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Particle–water interactions of organic nitrogen in turbid estuaries

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ABSTRACT

The hypothesis that particle–water interactions of organic nitrogen (ON) in turbid estuaries are controlled by specific fractions of suspended particulate matter (SPM) has been tested. Suspended particles from the turbidity maximum zone of a temperate, macro-tidal estuary were separated by gravitational settling to yield permanently suspended (PSPM; 46–51% of the total SPM load) and resuspendable (RSPM) particle fractions. Abiotic and biotic laboratory incubations were carried out in triplicate over 48 h, on the separate fractions to quantify the partitioning of ON under representative estuarine conditions, using acidic, basic and neutral ^{14}C-labelled amino acids as proxies for ON. Under abiotic conditions, about 10% of arginine (basic) sorbed to both SPM fractions within 48 h, while sorption did not occur for aspartic acid (acidic) and glycine (neutral). Partitioning of the amino acids with both SPM fractions under biotic conditions was much greater and most uptake had occurred by 24 h, when about 44% of added amino acid was in the particulate phase. By 48 h, 33–60% of activity on the particles had been lost as $^{14}CO_2$, and the mean amount of dissolved amino acid remaining, in all biotic experiments, was 14 ± 3%. For abiotic experiments with arginine, K_ds at 48 h were of the order 10^2 mL g^{-1}, although were much higher (10^3 mL g^{-1}) when RSPM concentrations were markedly increased. The biotic experiments consistently gave K_ds that were of the order 10^3 mL g^{-1}. Thus, uptake of DON onto the SPM was bacterially-controlled and the general paradigm, that the removal of DON to particles is an abiotic process that restricts its bioavailability, does not hold for estuarine SPM.

1. Introduction

Fluxes of reactive nitrogen (N) from terrestrial sources to estuaries are increasing, with a concomitant negative impact on coastal water quality (UNEP, 2004; Billen and Garnier, 2007). Approximately 50% of the annual global river flux of total N to estuaries is particulate organic N (PON), while dissolved organic nitrogen (DON) is a significant fraction of the remainder (Tappin, 2002; Berman and Bronk, 2003). The estuarine transport of ON is governed, to some extent, by particle–water interactions which are affected by various combinations of salinity, particle concentration and chemical composition, solution phase speciation and bacterial activity (Henrichs and Sugai, 1993; Hedges and Keil, 1999; Cottrell and Kirchman, 2000). However, the speciation and partitioning behaviour of ON in rivers and estuaries are generally poorly quantified (Aufdenkampe et al., 2001), giving rise to major uncertainty regarding fluxes of ON from estuaries to coastal seas.

Experiments quantifying the uptake and release of ON by natural and organic-free mineral particles have been conducted under abiotic conditions using elevated solid:solution (w:v) ratios typically encountered in sediments (Henrichs and Sugai, 1993; Wang and Lee, 1993; Montluçon and Lee, 2001; Ding and Henrichs, 2002; Wedyan and Preston, 2005). It is arguable, however, whether partition coefficients (K_d) obtained from experiments in highly concentrated sediment slurries can be extrapolated to sorption processes in the estuarine water column where solid:solution ratios are typically 1–1000 mg L^{-1} (Middelburg and Herman, 2007; Fitzsimons et al., 2006; Tappin et al., 2007). Organic nitrogen appears to partition preferentially on to the fine particle fractions of riverine SPM, with basic amino acids and organic nitrogen compounds sorbing to a greater extent than acidic and neutral forms (Hedges et al., 1994; Aufdenkampe et al., 2001). Sorption also appears to be reversible as about 60% of the organic matter (OM) associated with estuarine and deltaic SPM is lost, probably through desorption and subsequent remineralisation (Henrichs, 1995; Hedges and Keil, 1999). A clear understanding of the sorption behaviour of ON is hindered by a lack of experimental evidence obtained under realistic estuarine conditions.

Abbreviations: ARG, L-arginine; ASP, L-aspartic acid; ATP, adenosine-5′-triphosphate; CRM, certified reference material; DFAA, dissolved free amino acid; DIN, dissolved inorganic nitrogen; DOC, dissolved organic carbon; DOM, dissolved organic matter; DON, dissolved organic nitrogen; GLY, glycine; HDPE, high density polyethylene; HPLC, high performance liquid chromatography; K_d, partition coefficient; LDPE, low density polyethylene; OM, organic matter; ON, organic nitrogen; PAA, particulate amino acid; POC, particulate organic carbon; PON, particulate organic nitrogen; PSPM, permanently suspended particulate matter; RSPM, resuspendable suspended particulate matter; SPM, suspended particulate matter; TDN, total dissolved nitrogen; TMZ, turbidity maximum zone; TMZS, turbidity maximum zone summer; TMZW, turbidity maximum zone winter.

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Amino acids (in the free form, and as peptides and proteins) are the largest reservoir of ON in most aquatic organisms. They account for 3–97% of particulate N in rivers worldwide (Ittekot and Zhang, 1989) and 40–90% of N mineralised in coastal marine sediments (Henrichs and Farrington, 1987; Burdige and Martens, 1988). Amino acids comprise basic, acidic and neutral molecules, based on their side chain structure, and this chemical variability, coupled with their dominance of the ON pool, means that amino acids are meaningful proxies for studying the properties and behaviour of bulk ON in natural waters (Hedges et al., 1994; Aufdenkampe et al., 1999; Ding and Henrichs, 2002).

Separation of SPM on the basis of its intrinsic settling velocities, rather than size, is critical to an improved understanding of the influence of SPM on chemical cycling and transport, and concomitant impacts on the aquatic environment (Gustafsson and Gschwend, 1997). Suspended particles within turbid estuaries can be separated broadly into permanently suspended particulate matter (PSPM), and resuspendable suspended particulate matter (RSPM) which undergoes periodic resuspension and deposition (Alber, 2000). Generally, PSPM is more abundant than RSPM, and has a higher POC content and lower POC:PON ratios (Puls et al., 1988; Turner et al., 1994; Kerner and Krogmann, 1994; Alber, 2000). In the Columbia and Ogeechee River estuaries (USA), and the Tamar Estuary (UK), bacterial production and enzyme activity were higher in the PSPM relative to the RSPM, and both were much higher in the particle fractions than in the water (Plummer et al., 1987; Crump et al., 1999; Crump and Baross, 2000). Differences in the settling velocities of the PSPM and RSPM suggest that each fraction will have markedly different estuarine residence times and will thus influence the cycling and transport of constituents in contrasting ways.

The aim of this study was to test the hypothesis that the particle–water interactions of ON in turbid estuaries are significantly controlled by specific fractions of the SPM which have contrasting biogeochemical compositions and estuarine residence times. Samples of SPM were collected from the turbidity maximum zone (TMZ) of the Tamar Estuary (UK), fractionated into PSPM and RSPM and used, with ^{14}C-labelled dissolved amino acids, in abiotic and biotic partitioning experiments. The objective was to assess the relative roles of these SPM pools in the estuarine processing of DON.

2. Methods

Glass- and plastic-ware were cleaned by degreasing (2% Decon, >24 h) and acid-washing (1.2 M HCl, >24 h). Glass-ware was combusted (450 °C, 6 h) as a final step. All glass fibre filters (GF/F) were combusted and cellulose nitrate filters were acid-washed. Clean techniques were used throughout, and critical handling steps were performed in a Class 100 laminar flow cabinet.

2.1. Experimental design

Plummer et al. (1987) measured bacterial activity in PSPM, RSPM and water from the Tamar Estuary (SW England), and concluded that the numbers of freely suspended bacteria were small compared to those living on the particles. Therefore, our experiments focussed on the sorption behaviour of amino acids on these two particle fractions. Water samples were collected in 10 L carboys from the Tamar Estuary, a system representative of macro-tidal, turbid estuaries in the northern hemisphere (Haslett, 2008). Sampling was undertaken from the TMZ because of its role as a biogeochemical reactor (Uncles et al., 1998; Herman and Heip, 1999) and was conducted on spring tides, prior to high water slack, during summer (TMZS) and winter (TMZW). The conductivity and pH were monitored during sampling using calibrated sensors and the SPM concentration (dry weight) was obtained gravimetrically, following filtration of a known volume of water.

The partitioning experiments, using dissolved ^{14}C-labelled amino acids, were designed to emulate, as closely as possible, aquatic conditions at the time of sampling. The turbid suspensions were separated into PSPM and RSPM fractions by gravitational settling and slurries prepared by centrifugation. In order to differentiate between chemically- and biologically-mediated sorption processes, abiotic experiments were conducted using autoclaved particles. Particle slurries were diluted to field-measured SPM concentrations using filtered, autoclaved water from the relevant site, amended with a ^{14}C-labelled amino acid. The uniformly ^{14}C-labelled amino acids were L-arginine (ARG, basic), L-aspartic acid (ASP, acidic) and glycine (GLY, neutral). ARG and ASP are, respectively, the most basic and acidic of the 20 protein amino acids. The experiments were carried out in triplicate at natural pH and room temperature and the time-dependent behaviour of the dissolved, ^{14}C-labelled amino acids was monitored by liquid scintillation counting, following sampling at selected intervals.

2.2. Fractionation of SPM

The total SPM in ca. 8 L samples was separated into two fractions under gravity in settling tubes. The PSPM was defined as having a settling velocity <0.006 cm s^{-1}, following earlier work (Alber, 2000), and after a time interval approximately equivalent to high water slack (ca. 1 h) the upper water layer containing the PSPM was carefully decanted off (Williams and Millward, 1998). The RSPM had a small contribution from the PSPM which was accounted for in calculations, using the mixing algorithm described in Turner et al. (1994). The separated fractions were then concentrated into slurries by centrifugation (9000 rpm, 30 min), and stored in 30 mL aliquots at −20 °C until required for the partitioning experiments. Storage at −20 °C was chosen because the method is commonly used for the long-term preservation of aquatic bacteria with respect to genetic stability (Park et al., 2001; Hyun and Yang, 2003), and so would not unduly compromise the experimental design.

2.3. Amino acids

Dissolved and particulate amino acids were determined using the methods described in Tappin et al. (2007). Water samples for the analysis of dissolved free amino acids (DFAA) were vacuum-filtered through cellulose nitrate filters (0.2 μm pore size) and stored at −20 °C until analysis. Particulate amino acid (PAA) concentrations were measured in freeze-dried sediment (0.5–0.6 g). The sediment, in sealable vials, was wetted with high purity (18.2 MΩ cm^{-1}) water, and charge-matched internal standards added (3-methyl-L-histidine, basic; methylleucine, neutral; aminoadipic acid, acidic; final concentration 20 μmol L^{-1} of each), to quantify the recovery of the extractable amino acids (Cowie and Hedges, 1992; Tappin et al., 2007). Inorganic carbon was removed by the addition of 2 M HCl until effervescence ceased. After drying at 40 °C, 6 M HCl (4 mL) was added to each sample and the vial flushed with high purity argon. The samples were then hydrolysed in sealed vials at 150 °C for 90 min followed by cooling in an ice bath. The closed vials were centrifuged at 3000 rpm for 10 min and each supernatant transferred to a 50 mL round-bottomed flask for drying in a rotary evaporator at 50 °C. The remaining residue was dissolved in 0.4 mL high purity water then transferred to a 25 mL volumetric flask and made up to volume. The hydrolysed samples were stored at −20 °C until analysis. Sediment combusted at 450 °C for 6 h was used as a procedural blank (Arnason and Keil, 2000). Amino acids were analysed by reverse-phase HPLC using pre-column derivatization with *o*-phthaldialdehyde (OPA) and 2-mercaptoethanol (Lindroth and Mopper, 1979). Iodoacetic acid was added to improve detection of sulfhydryl-containing amino acids (Inglis, 1983). Analytes were separated on a 150×4.6 mm Spherisorb® ODS2 3 μm analytical