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FINAL REPORT

LOW MOLECULAR WEIGHT CARBOXYLIC ACIDS IN THE SEA: PHOTOOXIDATIVE PRODUCTION AND BIOLOGICAL CYCLING

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Project Summary

A large fraction of dissolved organic matter (DOM) in seawater is composed of biologically refractory (non-utilizable) substances. The formation and destruction pathways of this organic matter are still unknown. One potentially important removal pathway that has not been quantified is sunlight-induced (photochemical) break down of DOM in the sea surface. Important breakdown products include biologically utilizable compounds, especially low molecular weight (LMW) carboxylic acids, formate and acetate, and α-keto acids glyoxylate and pyruvate. Therefore, we used organic acid photo-production in seawater as a tool to evaluate the importance of photo-fragmentation of biologically refractory organic matter in the sea. Laboratory studies, integrated with a sea-going program, SOLARS, was used to establish a broad data base for the spatial and temporal distribution of organic acids in coastal and oceanic waters. With this data base, and associated biological turnover and photochemical production measurements, we determined that the photochemical production of these compounds, when completed to their biological turnover, plays a major role in the geochemical cycling in the sea.

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A large fraction of dissolved organic matter (DOM) in seawater is composed of biologically refractory (non-utilizable) substances. The formation and destruction pathways of this organic matter are still unknown. One potentially important removal pathway that has not been quantified is sunlight-induced (photochemical) break down of DOM in the sea surface. Important breakdown products include biologically utilizable compounds, especially low molecular weight (LMW) carboxylic acids, formate and acetate, and α-keto acids glyoxylate and pyruvate. Therefore, we used organic acid photo-production in seawater as a tool to evaluate the importance of photo-fragmentation of biologically refractory organic matter in the sea. Laboratory studies, integrated with a sea-going program, SOLARS, was used to establish a broad data base for the spatial and temporal distribution of organic acids in coastal and oceanic waters. With thi, data base, and associated biological turnover and photochemical production measurements, we determined that the photochemical production of these compounds, when completed to their biological turnover, plays a major role in the geochemical cycling in the sea.

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Photochemical source of biological substrates in sea water: implications for carbon cycling

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DISSOLVED organic carbon (DOC) in sea water represents one of the largest reservoirs of carbon on the earth[1]. The main fraction of this DOC is generally believed to be composed of old[2], biologically refractory material[3] such as humic substances, for which the removal mechanisms remain largely unknown. One potentially important removal process in the ocean that has not been investigated is the photochemical breakdown of this DOC in the photic zone to form biologically labile organic products. Here we show that biological uptake of pyruvate is highly correlated to its rate of photochemical production in sea water ($r = 0.964$), and that the photochemical precursor(s) of pyruvate is from the fraction of DOC having a nominal molecular weight of 500. This is the first evidence that photochemical breakdown of high-molecular-weight marine DOC, which is presumably biologically refractory, results in the production of a compound that is used by plankton as a substrate. Our results have important implications for the oceanic carbon cycle, particularly with respect to planktonic-food-web dynamics and the global carbon budget.

We collected surface seawater samples from a variety of coastal and oligotrophic stations (Fig. 1) during the SOLARS (Study of Light Activated Reactions in the Sea) cruises. Details of sampling procedures will be presented elsewhere (D.J.K. *et al.*, manuscript in preparation). Briefly, we determined the biological uptake of pyruvic acid by measuring its ambient substrate concentration S_n by high performance liquid chromatography[4] and determining the turnover time t_n of pyruvate at S_n using the respiration corrected kinetic approach[5]. Typical S_n values ranged from 0.2 to 2.0 nM for oligotrophic stations and 0.9 to 6.2 nM for coastal stations. We added [2-^{14}C] labelled pyruvate to unfiltered seawater samples to obtain concentrations in the range 10–120 nM; we added glutaraldehyde to pyruvate controls to account for abiological processes (such as adsorption onto particles). We incubated the samples in acid washed polycarbonate bottles placed in a water bath and exposed to sunlight. There is evidence that light inhibits uptake of organic substrates[6]; nevertheless, the incubations were done under light conditions because this most closely reflected ambient conditions in surface sea water. In cases where we made light–dark comparisons to estimate the magnitude of photoinhibition of uptake, the light rates of pyruvate uptake were within 20% of the corresponding dark rates (D.J.K. *et al.*, unpublished results). Samples were incubated from 0.2 to 1.7 h at coastal stations and from 3 to 13 h for offshore sites in the Gulf Stream and the Sargasso Sea. We chose incubation times such that, in most instances, the substrate uptake was <10% at the lowest addition; we measured the amount of label assimilated as well as the amount respired to carbon dioxide. The turnover time is the vertical-axis intercept of the plot of the incubation time divided by the fraction of isotope taken up as a function of concentration of added pyruvate. Time-course experiments performed at each station showed that the uptake of pyruvate in all cases was linear during incubations[7] (D.J.K. *et al.*, manuscript in preparation). After determining S_n and t_n, we can calculate the uptake rate

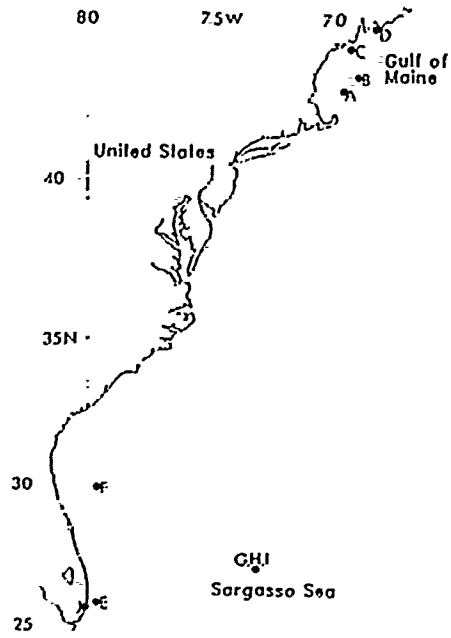

FIG. 1 Map of sampling locations. Gulf of Maine. *a*, Wilkinson Basin, 42°35.2′ N, 69°34.9′ W; *b*, Ammen Rock, 42°53.8′ N, 68°56.6′ W; *c*, Monhegan Island, 43°44.4′ N, 69°19.2′ W; *d*, Acadia National Park, 44°17.7′ N, 68°10.7′ W. Gulf Stream: *e*, 25°58.5′ N, 79°42.5′ W; *f*, 30°10.4′ N, 79°33.8′ W. Sargasso Sea: *g*, 27°21.4′ N, 73°12.6′ W; *h*, 27°20.9 N, 73°14.9′ W; *i*, 27°21.4 N, 73°16.4 W. Biscayne Bay, Florida. *j*, 25°41.0 N, 80°08.1′ W.

of pyruvate V_n at the ambient substrate concentration from the equation:

$$V_n = S_n / t_n$$

Concurrent with uptake studies, we exposed 0.22-μm-filtered seawater samples to sunlight to determine rates of photochemical production of pyruvate. Irradiations, ranging from 1 to 3 h, were carried out at midday between 10.00 and 14.00 local time. Sample filtration has no significant effect on photochemical production rates of pyruvate when performed carefully (such as gravity filtration through a Whatman GF/C filter followed by vacuum filtration through a 0.22-μm nylon filter at a pressure differential ≤100 mm Hg)[4].

Using data from several field locations, we plotted the uptake rate of pyruvate at S_n against its rate of photochemical produc-

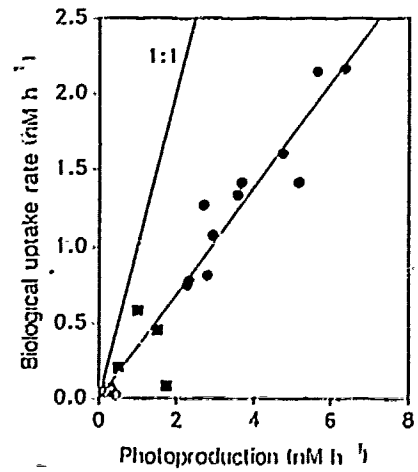

FIG. 2 Biological uptake rate of pyruvate plotted against its midday rate of photochemical production. The line drawn through the data was determined from linear regression analysis. The 1:1 line (slope = 1) depicts the case where photochemical production and biological uptake rates are equal.

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Determination of low-molecular-weight carboxylic acids in aqueous samples by gas chromatography and nitrogen-selective detection of 2-nitrophenylhydrazides

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(Received 4th January 1990)

ABSTRACT

A gas chromatographic method was developed for the determination of low-molecular-weight carboxylic acids in aqueous samples based on a derivatization procedure compatible with aqueous solutions. The technique uses nitrogen-selective detection with a thermionic-specific detector after derivatization of carboxylic acids as 2-nitrophenylhydrazides. The hydrazides were extracted with ethyl acetate prior to injection into the gas chromatograph. The derivatives appear to be stable in ethyl acetate at 0–5 °C for long periods and, therefore, can be stored for analysis at a later date. The detection limits of different short-chain acids are in the range 0.8–1.4 pmol per injected sample. The relative standard deviation is less than 10% at the 1 μM level. Examples of the use of the method are given for the determination of carboxylic acids in anoxic marine sediment pore waters, coastal sea water and Black Sea water samples.

Keywords: Carboxylic acids; Waters- Natural waters; Gas Chromatography

Low-molecular-weight carboxylic acids (aliphatic, short-chain, C_1–C_5) are important metabolites and intermediates in biological processes and are widely dispersed in the environment, including aquatic bodies and sediments. Several gas (GC) and liquid chromatographic (LC) methods have been reported for their determination at low nanomolar to micromolar concentrations in aqueous samples. Ion chromatography combined with conductivity detection can be used for analysis of relatively clean matrices such as rain [1] and glacial melt waters [2], but is not well suited for complex ionic matrices such as sea water or sediment pore water. Methods based on electrophoresis, such as isotachophoresis [3] and capillary zone electrophoresis [4] have also been used, but their detection limits are usually higher than those of chromatographic methods. GC and LC methods usually require chemical derivatization of carboxylic acids to enhance detectability, chromatographic separation and solute volatility. For GC, carboxylic acids have been converted to alkyl esters such as methyl [5], benzyl [6,7] or pentafluorobenzyl esters [8–11]. In LC methods, derivatizations with absorbing or fluorescent labels such as *p*-bromophenacyl bromide [12], pentaflurobenzyl bromide [13] or coumarins [14] have been commonly used.

In almost all previous studies, it was necessary to use a phase-transfer step prior to the derivatization of carboxylic acids because an organic medium was required for the labelling reaction.

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Free amino acids in marine rains: evidence for oxidation and potential role in nitrogen cycling

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Previous studies of dissolved organic nitrogen (DON) in precipitation have addressed various aspects of nutrient transport and global nitrogen cycling[1]. In most of these studies however, the detailed chemical composition of DON was not determined. Analyses of specific organic nitrogen compounds within precipitation can yield new information about sources and transformations of DON as well as about heterogeneous oxidative processes in the atmosphere. Dissolved free amino acids (DFAA) are a class of compounds for which surprisingly few anniyses in precipitation have been published[1]. We report here the first detailed analyses of DFAA and primary amines in marine rains. Unexpectedly high concentrations of total DFAA were measured, averaging about 6.5 μM (16 marine rain samples) and ranging from 1.1 to 15.2 μM (0.015 to 0.21 p.p.m. N); these values are similar to those obtained for inorganic nitrogen species[2]. Amino acids are predominantly found in rain as their L-optical isomers and therefore are most likely biological in origin; however, the exact sources and modes of enrichment in rain over the open ocean are not known. There is evidence that some of the amino acids, in particular methionine, are oxidized in the atmosphere possibly by a heterogeneous photochemical pathway. The finding of high DFAA and primary amine concentrations in marine rain may have important implications in global nitrogen cycling and also may contribute locally to available nitrogen at the sea surface.

Rain, air, and seawater samples were analysed at sea for one year during four recent cruises and at the home laboratory in Miami as part of the SOLARS programme (Studies of Light Activated Reactions in the Sea). These cruises were located in the Gulf of Mexico (RV *Cape Florida*) and in different locations in the northwest Atlantic Ocean (RV *Columbus Iselin*) (Table 1). Amino acids, primary amines and ammonium were analysed on board by high performance liquid chromatography in conjunction with precolumn fluorescence derivatization with *o*-phthalaldehyde and a thiol[3]. For some samples two different thiols, 2-mercaptoethanol (ME) and *N*-acetyl-L-cysteine (NAC), were employed in separate derivatizations. This was done to verify the identification of specific amino acids, since the thiol used in the reaction greatly alters the chromatographic selectivity of the fluorescent derivatives. In addition, the use of NAC results in the separation of L and D enantiomers[4] and also permits more sensitive detection of ammonium than ME. Secondary and tertiary amines cannot be determined by this technique.

Extreme care was exercised to eliminate contamination during sampling. Rain samples were collected with acid washed glass funnels and bottles that were rinsed several times with rain before the actual samples were taken. Air was sampled using an all glass impinger filled with deionized water; the air flow was 1–2 litre min^{-1} and approximately 0.1–0.2 m^3 of unfiltered air were sampled. Most rain samples were analysed unfiltered and within one hour of collection. Several samples were filtered (0.7 μm glass fibre or 0.2 μm membrane) and stored frozen. Filtration and freezing were found not to alter significantly the composition of samples. A summary of the results and background information on a few representative rain and air samples are given in Table 1; detailed DFAA and primary amine compositions of these samples are given in Table 2. Concentrations up to 15 μM were observed in marine rains. Procedural blanks (deionized water or sea water put through the above collection and sample handling procedures) showed no evidence of amino acid contamination from either the sampling personnel or the ship. Furthermore, the sample handling and derivatization procedures were identical to those routinely used on the same cruises for the determination of DFAA and ammonium in seawater, where total concentrations were 2–3 orders of magnitude lower than in most marine rain samples (Table 2).

The DFAA pattern of marine rain is dominated by glycine,

Table 1 Amino acids, primary amines, ammonium and background data for rain and air samples

Sample	Date	Location	Local Time (EST)	Prevailing wind direction	Amino acids (μM)	Primary amines (μM)	NH_4^+ (μM)	NO_3^- (μM)	Cl^- (μM)	SO_4^{2-} (μM)	H_2O_2* (μM)
				Gulf of Mexico							
B	9/16/85	26°22′ N 89°52′ W	19:30	NNE	13.22	0.10	13.24	18	3,024	191	38.6
D	9/19/85	24°38′ N 82°57′ W	12:17	E	15.17	0.08	ND	ND	ND	ND	20.2
				Miami, Florida							
F	10/2/85	Miami	11:00	SE	0.61	0.25	1.2	282	3,945	366	ND
I-3			17:10	NW	0.39	0.035	3.79	30	82	12	ND
I-3 Hydrolysis			17:10		3.03	1.32	13.14	ND	ND	ND	ND
				Northwestern Atlantic Ocean							
O-3	2/23/86	25°55′ N 78°13′ W	22:55–23:21	SW	10.80	1.35	13 06	1,169	666	52	8.4
Q-1	2/26/86	20°31′ N 68°44′ W	10:25–10:30	N	4.91	0.22	16.36	80	883	70	18.7
T	6/22/86	26°46′ N 75°24′ W	05:45–06:00	E	1.71	0.12	2.81	23	206	ND	ND
U	9/26/86	26°30′ N 76°04′ W	19:00–19·05	E	4.04	0.65	17.52	ND	ND	ND	80
Marine air 11	2/28/86	13°12′ N 66°04′ W	11:45–16:40	E	2.18	0.022	1.08	527	265	29	ND
Surface sea water (μM)	10/25/86	26°30′ N 76°04′ W	14:40	E	0.062	—	0.42	ND	ND	ND	0.012

ND, not determined.
* See ref. 17.

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PHOTOCHEMICAL FORMATION OF GLYOXYLIC AND PYRUVIC ACIDS IN SEAWATER

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ABSTRACT

Kieber, D.J. and Mopper, K., 1987. Photochemical formation of glyoxylic and pyruvic acids in seawater. Mar. Chem., 21: 135–149.

Glyoxylic and pyruvic acids were formed when filter-sterilized seawater was exposed to solar radiation. Production rates varied from samples collected from distinctly different regions of the sea. Humic-rich seawater from the Florida Bay exhibited net photochemical production rates (glyoxylate, 27.5 nM/W·h m^{-2}; pyruvate, 12.9 nM/W·h m^{-2}) that were significantly greater than net production rates for humic-poor water (glyoxylate, 3.1 nM/W·h m^{-2}; pyruvate, 1.8 nM/W·h m^{-2}) collected in the Gulf Stream. When seawater was not filtered, the concentrations of glyoxylate and pyruvate were found to undergo diurnal variations resulting from an imbalance between biological and photochemical processes.

A depth profile of the glyoxylate concentration from several oceanic stations showed a pronounced daytime maximum in the upper 10 m; this finding is consistent with laboratory results that demonstrated that glyoxylate is formed photochemically in seawater. Pyruvate, in contrast, showed no clear trend with depth; its distribution in the water column may be primarily controlled by biological processes rather than by photochemical processes.

Biological processes are generally thought to control the spatial and temporal distribution of simple organic metabolites in seawater. Our results show that photochemical processes may also be important in the marine cycling of some biochemical compounds.

INTRODUCTION

Sunlight penetrating the sea surface can promote transformations of dissolved organic matter (DOM) in the photic zone. In particular, sunlight can initiate condensation reactions leading to the formation of marine humic substances (Harvey et al., 1983; Momzikoff et al., 1983), and can induce a variety of primary and secondary photochemical reactions yielding small, oxidized organic and inorganic products such as aldehydes and ketones (Mopper and Stahovec, 1986), or carbon monoxide (Conrad and Seiler, 1980). Significant oxygen consumption can result from these photoprocesses (Laane et al., 1985). However, as indicated by a recent review by Zafiriou (1983), our present understanding of the nature and rates of these photoprocesses is still quite rudimentary.

Photochemical transformations of DOM may have an important impact on

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Short Communication

Determination of nanomolar levels of formate in natural waters based on a luminescence enzymatic assay

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(Received 17th August 1989)

ABSTRACT

A light-producing reaction utilizing three enzymes, bacterial luciferase, formate dehydrogenase and diaphorase, can be used to determine formic acid in natural waters at nanomolar concentrations. The method is rapid and convenient, requiring no preconcentration, desalting or derivatization procedures. Determinations can be done on small sample volumes (25 μl) at room temperature and pH 7. The precision (relative standard deviation) for sea water samples containing 1.0 μM formate was 9.0% ($n = 15$). The reaction is specific for formate with a detection limit of 20 nM (signal-to-noise ratio = 3). Results for applications of the method to sea, estuarine and rain water are given.

Formic acid and other volatile fatty acids play important roles in atmospheric chemistry and biochemical pathways. Formic and acetic acid are formed by photochemical oxidation in the atmosphere [1], and are known to be important substrates for bacteria in oxic and anoxic sediments and pore waters [2,3]. Volatile fatty acids are a potentially important component of dissolved organic matter in sea water. In the last decade, extensive research has been done to characterize dissolved organic matter, which plays an important role in chemical and oceanographic processes.

The determination of trace levels of volatile fatty acids, in particular formic acid, in aqueous samples has proved a difficult task owing to their high solubility and volatility. They are also not easily extracted or detected. Natural waters give rise to further difficulties, especially where the sample matrix is either saline or variable in ionic background and contains many other trace organic compounds. Methods which work for rain in general do not work for other natural waters, especially sea water. With very few exceptions [4,5], levels of formate in sea water, which are expected to be in the μM to nM range, have not been reported as existing methods do not have the required sensitivity. Most methods also involve either complicated, time-consuming sample preparation or have insufficient sensitivity or selectivity. Direct detection in natural water samples by chromatographic techniques is prone to interferences, mainly from inorganic salts, which in turn reduces the detection limit. Commonly used gas chromatographic methods involve concentration, extraction and derivatization steps [6–8]. Ion-exclusion chromatography with conductivity detection suffers from anion interferences which mask the formate peak at the levels expected in sea water [2]. Reversed phase liquid chromatography (RP-LC) is subject to low retention and poor detector response (UV and refractive index detection). Derivatization with chromophores [9–11] can help overcome this, but high blanks still cause major problems. A recent LC method described by Miwa et al. [11] and adapted for sea water samples [12] can detect formic acid and other volatile fatty acids at low micromolar levels, but is

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Determination of Formate in Natural Waters by a Coupled Enzymatic/High-Performance Liquid Chromatographic Technique

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An enzymatic method was developed to quantify formic acid in natural water samples at submicromolar concentrations. The method is based on the oxidation of formate by formate dehydrogenase with corresponding reduction of β-nicotinamide adenine dinucleotide (β-NAD^+) to reduced β-NAD^+ (β-NADH); β-NADH is quantified by reversed-phase high-performance liquid chromatography with fluorometric detection. An important feature of this method is that the enzymatic reaction occurs directly in aqueous media, even seawater, and does not require sample pretreatment other than sample filtration. The reaction proceeds at room temperature at a slightly alkaline pH (7.5–8.5) and is specific for formate with a detection limit of 0.5 μM ($S/N = 4$) for a 200-μL injection. The precision of the method was 4.6% relative standard deviation ($n = 6$) for a 0.6 μM standard addition of formate to Sargasso seawater. Average recoveries of 2 μM additions of formate to seawater, porewater, or rain were 103, 103, and 87%, respectively. Intercalibration with a Dionex ion chromatographic system showed an excellent agreement of 98%. Concentrations of formate present in natural samples ranged from 0.2 to 0.8 μM for Biscayne Bay seawater, 0.4 to 10.0 μM for Miami rain, and 0.9 to 8.4 μM for Biscayne Bay sediment porewater.

There is considerable interest in the role of formic acid and other volatile fatty acids in the early diagenesis of organic matter in lacustrine and marine sediments (*1*, *2*). Formic acid is an important fermentation product or substrate for many aerobic and anaerobic bacteria and for some yeasts (*3*). In the atmosphere, formic acid is an important product in the photochemical oxidation of organic matter (*4*).

Despite its potential importance, formic acid has proven difficult to quantify at submicromolar levels in natural water samples. Formidable analytical difficulties are associated with its detection in highly saline samples (*5*). Ion exclusion, anion exchange, and reversed-phase high-performance liquid chromatography (RP-HPLC) techniques based on the direct detection of formic acid in aqueous samples are prone to interferences (especially from inorganic salts) that ultimately limit the sensitivity of these methods.

A potentially more sensitive and selective approach involves reaction of formic acid with a reagent to form a chromophore or fluorophore, followed by chromatographic analysis. A wide variety of alkylating and silylating reagents have been used for this purpose (*6*). Two serious drawbacks to this approach are that inorganic salts and/or water interfere with the derivatization reaction, and these reactions are generally not specific for formic acid or other carboxylic acids. These techniques are prone to errors from adsorption losses, contamination, and decomposition of the components of interest (*7*). Enzymatic techniques, in contrast, are ideal for the analysis of natural water samples, since they are compatible with aqueous media and involve little or no chemical or physical alterations of the sample (e.g., pH, temperature) that

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