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Fractionated algal organic materials as precursors of disinfection by-products and mutagens upon chlorination

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ABSTRACT

Cells and proteins of *Chlamydomonas* sp. (a common green alga in local reservoirs) were separated by ultrafiltration respectively into 3 fractions with variable molecular weights (MW: >100, 10–3 and <3 kDa). After chlorination (20 °C, pH 7, Cl_2/DOC ratio of 20 mg Cl_2 mg^{-1}, 120 h), levels of disinfection by-products (DBPs) and mutagenicity (via *Salmonella* T100 mutation assay, −S9) were analyzed. The highest yields of chloroform (2571 μmol mol C^{-1}), DCAA (19,083 μmol mol C^{-1}) and TCAA (4939 μmol mol C^{-1}) were observed from the fraction of MW > 100 kDa, while the fraction of 3–10 kDa was potent DCAN precursor. In contrast, the chlorinated MW 3–10 kDa cell fraction showed high mutagenicity (maximum level of 93 rev μL^{-1} at 2 min), while the MW > 100 kDa cell fraction showed low mutagenicity (maximum level of 16.6 rev μL^{-1} at 7200 min) after chlorination. This indicated that unmeasured DBPs or possible interactions among the DBPs contributed to the mutagenicity. Comparing between the cell and protein fractions, the former was more potent in forming chloroform, DCAA, TCAA, DCAN and TCAN. This is the first study that fractionated algal cells and proteins were examined for DBP formation and mutagenicity.

1. Introduction

Chlorination has been applied in drinking water treatment as a major chemical disinfection measure throughout the world, including Hong Kong and mainland China. Disinfection by-products (DBPs) are generated during chlorination as organic matters in raw water may react with chlorine. A number of epidemiological and animal evidence has shown that DBPs could cause cancer and give adverse effect on reproductive system [1]. In Hong Kong and the Pearl River Delta region (China), our 3-year monitoring data revealed that major source of the organic precursors of DBPs was algal-derived organic matter (AOM), showing a low specific UV_{254} value (SUVA, 1.78 L mg^{-1} m^{-1}) [2]. This is different from many other natural waters where the dissolved organic matter was primarily derived from humic substances, with high SUVA (>4 × 1.78 L mg^{-1} m^{-1}) [3].

Algal-derived organic materials generally were small molecules, exhibiting very different properties [4], while humic substances generally were large. In water treatment, large molecules could be easily removed by coagulation [5,6], while small hydrophilic molecules were difficult [7–9]. It was reported that with an increase in the percentage of low-molecular-weight dissolved organic carbon (DOC, <5 kDa) the removal efficiency of the total DOC decreased [10]. Eventually, these hydrophilic low-molecular-weight molecules can serve as effective precursors of DBPs especially dihaloacetic acids [11,12]. On the other hand, AOM can also contribute substantially to the generation of nitrogen-containing DBPs (N-DBPs, e.g. haloacetonitriles [HAN] and halonitromethanes) [13], more carcinogenic than the commonly regulated DBPs (i.e., trihalomethanes, THMs and haloacetic acids, HAAs) [14].

Information on DBPs generation upon chlorinating AOM with variable molecular weight has not been available. Our previous studies on AOM of a local alga (*Chlamydomonas* sp.) showed that upon chlorination algal hydrophilic proteins were more potent chloroform precursors than the pooled whole algal cellular biomass [15]. Whether small-sized algal proteins, probably containing more hydrophilic algal proteins, were more effective chloroform precursors in algae remains unknown. On the other hand, we also noted that the time-dependent variation in the level of mutagenicity was not correlated with that in the concentrations of chloroform or other commonly measured DBPs [15], which corroborated that organic substances (<100 Da) with sufficient lipophilic characters may serve as potent mutagens [16]. Yet, it is not clear whether different molecular sized AOM display a similar pattern, or whether different size fractions of AOM show varied time-dependent mutagenicity upon chlorination.

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Bearing the above information in mind, the present study employed ultrafiltration, one of the most common membrane technologies, to separate AOM (whole algal cellular materials and proteins) into different molecular weight fractions and conducted chlorination experiments. Yields of DBPs and mutagenicity in the chlorinated solutions, sampled from varied chlorination time slots, were analyzed, respectively. The main objectives were: (1) to identify potent DBP precursors in AOM among varied molecular sized fractions; (2) to identify potent mutagen precursors in AOM among varied molecular sized fractions; and (3) to clarify contribution of the measured DBPs to the mutagenicity.

2. Materials and methods

Information on algal culture and harvest, proteins extraction, chlorination experiments, DBPs analyses (including chloroform, dichloroacetic acid (DCAA), trichloroacetic acid (TCAA), dichloroacetonitrile (DCAN), trichloroacetonitrile (TCAN), 1,1-dichloropropanone (1,1-DCP) and 1,1,1-trichloropropanone (1,1,1-TCP)), *Salmonella* mutagenicity assay and effect of protein extraction on DBP yields and mutagenicity was described in our previous report [15]. Briefly, $CHCl_3$ was extracted by pentane and determined according to the Standard Method [17]. DCAA and TCAA were extracted by methyl tert-butyl ether (MTBE) and methylated by acidic methanol, and determined following USEPA Method 552-3 [36]. DCAN, TCAN, 1,1-DCP and 1,1,1-TCP were extracted by MTBE, and analyzed according to the USEPA Method 551.1 [37]. 1,2-Dibromopropane was used as the internal standard. The DBPs were determined by a GC-ECD system (with a 30 m × 0.25 mm × 0.25 μm HP-5 capillary column) using nitrogen as the carrier gas. The recovery rates for $CHCl_3$, DCAA, TCAA, DCAN, TCAN, 1,1-DCP and 1,1,1-TCP were 97.5 ± 6.34, 101 ± 8.4, 97.3 ± 5.3, 90.7 ± 4.7, 87.7 ± 10.4, 102.7 ± 10.4 and 105.6 ± 8.2, respectively. The detection limits for these compounds were 9.38, 10.5, 8.94, 9.00, 9.42, 1.53 and 1.21 nmol L^{-1}, respectively. As protein extraction solvents contained precursors of DCAN, TCAN, 1,1-DCP and 1,1,1-TCP [15], data of these DBPs from algal protein were not reported.

Salmonella typhimurium tester strain TA100 (hisG46, rfa; DuvrB-bio; pKM101, ampr), capable of detecting base pair substitution-type mutagenicity, was selected for the mutagenicity assay. Plate incorporation test was conducted following the Standard Method [17]. Each chlorinated solution was diluted by Milli-Q water into five different doses (6, 12, 25, 50 and 100%), and plating was conducted in duplicate for each dose. Each 2 mL top agar (supplemented with biotin and histidine), containing 100 μL bacterial cell suspension (with the cell number adjusted to an optical density of 0.1 at 600 nm) and 100 μL dosed chlorinated solution, was prepared in a 10 mL glass vial. After pouring plate on minimal agar, the plates were sealed with parafilm, and incubated at 37 °C for 2 days. Negative controls received Milli-Q water solely, while positive controls received sodium azide (100 μL of 1 mg L^{-1} sodium azide per plate).

For the fractionation, briefly, the harvested algal cells frozen in liquid nitrogen were ground in a mortar and pestle for 10 min. The solution was then diluted to 1 mg C mL^{-1} with 1× phosphate buffer saline (pH 7.4). The solution containing the algal total proteins was diluted to 1 mg protein mL^{-1} with 1× phosphate buffer saline (pH 7.4). Both solutions underwent ultrafiltration by Millipore Amicon Ultra 15 centrifugal filter units (ultracel regenerated cellulose membranes) to give a series of fractions with the following ranges of molecular weight (MW): <3 kDa, 3–10 kDa and >100 kDa. Both retentate and filtrate were then analyzed for the concentrations of total organic carbon (TOC), whereas protein levels in the algal fractionated proteins were quantified by Bio-Rad DC Protein Assay kits. The TOC levels in the prepared AOM solutions were analyzed by a Shimadzu TOC 5000 carbon analyzer, and UV absorbance at 254 nm was measured for the proteins using a UV–visible spectrophotometer (Shimadzu UV-1601) according to the Standards Methods [17]. All the measurements were conducted in duplicate and the coefficient of variations were less than 2%. Milli-Q water was used as the blank control.

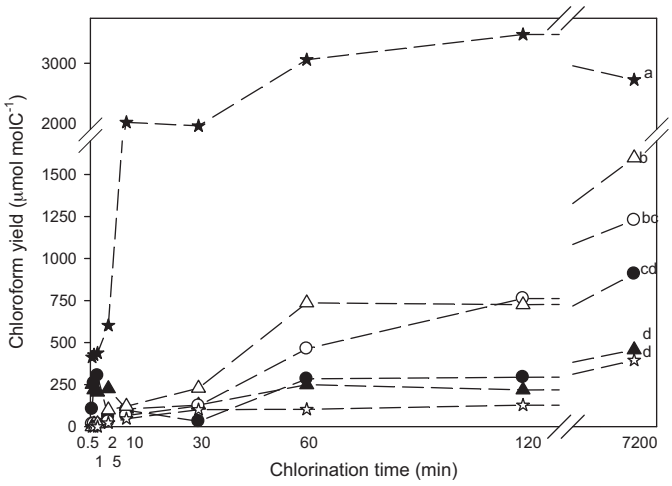

Fig. 1. Chloroform formation (μmol mol C^{-1}) at different reaction time, upon chlorination of different algal cell and protein fractions ((-●-) cell < 3 kDa, (-○-) protein < 3 kDa, (-▲-) cell 3–10 kDa, (-△-) protein 3–10 kDa, (-★-) cell > 100 kDa, (-☆-) protein > 100 kDa). At 7200 min, means with the same letter(s) are not significantly different ($p > 0.05$), according to multiple-comparison of means by one-way ANOVA.

3. Results and discussion

3.1. DBP generation from variable MW algal cells and proteins

Yield of chloroform in all fractions increased with chlorination time, and reached maxima within 120 h (Fig. 1). This is expected as chloroform is one relatively stable end product during chlorination [18–20]. However, fluctuations of the yields were also observed, especially in the fractioned cells. For example upon chlorinating the cell fraction with MW < 3 kDa, chloroform formation at 2 min reached 304 μmol mol C^{-1}, but another 3 min chlorination resulted in 5 times less chloroform generation (59.9 μmol mol C^{-1}). For the cell fraction of 3–10 kDa, higher level of chloroform was produced right after chlorination (0.5 min, 252 μmol mol C^{-1}), 2 times more than that at 10 min (105 μmol mol C^{-1}). As chlorination of algal organic material such as amino acids may generate chloropicrin, which may be converted into chloroform in the presence of reducing agents [21], it is possible that the fluctuations resulted from chloropicrin generation in chlorination and quenching process.

Among the six fractions, the algal cellular materials with MW > 100 kDa was the most potent precursor of chloroform. For example, at end of the reactions, it formed significantly more chloroform (2724 μmol mol C^{-1}, $p < 0.05$) than the other fractions. In contrast, the algal proteins of the same size (>100 kDa) were the least reactive (395 μmol mol C^{-1}). The pattern observed in the fractionated proteins was in consistent with previous observations, that chloroform was the main DBP in chlorinating small-molecular sized precursors [11,23,28]. However, data of the cell fractions did not support this pattern. One probable explanation is that some small algal compounds (<100 kDa), reactive chloroform precursors, may have failed to penetrate the membrane. Also, there were more larger-sized humic substances retained by the membrane. This phenomenon was also reported previously [24], due to a repulsion with ultrafiltration membrane surface that some negatively

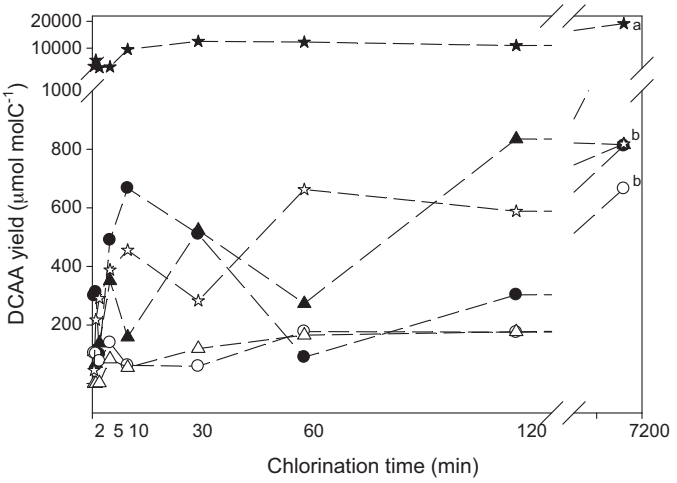

Fig. 2. Dichloroacetic acid (DCAA) formation (μmol mol C^{-1}) at different reaction time, upon chlorination of different algal cell and protein fractions ((-●-) cell < 3 kDa, (-○-) protein < 3 kDa, (-▲-) cell 3–10 kDa, (-△-) protein 3–10 kDa, (-★-) cell > 100 kDa, (-☆-) protein > 100 kDa). At 7200 min, means with the same letter(s) are not significantly different ($p > 0.05$), according to multiple-comparison of means by one-way ANOVA.

charged molecules might not be able to pass through the membrane [25].

The cell fraction of MW > 100 kDa had an SUV_{254} value of 4.52 L mg^{-1} m^{-1} (Supplementary data, Table S1), indicating a rich aromatic contents similar to humic substances, was also the most reactive in generating chloroform. The link between the SUV_{254} values and chloroform yields was not surprising. However, the 3–10 kDa algal protein fraction, with a low SUV_{254} value (0.478 L mg^{-1} m^{-1}), was the most reactive in forming chloroform among the three algal protein fractions. This suggested that tryptophan-like proteins, with low $SUVA_{254}$ values, predominated in the protein fractions [26]. The results supported that $SUVA_{254}$ is only indicative of humic or fulvic type aromaticity [4], but not for predicting chloroform yields in proteins.

Variation in DCAA and TCAA was similar to that of chloroform, that the fraction of larger MW (>100 kDa) generated the most DCAA and TCAA (Figs. 2 and 3), which likewise indicating that

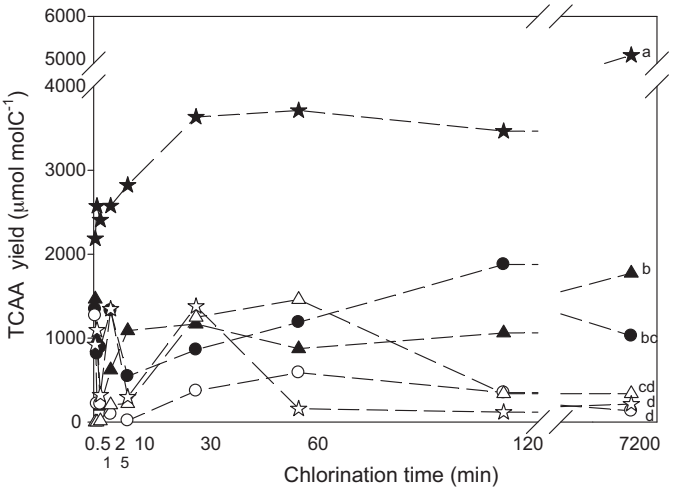

Fig. 3. Trichloroacetic acid (TCAA) formation (μmol mol C^{-1}) at different reaction time, upon chlorination of different algal cell and protein fractions ((-●-) cell < 3 kDa, (-○-) protein < 3 kDa, (-▲-) cell 3–10 kDa, (-△-) protein 3–10 kDa, (-★-) cell > 100 kDa, (-☆-) protein > 100 kDa). At 7200 min, means with the same letter(s) are not significantly different ($p > 0.05$), according to multiple-comparison of means by one-way ANOVA.

Table 1
Dichloroacetonitrile (DCAN) and trichloroacetonitrile (TCAN) formation (mean ± SD, μmol mol C^{-1}) at different reaction time, upon chlorination of different algal cell fractions. Means within each row with the same superscript are not significantly different ($p > 0.05$), according to multiple-comparison of means by one-way ANOVA. N.D.: below detection limit. Detection limit for DCAN was 2.16 μmol mol C^{-1} and for TCAN was 2.26 μmol mol C^{-1}.

Cell	Chlorination time (min)	<3 kDa	3–10 kDa	>100 kDa
DCAN	0.5	765 ± 97.7^{a}	591 ± 21.4^{a}	223 ± 15.6^{b}
	1	465 ± 9.96^{a}	489 ± 69.6^{a}	145 ± 8.29^{b}
	2	506 ± 12.4^{a}	594 ± 49.2^{a}	200 ± 17.7^{b}
	5	428 ± 2.19^{b}	642 ± 18.0^{a}	157 ± 2.77^{c}
	10	267 ± 8.75^{b}	602 ± 2.52^{a}	312 ± 24.2^{b}
	30	13.7 ± 3.63^{b}	133 ± 28.6^{a}	157 ± 20.4^{a}
	60	16.8 ± 2.17^{b}	65.0 ± 0.71^{a}	N.D.^{c}
	120	454 ± 128^{ab}	674 ± 148^{a}	118 ± 11.7^{b}
	7200	127 ± 23.9^{b}	670 ± 10.5^{a}	N.D.^{c}
TCAN	0.5	435 ± 79.3^{a}	318 ± 28.3^{a}	265 ± 28.5^{a}
	1	289 ± 57.6^{a}	325 ± 20.1^{a}	195 ± 5.45^{a}
	2	286 ± 16.4^{a}	309 ± 27.0^{a}	203 ± 48.5^{a}
	5	196 ± 22.8^{a}	261 ± 37.2^{a}	229 ± 27.1^{a}
	10	134 ± 3.10^{a}	314 ± 60.5^{a}	205 ± 44.1^{a}
	30	N.D.^{b}	135 ± 24.8^{ab}	199 ± 58.6^{a}
	60	N.D.^{b}	N.D.^{b}	101 ± 0.67^{a}
	120	272 ± 47.1^{a}	360 ± 57.7^{a}	N.D.^{b}
	7200	N.D.^{c}	414 ± 30.6^{a}	228 ± 62.7^{b}

biomolecules other than proteins in algal cells were potent DCAA or TCAA precursors. Yields of TCAN, DCP and TCP fluctuated during the chlorination (Table 2), which may be due to that these DBPs were reaction intermediates during chlorination. For instance, DCAN and TCP generated from algal cell fraction of <3 kDa generally decreased with time (Table 1), but chloroform increased with time (Fig. 1). DCAN, TCAN and TCP probably served as intermediate precursors for DCAA, TCAA and chloroform, respectively [3,24]. Hydrolysis of DCAN can also lead to the formation of chloroform [19]. Thus, in the complex mixtures of chlorinating solutions, it is envisaged that intermediates generated followed by hydrolysis and decomposition, and a longer chlorination gave rise to their transformation into more stable end-products such as chloroform, DCAA and TCAA.

Comparing different fractions, the result showed that out of the 9 chlorination periods, the yields of DCAN from 8 different

Table 2
1,1-Dichloroacetone (DCP) and 1,1,1-trichloroacetone (TCP) formation (mean ± SD, μmol mol C^{-1}) at different reaction time, upon chlorination of different algal cells fractions. Means within each row with the same superscript are not significantly different ($p > 0.05$), according to multiple-comparison of means by one-way ANOVA. N.D.: below detection limit. Detection limit for DCP was 3.26 μmol mol C^{-1} and for TCP was 2.66 μmol mol C^{-1}.

Cell	Chlorination time (min)	<3 kDa	3–10 kDa	>100 kDa
DCP	0.5	786 ± 36.6^{a}	765 ± 82.8^{a}	733 ± 98.3^{a}
	1	743 ± 27.2^{a}	817 ± 78.2^{a}	505 ± 27.9^{b}
	2	760 ± 27.6^{a}	849 ± 32.8^{a}	549 ± 61.3^{b}
	5	622 ± 108^{ab}	835 ± 38.7^{a}	540 ± 24.2^{b}
	10	444 ± 11.1^{c}	791 ± 8.14^{a}	734 ± 17.2^{b}
	30	245 ± 47.5^{b}	617 ± 75.1^{ab}	829 ± 133^{a}
	60	262 ± 51.7^{b}	463 ± 44.1^{a}	68.2 ± 1.79^{c}
	120	751 ± 41.3^{ab}	865 ± 97.6^{a}	586 ± 41.9^{b}
	7200	90.7 ± 8.00^{c}	795 ± 27.1^{a}	425 ± 64.4^{b}
TCP	0.5	621 ± 119^{a}	335 ± 45.0^{ab}	219 ± 46.2^{b}
	1	502 ± 130^{a}	563 ± 153^{a}	268 ± 16.4^{a}
	2	355 ± 41.7^{b}	821 ± 17.2^{a}	370 ± 65.7^{b}
	5	366 ± 190^{a}	292 ± 14.7^{a}	424 ± 64.3^{a}
	10	341 ± 9.21^{a}	386 ± 0.83^{a}	611 ± 168^{a}
	30	155 ± 14.6^{a}	160 ± 42.0^{a}	286 ± 34.1^{a}
	60	86.7 ± 22.8^{a}	241 ± 53.2^{a}	210 ± 35.3^{a}
	120	292 ± 59.5^{a}	96 ± 4.13^{b}	243 ± 6.60^{a}
	7200	N.D.	367 ± 14.0^{a}	125 ± 12.3^{b}

chlorination intervals (except 30 min) of small MW algal cell fractions (<10 kDa) were significantly higher ($p < 0.05$) than those of large MW algal cell fraction (>100 kDa) (Supporting Information, Table S1). The pattern was opposite to that observed in chloroform formation (Fig. 1). Nevertheless, algal cell wall, composed of cross-linked peptide chains of N-acetylglucosamine and N-acetylmuramic acids, may have served as potent DCAN precursors [27]. However, whether the small MW algal cell fractions contained larger amounts of these cell wall materials needs further investigations.

We compared the DBPs formation observed in this study with that reported in other algal species and humic substances (humic and fulvic acid) (Table 3). Chloroform formation from the *Chlamydomonas* cell fractions (5–27 $\mu g\,mg\,C^{-1}$) was lower than the other algae species (26–61 $\mu g\,mg\,C^{-1}$) and humic substance (31–68 $\mu g\,mg\,C^{-1}$). However, the *Chlamydomonas* cell fraction with MW > 100 kDa was the most reactive in producing DCAA (206 $\mu g\,mg\,C^{-1}$) than the other algal species and humic substance ($\leq$71 $\mu g\,mg\,C^{-1}$). In contrast, the TCAA formation from the *Chlamydomonas* cell fractions (14–69 $\mu g\,mg\,C^{-1}$) was similar to that from humic acid (24–67 $\mu g\,mg\,C^{-1}$), and fulvic acid (63–99 $\mu g\,mg\,C^{-1}$). As for *Chlamydomonas* proteins, the chloroform (3.9–16 $\mu g\,mg\,C^{-1}$), DCAA (7.1–13 $\mu g\,mg\,C^{-1}$) and TCAA (1.8–4.6 $\mu g\,mg\,C^{-1}$) formations were within the ranges from the other algal species and humic substance (chloroform: $\leq$147 $\mu g\,mg\,C^{-1}$; DCAA: <72.4 $\mu g\,mg\,C^{-1}$; TCAA: $\leq$99 $\mu g\,mg\,C^{-1}$).

When the yields of chloroform, DCAA and TCAA from different MW fractions were compared with those from other studies, the HAA (DCAA + TCAA) production (275 $\mu g\,mg\,C^{-1}$) from algal cells (>100 kDa) was much higher than that previously reported of an MW fraction (>100 kDa) originated from Myrtile Beach Intercoastal Waterway (125 $\mu g\,mg\,C^{-1}$) [28] (Table 4). On the contrary, algal cell and protein fractions (<3 kDa) produced much less HAAs (than those from the other studies). Similar pattern was observed by Hua and Reckhow [12], that compounds in raw water with MW > 3 kDa produced more HAAs than low MW (<3 kDa).

3.2. Correlations among the DBPs

Generally, algal DOC is more reactive to form DCAA to TCAA with the ratio of TCAA to DCAA of 0.5 $\mu mol\,\mu mol^{-1}$ (pH 7, 20 °C, 7-day reaction) [29], while humic substances have a preference in forming TCAA (TCAA:DCAA = 2.0 $\mu mol\,\mu mol^{-1}$, pH 7, 20 °C, 72 h) [30]. In this study, the ratios of TCAA over DCAA (120 h) ranged from 0.20 to 0.27 $\mu mol\,\mu mol^{-1}$, except for the fractions of <10 kDa from algal cells (TCAA/DCAA: 1.26–2.17 $\mu mol\,\mu mol^{-1}$). A generalized pattern was not observed, and it seems that the ratios of TCAA to DCAA vary with reaction time. Nevertheless, for the algal cell fraction of >100 kDa, the formations of DCAA and TCAA are significantly closely associated ($p < 0.05$) (Fig. 4), with preferential of DCAA formation over TCAA (TCAA:DCAA = 0.16 $\mu mol\,\mu mol^{-1}$).

High correlation coefficients were observed among chloroform, DCAA and TCAA (Supporting Information, Table S2). Along all the 9 chlorination time, chloroform had significant correlations ($p < 0.001$) with DCAA and TCAA, respectively. Additionally, 8 out of 9 chances that DCAA and TCAA were significantly correlated ($p < 0.001$). These correlations were consistent with previous studies analyzing relationships among chloroform, DCAA and TCAA [31,32]. Therefore, it indicated that chloroform concentration could be used to predict the concentrations of DCAA and TCAA, respectively. Moreover, among the measured DBPs, TCP and DCAN were possible intermediate precursors of chloroform, while DCAN and TCAN likely served as intermediate precursors of DCAA and TCAA, respectively [3,24,25]. Bull et al. [31] also observed a moderate correlation between levels of DCAN and chloroform in chlorinated waters across varied source waters in USA. However, correlation analyses in this study did not reveal relevant relationships among these DBPs in this study. On the other hand, significant correlations ($p < 0.05$) were observed between DCAN and TCAN (Fig. 5a), as well as between DCP and TCP (Fig. 5b). These relationships implied these two pairs of DBPs shared similar generation pathways, that TCAN and TCP were generated via additions of a Cl to DCAN and DCP, respectively.

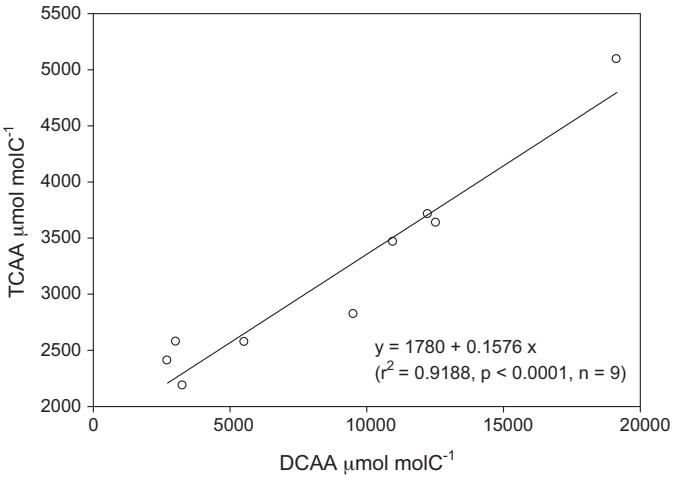

Fig. 4. Regression of TCAA on DCAA base on the data from chlorination of algal cell fraction > 100 kDa in the 9 reaction times.

3.3. Mutagenicity of different algal cell and protein fractions

Algal cell fractions developed two fold increases in the revertants with at least two consecutive dose levels (up to 4512 revertants per plate), compared to the negative control levels (113 revertants per plates) (Fig. 6). On the other hand, chlorinated algal proteins were not found to be mutagenic under the *Salmonella* assays. In order to quantify the mutagenicity of different fractions, mutagenic potency (MP), expressed as induced revertants per liter (rev μL^{-1}) of a sample, was therefore calculated as the slope of the initial linear regression over the linear region of the dose–response curve for each of the chlorinated solutions. All the linear regressions used for the calculation of mutagenic potencies were statistically significant ($p < 0.05$).

The order with the increase of MP value of the three algal cell fractions was: <3, >100 and 3–10 kDa. However, the MP values were not significantly correlated ($p > 0.05$) with the corresponding DBP levels. Further analyses showed that the levels of the DBPs were also insufficient to induce such high MP values [15]. Particularly, even though the algal cell fraction with MW > 100 kDa produced the most amount of total quantified DBPs among the three algal cell fractions throughout the chlorination process (Supporting Information, Fig. S1), the mutagenic potencies of this fraction were the lowest among the three algal cell fractions. This suggests that other unmeasured DBPs or any interactions among the DBPs contributed to the mutagenicity. On the other hand, high mutagenicity developed in the cell fraction of 3–10 kDa (Fig. 6). Hua and Rechow [12] showed that NOM with MW of 3–10 kDa produced the highest percentage of unknown total organic halogen over total organic halogen after chlorination. Whether any unknown organic halogen contributed to the high mutagenicity from the algal cell fraction (3–10 kDa) needs further investigation.

The pattern of variation of MP values with time was consistent with that observed previously [15]. However, fractionated algal proteins after chlorination were not mutagenic during the whole reaction time, different from what we observed previously that chlorinated algal proteins/hydrophobic proteins were

Table 3
Comparisons of DBP formation from algal fractions in the present study with other algae species from previous studies. N.A.: data not available.

	Chlorination time (h)	$CHCl_3$ ($\mu g\,mg\,C^{-1}$)	DCAA ($\mu g\,mg\,C^{-1}$)	TCAA ($\mu g\,mg\,C^{-1}$)
Algal-derived organic materials				
Intra-/extra-cellular materials				
Scenedesmus quadricauda [29]	168	48	35	23
Chlamydomonas [33]	96	34	29	33
Oscillatoria prolifera [29]	168	30	N.A.	N.A.
Oscillatoria [33]	96	26	34	39
Anabaena [34]	168	50	29	49
Microcystis [34]	168	61	71	93
Chaetoceros mulleri [29]	168	29	N.A.	N.A.
Nitzschia [33]	96	48	25	19
Chlamydomonas cell fraction[b]				
>100 kDa	120	27	206	69
3–10 kDa	120	5	9.6	24
<3 kDa	120	8.7	8.3	14
Cellular biomolecules				
5 aromatic a.a.[a] [32]	96	2.57–147	17.3–58.2	4.92–60.9
15 non aromatic a.a. [32]	96	<4.19	<72.4	<17.63
5 aromatic a.a. [25]	1	N.A.	N.A.	N.A.
18 non aromatic a.a. [25]	1	N.A.	N.A.	N.A.
Plant starch [33]	96	3	5	0
Chlamydomonas protein fraction[b]				
>100 kDa	120	3.93	8.79	2.91
3–10 kDa	120	15.9	13.2	4.60
<3 kDa	120	12.2	7.15	1.80
Humic substances				
Humic acid [30]	72 h	31–51	12–25	24–67
Fulvic acid [30]	72 h	47–68	24–32	63–99

[25]: Cl_2 dose: 10 mg L^{-1}; [32]: Cl_2 dose: 10:1 mg Cl_2:mg C; [29]: Cl_2 dose: 5:1 mg Cl_2:mg C; [30]: Cl_2 dose: 20 mg L^{-1}); [33]: Cl_2 dose: 10:1 mg Cl_2:mg C); [34]: residual Cl_2 > 0.5 mg L^{-1}; present study (Cl_2 dose: 20:1 mg Cl_2:mg C). All the chlorination experiments from the above references were conducted at pH 7, 20 °C.
[a] a.a.: amino acids.
[b] Present study.

Table 4
Comparison of DBPs formation from algal MW fraction with other MW fractions.

Fractions (kDa)	Molecular weight (kDa)	Source	THM ($\mu g\,mg\,C^{-1}$)	DCAA ($\mu g\,mg\,C^{-1}$)	TCAA ($\mu g\,mg\,C^{-1}$)	HAA_9 ($\mu g\,mg\,C^{-1}$)
>100 kDa	>100	MBIW [28]	92			125
		Algal cell[b]	27	206	69	275[a]
		Algal protein[b]	12	7.2	1.8	9.0[a]
3–10 kDa	5–10	PHRW [35]	12	14	8	
		TCHC [35]	12	10	13	
		AHC [35]	6	8	5	
		MBIW [28]	>72			>100
	6	IRW [22]	1.3			
	>5	TRW [28]	>20			>30
	3–5	MBIW [28]	>90			>110
		TRW [28]	>55			>60
	3	IRW [22]	5.3			
	Min–max		1.3–90	8.0–14	5.0–13	30–110
	3–10	Algal cell[b]	5	9.6	24	34[a]
		Algal protein[b]	16	13	4.6	18[a]
<3 kDa	1–3	MBIW [28]	>60			>70
		TRW [28]	>65			>75
	1.7	IRW [22]	2.98			
	1	IRW [22]	0.28			
	<1	PHRW [35]	55	65	60	
		TCHC [35]	16	15	18	
		AHC [35]	17	21	10	
		IRW [22]	1.01			
		MBIW [28]	>50			>50
		TRW [28]	>55			>60
	Min–max		0.28–65	15–65	10–60	50–75
	<3	Algal cell[b]	8.7	8.3	14	22[a]
		Algal protein[b]	3.9	8.8	2.9	12[a]

MBIW: Myrtile Beach Intercoastal Waterway; TRW: Tomhannock reservoir water; IRW: Iowa River water; PHRW: Pan-Hsin raw water; TCHC: Tokyo chemical-humic acid; AHC: Aldrich humic acid.
[a] HAA yield is equal to the sum of DCAA and TCAA.
[b] Present study.

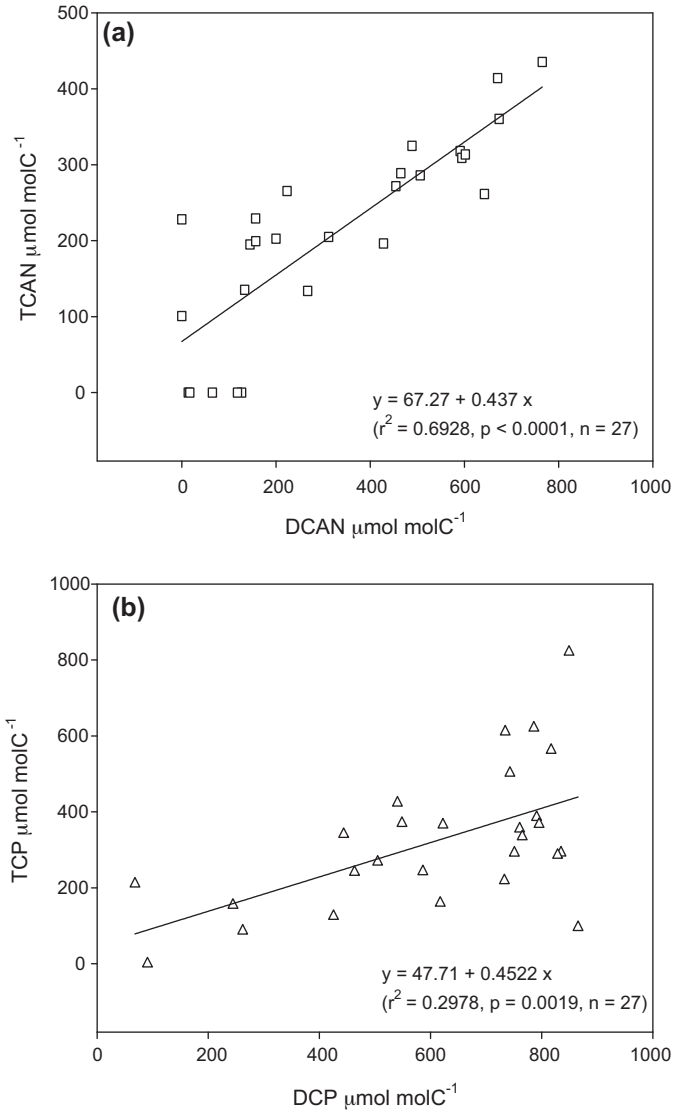

Fig. 5. Correlations between (a) DCAN and TCAN based on data from chlorination of both algal cell fractions and (b) DCP and TCP based on data from chlorination of algal cell fractions, in 9 reaction times.

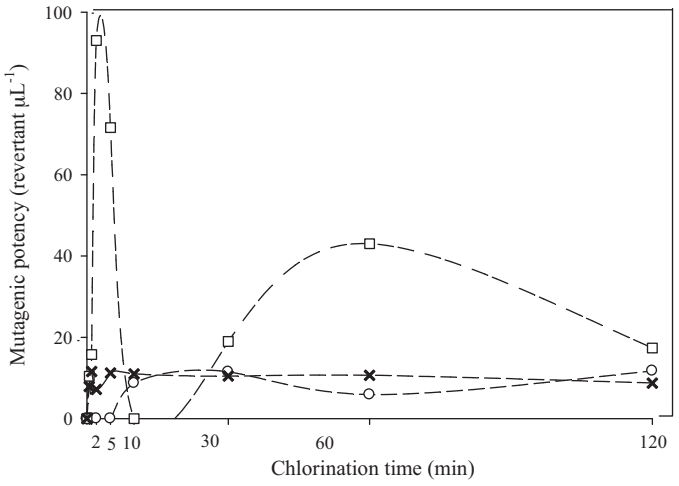

Fig. 6. Time-dependent changes in mutagenic potency of chlorinated algal cell fractions ((×) >100 kDa; (□) 3–10 kDa; (○) <3 kDa).

highly mutagenic [15]. This infers that mutagenic intermediates generated in chlorinating algal hydrophobic protein might be a result of chlorination of a mixture of precursor consisting variable sizes instead of just one single size. It is also possible that some of the mutagens in chlorinated hydrophobic protein were lost or degraded during protein fractionation. Whether fractionation caused a dilution or loss in mutagenicity remains unknown.

4. Conclusions

This is the first study that fractionated algal cells and proteins were examined for DBP formation and mutagenicity. Algal cells of varied molecular sizes were more reactive than fractionated algal proteins as the precursors of DBPs such as HAAs indicating that these DBPs production from the algal cells is not simply contributed by the algal proteins but some other algal biomolecules. Further comparisons with previous studies show that the *Chlamydomonas* cells could serve as the precursors of HAAs, DCAN and DCP, as important as humic and fulvic acids. Generally, large MW fractions (>100 kDa) produced more stable DBPs (chloroform, DCAA and TCAA), while small fractions of 3–10 kDa were the major precursors of HANs and HKs upon chlorination. Similar to previous studies, yields of chloroform, DCAA and TCAA from chlorinated algal fraction were closely correlated. For mutagenicity by *Salmonella* Ames assay, algal cell fraction of 3–10 kDa was the most mutagenic, followed by the >100 kDa fraction and lastly the <3 kDa fraction. Fractionated algal proteins however were not found to be mutagenic. The high levels of DBPs quantified in large MW fractions did not necessarily lead to higher mutagenicity; instead level of unstable DBPs such as DCAN and TCAN generated played a more important role. The mutagenicity of the chlorinated solutions changed with chlorination time, indicating the presence of potent mutagenic intermediates after short chlorination period.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhazmat.2012.01.023.

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