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Photodegradation of strong copper-complexing ligands in organic-rich estuarine waters

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Abstract

Exposure to solar radiation substantially decreased the strong copper-complexing capacity of samples collected from the organic-rich Cape Fear estuary, North Carolina. Samples exposed to natural sunlight for 1–2 d experienced a loss of strong Cu ligand (modeled at fixed $K'_{\mathrm{CuL}} = 10^{13.5}$) ranging from 15–33%, and >90% using long-term exposures under controlled conditions with a Xe arc solar simulator light (14 d summer sunlight). Pseudo first-order rate constants of strong Cu ligand photodegradation averaged 0.28 d^{-1} for two separate Cape Fear samples exposed to simulated solar radiation, much higher than corresponding dissolved organic carbon photooxidation rate constants ($<0.01\ \mathrm{d}^{-1}$). Degradation rates measured in solar simulator experiments predicted ligand concentrations in separate samples exposed to natural sunlight within 30% after rates were normalized to the ultraviolet (UV) light absorption coefficient at 300 nm. UV light is not solely responsible for photodegradation because exposure to photosynthetically active radiation also decreased strong Cu ligand levels, but with a smaller rate constant (0.06 d^{-1}). Although photodegradation appears to be a relatively minor sink within the Cape Fear estuary because of a shallow light penetration depth (<2% of water column) and short residence times (<1 week), strong Cu ligand levels in optically clearer South Atlantic Bight (SAB) surface waters are likely to be substantially reduced by photodegradation. Ligand degradation may be especially important to Cu-sensitive ecosystems where large increases in bioavailable and potentially toxic levels of free Cu^{2+} ions are possible.

Strong complexing ligands control the bioavailability and potential toxicity of free Cu^{2+} ions in most estuarine and marine waters (Croot 2003). These ligands typically exist in excess of dissolved Cu concentrations in coastal waters (Shank et al. 2004*c*) and near equal levels in the open ocean (Coale and Bruland 1990; Moffett 1995). Any process with the potential to alter or destroy the binding capacity of these organic complexants has the potential to substantially influence Cu speciation and diminish the free Cu^{2+} buffering capacity. Distributions of Cu^{2+} ions can significantly affect the ecology of Cu-sensitive cyanobacteria populations (Mann et al. 2002). If organic ligands are photoreactive, absorption of solar radiation could be a primary pathway by which their Cu^{2+} buffering capacities are degraded. Croot et al. (1999) showed that strong Cu ligands produced by the marine cyanobacteria *Synechoccocus* degraded in natural sunlight, causing weaker ligands to dominate Cu speciation. Additionally, photodegradation of strong Cu ligands was deemed responsible for increases in free Cu^{2+} : total Cu ratios observed in surface waters of the Sargasso Sea (Moffett et al. 1990). The photoreactive nature of organic iron complexing ligands in marine waters has also been demonstrated (Barbeau et al. 2001, 2003; Powell and Wilson-Finelli 2003).

Solar radiation influences the geochemical cycling of many chemical constituents in marine systems, including organic moieties and trace metals. Organic compounds bound to redox active trace metals such as Cu can be especially susceptible to photodegradative processes (Moffett and Zika 1987; Waite 1988). Cu(II) bound to photoreactive organic matter may be reduced to Cu(I) in a ligand to metal charge transfer reaction (LMCT). The ability of Cu to cycle between its +2 and +1 oxidation states allows it to participate in LMCT reactions during which the Cu(II)-bound ligand is oxidized and Cu(I) is produced (Waite 1988; Voelker et al. 2000). Photochemically induced Cu(I) production has been observed in Cape Fear (North Carolina coast) estuarine waters (M. L. Smith unpubl. data) and offshore Atlantic Ocean waters (Moffett and Zika 1988). However, because strong Cu complexing ligands typically exist in large excess of total dissolved Cu (TDCu) levels in estuarine waters, additional photodegradation pathways might be necessary to degrade significant quantities of ligands and alter Cu speciation provided the excess ligands are not bound to other redox active metals such as Fe, which also participate in LMCT reactions.

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Ultraviolet radiation (300–400 nm wavelengths) is the primary energy source driving photochemical reactions in marine waters. The primary sink for ultraviolet (UV) light in natural waters is absorption by chromophoric dissolved organic matter (CDOM; Zepp 2003). CDOM absorbs photons of UV light energy via numerous photoreactive functionalities, including phenolic and carboxylic groups. Upon absorption of UV photons, CDOM experiences numerous chemical reactions, including mineralization to CO_2 (Gao and Zepp 1998; Johannessen and Miller 2001) and loss of aromaticity (Vähätalo et al. 1999). In most estuaries along the southeast U.S. coast, the CDOM pool is dominated by terrestrially derived humic substances (Gao and Zepp 1998; Moran et al. 2000). Humic substances also serve as the primary strong Cu complexants in these organic-rich estuaries (Shank et al. 2004*c*), whereas river discharge provides large quantities of strong Cu binding agents to South Atlantic Bight (SAB) coastal ocean waters (Shank et al. 2004*a*). Thus, if the strong Cu complexation capacities of estuarine and marine waters within the SAB region reside within the CDOM pool as we hypothesize, they could be vulnerable to photodegradation processes.

Despite the potential importance of photochemistry to the speciation of Cu, the effect of irradiation on the strong Cu complexation capacity of estuarine and coastal waters has received little attention. We present photodegradation rates of strong Cu complexing ligands in organic-rich Cape Fear estuarine waters and discuss the potential importance for photochemical degradation of strong Cu ligands within SAB shelf waters. In addition to explaining the geochemical cycling of strong Cu binding ligands, determining the photochemical reactivity of these moieties may also help predict their chemical structures.

Methods

Field sampling—Water samples were collected during the summer months of 2000, 2001, and 2003, within the typically mesohaline region of the Cape Fear estuary, located just downstream of Wilmington, North Carolina (Fig. 1). Sample locations were chosen to coincide with simultaneously conducted Cu speciation studies in this area of the Cape Fear estuary (Shank et al. 2004*b,c*), as well as to ensure nearly equivalent salinities for each experiment. Due to high fluctuations in river flow, environmental conditions including physical and chemical parameters can vary widely in this estuary (Avery et al. 2003). However, for this study sampling was conducted only during summer months when flow conditions and environmental parameters were similar. Previous research in this estuary has shown that DOC and Cu speciation data are very consistent in the Cape Fear estuary under comparable flow conditions, although TDCu variability is less predictable (Avery et al. 2003; Shank et al. 2004*c*).

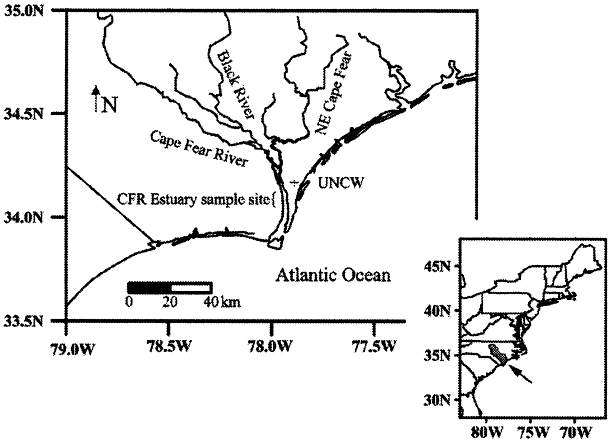

Fig. 1. Map of Cape Fear estuary in southeastern North Carolina. Shaded area on U.S. East Coast inset map shows the CFR drainage basin. All samples were collected within noted sampling area.

Copper speciation (including TDCu) and DOC samples were collected at a depth of 1.0–1.5 m using trace metal clean techniques. Field sampling was performed using a peristaltic pump connected to Teflon-lined Tygon tubing extended approximately 3 m away and upstream from the boat with a PVC pole. Sample water was filtered upon collection using trace metal clean in-line cartridge filters (Meissner), beginning with a 1.2 μm polypropylene filter followed by a 0.2 μm polyethersulfone filter (nominal ‘‘dissolved″ filter sizes). Speciation bottles (fluorinated high-density polyethylene) were placed within two separate clean plastic bags to prevent contamination and stored frozen at -20°C. Duplicate analyses on frozen and nonfrozen samples revealed no significant storage artifacts. Filtered DOC samples were stored in muffled amber glass vials in the dark at 2–4°C. Salinity and water temperature data were collected in situ using a YSI 85 temperature/conductivity probe. Sampling bottles, tubing, filters, and other implements were washed extensively with acid to prevent contamination.

Photodegradation experiments: natural sunlight—Three 0.2 μm filtered samples were collected on separate occasions (July 2000, June 2001, July 2001) from the same location in the Cape Fear estuary and exposed to natural sunlight. Two experiments consisted of a single day exposure (July 2000 and June 2001, 6 h of summer sun), and one experiment used a 2-d exposure (July 2001, 12 h of summer sun). Duplicate filtered samples were placed outdoors in a shallow pool during peak daylight hours in 250 mL trace metal clean quartz tubes (3 cm diameter) fitted with Teflon stoppers. Duplicate dark controls in 130-mL quartz tubes were also placed outside in the shallow pool but were covered with aluminum foil to prevent exposure to natural light. Because dark control tubes contained only 130 mL, these samples were combined prior to the various analyses. Fresh water from a hose flowed gently over the top of the tubes to maintain ambient temperatures. Integrated light exposure was measured using an International Light model IL1700 radiometer equipped with a broadband SUD033 UVA + UVB photodetector. Light absorption coefficients of filtered samples were determined using a Varian Cary 1E scanning spectrophotometer.

Table 1. Results of photodegradation experiments for 0.2 μm filtered Cape Fear estuarine samples exposed to natural sunlight. Samples were collected on three separate occasions shortly before exposure. Initial and dark control ΣL_i values represent average ± range for duplicate CLE-CSV analyses of a single sample. Post-exposure ΣL_i values are reported as average ± range for individual CLE-CSV analyses of duplicate exposed samples.

Sample	UVA+UVB exposure (J cm^{-2} × 10^2)	Salinity	pH	TDCu (nmol L^{-1})	Initial ΣL_i (nmol L^{-1})	Dark control ΣL_i (nmol L^{-1})	Postexposure ΣL_i (nmol L^{-1})
Jul 2000	1.4 (1 d)	23	7.9	6.4	128±9*	128±9	85±11 (33% loss)
Jun 2001	1.8 (1 d)	23	7.9	6.4	132±7	122±7	94±9 (23% loss)
Jul 2001	3.5 (2 d)	24	7.9	5.6	116±6	111±7	94±4 (15% loss)

* Assumed equal to dark control ΣL_i because of analytical error when measuring initial ΣL_i.

Photodegradation experiments: solar simulator—Two photodegradation experiments were conducted using a Spectral Energy solar simulator (1 kW Xe arc light source) equipped with an AM1 filter to remove wavelengths not found in the solar spectrum. Filtered samples used in these experiments were collected during a single field excursion in June 2003 at locations approximately 8 km apart in the Cape Fear estuary (salinities of 22 and 25). Both of these sites were within 5 km of the sampling location utilized for the natural sunlight exposures. Following the full-spectrum solar simulator exposure of the lower salinity sample, an additional aliquot of this same sample was exposed under the simulator with longpass cutoff filters in place to eliminate UV wavelengths to isolate the effect of photosynthetically active radiation (PAR) on Cu ligand degradation. The light dose received during a 6.5 h full-spectrum exposure in the solar simulator (total UV-visible irradiance 53 mW cm^{-2}) was approximately equal to that of 1 d of natural summer 34°N latitude sunlight (total solar noon UV-visible irradiance 66 mW cm^{-2}). Samples were placed under the solar simulator in 1 L quartz round bottom flasks. The flasks were placed in a temperature-controlled water bath to maintain ambient temperature (20°C). Dark controls were placed under a black box near the solar simulator. All samples were stirred continuously with Teflon-coated magnetic stir bars. Total exposure time was 94 h for all solar simulator experiments, equivalent to approximately 14 d of summer sunlight at 34°N latitude. Four sets of subsamples were collected at shorter intervals during each experiment. Light intensity measurements and light absorption coefficients were measured as described above.

Cu speciation and TDCu analyses—Copper speciation analyses were performed using competitive ligand equilibration–cathodic stripping voltametry (CLE-CSV) at a hanging mercury drop electrode (HMDE) with 8-hydroxyquinoline (8-HQ) as the competing ligand (Donat et al. 1994). The detection window chosen ($\alpha_{\mathrm{Cu8HQ}} = 10^5$) was equivalent to that used in a study of Cu speciation in Cape Fear estuarine waters conducted simultaneously (Shank et al. 2004*c*). Details of the CLE-CSV technique employed in this study are reported in Shank et al. (2004*c*), as are TDCu and DOC analyses. Ligand concentrations (ΣL_i) were calculated with SigmaPlot using a nonlinear fit to a one-ligand model with the following equation and a fixed conditional stability constant (K'_{CuL}) of $10^{13.5}$:

$$[\mathrm{Cu}^{2+}][\mathrm{CuL}]^{-1} = [\mathrm{Cu}^{2+}][\Sigma L_i]^{-1} + (K'_{\mathrm{CuL}}[\Sigma L_i])^{-1} \quad (1)$$

By fitting the titration data to a one-ligand model, the model becomes a representation of all ligands within the detection window, requiring the term ΣL_i to be used. Ligand concentrations (ΣL_i) were calculated in this manner for consistency with the coincident Cape Fear estuarine study (Shank et al. 2004*c*). Caveats associated with fitting speciation data to one-ligand models as well as potential sources of calibration errors when examining titration data have been discussed previously (Shank et al. 2004*c*).

Results

Natural sunlight exposures—Substantial degradation of strong Cu binding ligands (calculated at fixed $K'_{\mathrm{CuL}} = 10^{13.5}$) occurred in all three samples exposed to natural sunlight (Table 1). An initial concentration of strong binding ligand (initial ΣL_i) in the July 2000 experiment was unavailable because of a CSV analytical error. Assuming the initial ΣL_i was equivalent to the dark control ΣL_i for July 2000 (initial and dark controls were statistically equivalent in both 2001 experiments), this 1-d exposure resulted in a 33% loss of the strong Cu complexation capacity (128 → 85 nmol L^{-1}). Approximately 23% of the strong Cu ligand concentration was photodegraded during the 1-d June 2001 exposure (132 → 94 nmol L^{-1}). Results from a 2-d exposure in July 2001 revealed only a 15% reduction (116 → 94 nmol L^{-1}) in the strong ligand concentration. All experiments were conducted under ‘‘sunny’’ conditions, and daily irradiance levels were similar (Table 1). Potential variations due to the light absorption characteristics of individual samples will be discussed later.

Solar simulator exposures—Simulated full-spectrum solar radiation degraded >90% of initial strong Cu ligand concentrations in separate 94-h exposures of two Cape Fear estuarine samples using the solar simulator (Table 2, experiments 1 and 2). A simulated exposure of 94 h is equivalent to slightly more than 2 weeks of natural sunlight during the summer months at 34°N. During solar simulator experiment 1, the sample ($S = 22$) displayed a ligand reduction from 106 to 6 nmol L^{-1}. The concentration of strong ligand in the higher salinity sample ($S = 25$) diminished from 60 to 5 nmol L^{-1} during the second full-spectrum solar simulator experiment. During this second experiment, the strong Cu

Table 2. Results of August 2003 photodegradation experiments for 0.2 μm filtered Cape Fear estuarine samples exposed to Xe arc lamp simulated solar radiation. Experiment 3 was conducted with a separate aliquot of the same Cape Fear sample used in experiment 2 but with UV cutoff filters to eliminate <400 nm wavelengths. ΣL_i values represent average ± range for duplicate CLE-CSV analyses of a single sample. Dark controls are not listed because there were no changes from initial ΣL_i values.

Solar simulator experiment	Sample parameters	Exposure type	Exposure time (h)	Equivalent days of summer sun	ΣL_i (nmol L^{-1})
1	Salinity=22	Full spectrum	0	0	106±1
	TDCu=4 nmol L^{-1}		5	0.77	74±5
	pH=7.8		10	1.5	55±12
			28	4.3	45±6
			94	14.5	6±1
2	Salinity=25	Full spectrum	0	0	60±1
	TDCu=7 nmol L^{-1}		5	0.77	50±5
	pH=7.8		10	1.5	42±8
			28	4.3	16±1
			94	14.5	5±1
3	Salinity=25	PAR only	0	0	61±1
	TDCu=7 nmol L^{-1}		5	0.77	53±3
	pH=7.8		10	1.5	52±5
			28	4.3	47±2
			94	14.5	27±5

ligand concentration was degraded below the ambient TDCu level. Under this scenario, the concentration of free Cu^{2+} could increase substantially in the water column or weaker ligands may begin to dominate Cu speciation. Strong ligand concentrations did not significantly change from initial values for samples kept in the dark over the duration of the experiment.

A third simulated solar radiation exposure was conducted using a separate aliquot of the sample used in experiment 2 ($S = 25$), but with cutoff light filters in place to eliminate all UV wavelengths (<400 nm) reaching the sample. Results from this experiment (Table 2, experiment 3) show a ligand decrease from 61 to 27 nmol L^{-1}, a loss of 56% of the initial concentration, indicating that Cu ligand degradation is not solely driven by the absorption of UV light.

Discussion

Photodegradation rates of strong Cu ligands—Results from the full-spectrum solar simulator experiments (Table 2, experiments 1 and 2) were used to calculate (SigmaPlot) pseudo first-order photodegradation rate constants (k) of strong Cu binding ligands in Cape Fear estuarine waters using the following equation:

$$\Sigma L_i = \Sigma L_{io} e^{-kt} \tag{2}$$

where ΣL_{io} represents the initial ligand concentration and t is measured in equivalent days of light exposure. Calculated degradation rate constants (Fig. 2A,B, full-spectrum data) were quite similar in these two experiments, measuring 0.29 ± 0.09 d^{-1} in experiment 1 and 0.27 ± 0.03 d^{-1} in experiment 2, although initial ligand concentrations differed by almost a factor of 2 (106 vs. 60 nmol L^{-1}). The reported uncertainties represent the calculated (SigmaPlot) standard error of the mean k for the exponential fit to Eq. 2 (standard error = SD[$n^{-0.5}$]). Ligand photodegradation rate constants were approximately 3 times those of the photodegradation rate constants for absorbance loss in the samples (0.098 ± 0.001 and 0.119 ±0.001 d^{-1} for a_{300} and a_{350}, respectively). However, the ligand degradation values were approximately 20–35 times greater than the corresponding DOC photooxidation rate constants (0.015 and 0.008 d^{-1}) as DOC concentrations decreased only 11% (Fig. 2A) and 18% (Fig. 2B) during the 94 h full-spectrum solar simulator exposures. These results indicate Cu binding is intimately associated with photoreactive functional groups and that photochemical processes could play a pivotal role in the cycling of strong Cu binding ligands in coastal waters. Also modeled in Fig. 2B is the photodegradation of strong Cu ligands resulting from exposure to PAR. The decay rate of strong Cu binding due to PAR exposure measured 0.06 ± 0.02 d^{-1} (average ± range as described for full-spectrum k calculation), or 20% of the full-spectrum decay rate. Consistent with strong Cu ligands, DOC loss rates in the PAR exposure measured approximately 20% of the full-spectrum DOC photooxidation rate (Fig. 2B), although photomineralization of DOC is likely a distinctly different chemical process than is transformation of strong Cu binding sites.

Photodegradation rate constants calculated for the solar simulator exposures are specific to the estuarine samples analyzed. To determine whether these rate constants could predict the photodegradation of strong Cu binding ligands exposed to natural sunlight, a normalized constant accounting for the light absorption capacity of each particular sample was calculated. Since UV light is the primary energy source driving photochemical processes in estuarine waters (Zepp 2003) and our results indicate that full-spectrum decay rates are 4–5 times larger than those corresponding to PAR, we used the light absorption coefficient of each sample at $\lambda = 300$ nm (a_{300} in units m^{-1}) to account for variations in light absorption capacity. Values of a_{300} were calculated from spectrophotometric absorbance (A) measurements ($a_{300} = 2.303 A_{300} L^{-1}$, where L represents light pathlength). Normalized photodegradation rate constants (k_{norm}) for each sample

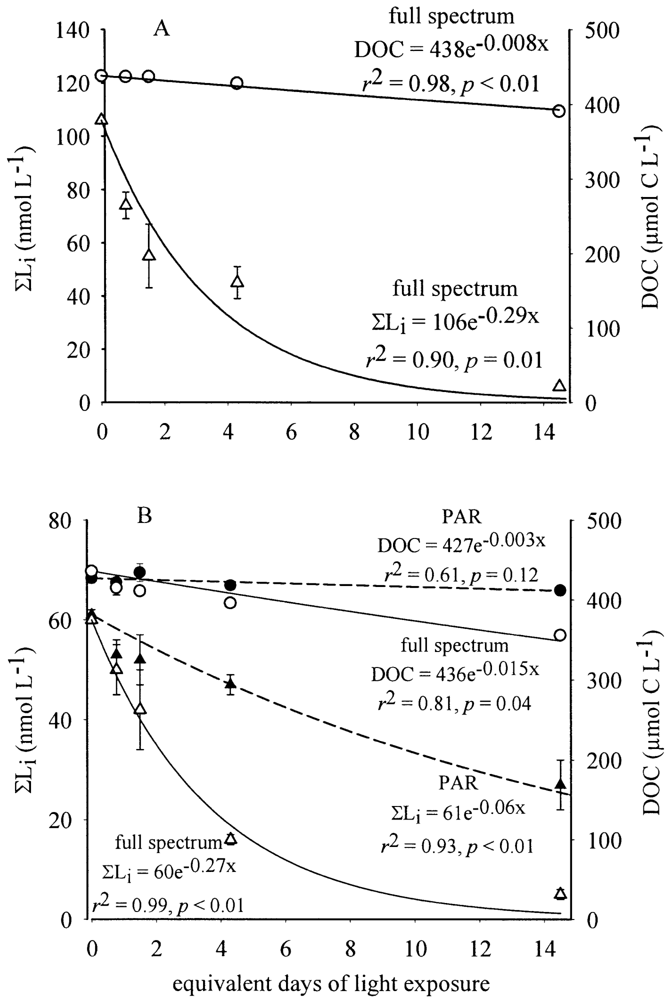

Fig. 2. (A) Photodegradation of strong Cu ligand (ΣL_i) and DOC concentrations resulting from exposure to full-spectrum simulated solar radiation using Xe arc lamp (sample parameters, $S = 22$, pH = 7.8, TDCu = 4 nmol L^{-1}). (B) Photodegradation of strong Cu ligand and DOC concentrations resulting from exposure to simulated solar radiation using Xe arc lamp with and without UV cutoff filters (sample parameters, $S = 25$, pH = 7.8, TDCu = 7 nmol L^{-1}). Results from dark controls omitted since ΣL_i and DOC concentrations did not change significantly over the course of the experiments. ΣL_i data points represent average ± range for two CLE-CSV analyses of a single sample.

were then calculated using the average ligand degradation rate constant (k_{ss}) of 0.28 d^{-1} determined from the two full-spectrum solar simulator exposures in combination with the following equation:

$$k_{\mathrm{norm}} = (k_{ss})(a_{300}\ \text{sample}) \times (\text{mean}\ a_{300}\ \text{solar simulator samples})^{-1} \quad (3)$$

where the mean a_{300} of the solar simulator samples was 22 m^{-1}. The following equation was then applied to predict the decay of strong Cu ligands accounting for irradiance dose and light absorption capacity:

$$\sum L_i = \sum L_{io}\, e^{-k_{\mathrm{norm}}(J/180)} \quad (4)$$

where J represents UVA + UVB exposure dose and 180 joules cm^{-2} (equal to June 2001 daily irradiance) was assumed to be the daily dose of summer UV light at our latitude (Table 3). Using this model, predicted photooxidation of strong Cu ligands (Table 3) for the June 2001 sample (90 ± 5 nmol L^{-1}) was remarkably similar to the measured postexposure value (94 ± 9 nmol L^{-1}). For the July 2000 sample, our model predicted a final ligand concentration that was 24% greater than the measured value (105 ± 7 vs. 85 ± 11 nmol L^{-1}), although given the range of measured and predicted ΣL_i concentrations the model results appear reasonable. Less promising results were obtained applying the model for the July 2001 sample as the predicted ΣL_i concentration was 30% lower than the measured postexposure ΣL_i concentration (66 ± 3 vs. 94 ± 4 nmol L^{-1}).

Photodegradation rates (k) measured in the full-spectrum solar simulator exposures and used in the model predictions above exhibited standard error ranges of 0.9 d^{-1} ($k = 0.29$ d^{-1}) for experiment 1 and 0.2 d^{-1} ($k = 0.27$ d^{-1}) for experiment 2. Some of the uncertainty in calculating k values results from analytical artifacts related to the Cu speciation procedure where it is not uncommon for duplicate analyses of a single sample to exhibit differences of 10–20%, especially for organic-rich estuarine samples (Shank et al. 2004*c*). Examining the data in Table 2, it is clear that duplicate analyses of individual samples exposed to simulated full-spectrum sunlight exhibited relatively large variability in a few instances in this study (e.g., experiment 1, 10 h exposure). Assuming the mean k_{ss} value used to calculate k_{norm} (Eq. 3) had an error as large as 0.9 d^{-1}, and adjusting the k_{norm} values listed in Table 3 by ±0.9 d^{-1} for use in Eq. 4, predicted ligand concentrations could be altered by a maximum of ±7% for the July 2000 1-d exposure and ±11%

Table 3. Predicted photodegradation of strong Cu ligands for 0.2 μm filtered Cape Fear estuarine samples exposed to natural sunlight based on average rate constant of samples exposed to full-spectrum simulated solar radiation ($k_{ss} = 0.28$ d^{-1}) and adjusted for UV light absorption capacity (a_{300}) of the individual sample. ΣL_{io} values represent average ± range for duplicate CLE-CSV analyses of a single sample (initial ΣL_i data from Table 1). Postexposure ΣL_i values are reported as average ± range for individual CLE-CSV analyses of duplicate exposed samples (postexposure ΣL_i data from Table 1). Predicted ΣL_i values (italic) were calculated with Eq. 4 using k_{norm} and range of listed ΣL_{io} values and are reported as average ± expected range.

Exposure date	UVA+UVB exposure (J cm^{-2} × 10^2)	a_{300} (m^{-1})	k_{norm} (d^{-1})	ΣL_{io} (nmol L^{-1})	Postexposure ΣL_i (nmol L^{-1})	Predicted ΣL_i (nmol L^{-1})
Jul 2000	1.4 (1 d)	20	0.25	128±9	85±11	*105±7*
Jun 2001	1.8 (1 d)	30	0.38	132±7	94±9	*90±5*
Jul 2001	3.5 (2 d)	23	0.29	116±6	94±4	*66±3*

for the July 2001 2-d exposure. Thus, analytical artifacts associated with speciation measurements could represent almost 50% of the model's uncertainty for these two samples, although applying the same increase or decrease in photodegradation rate to both experiments 1 and 3 would worsen the prediction for one of the samples. Another caution when using our model to predict the degradation of the samples exposed to natural sunlight is that there are several inherent difficulties in comparing solar simulator results with outdoor incubations. Differences in internal reflection of light within the flasks may account for some variability, but these are difficult to quantify. Likewise, differences in radiation geometry between experimental setups can be a potential source of variability. However, a reasonable estimate of path lengths within the tubes and flasks in conjunction with the respective radiated area to volume ratios suggests the two setups were comparable in terms of radiation geometry.

Although analytical artifacts may exert some influence on our model, structural variability of Cu ligands is likely to be the primary factor governing photodegradation rates and will be a function of numerous environmental parameters. The chemical nature of these ligands will be closely related to their specific sources as well as the parameters that control changes to the ligands as they are transported to estuarine waters, such as bacterial activity and residence time in the photic zone. Thus, an important limitation of our photodegradation model is that the structures of these ligands are unknown. Yet, as mentioned before, strong Cu ligand and DOC concentrations in the Cape Fear estuary are very consistent under "normal" flow patterns and nearly always exhibit conservative mixing patterns (Avery et al. 2003; Shank et al. 2004*c*), so that structural variability may be smaller in the Cape Fear than in other systems. It must also be noted that normalizing the decay rate to a_{300} may only be valid when examining the photoreactivity of ligands derived from terrestrial humic sources. To this point, no studies have presented degradation rates for in situ biologically produced ligands in marine waters. Despite analytical concerns and potential chemical variability among samples, it is compelling that photooxidation rates measured in solar simulator experiments in conjunction with the absorption capacity for UV light in each particular sample allowed for a reasonable prediction of the strong Cu ligand concentrations in Cape Fear samples exposed to natural sunlight.

Photodegradation of strong Cu ligands within the Cape Fear estuary—To assess potential losses of strong Cu complexation capacity due to photodegradation processes within the Cape Fear estuary, we assumed an estuarine residence time of 3 d, an average depth of 8 m (Shank et al. 2004*b*), and a pseudo first-order degradation rate constant (k) for strong Cu ligands in Cape Fear estuarine waters of 0.28 d^{-1}. Given that UV light penetration (1% light level) in the darkly colored Cape Fear is <15 cm (R. F. Whitehead unpubl. data), the strong ligand photodegradation rate (k) must be adjusted to an average rate appropriate for the entire water column. A 1% light level at 15 cm light corresponds to a diffuse light attenuation coefficient (K_d) of 30 m^{-1}. A K_d value is calculated from irradiance measurements using $E_d = E_o e^{-K_d z}$, where E is irradiance level and z is light penetration depth. An average photodegradation rate (k_{avg}) for the entire water column can then be calculated using the following equation (Hu et al. 2002) if UV light is assumed solely responsible for the degradation process:

$$k_{avg} = k(1 - e^{-K_d z})K_d^{-1}z^{-1} \tag{5}$$

where z is water depth. Using the assumptions provided above, k_{avg} for the 8-m deep water column is 0.0012 d^{-1}. Applying Eq. 2 with k_{avg}, strong Cu ligands would be <1% degraded by UV light during a 3-d transit through the estuary. It is possible that a larger portion of the freshwater-derived ligands will remain in lower saline and less dense surface waters upon entering the estuary, so that the magnitude of photodegradation may be underestimated in our calculation. More likely, however, the calculated degradation is an overestimate because it is based on irradiation of filtered estuarine water under midsummer conditions and the potential importance of radiation absorption and scattering by particulate matter was not considered (Nelson and Guarda 1995; Sosik 1999). In any case, because of short estuarine residence times and limited light penetration, <1% of the mean standing stock of strong Cu ligands within Cape Fear estuarine waters is likely to be degraded.

Although photodegradation does not appear to significantly influence strong Cu complexation capacity within the Cape Fear estuary, this process may be very important in more optically clear estuaries or those with longer residence times. For example, residence times of freshwater entering the Chesapeake Bay are typically 6–12 months (Skrabal et al. 1997). Although strong Cu ligands in that system may be of biological origin rather than dominated by light-absorbing humic compounds, Chesapeake Bay is a shallower and less-colored estuary than Cape Fear, thus promoting a relatively deeper light penetration depth within the water column. Based on data provided by Moffett et al. (1990) and Croot et al. (1999) in which the photoreactivity of strong ligands of biological origin was demonstrated, it is possible a substantial amount of in situ biologically produced ligands could be photodegraded over periods of several months in Chesapeake Bay estuarine waters. Thus, even in estuaries where the strong ligand pool is not dominated by highly absorbing terrestrially derived DOM, it is possible these systems experience a substantial loss of strong Cu complexation capacity due to photochemical processes.

Photodegradation of strong Cu ligands within SAB waters—Results presented in Shank et al. (2004*a*) predicted up to 50% of the strong Cu ligand pool in SAB waters is delivered by organic-rich river discharge. Given the short residence times (1 week) of freshwater in most estuaries along the SAB (Moran et al. 1999), photochemical processes are likely negligible within estuarine boundaries. However, once discharged into the SAB, this water has a shelf residence time of 80 d (Atkinson et al. 1978). Miller and Moran (1997) and Moran et al. (1999) estimated an 80-d residence time corresponded to 16 h of noontime solar radiation (4–5 total sunlight days) for a well-mixed SAB system with a 2 m UV penetration depth. In the natural sunlight exposures discussed earlier, considerable quantities of strong Cu ligand had been photodegraded after 1–2 d of summer sun, suggesting photodegradation processes may be important sinks of strong Cu ligands in SAB waters.

To determine the potential importance of photooxidation as a sink for strong Cu ligands in SAB shelf waters, it was necessary to calculate a photodegradation rate constant applicable to this system. During a previous examination of the optical properties of SAB waters, Nelson and Guarda (1995) reported a_{300} values that ranged from <1 to 8 m^{-1} along transects extending 60–70 km offshore from Savannah, Georgia. UV absorption coefficients exhibit large fluctuations within SAB shelf waters because of inputs from Gulf Stream waters and fluctuations in river discharges (Nelson and Guarda 1995). By comparison, Del Vecchio and Blough (2002) reported a_{300} values of <2 m^{-1} for Middle Atlantic Bight waters. Based on these studies, we assumed an average a_{300} value of 4 m^{-1} for terrestrially derived DOM residing within SAB waters (22 m^{-1} in Cape Fear estuarine samples used in solar simulator experiments). Using this a_{300} value and Eq. 3, we calculated a normalized photodegradation rate constant (k_{norm}) of 0.05 d^{-1} for strong Cu ligands in SAB waters delivered by river discharge. Assuming strong Cu ligands experience the equivalent of 4–5 d of solar radiation while in residence within the SAB system (Miller and Moran 1997; Moran et al. 1999), a loss rate of 0.05 d^{-1} indicates photodegradation may account for an 20% loss of the river-borne strong Cu complexation capacity, or 10% of the total ligand pool assuming rivers provide 50% of the ambient pool (Shank et al. 2004*a*). Because river discharge will be of lower salinity than receiving coastal ocean waters, terrestrially derived ligands may experience more than 4–5 d of sunlight under some conditions. However, as discussed for similar calculations regarding Cape Fear estuarine waters, the predicted magnitude of photooxidation we presented for shelf waters may be a maximum value because of the potential importance of particulate matter in scattering and absorbing some portion of the available light (Nelson and Guarda 1995). Nonetheless, given our results and that previous research has shown biologically produced strong Cu ligands are also photoreactive (Moffett et al. 1990; Croot et al. 1999), solar radiation should be a primary sink for a considerable portion of the ambient strong Cu ligand pool in SAB shelf waters, although photodegradation rates of biologically produced strong Cu ligands have not been calculated.

Provided photodegradation is an important sink for both in situ biologically produced and terrestrially derived strong Cu ligands as we hypothesize, there are two important geochemical ramifications. First, photolytic destruction of strong binding sites increases the importance of weaker binding ligands in controlling the speciation of dissolved Cu (Croot et al. 1999). Strong Fe(III) binding ligands produced by marine bacteria can be photochemically altered to form ligands with weaker Fe(III) affinities directly affecting the biogeochemical cycling of Fe in oceanic waters (Barbeau et al. 2001, 2003). Terrestrially derived or biologically produced strong Cu ligands may experience a similar chemical alteration. Alternatively, photodegradation of strong Cu ligands may induce a substantial increase in free Cu^{2+} levels as reported by Moffett et al. (1990) for surface waters in the Sargasso Sea. The ecology of Cu-sensitive marine organisms would be directly affected by the release of free Cu^{2+} ions (Moffett et al. 1990; Mann et al. 2002).

Predicted binding functionalities of strong Cu ligands in the Cape Fear estuary—Humic substances are a large constituent of the CDOM pool and are responsible for much of the UV light absorption in organic-rich estuaries along the southeast U.S. coast (Gao and Zepp 1998; Moran et al. 2000). Results presented in this paper show strong Cu ligands exist within the pool of light absorbing organic matter in Cape Fear estuarine waters. Similarity between the high photoreactivity of humics reported by other researchers and the high photoreactivity of strong Cu ligands further supports the argument presented by Shank et al. (2004*c*) that humics are the dominant pool of strong Cu binding ligands in the Cape Fear system. Terrestrial CDOM is characterized by a relatively large proportion of aromatic functional groups compared with oceanic CDOM (Zepp 2003). Additionally, research has shown terrestrial CDOM loses much of its aromatic character when oxidized or photobleached by solar radiation (Vähätalo et al. 1999). Therefore, correlation between the humic nature of strong Cu ligands and the photochemically induced loss of binding functionalities of Cape Fear samples suggests aromatic functional groups within humic substances may be primary binding sites for Cu in this system, and potentially in other humic-dominated southeastern U.S. estuaries.

The pathway by which strong Cu ligands are photodegraded in the Cape Fear estuary appears to be through direct photooxidation of humics, rather than through a Cu(II)-LMCT mechanism. Evidence supporting this hypothesis includes the fact that strong Cu ligands exist in substantial excess of TDCu levels, limiting the participation of Cu in the LMCT process. Additionally, Voelker et al. (2000) reported that strong Cu ligand complexes may be less susceptible to photolysis by superoxide radicals than weaker Cu-ligand associations. The LMCT process may become important as stronger ligands degrade during irradiation and weaker ligands begin controlling Cu speciation (Croot et al. 1999) or in oceanic waters where TDCu and strong ligand concentrations are approximately equivalent. Although a Cu(II)-LMCT mechanism may not be the dominant photodestructive pathway, Cu(I) ↔ Cu(II) redox cycling may act as a catalyst for the photodestruction of these binding sites. Humic substances contain catechol functional groups, and catechol can be oxidized by molecular oxygen in a nonphotochemical reaction catalyzed by Cu(II) ions (Balla et al. 1992), although it is unclear how important this process may be in natural waters at nanomoles per liter concentrations of Cu and how sunlight would affect this process. In Cape Fear waters, Fe exists in micromoles per liter quantities (C. O’Connell unpubl. data), and this metal has been shown to participate in numerous types of photochemical reactions that produce radical species including peroxyl, superoxide, and hydroxyl radicals (Faust 1994). Thus, the most probable pathways for the photodestruction of terrestrially derived strong ligands not bound to Cu ions are through direct reactions with a variety of radical species produced during photolysis. More studies are necessary to determine the roles of superoxide and hydroxyl radicals in the photodestruction of terrestrially derived strong Cu ligands. Additionally, valuable information could be gained by simultaneously comparing photodegradation rates of Fe and Cu binding ligands in coastal waters.

In summary, strong Cu binding ligands in the Cape Fear River estuary are extremely photoreactive. However, short estuarine residence times coupled with a relatively shallow light penetration depth limit the magnitude of photodegradation (<1%) within Cape Fear estuarine waters. Calculated photooxidation rates indicate 20% of the river-borne strong Cu ligand pool in SAB shelf waters could be degraded by photochemical processes, likely much higher in surface waters where Cu-sensitive organisms may reside. Degradation rates were considerably higher in full-spectrum solar radiation exposures than in PAR exposures, indicating that UV light is the primary energy source driving ligand destruction. Correlation between the loss of aromaticity in terrestrial DOM during photobleaching reported by other researchers and the high photoreactivity of strong Cu binding ligands in the humic-rich Cape Fear estuary suggests aromatic functional groups may be the primary Cu binding sites in terrestrial humic substances. Combined with previous studies where the photoreactive nature of strong ligands produced by marine bacteria was demonstrated, photochemical reactions appear to be a major ligand sink in oceanic waters and may exert an important influence on the ecology of Cu-sensitive organisms.

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