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Cyanogen Chloride Precursor Analysis in Chlorinated River Water

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Amino acids have been cited as potential precursors of the disinfection byproduct cyanogen chloride in chlorinated drinking water. Screening experiments with 17 amino acids were performed in this study to comprehensively identify important CNCl precursors. Among this set, only glycine was found to yield detectable CNCl (i.e., > 0.6% yields). Additional experiments were conducted to estimate the relative significance of glycine as a CNCl precursor in water samples collected from the Huron River, Michigan, by concurrently characterizing the amino acid content and monitoring CNCl yields after chlorination. Chlorine was added at slightly less than the sample breakpoint dose to optimize CNCl formation and stability in the samples. On the basis of previous determinations that glycine-nitrogen is stoichiometrically converted to CNCl−N at pH > 6, it was estimated that glycine may account for 42−45% of the CNCl formed in the river water samples (pH 8.2). The kinetic profile of CNCl formation in the sample, with a half-life of about 20 min, indicated that both rapid and slower formation pathways were important. Glycine formation of CNCl, with a half-life of 4 min, is likely to contribute significantly to the rapidly formed CNCl, while unidentified precursors must account for the slower pathway. Non-glycine-derived CNCl precursors in this water source were further examined to determine if they were largely proteinaceous in character using a technique known as immobilized metal ion affinity chromatography (IMAC). These experiments demonstrated that copper-loaded IMAC resins were much more effective in removing glycine than other CNCl precursor compounds in the sample matrix. The unidentified CNCl precursor components, therefore, are not likely to be proteinaceous and are more likely to be associated with the fulvic/humic fraction of organic matter.

Introduction

Cyanogen chloride (CNCl) is a toxic disinfection byproduct (DBP) formed by the reaction of free chlorine or chloramines with natural organic matter (NOM) (*1*). In 1991, CNCl was listed on the USEPA Drinking Water Priority List. It was also monitored for as part of the USEPA Information Collection Rule (ICR) program during 1997 and 1998. Drinking water standards for CNCl have not been established, however, the World Health Organization (WHO) has recommended that a maximum of 70 μg L^{-1} as cyanide be used as a guideline for total cyanogen compounds (*2*).

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Occurrence data for CNCl from 35 water treatment utilities were collected and analyzed by Krasner et al. (*1*). Median CNCl concentrations for utilities that used only free chlorine or only chloramines were about 0.4 μg L^{-1}, while utilities that prechlorinated and post-ammoniated had median CNCl concentrations of 2.2 μg L^{-1}. The data suggest a phenomenological association of higher CNCl concentrations with the combined use of chlorine and chloramines. Possible explanations for the disinfectant-dependent CNCl concentration trends may be related to differences in the stability of CNCl in the presence of these disinfectants, as well as CNCl formation. Earlier model system studies in our laboratory and elsewhere have established, for example, that free chlorine as hypochlorite ion can significantly catalyze the hydrolysis of CNCl over the time scale of disinfection, while monochloramine does not (*3*−*6*). If indeed the growing practice of post-chloramination has the hypothesized effect of stabilizing CNCl formed during chlorination, then it is important to understand the chlorination pathways that form CNCl.

Amino acids have been cited as potential CNCl and halogenated nitrile precursors (*7*, *8*) and prior to this study only a few have been assessed for their potential to form CNCl (*9*−*12*). Of those tested, glycine appears to have the greatest yields (*12*). In a recently completed study of the mechanism of CNCl formation from glycine in our laboratory, conversion of glycine-nitrogen to CNCl−N was shown to be 100% at pH > 6 after accounting for CNCl losses due to hydrolysis (*13*). To determine if other amino acids are important precursors, a comprehensive screening of DNA-derived amino acids was undertaken in this study.

The abundance of dissolved amino acids in natural surface waters has been characterized only to a limited extent, primarily because the required analytical determinations are onerous (*14*−*17*). While amino acids account for a small fraction of the dissolved organic carbon (DOC) in surface waters, they account for a much higher proportion, 15−35%, of the dissolved organic nitrogen (DON) (*18*). Watersheds draining agricultural areas contain the highest concentrations of DON and thus higher amino acid concentrations would be expected in basins with this type of land use. In addition, free amino acids represent a small fraction of the total dissolved amino acid (combined and free) content. In the pristine Canadian MacKenzie River system, for example, combined amino acids were about 10 times more abundant as free amino acids, which ranged from 3 to 12 μg L^{-1} (*16*). Higher concentrations of dissolved free amino acids have been reported in more agricultural- and runoff-impacted surface waters supplying the Southern California Metropolitan Water District; State Project water and Colorado River water samples contained 30 and 40 μg L^{-1}, respectively (*14*).

The relative order of abundance of free amino acids in natural waters is quite variable, but glycine has been described as one of the predominant amino acids after reviews of the literature (*8*). In the MacKenzie River, glycine was the most abundant dissolved amino acid and total glycine ranged from 2.3 to 17.7 μg L^{-1}. Glycine was also the predominant dissolved amino acid in a Pennsylvania creek (*15*). In the two primary water sources of the Southern California Metropolitan Water District, both contained 2.3 μg L^{-1} free glycine, which was the fourth most abundant free amino acid (*14*). Wu and Tanoue did not report absolute glycine concentrations, but found that glycine was the third most abundant total dissolved amino acid in Lake Biwa, Japan (*17*). Assuming a 2 μg L^{-1} free glycine concentration, and if 100% of the glycine-N in a sample were converted to CNCl−N upon chlorination,

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approximately 1.7 μg L^{-1} CNCl would be glycine-derived. Since CNCl concentrations in finished drinking water are generally less than 10 μg L^{-1}, these calculations show that glycine-derived CNCl could be a substantial fraction of the CNCl formed.

Unfortunately, simultaneous measurements of amino acids and CNCl formation in natural water samples are unavailable, and a comprehensive evaluation of significant amino acid CNCl precursors is lacking as well. In this research, screening studies were conducted to identify important amino acid CNCl precursors. Concurrent characterizations of both the amino acid content and the CNCl formed after chlorination were also performed with water samples from the Huron River, Michigan, which drains a predominantly agricultural basin and has high nutrient loadings (*19*).

Other nitrogenous components of NOM are thought to form cyanogen halides in the presence of chlorine. Chlorinated proteins and polypeptides, for example, have been shown to give small CNCl yields (*11*). Modest to small yields of CNCl were obtained with purine bases (adenine and guanine) in the presence of chloramines (*20*). Humic matter may also contain CNCl precursor compounds. Reactions of humic acid with chlorine in the presence of ammonium ion were reported to produce CNCl (*21*). Heller-Grossman et al. also found that modest amounts of CNBr, another cyanogen halide formed in bromide-rich waters, were produced when fulvic acid/bromide solutions were chlorinated (*22*). To better understand which of these complex components of NOM may be more important as CNCl precursors in natural water samples, a fractionation technique known as immobilized metal ion affinity chromatography (IMAC) was applied in this study to a Huron River water (HRW) sample.

Column resin materials in IMAC systems were originally developed to purify proteins (*23*, *24*), but their use has recently been extended to fractionate and characterize NOM in surface water (*25*−*27*). The IMAC resins are loaded with chelating divalent metal ions, and the principle of organic matter separation and fractionation is based on the chelation strength of ligands in the matrix. Because the IMAC technique can separate organic matter fractions nondestructively, we have applied it to examine the relative CNCl formation potentials of highly vs weakly retained HRW organic matter.

Materials and Methods

Reagents. All chemicals in this study, unless otherwise noted, were ACS reagent grade, purchased from either Fisher Scientific or Sigma-Aldrich, and used without further purification. Glycine, arginine, histidine, and tyrosine were obtained from Alfa Aesar, Acros Organics, and ICN Biomedicals, Inc. Waters (Milford, MA) AccQ-Fluor Reagent kit (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate, acetonitrile, and borate buffer) was used to derivatize the amino acids prior to HPLC analysis. Waters AccQ-Tag Eluent A (sodium acetate trihydrate, phosphoric acid, triethylamine, sodium azide) and HPLC-grade acetonitrile (Fisher Scientific) were used as solvent eluents for amino acid HPLC analyses. Cyanogen chloride standards were synthesized immediately prior to use according to Standard Method 4500-CN-E (*28*). Stock sodium hypochlorite solutions were made fresh daily from approximately 5% commercial solutions and standardized by DPD titration (*29*).

Amino Acid Screening Experiments. Screening experiments to identify CNCl precursors were performed at pH 7 and 25 °C with the following 17 amino acids: alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine, and valine. Chlorination of individual 340 μM amino acid solutions was conducted in a sealed, dark 100-mL reactor with less than 1 mL of headspace. Sodium phosphate salts (mono- and di-basic) were used to buffer the pH, wherein the total phosphate concentration was 100 μM. Chlorine concentrations were varied from 0.5 to 5 times the amino acid concentration. Analysis for cyanogen chloride was conducted by membrane introduction mass spectrometry (MIMS). Method details are published elsewhere (*3*). The MIMS method detection limit for CNCl was approximately 120 μg L^{-1}.

River Water Characterization and Chlorination. Grab samples were collected from the Huron River at the Ann Arbor, Michigan Water Treatment Plant intake line in a clean Cubitainer (I-Chem certified 300 series) on 2 days in June 2004 for later amino acid characterization and use in chlorination experiments. Preliminary grab sampling and testing at this site had also been performed to characterize the amino acid content of the river water in July 2003, and to determine appropriate chlorine reaction times in May 2004 for CNCl yield measurements. All samples were immediately filtered with a Millipore 0.45-μm hydrophilic membrane filter under vacuum, and then stored at 4 °C. Total organic carbon (TOC) concentrations (Shimadzu, model TOC-500), breakpoint chlorine dose, and specific ultraviolet absorbance (SUVA-254) (Hitachi, model U-2000 spectrophotometer) of the filtered samples were measured within 24 h. Breakpoint chlorine doses of the river samples, defined here as the minimum chlorine dose leading to free chlorine residual, were determined by standard DPD titrimetric methods (*29*).

Amino acid analyses were performed after concentrating 500 mL of pre-filtered river water; first to approximately 10 mL by rotary evaporation (Buchi, Rotovap) for 7 h at 67 °C, 50 rpm under vacuum, and then by freeze-drying (Labconco, Freezezone6) to a final volume of 2 mL. Concentrated samples were immediately derivatized using a Waters AccQ-Fluor reagent kit (Millipore Co., Milford, MA, containing borate buffer, 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate, and acetonitrile), followed by analyte separation on a Waters Accu-Tag HPLC column (3.9 mm × 150 mm), and fluorescence detection (Hewlett-Packard, model 1100). HPLC elution (Hewlett-Packard, model 1090 HPLC system) and separation of the derivatized amino acids were achieved with a mobile phase composed of A/B/C ratios of (A) AccQ-Tag Eluent A, (B) acetonitrile, and (C) deionized water at a flow rate of 1.0 mL min^{-1}. The following gradient program was employed at room temperature: 0 min (100:0:0), 0.5 min (99:1:0), 18 min (95:5:0), 19 min (93:7:0), 29.5 min (86:14:0), 33 min (80:20:0), 42 min (0:60:40), and 45 min (100:0:0), for a total run time of 75 min. The general detection limit for amino acids was approximately 2 nM.

Pre-filtered river water samples were chlorinated in the dark by adding 90% of the sample chlorine breakpoint dose. The source of chlorine was a standardized, stock NaOCl solution. Since the MIMS method was not sufficiently sensitive for natural sample analysis, a headspace, gas chromatography (GC)-μECD (Hewlett-Packard, model 6890) method was used to determine the resulting CNCl concentrations. After 60 min, 15 mL of the chlorinated samples were transferred to 22 mL autosampler headspace vials, ascorbic acid was added (500 mg L^{-1}) to remove any remaining hypochlorite, and the pH was adjusted to pH ~4 with NaH_2PO_4 and HCl to hinder the remaining dichloroglycine from decomposing and forming more CNCl during the GC analysis. In our previous mechanistic studies of CNCl formation from glycine, it was established that the yield of CNCl from dichloroglycine at pH 4 was slightly less than 20% (*13*). Sealed vials were equilibrated at 40 °C for 20 min in a headspace autosampler (Tekmar 7000) prior to injection in the GC. A capillary GC column (J&W Scientific, db625, 60 m × 320 μm) was used, with inlet and detector temperatures both set at 250 °C, and the following temperature program: 45 °C for 6 min, 20 °C min^{-1} to 150 °C over 5 min, with a total run time

of 16.25 min. The GC was operated in split mode (10:1). The method detection limit for CNCl was estimated as 0.1 μg L^{-1}.

IMAC Sample Processing and Chlorination. The pre-filtered HRW sample collected on June 29, 2004 was further processed with an immobilized metal ion affinity chromatography column (IMAC). The IMAC column consisted of a copper-loaded polyvalent metal ion binding (PMIB) resin (Affiland, Belgium), in a 10-mm-diameter glass column (Omnifit). The packed height and volume of the resin were 10 cm and 7.9 mL, respectively. Copper loading of the PMIB resin binding sites was achieved by flowing 1 L of 5 mM $CuSO_4$ (pH 4.7) at 3 mL min^{-1} through the column. On the basis of mass balance analyses, the copper binding capacity of the resin was estimated as 185 μmol mL^{-1} resin.

A 1.2-L volume of pre-filtered HRW was processed in the IMAC column at a loading rate of 3 mL min^{-1} and the effluent was collected for amino acid analysis and breakpoint chlorine dose evaluation. After determining the breakpoint chlorine dose, the effluent sample was immediately chlorinated at a dose corresponding to 90% of the breakpoint chlorine dose and analyzed for CNCl by the previously described headspace-GC method.

Results and Discussion

Amino Acid Precursor Screening. Among the 17 amino acids studied in the screening experiments, only glycine produced detectable concentrations of CNCl at pH 7 and 25 °C. On the basis of the MIMS method detection limit for CNCl, 2 μM, yields of CNCl for all amino acids other than glycine were estimated therefore to be less than 0.6%. This finding generally agrees with literature studies of amino acid chlorination. Significant CNCl yields were reported for glycine (*12*), minor to trace yields for threonine 8.0% (*10*), tyrosine, 4.0% (*11*), serine, 1.2% (*10*), cysteine, 0.7% (*12*), and asparagine, 0.5% (*12*), and no CNCl was detected when alanine, leucine, phenylalanine, and tryptophan were chlorinated (*11*).

Threonine and tyrosine yields of CNCl as large as 8 and 4%, respectively, were not observed in our experiments, in contrast to these earlier reported by Hirose et al. (*10, 11*). The method used in this earlier study to detect and quantify CNCl production was described as a modified pyridine–pyrazolone method that relied on derivatization and visible light absorbance detection. The MIMS method of detecting CNCl used in our study is likely to be more specific and less vulnerable to interference than this spectrophotometric technique. On the basis of our MIMS screening studies and a review of the available literature, glycine is the most important amino acid precursor of CNCl. An analysis of glycine's role as a CNCl precursor became the focus of subsequent experiments with HRW samples.

Observed CNCl yields in model glycine solutions, shown in Figure 1, were a complex function of chlorine dose. The percentage CNCl yields are defined here as the percentage of initial glycine-N that was observed as CNCl-nitrogen. A maximum *observed* yield of approximately 55% was found when the initial molar chlorine/glycine ratio was 2.0. Shang et al. (*12*) also observed that the observed CNCl yield after 30 min was a maximum at a chlorine/glycine ratio of about 3.

Studies of the CNCl formation mechanism from glycine in our laboratory have provided explanations for the dose-dependent observed yields in Figure 1 (*13*). The increasing ultimate yields up to a chlorine/glycine ratio of 2 reflect the mechanistic requirement that dichlorinated glycine be formed to produce CNCl. At higher chlorine/glycine ratios, however, the presence of free chlorine catalyzes the hydrolysis of CNCl and the *observed* yields appear to decrease. After accounting for the decay by hydrolysis, however, *actual* yields of CNCl-N formed from glycine at pH > 6 were shown to be 100%.

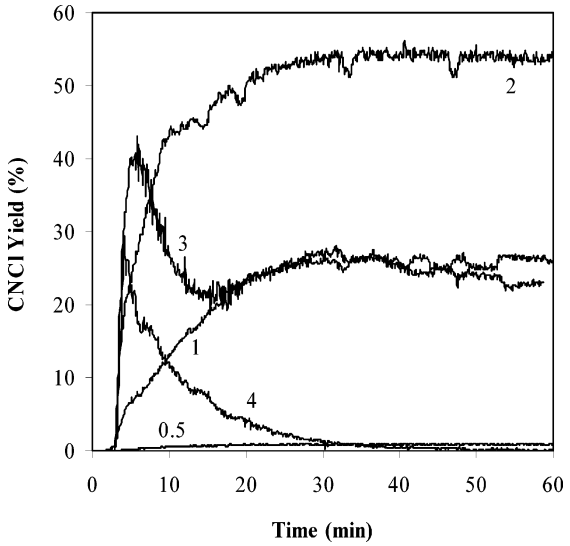

FIGURE 1. Time profiles of CNCl yield with glycine as measured by MIMS at varying initial free chlorine-to-glycine molar ratios ($[Cl_2]_T/[Gly]_0$). $[Gly]_0 = 340$ mM at pH 7 and 25 °C. Chlorine-to-glycine ratios are labeled next to curves.

TABLE 1. Free Amino Acid Characterization of Huron River Water Samples

	concentration ($\pm\sigma$) (μg L^{-1})[a]		
amino acid	7/30/2003	6/1/2004	6/29/2004
aspartic acid	1.2 (0.11)	0.8 (0.06)	0.3
serine	1.7 (0.11)	1.4 (0.24)	0.7
glutamic acid	1.3 (0.25)	0.4 (0.04)	ND
glycine	2.3 (0.04)	1.5 (0.14)	1.2
histidine	ND	ND	ND
arginine	2.1 (0.22)	ND	ND
threonine	0.8 (0.15)	2.3 (0.10)	4.4
alanine	0.6 (0.09)	0.2 (0.02)	0.3
proline	4.2 (0.13)	2.8 (0.69)	ND
cysteine	ND	ND	ND
tyrosine	ND	5.3 (0.38)	2.2
valine	ND	0.3 (0.03)	ND
methionine	4.3 (0.15)	1.1 (0.05)	1.1
lysine	ND	2.6 (0.25)	ND
isoleucine	0.4 (0.28)	0.1 (0.01)	ND
leucine	ND	0.6 (0.04)	0.2
phenylalanine	0.7 (0.3)	ND	ND
TOTAL	19.6	19.4	10.4

[a] ND = Not detected.

Since the instability of CNCl in the presence of hypochlorite can confound the determination of "CNCl yields", special considerations with respect to the chlorine dosing strategy are necessary to evaluate CNCl precursors in natural water samples. Subsequent experiments to estimate glycine's importance as a CNCl precursor in HRW samples were designed with these factors in mind. In particular, sample breakpoint chlorine doses were first characterized to select chlorine doses that would minimize CNCl instability and maximize its yield.

River Water Characterization. The amino acid content of the river water was determined on three summer occasions: one preliminary sample in 2003, and two sampling dates in 2004. The latter two 2004 samples were also examined in terms of their CNCl formation potential. The amino acid analysis results are presented in Table 1. Total concentrations of free amino acids in HRW ranged from 10.4 to 19.6 μg L^{-1}. These total concentrations are generally higher than those reported for the more pristine MacKenzie River drainage basin (*16*), but are less by a factor of about two than the free

TABLE 2. General Water Quality Parameters of Filtered HRW Used in Chlorination Experiments

parameter	6/01/2004	6/29/2004
pH	8.2	8.2
DOC (mg L^{-1})	8.7	7.4
SUVA 254 nm (L mg^{-1} C m^{-1})[a]	NM[b]	4.3
breakpoint chlorine dose (mg Cl as Cl_2 L^{-1})	2.4	2.3

[a] SUVA = Specific UV Absorbance = UV-254 nm/DOC. [b] NM = not measured.

amino acid contents of the Colorado River and State Project Water reported by Chinn and Barrett (*14*). Glycine concentrations in HRW ranged from 1.2 to 2.3 μg/L, which is similar to the free glycine abundance reported in Chinn and Barrett's study (*14*). Among the three HRW samples tested, glycine ranked second to fourth in abundance relative to the other free amino acids.

Excessive nutrient loadings in the Huron River drainage basin are known to cause nuisance algal blooms. Sections of the river both immediately upstream and downstream from the sampling site used in this study were in fact being separately monitored weekly over this period for nutrient loadings and other related water quality parameters by another research group at the University of Michigan (*30*). Lehman's data indicate that total dissolved nitrogen concentrations at both upstream and downstream locations were approximately 80 μM on June 1, 2004 and were generally declining to a value of about 55−60 μM on July 1, 2004. The fluctuations in dissolved nitrogen over this reach and time period, however, were primarily due to nitrate concentration changes. Approximately $^1/_3$−$^1/_2$ of the dissolved nitrogen was present as nitrate, while the remainder was probably predominantly DON. Differences between dissolved total nitrogen and nitrate concentrations during June 2004 imply that DON varied only from about 38 to 35 μM.

Other water quality parameters that were characterized for the HRW samples in our CNCl precursor analysis experiments are reported in Table 2. The sampling period was preceded on May 22, 2004 by a fairly large rainfall event that resulted in relatively high DOC levels. The SUVA value measured in the June 29 sample was also relatively elevated for a river water sample. In a survey of natural and agricultural drainage streams in California, for example, only 16% of samples with SUVA > 4 L mg^{-1} m^{-1} were collected from natural streams (*31*), whereas the majority of samples in this class were from agricultural runoff. The high DOC and SUVA values during this period were likely due to the leaching of humic matter from suspended sediments brought by the preceding storm and flooding. Data collected by Lehman's group also indicated that the UV-254 absorbance of river samples achieved an annual peak during the week prior to June 1, 2004 and remained elevated throughout the month (*30*).

The samples characterized in Table 2 were similar in pH and breakpoint chlorine doses. The somewhat alkaline pH values are apparently typical; mean pH values for the Huron have been cited as 8.2 (*19*). Lehman et al. also reported pH values near 8.2 for this period and reach of the river (*30*). Since CNCl decays more rapidly at higher pH, due to the effect of hypochlorite ion, the similar pH values of these samples imply that the decay rates of CNCl in the two samples should be similar, but potentially substantial, since OCl^- is the predominant form of free chlorine. The comparable breakpoint chlorine doses of these samples suggested that they could also be chlorinated similarly in CNCl formation potential experiments. The dosing rationale, described later herein, was based on these breakpoint dose determinations, studies of the CNCl yield−chlorine dose dependence, and the ultimate stability of CNCl in kinetic studies of its formation in HRW.

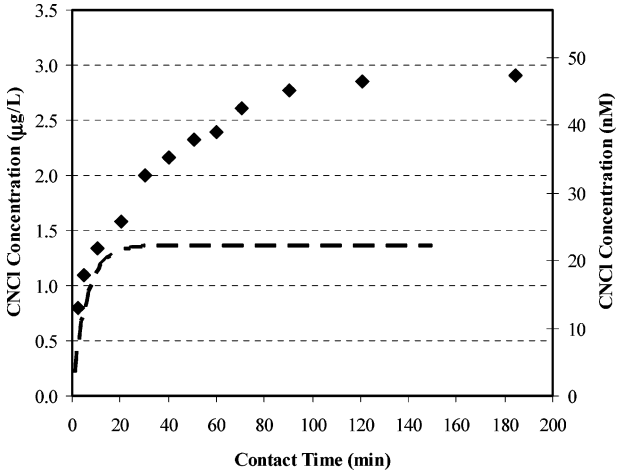

FIGURE 2. Cyanogen chloride formation kinetics in HRW sample (data points) collected May 24, 2004 with 2.3 mg Cl as Cl_2 L^{-1}, pH 8.3. Sample breakpoint chlorine dose was 2.5 mg Cl as Cl_2 L^{-1}. Dashed line is a calculated prediction of glycine-derived CNCl formed assuming a typical initial concentration of 1.7 μg L^{-1} (22 nM) glycine and eq 1.

CNCl Formation Kinetics and Chlorine Dose Dependence. To establish a contact time for CNCl yield determinations in precursor analysis experiments, the kinetics of CNCl formation was first studied in a HRW sample collected on May 24, 2004 HRW. A chlorine dose corresponding to 90% of the sample breakpoint dose was also selected to assess whether this dose was sufficient to minimize CNCl decay. The temporal production of CNCl in the sample is shown in Figure 2.

Cyanogen chloride formation was approximately 83% complete after 1 h and achieved a stable plateau value after 2 h. By the first sampling time, 2 min, more than 25% of the ultimate CNCl was measured, indicating that there were both fast and slow pathways for CNCl formation in this river water sample.

On the basis of the observed stable CNCl plateau, subsequent precursor analysis experiments were designed with the same chlorine dosing strategy, wherein the chlorine dose was selected as 90% of the breakpoint dose. A reaction time of 60 min was also selected for CNCl yield determinations in subsequent precursor analysis experiments, since it represents an upper limit for most disinfection chamber contact times and because this reaction period allows at least 80% of the ultimate CNCl to form.

A separate study of the chlorine dose dependence of CNCl formation was conducted with HRW samples collected on June 29, 2004 to verify the breakpoint dose effect on CNCl formation. After 30-min reaction periods, maximum yields were observed near the sample breakpoint dose of chlorine, as shown in Figure 3. The breakpoint dose of this sample was 2.3 mg Cl as Cl_2 L^{-1}. As in the glycine chlorination experiments of Figure 1, the presence of free chlorine in the river water samples at chlorine doses greater than 2.3 mg L^{-1} would be expected to catalyze the hydrolysis of CNCl. On the basis of several model system studies of CNCl decay in the presence of free chlorine, residual chlorine was previously hypothesized to be an important determinant of CNCl stability in chlorinated water (*3*−*6*). The data in Figure 3 conclusively demonstrate this relationship in a natural water sample.

Glycine Precursor Analysis. After characterizing the breakpoint chlorine dose and the glycine content of prefiltered HRW samples on two different dates in June 2004,

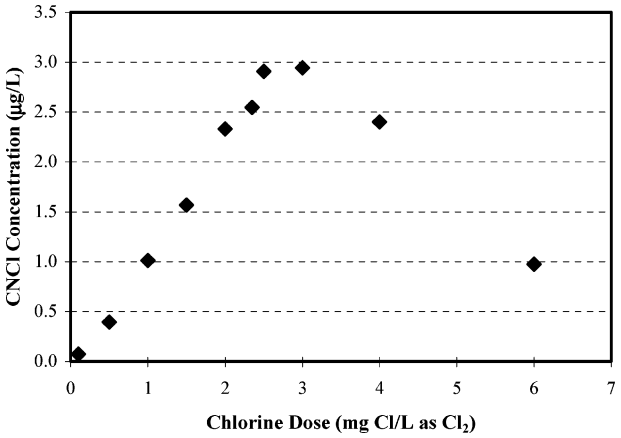

FIGURE 3. Effect of chlorine dose on CNCl formation in pre-filtered HRW after a 30 min reaction period.

TABLE 3. Calculated Contributions of Glycine to Observed CNCl Yields in Chlorinated HRW at 60 min, pH 8.2

sample date	glycine, nM	chlorine dose, mg L^{-1}	observed CNCl[a] $\mu g\ L^{-1}$	observed CNCl[a] nM	estimated % CNCl yield from glycine[b]
June 1, 2004	20.2	2.3	2.7 (0.05)	44	45
June 29, 2004	16.2	2.1	2.4 (0.06)	39	42

[a] Values in parentheses are standard deviation of the measurement, $N = 3$. [b] Estimated as $([\text{glycine}]/[\text{CNCl}]_{obsd}) \times 100$.

the samples were chlorinated for 60 min at 90% of their breakpoint dose and the resulting CNCl yields were determined. Estimates of the percentage contribution of glycine to the observed CNCl yield were made by assuming that 100% of the glycine-nitrogen was converted to CNCl-N. The results of these calculations, provided in Table 3, indicate that glycine could account for 42−45% of the observed CNCl. Since the chlorination reaction was quenched at 60 min, glycine's contribution to the *ultimate* formation potential of CNCl is likely to be slightly smaller than this estimate, however, these calculations suggest that glycine is a significant precursor and probably the most important one.

On the basis of the observed kinetics of CNCl formation in Figure 2, it is apparent that other nonglycine precursors must be responsible for the more slowly formed CNCl fraction. In model system studies, the formation rate of CNCl from glycine was shown to have a half-life of approximately 4 min (*13*). At pH > 6, the formation of CNCl from glycine (with chlorine in excess) can be modeled as

$$[\text{CNCl}] = [\text{Gly}]_0[1 - e^{-kt}] \quad (1)$$

where $k = 3.0 \times 10^{-3}\ s^{-1}$ at 25 °C. Assuming a typical HRW concentration of 1.7 μg/L initial glycine (the average value of samples in Table 1) and using eq 1, the predicted formation profile of glycine-derived CNCl would be as shown in Figure 2 (dashed line). These calculations suggest that glycine may be particularly important as a precursor in terms of the fraction of CNCl that forms in disinfection contact chambers, which generally have contact times less than 60 min, while other precursors would be responsible for CNCl that continues to form in distribution systems.

From previous research, the yields of CNCl from glycine are pH-independent at pH > 6 (*13*). The conditions of drinking water disinfection are typically within this pH range and hence the amount of glycine-derived CNCl formed should depend only on the glycine concentration of the water supply, and not the pH. If CNCl formation were pH-dependent, it would be due only to other as yet unidentified precursors.

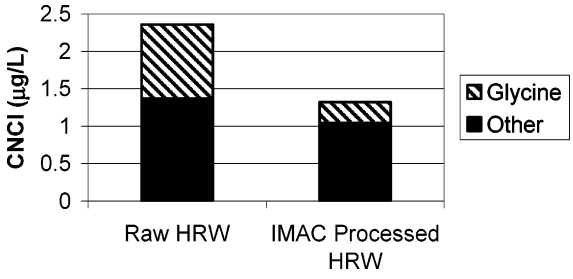

FIGURE 4. Comparison of calculated CNCl formed due to glycine and all other precursors in chlorinated HRW and HRW processed with a Cu-loaded IMAC column. Sample was collected 6/29/2004.

Possible precursors of the nonglycine-derived CNCl could be proteinaceous compounds or peptides that are more slowly cleaved to give CNCl-precursor residues, perhaps even glycine. Purine bases have additionally been identified as potential precursors, although little is known about the kinetics of CNCl formation from these compounds or the abundance of these precursors in natural waters. Given the appreciable DOC and SUVA of the water samples used in this study, fulvic or humic matter could also be CNCl precursors. Since both proteinaceous and humic matter classes of compounds are difficult to characterize, a method to distinguish which was more important in producing CNCl was pursued. This technique involved using IMAC columns to examine the character of nonglycine CNCl precursors.

Chlorination of IMAC−Processed River Water. Treatment of a pre-filtered HRW sample (taken 6/29/04) in an IMAC column resulted in significant removal of free amino acids and a reduction in the CNCl formation potential. Serine and glycine were the only free amino acids detected in the IMAC-processed sample; serine was reduced from 6.2 to 1.4 nM and glycine was reduced from 16.2 to 4.5 nM. A 48% reduction (from 2.4 to 1.3 $\mu g\ L^{-1}$ CNCl) in the sample's CNCl formation potential was observed after a 60 min chlorination period. On the basis of before and after glycine contents of the IMAC-processed sample, precursor contributions to CNCl formation in the chlorinated raw and IMAC-processed HRW were estimated as shown in Figure 4. The IMAC column removed 72% of glycine-derived CNCl, but only 20% of the remaining CNCl precursor compounds. The unidentified CNCl-precursor compounds appear to bear more weakly copper-binding ligands than glycine. Assumptions of 100% glycine-to-CNCl conversion were again made in this calculation.

Proteins or peptides would generally be more favorably retained by copper-loaded IMAC resins than free amino acids (*24*). Given that the nonglycine CNCl precursor material in the HRW sample was more weakly retained in the IMAC column than glycine, it is unlikely that these precursors are largely proteinaceous. Components of fulvic or humic acids, on the other hand, are more likely contributors to CNCl formation. Unpublished comparisons in our laboratory of the retention of glycine and IHSS Suwannee River fulvic acid in the same type of IMAC column, for example, showed much less retention of fulvic acid than glycine (see Supporting Information). Other studies of ligand characteristics in streamwater using Cu-loaded IMAC columns have demonstrated that strong Cu-complexing ligand fractions are protein-like while weak ligands are generally humic-like (*26*).

Drinking Water Disinfection Implications. Concurrent measurements of amino acid content and CNCl formation in a chlorinated natural river water sample suggest that glycine is likely to be the most important single precursor, forming potentially at least 40% of the observed CNCl. Few, if any, DBPs have been previously shown to have such a significant single precursor. This relationship between glycine and CNCl formation, therefore, offers opportunities for

prediction and control of this DBP. Greater characterization of the temporal nature of glycine abundance, for example, would facilitate an understanding of the seasonal conditions favoring CNCl formation. Glycine, however, is not likely to be effectively removed by most conventional water treatment processes (softening, coagulation, filtration) prior to chlorination. Ozone, which is becoming more common as a primary disinfectant, may be quite effective in removing glycine. Based on available kinetic studies of its reaction with ozone, the half-life of glycine is estimated to be about 90 s at pH 7 for an assumed ozone concentration of 1 mg L^{-1} (*32*). More research is needed, however, to determine if the use of ozone in combination with secondary disinfectants such as chloramines leads to lower finished water concentrations of cyanogen halides.

The kinetics of CNCl formation in HRW suggests that the most rapidly produced CNCl in chlorination processes is even more significantly due to glycine. Since glycine-derived CNCl was shown to be rate-limited by the first-order decomposition of dichlorinated glycine (*13*), which forms very rapidly, there are few options for controlling the conversion of glycine to CNCl in an actual chlorination process. More options exist, however, for controlling the *stability* of the CNCl that forms. In particular, the presence of residual free chlorine and more alkaline pH are conditions that promote CNCl hydrolysis to cyanate, a less toxic byproduct. The dechlorination agent sulfite is also known to accelerate CNCl hydrolysis (*4*).

Unidentified CNCl precursors contributed to more than half of the observed CNCl in HRW. On the basis of the kinetics of CNCl formation in the sample, it is likely that they form CNCl more slowly and these precursors may be more significant contributors to the CNCl that forms in distribution systems. Based on the IMAC studies herein, this precursor pool is not likely to be proteinaceous, but could be humic in nature. There may be control opportunities for pre-removing this fraction of the precursor pool in conventional water treatment facilities, but more studies are needed to answer this question.

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Supporting Information Available

Details and results of a chromatographic retention study of glycine and fulvic acid in an IMAC column. This material is available free of charge via the Internet at http://pubs.acs.org.

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