmente importanti è la matrice composta da sostanze polimeriche extracellulari che sono raggruppate in fibrille di dimensioni colloidal. Queste fibrille di diametro 0.005 μm sono unità fisiche di mucillagino che possono essere facilmente visualizzate al microscopio elettronico (TEM). Queste unità formano dei ponti di polimeri tra i vari componenti biotici e minerali della neve marina, creando una rete tridimensionale che influisce sulla porosità, densità e processi di sedimentazione dei flocchi. Recenti osservazioni della matrice effettuate al TEM rivelano canali e sistemi capillari complessi delimitati da fibrille che parzialmente attraversano la neve marina e che giocano un ruolo importante in tassi di sedimentazioni anomali. Sono state investigate le relazioni tra ultrastruttura, chimica e proprietà ambientali della neve marina considerandola come un micro-ecosistema. Assumendo che i materiali della matrice colloide, compresi quelli che vengono dispersi nel mare, possano dare informazioni in anticipo su un anomalo comportamento dei flocchi, si propongono due nuovi metodi per osservare il mare Adriatico settentrionale. Un metodo usa una tecnica per la quantificazione chimica del carbone organico colloide, l’altro usa il TEM per osservare i derivati raccolti da ultracentrifugazione “in cascade” con lo scopo di valutare le fibrille come una frazione del carbone organico colloide.

Parole chiave: mucillagino, neve marina, fibrille, carbone organico colloide, microscopia elettronica.

Introduction

The mucilage phenomenon

The mucilage phenomenon in the northern Adriatic Sea is a sporadic but massive accumulation of gelatinous or mucilaginous material at the sea surface [1]. In 1989 it caused billions of dollars of economic disruption as estimated by authorities in Italy. Among the scientific questions posed since that time have been the following: 1) what is the basic material of the mucilaginous aggregates?; 2) assuming the action of environmental triggering mechanisms, exactly what aspects of mucilage structure and quantity might have been altered by the triggers?; 3) what kind of combined changes in structure and aggregated particle properties could promote excessive aggregation (macroaggregate and surface layer formation) and anomalous sedimentation (flotation)?

This paper addresses these specific questions. It focuses on new information about the structure of mucilage-rich suspended particles in relation to their general characterization and to their examination with respect to hypotheses on anomalous behaviour. New technology is available for characterization and experimentation, with respect to new information needs, and it is outlined below to stimulate new research thrusts.
Marine snow in relation to the muclilage phenomenon

Biological, chemical and physical factors interact to determine muclilage production and molecular speciation of muclilage by secreting biota [2]. Accumulation at the sea surface involves many kinds of interacting physico-chemical processes and biological materials; these materials include a variable mixture of macromolecular species of polysaccharidic muclilage. The phenomenon is in part an accumulation of marine snow in various stages of evolution [3], but, floculating colloidal particles [4, 5] and materials produced by the bacterial degradation and alteration of suspended biotic particles are likely to contribute also [6]. The general characteristics, dynamics and significance of marine snow have been well described [7], as have some microbial activities and transformations of organic matter within suspended mucilaginous material [8]. Observations specific to northern Adriatic Sea marine snow have been published recently on structure [9], activity [10], microbial ecology [11, 12] and floc/ ecosystem interactions [4]. New information on polysaccharide-rich TEP or transparent exopolymer particles [13, 14], suggests that TEP is responsible for much of the muclilage phenomenon. Also relevant may be the model of Chin et al. [15] who show that free marine organic polymers can spontaneously entangle to form polymer microgels, whose formation mechanism can be described in terms of polymer gel theory [16]. Both TEP and polymer microgels have materials and properties in common with mucilaginous marine snow; while it is likely that all three entities contribute to the muclilage phenomenon, only marine snow will be considered in detail here.

Marine snow structure and matrix fibrils

Because of the important role for marine snow and marine snow precursors, and because of the colloidal aspects of the fine structure of marine snow, these floes have recently been analyzed by high-resolution electron microscopy in an attempt to link structure to biological function and chemical activity. Protocols have been established and refined to prepare (fixation/stabilization) representative floc particles and their subcomponents [4, 5, 9, 17] by transmission electron microscopy (TEM), with a focus on visualizing the matrix, and associated biotic and abiotic particles/colloids. The marine snow matrix holds individual particulate subcomponents together, via bridging structures (fibrils), and contributes to important physical properties such as porosity, density, settling and flotation.

For northern Adriatic Sea samples in mid-summer, the principal matrix colloidal material of marine snow (Fig. 1) is a fibrillar extracellular polymeric substance, or a "fibril" of ca. 0.005 μm diameter and great length-to-width ratio [5, 4, 9, 17]. Fibrils correspond approximately to the exopolymer secretions reviewed by Decho [18]; individual fibrils have the dimensions, morphology and staining properties of acid polysaccharide aggregates described elsewhere for flocculated particles rich in bacteria and/or algae [2, 19]. Such aggregates are mixtures which contain other molecular species and sorbed substances [18, 19]. The relationships of fibrils to other marine snow components and to the adjacent aquatic environment are shown in Fig. 2, which addresses structures covering seven orders of magnitude [4]. Passow et al. [14] consider fibrils to be a major component of the abundant oceanic TEP. Additionally, fibril dimensions and physico-chemical characteristics [19] are in accord with the mucilaginous gel formation ideas of Chin et al. [15] and of Wells [20].

Observations from correlative electron microscopy [21, 22] show that most fibrils are associated in threedimensional arrays (Fig. 1) to provide a gel structure of generally high porosity and water content for small floes with a diatom core; superimposed on this basic structure are localized differentiations of fibril type and degree of fibril packing [4, 9]. Individual fibrils are revealed as polymer bridges [23] between bacteria, algae, organic debris, biogenic silicates, clay minerals, and other fibrils. There is evidence for some of the associations being under direct biological control [4]. There is also evidence that fractal aggregates of humic substances are excluded from the matrix architecture of marine snow, at least for small bacteria-rich floes [5].

For large marine snow floes, the porosity established by the fibrillar matrix can be highly variable. Heterogeneity in fibril packing can also be great and
can allow for internal regions of very dense packing, as has recently been demonstrated for a variety of non-marine flocs [24, 25]. Gradients of fibril packing suggest a physical basis for gradients of chemical activity within a floc (e.g., oxygen and pH gradients), controlled by the biological secretion and positioning of fibrils. Gradients of biological function could be established as a consequence of these physical barriers (e.g., cooperative metabolism between microbes associated intimately in a densely-packed fibrillar matrix, and the establishment of protective barriers to limit the approach of predators). The roles of densely-packed fibril zones are currently under intensive investigation in the hope of finding correlates of function and activity with structure.

**Structure/function/activity relationships**

For fibrils, relationships between physical structure, biological function and chemical activity have been examined recently by Leppard [2, 19, 26]. For exopolymers in general, there is an excellent review by Decho [18]. For biota-rich flocs, such relationships are being explored with new technology by Liss et al. [24] and Droppo et al. [25]; floc function in relation to fibrils and contaminant transport is explored in Leppard [26]. Researches specific to marine snow and marine snow colloids in the northern Adriatic Sea have been carried out recently [4, 5, 17].

Some relevant general relationships at the level of fibrils are listed as follows. Fibril adhesion properties play a major role in aggregation phenomena, such as floc formation (Fig. 2). The amount of fibrils secreted can be dictated by the nutrient phenomena. Fibril production can be induced by phosphate deprivation. Fibrils can scavenge the bulk water for mineral nutrients. Fibril production can be greatly influenced by aging. Fibrils can be assembled by microbes into specific attachment structures to facilitate colonization of a surface, including surfaces within flocs. Several different kinds of fibrils may be produced by a given microbe. Some microbes shed their fibrils without concomitant intracellular structural damage. Fibrils can be released into water as free fibrils in massive numbers. Fibrils can change their own composition by sorption of chemicals from water. Fibrils can sorb enzymes whose activity is retained. The immobilization of toxic substances can occur on fibrils. Fibrils can spontaneously form large aggregates so that fibril/contaminant associations can change their status from virtual solutes to sedimenting aggregated particles. By way of these phenomena, a fibril can exhibit many functions in relation to living cells, while displaying a variety of physicochemical activities.

Flocs with fibrillar matrices are now being conceptualized as irregularly-structured suspended particles, with individual structural components (e.g., bacteria, algae, fibrils, clay minerals, silicates, heavy

---

**Fig. 2.** - Suggested scheme of mucilage particle formation in the sea, and dynamic relations between mucilage particles and their aquatic environment. A size continuum is proposed from single mucilage fibrils (in the nm range), released by microorganisms, to the large marine snow (in the dm range). At all levels of aggregated particle size, the particles experience various physically, chemically and biologically mediated modifications. Reproduced with permission from Heissenberger et al. [4].
metal oxides, organic debris, extracellular enzymes) contributing in specific ways to floc function. The floc, as microecosystem, functions as a sedimenting unit which imposes its active internal colloidal associations on interactions with the bulk water [25]. This sedimenting unit acts as a vehicle for organic carbon transport and as an agent of water decontamination, with specific components in addition to cells carrying out specific activities. The concept of the floc as an amorphous indescribable entity, or “black box”, is now outmoded. The relationship of non-living components of floc architecture to specific activities and specific chemical fine structure has become a new field of research [24, 25].

Fibrils and anomalous marine snow behaviour; speculations

Of special interest to the role(s) of marine snow in the mucilage phenomenon are correlational comparative electron-optical analyses of flocs which display anomalous behaviour. For flocs in engineered water treatment systems [24, 25], there is interest in the mechanism whereby a sedimenting floc becomes transformed into a floc capable of flotation, as is the case for the Adriatic mucilage phenomenon. One can speculate that the mechanism could be the stabilization of microbubbles by specific arrays of specific fibril types, making such a mechanism directly relevant to the mucilage phenomenon and its biological triggers [2]. Research on fibril arrays in matrices is at an early stage with engineered flocs but technological and conceptual advances should be transferable to the analysis of marine snow. Microbubble stabilization is likely to correlate with the chemical fine structure of mucilage macromolecules comprising a fibril. This chemical fine structure is likely a function of environmental interactions with principal secretor organisms. Chemical fine structure in relation to fibril density is currently being probed with molecular markers which allow one to correlate electron-optical images of fibril-rich zones with “whole floc” images provided by SCLM, or scanning confocal laser microscopy [27, 28].

New observations and possible explanations

The previous paragraph, which mixed fact with hypothesis and an optimistic outlook, indicates how observations from transmission electron microscopy (TEM) can be used to guide [2] an interdisciplinary scientific investigation of marine snow structure, chemistry, microbiology and physical properties. Using correlative preparatory approaches to TEM [9], in combination with an overall correlative use of various microscopies (conventional optical microscopies, or COM, plus SCLM) which address volume and resolution gaps complementarily, one can examine the total volume of a representative floc at low resolution so as to establish which associations should be analyzed cost-effectively at high resolution [25]. These analyses are readily coupled to information on chemistry, biology and settling behaviour for a given population of flocs described in terms of average shape, size and density, as demonstrated recently for freshwater flocs [25]. For the northern Adriatic Sea, preliminary explorations of this type have begun [4].

Below are three major observations on flocs, from recent electron-optical investigations by this author and his collaborators [4, 24, 25, 29], of relevance to the mucilage phenomenon:

1) the presence of some open channels running from the floc/bulk water interface into the floc interior, channels of greater than colloidal diameters which might allow for a rapid movement of nutrients, bacteria and viruses into the floc interior;

2) the presence of similar channels which are plugged with fibrils, thus converting an apparently open (by COM) channel into a network of fine fibril-bound cavities small enough to obstruct bulk water flow into the floc interior;

3) the presence of fibril-dense zones which may be dense enough and thick enough to permit embedded microbes to establish chemical gradients which in turn promote symbiotic microbe-microbe interactions, as is the case for biofilms.

The relevance of the first two observations is that fibril positioning might be part of a density regulation mechanism, not previously considered. A dysfunction of a fibril system (e.g., unusual packing and/or unusual richness in an otherwise rare chemical species of fibril), in conjunction with microbubble formation, might lead to excessive microbubble formation/stabilization followed by flotation. Alternatively, a low density mucus matrix, rich in fibrils and resistant to diffusion of salts and water, might allow mucus-rich marine snow to attain neutral buoyancy and accumulate at even modest pycnoclines [30] to disturb settling.

The significance of the third observation is that microbe-microbe interactions may provide more physiological versatility to marine snow activities than previously imagined, with impact on settling. The use of the latest generation of molecular probes should permit one to explore, by correlative microscopy [22], the versatility of function in terms of fibril associations and fibril packing density. As microelectrodes become more miniaturized and more refined, and as the capacity to position them within mucilaginous materials becomes more precise, the channels and the densely-packed fibril zones should become more amenable to (i) activity-based analyses of gradients and to (ii) delineating structural correlates of anomalous behaviour.
Marine snow as a transport “vehicle”

The marine snow particle is currently being described by electron-optics at near-nanometre resolution as a microecosystem [4]. Additionally, like freshwater flocs [25], it can be considered as an environmental “machine” or “vehicle” which (i) provides beneficial habitat for biota, (ii) extracts the surrounding bulk water for nutrients and (iii) transports organic carbon on a sometimes massive scale. It might be useful conceptually to consider the mucilage phenomenon layer, at the surface of the northern Adriatic Sea, to be the result of a dysfunctional environmental machine, be it marine snow per se, an overabundance of active marine snow parts (e.g., fibrils) or an active material derived from a late stage of marine snow seasonal evolution or decomposition.

It is reasonable to assume that a dysfunctional environmental machine provides advance warning of dysfunction (e.g., unwanted flotation). This hypothesis is currently being pursued vigorously by scientists attempting to understand costly dysfunction in engineered biological treatment systems for decontaminating industrial waste waters [24, 25]. Floc populations, and individual representative flocs from these engineered decontamination systems, resemble marine flocs in terms of matrix structure (Leppard, personal observations on many ecosystems rich in mucilaginous flocs); thus the examination of entire flocs may be amenable as a tool for predicting dysfunction. Since adhesion and extended colloidal surfaces play important roles in marine snow structure (and almost certainly in marine snow activities as well), and since the principal adhesives and contributors to surface extension are the organic fibrils of colloidal dimensions, it might be relevant to monitor the sea for fibrils, or, given the recent demonstration of spontaneous microfloc generation by Chin et al. [15], at least for organic colloids in general. There is a field-tested technology available to do this for marine ecosystems; it might be valuable to integrate it with current monitoring programs.

Monitoring the sea for organic colloids: some suggestions

The difficulties of routine quantitative measures for colloidal organic fibrils are outlined in Leppard [19]. For practical purposes, a quantitative measure of colloidal organic carbon (COC) might be a good starting point. The general problems associated with COC measures are described in Buffé and Leppard [31, 32] and elaborated further in Leppard and Buffé [33]. For specific techniques, cross-flow ultrafiltration [34] and differential cascade centrifugation and ultracentrifugation [35] have great potential, but are complicated in some ways. A new ultracentrifugation approach, which is both quantitative and cost-effective, has been developed by the Perret group [36] for monitoring fibrils as a portion of total colloids; it has been field tested in the northern Adriatic Sea.

Recently, Buesseler et al. [37] published an intercomparison of cross-flow filtration techniques used for sampling marine colloids, with a focus on COC defined operationally as the fraction whose size is between 1 kDa and 0.2 μm. In the same year, Guo and Santschi [38] prepared a critical evaluation of the cross-flow ultrafiltration technique for sampling COC in seawater, in which they examined retention characteristics, integrity and performance. With concentration factor and sample storage known generally to play important roles in artifact production by membranes [38] especially with regard to provoked aggregation of colloids, Guo and Santschi [38] also assessed the effects of these two variables. The percentage of COC retained by the ultrafilter changed with the concentration factor during the ultrafiltration process, which meant that the calculation of COC was not straightforward. As anticipated, they showed that reproducible and accurate results for size fractionations required rigorous cleaning and strict sampling protocols. The protocols are delineated and they should yield useful information; however, interpretations will have to consider that colloids in marine ecosystems are abundant in the size range from 0.2 μm up to an order of magnitude greater in least dimension [5]. Further support for the use of cross-flow ultrafiltration comes from Guo and Santschi [39], who outline the contribution that it has already made to understanding the cycling of COC, and by Gustafsson et al. [40] who analyzed the integrity of cross-flow filtration for collecting marine organic colloids, with an emphasis on detecting and understanding membrane fouling within the apparatus.

Conclusions and recommendations

Adriatic marine snow, in mid-summer, is a biota-rich floc with a matrix of fibrils contributing to a mucilaginous aspect. In an incompletely-understood altered form, it contributes to the economically-devastating mucilage phenomenon. In its general aspects, marine snow resembles flocs from many other aquatic ecosystems studied by this author (riverine, lacustrine, and wastewater treatment systems). Research on these other systems currently benefits from the application of new technology (especially the simultaneous multi-method approach involving the correlative use of TEM, COM, SCLM, molecular markers, computerized image analysis and sedimentation data for selecting/isolating representative flocs). Some of this new technology has
been applied successfully to the ultrastructural analysis of marine snow, and the fibril associations within it. Thus the riverine, lacustrine and wastewater floc literatures might profitably be consulted by floc specialists whose interests normally confine them to the marine literature. The idea of the marine floc as an unknowable "black box" should be abandoned. Structure, function and activity relationships can be ascertainment within flocs; correlates of dysfunction are achievable.

Recommended for future research on Adriatic marine snow and colloids released from it, as well as on the increasingly interesting TEP and microfloc/organic polymer transitions, is the following analytical approach. To compare normal flocs with abnormal flocs, when an advance warning exists, one should use the recent protocols for microscopical and microchemical analyses of representative whole flocs, in a search for ultrastructural/chemical correlates of floc dysfunction [24, 25, 27-29]. A simple test could conceivably be developed from their multi-method high technology, one sufficiently simple and cost-effective for routine use in monitoring.

Two methods can be recommended now for general use. To monitor the Adriatic Sea for total organic colloids, which might be informative about important changes in marine snow, the chemical quantitative method of Guo and Santschi [34] should be helpful. To obtain information on the relative contribution of fibrils to total COC, the electron-optical protocol of Lienemann et al. [36] should be helpful.

Acknowledgments

The ideas expressed above owe a debt to research and technical contributions from: (Canada) J. Carson, I.G. Droppo, D.T. Flannigan, B.C. Lee, S.N. Livs, J.N.A. Lott & M.M. West; (Austria) A. Heissenberger & G.J. Herndl; (Switzerland) J. Buffle, C.P. Lienemann, D. Mavrocordatos & D. Perret. Am grateful for institutional support from Environment Canada (NWRI, Burlington), McMaster University (Hamilton, Canada), the Natural Sciences and Engineering Research Council of Canada (Ottawa), the Rudjer Boskovic Institute’s Centre for Marine Research (Rovinj, Croatia), the Austrian Science Foundation (Vienna), the New Energy and Industrial Technology Development Organization (Japan); the Research Institute of Innovative Technology for the Earth (RITE, Kyoto, Japan), the Swiss National Science Foundation (Bern); and the Laboratory of Marine Biology (Trieste, Italy).

Submitted on invitation.
Accepted on 25 January 1999.

REFERENCES


