Carbohydrate polymers as constituents of exopolymer substances in seawater, their complexing properties towards copper ions, surface and catalytic activity determined by electrochemical methods

Marta Plavšić [*], Slađana Strmečki

Ruđer Bošković Institute, Division for Marine and Environ. Research, Zagreb, P.O. Box 180, 10002 Zagreb, Croatia

ABSTRACT

The goal of this study was to investigate to which extent polysaccharides (PS) contribute to the complexing capacity for copper ion (L_T), to determine their property of surface activity and evaluate their capability to cause the catalytic hydrogen evolution wave (peak "H") due to their adsorption and the catalytic groups in their structure. Complexing capacities and apparent stability constants (K_{app}) were measured electrochemically for model polysaccharides (PS): carrageenans (κ-, ι- and λ-), chondroitin sulfate, dextran, dextran sulfate, Na-alginate and humic material. Cu-complexing capacities were determined for Na-alginate ($\log K_{app} = 8.32$) and chondroitin sulphate ($\log K_{app} = 8.14$). PS adsorb on different surfaces due to their amphyphylic properties and on that way they could increase the interaction of copper ions with these surfaces by forming the surface complexes with Cu ions.

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1. Introduction

Several substance classes including, polysaccharides, humic substances originated from aged and microbially modified organic matter and different exopolymer substances (EPS) have been postulated to play a central role in trace metal binding and their biochemical cycling (Hassler, Schoemann, Mancuso Nicols, Butler, & Boyd, 2011; Hassler et al., 2015; Zhang, Xu, & Santschi, 2008). Data from Hassler et al. (2015) suggest that natural phytoplankton growth enhancement in the presence of EPS was not solely due to highly bioavailable iron forms, but also to the presence of other micronutrients. Laboratory and field experiments demonstrated that EPS produced by marine microorganisms are efficient in sustaining biological iron uptake as well as algal growth, and can affect natural phytoplankton community structure.

Stronger ligands were detected following iron-siderophore enrichments ($\log KFe'L = 12.0$) and weaker ligands were measured in the presence of EPS ($\log KFe'L = 10.4$–11.0). This study advances the understanding of the regulation of oceanic iron bioavailability from bacterial siderophores to EPS produced by photosynthetic eukaryotes.

Phytoplankton exudates and their derivates contribute appreciably to the pool of metal ligands (Bhaskar & Bhosle, 2006; Lorenzo, Nieto-Cid, Alvarez-Salgado, Perez, & Beiras, 2007), but metal complexing properties differ in organic matter derived from different sources. Marine metal ligands are specific for certain metals, and ligands that bind different metals differ physico-chemically. As a result of complexity in origin and temporal and spatial variability individual ligands are not well characterized.

Transparent exopolymer particles (TEP), which are formed abiotically from dissolved precursors released by phytoplankton and bacteria (Alldredge, Passow, & Logan, 1993; Passow, 2002) are rich in acidic PS (especially sulfated ones), which make them surface active (Mopper et al., 1995; Zhou, Mopper, & Passow, 1998).

The discovery of ubiquitous, abundant, exopolymer, transparent gel-like particles, containing polysaccharides, has changed the conception of particle–organism interaction and created new questions about the origin, composition and role of these particles in aquatic systems (Cisternas-Novoa, Lee, & Engel, 2015). TEP and their dissolved precursors exhibit a high affinity to thorium and iron binding. [234]Th have been widely used as tracer to quantify the fluxes of particulate organic carbon (POC) in the upper ocean and to constrain the cycling of dissolved organic carbon (DOC) in the marine environment. It was proposed that the observed variability of OC: [234]Th ratios in suspended and sinking matter in the ocean might be caused by the variability of PS content (Passow, Dunne, Murray, Balistrieri, & Alldredge, 2006; Quigley, Santschi, Hung, Guo, & Honeyman, 2002; Santschi et al., 2006).

Surface active substances (SAS), which are derived from different sources include a variety of organic substances (proteins, PS, lipids, humic type substances) and possess hydrophobic (e.g. fatty acids chains, aromatic rings, hydrocarbons) and hydrophilic functional groups (e.g. NH_2, COOH, OH, SH) and therefore participate in electrostatic and hydrophobic interactions (Ćosović, 1985, 2005). SAS act as metal ligands and bind Cu ions well (Plavšić, Lee, & Ćosović, 2006). SAS accumulate at different phase boundaries determined by their hydrophilic/hydrophobic properties (Liss & Duce, 1997).

The existing methods for these phytoplankton derived exudation products, that appear to play such a large role in trace element cycling, are semi-quantitative and none of these substance classes is well enough characterized.

We present data on the complexing capacity of polysaccharide solutions used as models for EPS and precursors, to test the hypothesis that substances characterized as EPS, TEP, PS and SAS bind the copper ions and to determine the degree of overlap between these different substance classes. Specifically, we determined (1) the complexing capacity for copper ions (Plavšić, Krznarić, & Branica, 1982; Scoullos, Plavšić, Karavoltsos, & Sakellari, 2006) using electrochemical methods (differential pulse anodic stripping voltammetry, DPASV). In parallel, we characterized the organic matter: (2) we measured catalytic groups, by chronopotentiometric stripping analysis (CSA) (Strmečki & Plavšić, 2012); (3) we determined the surface activity on the mercury electrode by alternating current voltammetric method (Ćosović, 1985).

2. Materials and methods

2.1. Model substances

Na-alginate, chondroitin sulfate, dextran, dextran sulfate, carrageenans (κ-, ι- and λ-, of types III, V, and IV, respectively) and Na-humate (all from Sigma–Aldrich, Germany) were used as model substances (Table 1).

Carrageenans are sulphated galactans found in the cell walls of red marine algae (Rodophyta). The carrageenans have a molecular mass of $3.5–7.5 \times 10^5$ Da.

Their structure consists of a linear chain of 3-β-D-galactose and 4-α-D-galactose. They are classified according to the position and the number of sulphate groups, and by the presence of 3,6-anhydro bridges in the α-D-galactose residues. κ-, ι- and λ-carrageenans are distinguished by the presence of one, two, and three esters sulphate groups per repeating disaccharide unit. Conformation of the 3,6-anhydrogalactose unit has a direct influence on the rheological difference between ι- and κ- on one side and λ-carrageenan on the other (Campo, Kawano, da Silva, & Carvalho, 2009). While in the structure of ι- and κ-carrageenan, this unit has $1C_4$ conformation, in λ-carrageenan its conformation is $4C_1$. Only $1C_4$ conformation

allows a helix in the secondary structure, which is essential for making gels.

Na- alginate consists of D-manuronic + L-glucuronic acids (molecular mass of $1.0–2.0 \times 10^5$ Da) to which sodium ions are bound. Na-alginate originated from brown algae. Chondroitin sulfate (CS) is a naturally found linear glycosaminoglycan (from shark cartilage) composed of repeating disaccharides of D-glucuronic acid and N-acetyl-D-galactosamine. Depending on source organism, tissue, and age, D-galactosamine can be sulphated at either C-4 or C-6 hydroxyl group. CS is abundant in vertebrates and invertebrates, where it is synthesised as a part of proteoglycans. Actinides (Pu and Am) have high affinity for making complexes with CS from bones' glycoproteins (Gorden, Xu, Raymond, & Durbin, 2003). Dextran is a polymer of glucose (molecular mass is 2×10^6 Da) and dextran sulphate is a polymer of glucose (molecular mass is $5x10^5$ Da) containing sulphate groups bound on PS skeleton. Na-humate was isolated from peat. Molecular mass distribution by separation on Sephadex columns showed a molecular mass of $1–5 \times 10^3$ Da and C-14 analysis showed that peat material was formed ~13 000 years before present (Ochs, Ćosović, & Stumm, 1994). Elemental composition of Na-humate showed the following percentages: C = 50.17%; H = 5.43%; N = 0.88% and S = 3.26%. Besides these model substances, natural seawater sample from North Adriatic, rich in polysaccharides which predominate in spring and early summer (Ahel, Tepić, & Terzić, 2005) was measured, as well as one winter sample in which the PS are not expected to be present. Myklestad (1974, 1995), Myklestad, Skanoy, and Hestmann (1997) determined PS as main component of organic matter in seawater present during the enhanced biological activities.

Some of these model PS (e.g. carrageenans, alginate) are found in marine environment and are known to abiotically form gel particles similar to natural TEP (Passow & Alldredge, 1995; Wurl & Holms, 2008). In solutions PS exist as a size continuum from truly dissolved to particulate, with gel particles several 10's to 100's of μm in size (Verdugo et al., 2004; Verdugo & Santschi, 2010).

Stock solution (0.5 g/L) of all model polymers were prepared by dissolving their powder in Milli-Q water and left overnight in the fridge. Final concentration of 10 mg/L in 25 mL volumetric flask was prepared by adding 2.5 mL of 5.5 M NaCl solution and filling the flask with Milli-Q water.

2.2. Analysis

2.2.1. Electrochemical instrumentation

L_T, AdTCSA and SAS measurements were performed with a μ-Autolab analyzer, Type III (Eco Chemie, The Netherland) connected to a 663 VA stand (Metrohm, Herisau, Switzerland), with a static mercury drop electrode (SMDE) as the working electrode. The reference electrode was an Ag/AgCl (3 M KCl). A platinum electrode served as the auxiliary electrode.

2.2.2. Complexing capacity and stability constants for copper complexation

Copper complexing capacities were determined by differential pulse anodic stripping voltammetry (DPASV) as described previously (Plavšić et al., 1982). DPASV was applied under the following conditions: modulation time 0.04 s, interval time 0.31 s, modulation amplitude 25 mV, and step potential 5 mV. The deposition time was 60 s at −0.6 V for copper vs. a Ag/AgCl (3 M KCl) reference electrode. We applied the direct titration method by gradually adding more metal ions to the sample. First, a solution of 0.55 M NaCl in MQ-water (or UV irradiated seawater, without organics) were titrated with copper ions, respectively, to determine the sensitivity S of the method for copper. Second, a 0.55 M NaCl solution with added model substance or natural seawater sample were titrated in the same way. The height of the Cu voltammetric peaks in 0.55 M