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Evaluation of tangential flow ultrafiltration procedures to assess trace metals bound to marine dissolved organic matter

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ABSTRACT

A procedure based on two-dimensional size exclusion chromatography (SEC) and anion exchange chromatography (AEC) with UV (205 nm) and ICP-MS detection was used to assess dissolved organic matter (DOM) and trace metals associated to DOM in surface seawater. Marine DOM was isolated by tangential ultrafiltration (UF) using two different polyethersulfone membranes exhibiting different molecular weight cut-off (MWCO), 3 and 10 kDa. The procedures require a volume of seawater sample of 100 L, and marine DOM of molecular weight higher than 3 and 10 kDa (UF membranes of 3 and 10 kDa MWCO) was pre-concentrated in 0.5 L (retentate), which implies a pre-concentration factor of 200. Retentate fractions obtained after the different UF procedures were further desalted by using HI Trap desalting mini-columns before two-dimensional SEC/AEC. Apparent molecular weights of isolated compounds after SEC with UV detection ranged from 1.5 kDa (close to the permeable volume of the SEC column fixed by injecting vitamin B12) to 16 and 22 kDa (UF membranes of 3 and 10 kDa MWCO, respectively). Further AEC/UV characterization of SEC fractions showed a large group of macromolecules eluted at 4.5 min, and small signals at shorter retention times (2.5 and 3.5 min). In addition, AEC experiments of the isolated SEC fractions when using 10 kDa MWCO UF membranes showed a group of substances eluted at high retention times (13 min). SEC hyphenation with ICP-MS proved the existence of several trace elements (Ni, Co, Cu, Zn, Mn, Mo and Sr) bound to the isolated marine DOM. Mass balance studies after analyzing the retentate and permeate fractions for trace elements indicate good recoveries (close to 100%) for elements such as Mo, Sr, Ba and Zn when performing the UF with both 3 and 10 kDa MWCO membranes. However, recoveries from 36 to 81% were obtained for the remaining studied elements after either UF procedure. SEC-ICP-MS experiments showed percentages of metals bound to the isolated marine DOM ranging from 0.055 and 0.077% (Zn) to 4.1 10^{-4} and 1.4 10^{-4}% (Sr).

1. Introduction

Marine dissolved organic matter (DOM) is a complex mixture of very different organic molecules from natural and anthropogenic sources. Marine DOM is one of the planet's most important reservoirs of carbon (approximately 700×10^{15} g), an amount similar to the carbon content in atmospheric carbon dioxide (750×10^{15} g) [1,2]. The ocean is an important sink for anthropogenic carbon dioxide because it can absorb one third of the carbon dioxide emissions from combustion of fossil fuels and from tropical deforestation by fire. Marine DOM presents, therefore, a considerable contribution to the global carbon cycle [3,4]. On the other hand, the presence of various highly reactive functional groups in marine DOM is responsible for the binding properties of many different types of contaminants, such as organic compounds and trace metals. Marine DOM plays, therefore, an important role for controlling the transport of toxic contaminants and nutrients and influencing toxicity and bioavailability of those compounds in the marine environment [5–7]. Among the different compounds contained in marine DOM, proteins are important components of high molecular weight. The study of marine proteins has increased because they are the most reactive substances present in marine DOM [8]. Trace metals can be bound to marine proteins and thus affect metal toxicity and availability.

The assessment of marine DOM of high molecular weight is difficult because this fraction occurs at low concentrations, while a large amount of inorganic salts are present in seawater. Some solid phase extraction (SPE) procedures have been applied for pre-concentrating marine DOM. These procedures have been shown to be successful when separating DOM of low molecular weight [9], a fraction which can account for 65–75% of DOM in surface seawater [4]. On the contrary, DOM of high molecular weight, such as marine proteins, is more easily isolated by tangential flow ultrafiltration (UF) procedures [5,8]. This technique offers the advantage of high pre-concentration factors and the possibility of using large sample volumes. UF procedures usually

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require membranes with a nominal molecular weight cut-off (MWCO) of 10 kDa, which guarantees the isolation of marine DOM of high molecular weight (marine proteins included) [10–20]. Nevertheless, marine DOM can be lost by adsorption onto the UF membranes [14,15], and this problem is especially important when dealing with marine proteins due to their highly adsorptive nature. Different reagents such as sodium azide and sodium dodecylsulphate (SDS) are commonly added to the filtered seawater before UF to prevent protein adsorption onto the ultrafiltration membrane [15–20]. On other occasions, UF procedures based on membranes with nominal MWCO of <10 kDa have been proposed, and 1 kDa MWCO membranes have been used in several studies [11,21–25]. UF procedures by using 5 kDa MWCO membranes have also been reported [20].

The aim of this work is the development of pre-concentration procedures for marine DOM of high molecular weight using tangential UF procedures. Isolation was performed by using two membranes with different MWCO (3 and 10 kDa). Differences of the isolated marine DOM were established by two-dimensional size exclusion chromatography (SEC) and anion exchange chromatography (AEC) with UV detection. Inductively coupled plasma-mass spectrometry (ICP-MS) was also used as a detector for assessing trace elements bound to the isolated marine DOM.

2. Material and methods

2.1. Apparatus

The tangential UF system consisted of a Masterflex I/P pump (Millipore, Bedford, MA, USA), a Prep/Scale-TFF Cartridge (Millipore) with two polyethersulfone membranes (nominal MWCO 10 kDa and 3 kDa), and a Pre/Scale-TFF Holder (Millipore) equipped with a pressure gauge.

A Sievers Innovox Laboratory TOC analyzer from General Electric Analytical Instruments (Boulder, CO, USA) was used for DOM quantification. The analyzer uses a super critical water oxidation (SCWO) technique to achieve superior wet DOM oxidation (persulfate in acid medium), and a Non-Dispersive Infra Red (NDIR) for CO_2 detection.

A Dionex P680 HPLC pump (Dionex, Sunnyvale, CA, USA) equipped with a Rheodyne 4097 injector (Cotati, CA, USA) with a 50 μL injection loop, and a Dionex UVD170U absorbance detector were used for HPLC-UV determinations. A 250 μL Hamilton Gastight 1725 syringe (Bonaduz, Switzerland) was used for manual injection.

A Dionex UltiMateO 3000 LC HPLC (Dionex), equipped with a GP50 gradient pump (Dionex), an AS50 thermal compartment (Dionex) and an AS50 autosampler (Dionex) was coupled to an ICP-MS Thermo Finnigan X Series (Thermo Fisher Scientific Inc., Waltham, MA, USA) for assessing metals bound to DOM. DOM fractions were separated with a TSK-G4000 SW_{XL} SEC column (30 cm × 7,8 mm I.D., 8 μm particle size, optimum separation range for Polyethylene Glycol (PEGs) between 2 and 250 kDa, and for proteins between 20 and 10,000 kDa) from Tosoh Bioscience (Tokyo, Japan) coupled to a 10 cm × 8 mm i.d. TSK-gel SW glass guard column (TosoHaas, Tokyo, Japan). A PRP-X100 anion-exchange column (250 mm × 4.1 mm i.d. × 10 μm) from Hamilton (Reno, NV, USA) coupled to a 25 × 2.3 mm I.D. PRP-X100 guard column (Hamilton) was also used.

Hi-Trap Desalting 5.0 mL columns containing Sephadex G-25 Superfine, cross-linked dextran (fractionation range between 1 and 5 kDa) from GE Healthcare (Buks, UK) were used for ultrafiltrate desalting. A Harvard Pump 11 Plus Single Syringe (Harvard Apparatus, Holliston, MA, USA) was used for ultrafiltrate loading into the Hi-Trap Desalting columns by using 5 mL BD Discardit™ II syringes (Becton, Dickinson and Company, Fraga, Huesca, Spain).

Other materials were an ORION 720A plus pH-meter with a glass-calomel electrode (ORION, Cambridge, UK), HAWP14250 Millipore 0.45 μm mixed esters of cellulose membrane filters (140 mm diameter), and Albet®LabScience 0.20 μm cellulose acetate syringe filters (25 mm diameter) from Albet–Hahnemuehle (Dassel, Germany).

2.2. Reagents

Ultrapure water, resistance 18 MΩcm, was obtained from a Milli-Q water-purification system (Millipore). Potassium hydrogen phthalate stock standard solution (1000 mg L^{-1}) was prepared from 99.5% potassium hydrogen phthalate supplied by Panreac (Barcelona, Spain). Potassium peroxodisulphate solution (30% (m/v)) and phosphoric acid solution (6.0 M) were prepared from 99% potassium peroxodisulphate and from 85% phosphoric acid (Panreac), respectively. Ready Calkit PEO/PEG (PSS) containing polyethylene oxides (molecular weights between 6.7 and 478 kDa) were from PSS Polymer Standard Services GmbH (Mainz, Germany). Blue dextran 2000 (molecular weight 2000 kDa) was from Pharmacia Biotech (Piscataway, NJ, USA). Carbonic anhydrase (29 kDa MW), ribonuclease A (13.7 kDa MW), ovalbumin (43 kDa MW), conalbumin (75 kDa MW), aldolase (158 kDa MW) and ferritin (440 kDa MW) were from GE Healthcare.

Mixed exchange AG 501-X8 resin was from Bio-Rad (Richmond, CA, USA). Other reagents were high purity 69% nitric acid (Panreac), high purity 25% ammonia (Merck, Darmstad, Germany), sodium hydroxide (Merck), diammonium sulphate (Panreac), and diammonium hydrogenphosphate (BDH, Poole, UK). Multi-element standard solutions were prepared by combining stock standard solutions (1.000 g L^{-1}) supplied by Merck (Poole, Dorset, UK).

2.3. Procedures

2.3.1. Seawater sample collection procedure

Surface seawater samples (100 L) were collected from the Ría de Arousa estuary (north-western Spain) in pre-cleaned 12 L non-metallic free-flushing Niskin bottles attached to a 1015 rosette multibottle array (General Oceanics, Miami, FL, USA). After collection, seawater samples were filtered (0.45 μm) and immediately subjected to the ultrafiltration procedure.

2.3.2. Seawater tangential flow ultrafiltration procedure

According to manufacturer's instructions, the UF system was cleaned before use by re-circulating 2 L of 0.1 M NaOH at 45 ± 5 °C for 60 min; and rinsing with 9 L of Milli-Q water, also at 45 ± 5 °C. The seawater (100 L) was then concentrated by tangential flow UF through two different polyethersulfone membranes (size 0.6 m^2, nominal MWCO of 10 kDa and 3 kDa) until obtaining a volume of retentate (ultrafiltrate containing substances of molecular weight higher than 10 and 3 kDa, respectively) of approximately 500 mL. The remaining sample (permeate, ~99.5 L) contained substances of molecular weight lower than 10 and 3 kDa (membranes of nominal MWCO of 10 kDa and 3 kDa, respectively), and it was reserved for further mass balance studies. No preservatives for avoiding marine DOM adsorption onto the UF membrane were added, because mass balance studies for marine DOM were performed through TOC determinations.

2.3.3. Retentate desalting procedure by using HI Trap desalting columns procedure

Hi Trap Desalting columns were used for removing the salts from the retentate. The Sephadex G-25 Superfine contained in the columns offer a fractionation range for globular proteins between 1 and 5 kDa, with an exclusion limit of approximately 5 kDa. This ensures the separation of bio-molecules exhibiting a molecular weight higher than 5 kDa from those molecules with molecular weights less than 1 kDa. The mobile phase used for desalting consisted of a 25 mM/25 mM ammonium sulphate/diammonium hydrogen phosphate buffer solution at pH 6.5. The column was connected to the chromatographic system with UV detection, and was first equilibrated by passing 25 mL of a buffer solution at a flow rate of 2 mL min^{-1}. Once equilibrated, 1.5 mL of