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Hydroxyl Radical Photoproduction in the Sea and Its Potential Impact on Marine Processes

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Photochemical production rates and steady-state concentrations of hydroxyl radicals (·OH) were measured in sunlight-irradiated seawater. Values ranged from 110 nanomolar per hour and 12×10^{-18} molar in coastal surface water to 10 nanomolar per hour and 1.1×10^{-18} molar in open ocean surface water. The wavelengths responsible for this production are in the ultraviolet B region (280 to 320 nanometers) of the solar spectrum. Dissolved organic matter (DOM) appears to be the main source for ·OH over most of the oceans, but in upwelling areas nitrite and nitrate photolysis may also be important. DOM in the deep sea is degraded more readily by ·OH (and its daughter radicals), by a factor of 6 to 15, than is DOM in open-ocean surface water. This finding may in part bear on major discrepancies among current methods for measuring dissolved organic carbon in seawater.

THE HYDROXYL RADICAL (·OH) IS the most reactive, photochemically produced free radical in the environment (*1–3*). It plays a central role in atmospheric chemistry (*4*), but its role in aquatic environments is less clearly understood (*3, 5*). Flash photolysis studies (*2*) demonstrated that ·OH is formed in seawater, and a few model calculations of ·OH production rates and concentrations in surface seawater have been reported (*6, 7*). However, there have been no actual measurements. We evaluated photoproduction of ·OH in seawater by two independent, well-characterized reactions. The first is based on H atom abstraction from an aliphatic alcohol, methanol (CH_3OH), by ·OH. The formation rate of the main stable product, formaldehyde (CH_2O) (*8, 9*), is then measured:

$$\cdot OH + CH_3OH \rightarrow \cdot CH_2OH + H_2O$$

$$\cdot CH_2OH + O_2 \rightarrow CH_2O + HO_2\cdot$$

The other reaction, which is more specific for ·OH, is based on addition of ·OH to the aromatic (Ar) ring of benzoic acid. The formation rates of the addition products *o*-, *m*-, *p*-hydroxybenzoic acids (*1, 8, 10*) are then measured:

$$\cdot OH + H\text{–}Ar \rightarrow \begin{matrix} H \\ \diagdown \\ HO \end{matrix}\!\!>\!Ar \xrightarrow{O_2} HO\text{–}Ar + HO_2\cdot$$

Similar, but less sensitive, techniques have been used to determine ·OH production rates in freshwaters (*6, 7, 10*). However, to our knowledge, these techniques have not been previously applied to seawater.

Details of experimental procedures and controls have been presented elsewhere (*11*). Samples (filtered and unfiltered) were irradiated in quartz flasks with natural sunlight (4 hours, solar noon, cloudless sky, 26°N). Production rates of both CH_2O and hydroxybenzoic acids were measured by liquid chromatography with ultraviolet (UV) detection. Formaldehyde was determined with about a 20-fold greater sensitivity as its 2,4-dinitrophenyl hydrazone (*12*). The reproducibility ($\pm 1\sigma$ SD for repeated measurements on the same sample) of ·OH production rates for coastal water ($n = 10$) was <5% for the CH_3OH probe and about 10% for the benzoic acid probe. Production rates obtained with these two different probe scavengers agreed to within ± 20% (1σ SD) for all seawater and freshwater samples tested (*11*). Because of its much higher sensitivity, we used the CH_3OH probe to measure ·OH production rates in open ocean samples where we anticipated much lower production rates than for the coastal samples. Photoproduction rates for ·OH were converted to steady-state concen-

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trations with an experimentally determined apparent rate constant for the reaction of ·OH with the natural scavengers in seawater. We used standard competition kinetics techniques (*13*) to evaluate this rate constant, as described elsewhere (*11*).

We measured ·OH steady-state concentrations and photoproduction rates in different seawater types to answer the following questions. (i) Are measured ·OH production rates in seawater in agreement with rates previously predicted from models based on known ·OH sources, such as nitrate (NO_3^-), nitrite (NO_2^-), and hydrogen peroxide (H_2O_2) photolysis? (ii) How important is ·OH in the oxidation of dissolved organic matter (DOM) in the sea? (iii) Are ·OH photoproduction rates sufficiently high to potentially affect chemical and biological processes at the sea surface?

Steady-state concentrations and production rates of ·OH in different samples of seawater are given in Table 1. Concentrations of ·OH in surface seawaters are one to two orders of magnitude lower than those reported for organic- and NO_3^--rich freshwaters (*6, 7, 10*). Steady-state concentrations and production rates of ·OH are much higher in upwelling and coastal waters than in open-ocean surface water (*14*). From past studies (*2, 6, 7, 10, 15*), the main sources of ·OH in seawater should be photolysis of NO_3^-, NO_2^-, and H_2O_2, and Fenton-type reactions. We measured the ·OH production rate from NO_3^- photolysis in sunlight to be 3.0×10^{-13} molar s^{-1} per micromolar of NO_3^-, which is in excellent agreement with the results of Zepp *et al.* (*7*) for freshwater. The corresponding ·OH production rates for NO_2^- and H_2O_2 photolysis were 2.3×10^{-11} molar s^{-1} per micromolar of NO_2^- and 4.1×10^{-12} molar s^{-1} per micromolar of H_2O_2, respectively. Photolyses of NO_3^- and NO_2^- should be important ·OH sources only in some upwelling areas, and at times in productive coastal waters (Table 1). Even when NO_3^--rich deep water from the Sargasso Sea was brought up to the surface and irradiated on deck (Fig. 1), only about 15 to 20% of the ·OH production rate was due to NO_3^- and NO_2^- photolyses. Furthermore, from the low steady-state concentrations of H_2O_2 (*16*) and dissolved Fe and Cu (*17*), we calculate that photolysis of H_2O_2 and Fenton-type reactions are insignificant sources of ·OH in seawater. When catalase was added (to destroy H_2O_2) to coastal and open ocean seawater, no significant change in ·OH photoproduction rates was observed. However, in Fe-rich freshwaters, the Fenton reaction may be a significant ·OH source (*18*).

Table 1. Measured and estimated ·OH steady-state concentrations and production rates in sunlight-irradiated seawater and freshwater; *n*, number of samples used for the experiment; UD, undetectable; N.D., not determined.

Sample	$[\cdot OH]_{ss}$* $\times 10^{-18}$ M	·OH production rate* $\times 10^{-12}$ M/s (nM/hour)	·OH production from different sources (%)			
			NO_3^-	NO_2^-	H_2O_2	Other (DOM)
			(concentration of sources, μM)†			
Open-ocean surface water (Sargasso Sea, *n* = 6)	1.1 ± 0.1	2.8 ± 0.2 (10.1)	<1 (<0.05)	UD	<4 (<0.05)	>95 (200)
Gulf Stream surface water (*n* = 1)	1.2	3.1 (11.2)		N.D.		
Deep-ocean water (Sargasso Sea, >700 m, *n* = 7)	6.3 ± 0.3	15.9 ± 0.7 (57.2)	19 (10)	1 (0.01)	3 (0.1)	77 (70)
Deep Gulf Stream water (700 m, *n* = 1)	5.8	14.7 (52.9)		N.D.		
Subtropical coastal water (Biscayne Bay, FL, high tide, *n* = 4)	9.7 ± 1.2	24.4 ± 3.0 (87.8)	2 (2.0)	UD	2 (0.2)	96 (300)
Subtropical coastal water (Biscayne Bay, FL, low tide, *n* = 5)	13.7 ± 1.7	34.5 ± 4.3 (124.2)		N.D.		
Temperate coastal water (Vineyard Sound, MA, *n* = 1)	10.6	26.5 (95.4)		N.D.		
Equatorial upwelled water (estimated)	7.4	18.6 (67.0)	3 (5)	25 (0.2)	3 (0.1)	65 (200)
Coastal upwelled water (estimated)	26.3	66.1 (238)	7 (15)	35 (1)	6 (0.1)	52 (300)
10% Everglades water in Biscayne Bay water (*n* = 1)	30.1	68.9 (248)		N.D.		
DOM-rich freshwater (Everglades, *n* = 2)	840‡	420 ± 58 (1.5×10^3)		N.D.		

*± 1σ SD. †Concentrations of H_2O_2 and DOM (mole carbon basis) were estimated from published values (*16, 26*). ‡This steady-state concentration was calculated with a measured scavenging coefficient of 5×10^5 s^{-1} (*15*).

From the preceding results, it is apparent that there is a major, unknown source or sources of ·OH in seawater. DOM has been shown to be a photochemical source for ·OH in some freshwaters (*1, 6, 7, 10, 19*). We believe that DOM is also a major source for photochemically produced ·OH in seawater. We found linear relations between DOM absorbance (at 300 nm) or fluorescence [360 nm, excitation; 460 nm, emission (*3*)] with ·OH photoproduction in a large number of seawater samples in Fig. 1. All slopes were significant at $P < 0.05$. Also, addition of humic-rich freshwater to humic-poor seawater significantly enhanced ·OH photoproduction rates (Table 1). The enhancement was in direct proportion to the DOM absorbance and to the fraction of humic-rich water present. Furthermore, the solar-normalized action spectra for photoproduction of ·OH in coastal seawater (Fig. 2) and for seawater containing purified humic substances (*20*) showed that the photoactive wavelengths in the solar spectrum responsible for this production are in the UV-B region of 280 to 320 nm (*21*), which corresponds to a 1/*e* light penetration depth of about 7 m in the open ocean (*22*). Nearly identical wavelengths were obtained for photobleaching (loss of DOM absorbance) in seawater (*23*).

These results suggest that the relation between the absorbance of light by DOM and the photoproduction of ·OH from DOM is not simply incidental. Photolysis of light-absorbing sites, in particular hydroquinolic and phenolic moieties, within humic substances appears to be responsible in part for ·OH photoproduction and absorbance photobleaching in natural waters (*19, 24*).

Many sinks exist for ·OH in seawater (*2, 3, 6, 7, 25*). On the basis of competition kinetics experiments (*11*), we estimate that scavenging by Br^- will consume about 93% of the ·OH production, which is in agreement with past estimates of 89 to 98% (*2, 25*). Thus, approximately 7% of ·OH produced reacts directly with other components of seawater, including DOM (*25*). Furthermore, reactive daughter products of ·OH, such as bromine-containing radicals, also attack DOM (*2*).

Thus, in seawater DOM plays a dual role as both source and sink for ·OH. The extent of attack on DOM depends on the pseudo–first-order rate constant, k'_{DOM}, for the reaction of ·OH with DOM. This constant is expected to vary with DOM source and the history of the water. Therefore, experiments were performed to measure k'_{DOM} for different seawaters. In these experiments, an ·OH source, H_2O_2, was added at various concentrations (0 to 150 μM) to coastal and

open ocean samples and photoproduction of identifiable low molecular weight (LMW) products, CH_2O, acetaldehyde, glyoxal, and keto acids, was monitored:

$$\text{DOM} + \cdot\text{OH} \rightarrow \text{LMW products} + \text{other products}$$

We estimated k'_{DOM} from the initial increase in photoproduction rate of products (ΔPRP) with increase in $[\cdot OH]_{ss}$:

$$d[\text{DOM}]/dt = -k'_{DOM}[\text{DOM}][\cdot OH]_{ss} = -k'_{DOM}[\cdot OH]_{ss} = \text{production rate of products}$$

$$k'_{DOM} = \Delta\text{PRP}/\Delta[\cdot OH]_{ss}$$

The ranges of k'_{DOM} obtained were (in units of 10^4 s^{-1}) 1 to 2 for surface oceanic water, 4 to 5 for deep oceanic water, and 5 to 8 for coastal surface water. Because the concentration of DOM (on a mole carbon basis) of open ocean surface water is about three times that of open ocean deep water (*26*), our k' results indicate that surface DOM is about 1/6 to 1/15 as reactive toward ·OH attack as deep-sea DOM. This finding may explain large discrepancies, especially for surface waters, in different methods currently being used for measuring the concentration of oceanic dissolved organic carbon (DOC) (*26*), since several of these methods rely on oxidation by ·OH and other reactive free radicals. The reasons for the relatively low reactivity of DOM in surface seawater toward ·OH are not known but may include extensive photobleaching in the photic zone (*27*) and differences in molecular size distributions (*26*).

Reaction of ·OH with DOM should speed up the degradation of biologically refractory organic matter at the sea surface, since LMW products from this reaction can be readily taken up and oxidized by organisms (*28*). This process could affect the geochemical cycling of organic carbon in the sea (*23, 27*). We have used our estimated ranges of k'_{DOM}; published spatial distributions of open ocean, coastal, and upwelling regimes (*29*); and typical DOM concentrations (on a mole carbon basis) in these different regimes (*26*) to calculate that the residence time for oceanic DOM is roughly 4×10^4 (± 30%) years. Although this value is about six to seven times the measured ^{14}C age of deep-sea DOM (*30*), it is still significant because our k'_{DOM} values are conservative, as only a few LMW products were measured. In addition, our estimate is based on attack of DOM by only ·OH and its daughter products; other DOM degradation pathways, such as direct photolysis, which produce LMW "fragments" at even higher rates (*12, 23, 28*), were not considered.

Photoproduction of ·OH and its reactive daughter products at the sea surface may also impact upon biota residing there. Biologically utilizable carbon produced from direct or indirect attack of DOM by ·OH may enhance secondary productivity (bacterial growth), especially in carbon-limited oligotrophic waters (*31*), in upwelling waters, and in regions with high or increasing UV-B light penetration. However, high production rates of reactive free radicals can also destroy key biomolecules in organisms (*32, 33*), thereby retarding growth and enhancing mutation (*34*). On the basis of ·OH production rates up to several hundred nanomolar per hour (Table 1), it can be argued that photoinhibition at the sea surface, especially in productive coastal and upwelling waters (*35*), is in part due to ·OH and its reactive daughter products (*33*).

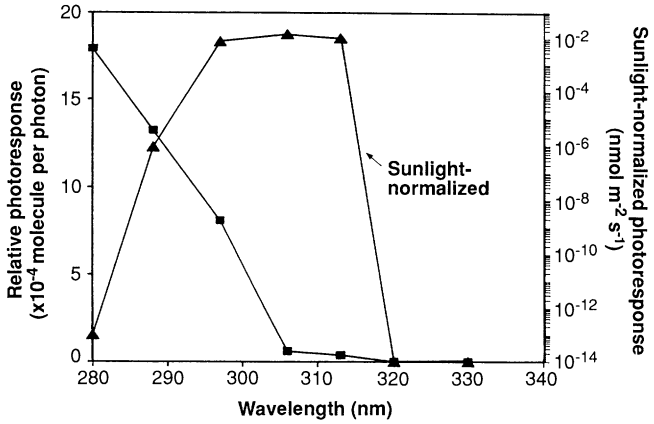

Fig. 2. (■) Action spectrum for ·OH production in coastal seawater (Biscayne Bay, Florida). (▲) Action spectrum normalized to downward solar irradiance incident at the sea surface. A logarithmic scale is used because of the large variation in solar irradiance between 280 to 320 nm (*22*). Details of the actinometry are given elsewhere (*21, 23*).

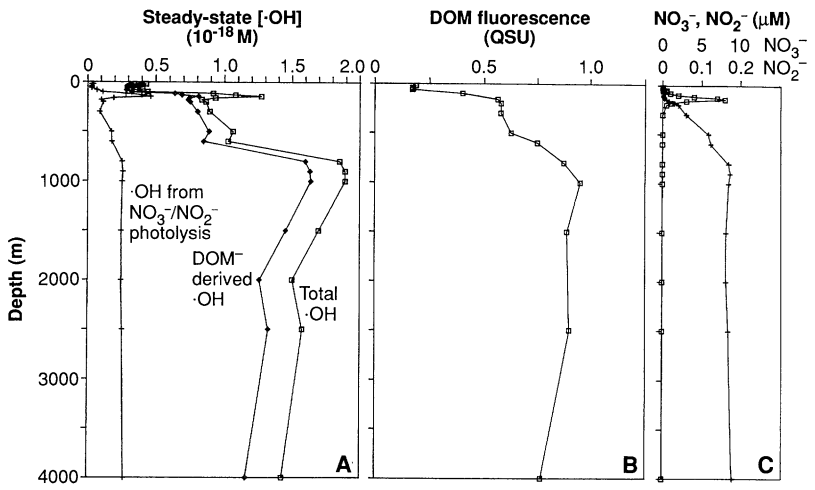

Fig. 1. (**A**) Steady-state ·OH concentrations in sunlight-irradiated Sargasso Sea water plotted against sampling depth. Samples were brought to the surface and irradiated on deck in quartz flasks with natural sunlight. At this station (26°00′N, 76°00′W), the mixed layer extends down to about 80 m, the chlorophyll and NO_2^- maxima occur at 100 to 140 m, and the oxygen minimum occurs at about 800 to 900 m. The spike in the total ·OH production curve at 100 to 140 m appears to be due entirely to NO_2^- photolysis. (**B**) Profile of DOM fluorescence (360 nm excitation, 460 nm emission), at the same station. The fluorescence was normalized to a quinine sulfate standard (1 QSU = 1 ppb in 0.05 M H_2SO_4). (**C**) Profiles of NO_3^- (+) and NO_2^- (□) at the same station.

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Intercalation of Sea Urchin Proteins in Calcite: Study of a Crystalline Composite Material

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Sea urchin skeletal elements are composed of single crystals of calcite. Unlike their synthetic counterparts, these crystals do not have well-developed cleavage and are consequently much more resistant to fracture. This phenomenon is due in part to the presence of acidic glycoproteins occluded within the crystals. By means of x-ray diffraction with synchrotron radiation, it is shown that the presence of the protein in synthetic calcite only slightly decreases the coherence length but significantly increases the angular spread of perfect domains of the crystals. In biogenic calcite, the coherence length is 1/3 to 1/4 as much as that in synthetic calcite and the angular spread is 20 to 50 times as wide. It is proposed that the presence of macromolecules concentrated at mosaic boundaries that are oblique to cleavage planes is responsible for the change in fracture properties. These results may be important in the material sciences, because of the unusual nature of this material, namely, a composite based on the controlled intercalation of macromolecules inside single-crystal lattices.

THE MINERAL PHASE OF SEA URCHIN spines and tests is composed of fenestrated Mg-bearing calcite. It is a unique material, as whole body plates and spines up to several centimeters in length diffract x-rays as single crystals. These skeletal elements are not, however, as fragile as single calcite crystals but appear to be made of a relatively strong material (*1*). Pure calcite cleaves easily along the well-developed {104} crystal planes. Sea urchin skeletal elements, however, break with conchoidal (glassy) fracture, typical of amorphous materials. The protein content of these skeletal elements is of the order of 0.5 mg per gram of calcite (*2, 3*). The possibility that organic macromolecules located within the crystals are responsible for the unusual fracture properties was first proposed by Merker (*4*). Direct experimental evidence supporting this proposal was obtained when calcite crystals were grown in the presence of acidic glycoproteins extracted from sea urchin skeletons (*5*). The macromolecules intercalate into single synthetic crystals, and, as a result, their fracture properties change. These protein-crystal composites break with conchoidal fractures similar to those of fractured sea urchin skeletal elements (*5*). The molecular structure of echinoderm skeletal elements may therefore be of interest to material scientists, as these materials are composed of large single crystals reinforced by proteins located within their lattice structure.

The aim of this study was to gain insight into this unique protein-crystal composite by determining how bulky proteins can be incorporated into single crystals. As calcite crystals are extremely well ordered, it was necessary to examine their x-ray diffraction profiles with the use of highly collimated synchrotron radiation. The study focuses on the internal texture of biogenic and synthetic calcite crystals, in terms of the mosaic spread and the domain size of the crystals. The following crystals were analyzed: two pure calcite crystals; three calcite crystals containing occluded protein ranging in concentration from approximately 250 to 750 ppm (w/w) (*6*); two small sea urchin spines and one sea urchin tooth element (*7*). The diffraction data were collected at the National Synchrotron Light Source (Brookhaven

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