Available online at www.sciencedirect.com
ScienceDirect
Marine Chemistry 106 (2007) 456–462
MARINE CHEMISTRY
www.elsevier.com/locate/marchem

Development of a fluorescence quenching assay to measure the fraction of organic carbon present in self-assembled gels in seawater

Yong-Xue Ding [a], Wei-Chun Chin [b,c], Pedro Verdugo [d,*]

[a] Friday Harbor Laboratories, University of Washington, 620 University Road, Friday Harbor, WA 98250, USA
[b] School of Engineering, University of California, Merced, CA 95344, USA
[c] College of Engineering, Florida State University, Tallahassee, FL 32310, USA
[d] Department of Bioengineering and Friday Harbor Laboratories, University of Washington, 620 University Road, Friday Harbor, WA 98250, USA

Received 21 December 2006; received in revised form 14 March 2007; accepted 6 April 2007
Available online 3 May 2007

Abstract

The dissolved organic carbon pool (DOC) is among the largest reservoir of reduced carbon on our planet. The demonstration that DOC polymers remain in assembly/dispersion equilibrium forming microscopic hydrogels has a broad range of critical implications. Previous studies estimate that ~10% of DOC could be assembled as gels, yielding values of $\sim 7 \times 10^{16}$ g of organic carbon present as microscopic hotspots of high substrate concentration. This huge mass of reduced carbon emphasizes the need to develop reliable methods to systematically investigate the budget of self-assembled marine gels (SAG), and their role in biogeochemical cycling. However, a quantitative method to measure SAG in seawater has not been available. Here we present the validation of a simple assay to measure the mass of organic carbon assembled as microgels in native seawater. This method is based on the ratio of Chlortetracycline (CTC) fluorescence quenching between Ca bound to non-assembled organic molecules and molecules assembled as microgels. This assay can be readily implemented on board using a low cost fluorometer and provisions to measure TOC.

Keywords: Fluorescence assay; CTC; Self-assembled gels; Fluorescence quenching

1. Introduction

Biopolymers present in seawater comprise one of the basic substrates for marine bacteria. However, to cross the bacterial cell membrane and be metabolized, polysaccharides, lipids, polynucleotides, and proteins must be cleaved to short chain oligosaccharides, oligopeptides, etc, of less than ~600 Da (Weiss et al., 1991). Bacteria carry out this process by releasing extoenzymes that cleave marine biopolymer. Available observations confirm that the biosusceptibility of marine organic molecules is indeed greatest for small biomolecules (<600 Da) that can be directly incorporated by bacteria but as their size increases they turn largely refractory. However, marine macromolecules reaching colloidal size become less mobile, easier to seize by bacteria and their

* Corresponding author. Fax: +1 206 543 1273.
E-mail address: verdugo@u.washington.edu (P. Verdugo).

0304-4203/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.marchem.2007.04.005

biosusceptibility increases again (Amon and Benner, 1994). Moieties too large to pass the bacterial cell membrane yet too small to be captured by bacteria comprise a huge refractory pool leading to the idea that the ocean could function as a massive repository of burial carbon (Hedges and Keil, 1995; Hedges and Oades, 1997). However, the discovery that about 10% of DOC remains in reversible self-assembly equilibrium forming microscopic gels introduced a powerful new set of implications regarding the processes connecting the ocean microbial loop and biological pump to the biogeochemical dynamics of the rest of the biosphere and the geosphere (Chin et al., 1998; Wells, 1998; Verdugo et al., 2004). Paramount among those is that microgels resulting from DOC self-assembly form porous structures of high substrate concentration that can be readily colonized, degraded, and metabolized by bacteria with the subsequent return of CO_2 to the global carbon cycle (Orellana and Verdugo, 2003). DOC self-assembly process comprises a huge shunt between a mostly refractory DOC pool of $\sim 7\times10^{17}$ g of reduced C and a highly biosusceptible supply of microscopic marine gels forming discrete hot spots of concentrated bacterial substrate that could reach up to $\sim 7\times10^{16}$ g of C (Chin et al., 1998; Wells, 1998; Orellana et al., 2000; Verdugo et al., 2004). These observations highlight the need to develop reliable quantitative methods to systematically monitor the budget of self-assembled organic moieties. Here we report the development and experimental validation of a simple and reproducible method to measure the fraction of total organic carbon (TOC) that is present as SAG in seawater. This method can be readily implemented on board using a low cost portable fluorometer to accurately monitor the SAG budget, its time variations, and its geographic and depth distribution.

2. Theory

The assay is based on our previous demonstration that self-assembled DOC polymers forming SAG are crosslinked by Ca^{2+} bonds. The concentration of Ca bound inside these gels is much higher than free ionized Ca^{2+} in seawater (Chin et al., 1998). Chlortetracycline (CTC) can be used as a reporter of bound Ca (Rudolf et al., 2003), and we previously validated its application to label SAG (Chin et al., 1998; Orellana et al., in press). This reagent selectively fluoresces ($\lambda_{ex}=374$ nm, $\lambda_{em}=560$ nm) when complexed with Ca^{2+} bound to free DOC polyanions, to DOC polyanions assembled in the matrix of microgels (Chin et al., 1998), or to polyanions found in cytoskeletal networks of dead cells. Chelation of Ca using ethylenediaminetetraacetic acid (EDTA) takes away the Ca^{2+} crosslinks, resulting in immediate dispersion of SAG. However, unlike SAG, cytoskeletal networks are chemically crosslinked and don't disperse by exposure to EDTA.

Quenching of CTC emission and the corresponding changes of quantum yield are different when Ca is bound to free polymers or to polymers assembled forming networks. Fluorescence emission is much higher when the CTC-Ca tag is bound to free polymers than when it is bound to networks (see Fig. 1). Since EDTA disperses only SAG, and not cytoskeletal networks, the observed increase of CTC emission resulting of EDTA-induced SAG dispersion results only from the assembled fraction of TOC. Thus, the fraction of TOC

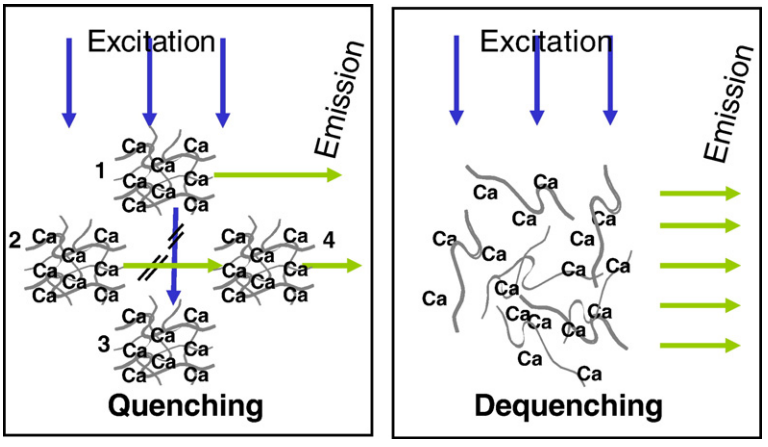

Fig. 1. Fluorescent quenching in these experiments results from topological constraints whereby SAG shadow each other by either blocking the excitation radiation from reaching its target—in this illustration SAG 1 shadows SAG 3—or the emission radiation from reaching the detector—SAG 4 shadows SAG 2. Quenching is proportional to the number and size of gels assembled resulting in a corresponding decrease in total quantum yield. Chelation of Ca with EDTA causes segregation of CTC-tagged polymers with a corresponding dequenching and increase of quantum yield.