Carbohydrates in size fractionated dissolved organic matter in a station of the Bay of Bengal

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Received 12 July 2006; revised 18 June 2007

Seawater samples were collected from 4 depths (2, 100, 500 and 1000 m) at one station in the Bay of Bengal. Very high molecular weight (VHMW) (>30 kDa to 0.2 µm) and high molecular weight (HMW) (10 to 30 kDa) fractions of the dissolved organic matter (DOM) were collected using Amicon stirred Ultrafiltration Cell, and analysed for dissolved organic carbon (UDOC), total carbohydrates (UTCHO) and neutral sugars (UNS). UDOC concentrations were relatively higher in HMW fraction whereas, UTCHO and UNS were relatively enriched in VHMW fraction. Concentrations of UDOC, UTCHO and UNS generally decreased with increasing water depth. The UNS-C accounted for 2.2 to 6.2% of UDOC and generally decreased with depth. The observed decrease suggests utilization by \textit{in situ} heterotrophic organisms. Monosaccharide composition data suggest that rhamnose, fucose and galactose were relatively more abundant in VHMW fraction, as well as glucose in HMW fraction. The weight percentage fractions of most of the monosaccharides of both VHMW and HMW did not show consistent decrease with depth. The yields and concentrations of UNS indicated a size-related diagentic sequence where VHMW represents the most reactive fraction.

\textbf{Introduction}

Carbohydrates are common structural and storage components of both marine and terrestrial organisms. These compounds are derived from various sources including algal extra cellular secretions and auto-lysis, viral and bacterial lysis of phytoplankton cells, sloppy feeding and egestion by grazers, microbial degradation of organic matter, and the release and physical transport of terrestrial organic matter\textsuperscript{1}. In natural seawater carbohydrates are mostly present as polymeric form\textsuperscript{2,3}, and may account for 20 to 30\% of dissolved organic carbon (DOC) in surface seawater\textsuperscript{2}, and \approx50\% of the ultrafiltered dissolved organic carbon (UDOC) in marine surface waters\textsuperscript{4}. Several functions have been ascribed to carbohydrates in many environmental processes. For example, carbohydrates are involved in formation of humic substances\textsuperscript{5}, removal of dissolved metal ions, enhancing and/or inhibiting metal deterioration\textsuperscript{6,7}, microbial adhesion and biofilm formation\textsuperscript{8,9}, flocculation of DOM and production of macroaggregates\textsuperscript{10}.

Rapid decrease in their concentration with increasing water column depth indicates their reactive nature. Therefore, they are useful substrates for heterotrophic metabolism\textsuperscript{2,3,11,12}. Recent studies\textsuperscript{13-16} on carbohydrate distribution in various size fractions of dissolved organic matter (DOM) collected from various seas and oceans suggest that carbohydrates in high molecular weight (HMW) fraction (>1 kDa cut off) of the organic matter are rapidly consumed in surface waters, whereas carbohydrates of low molecular weight (LMW) fraction (<1 kDa cut off) persist at greater depths of the ocean. This indicates that HMW fraction of organic matter is more labile and bio reactive, and is of more recent origin compared to the LMW fraction. Furthermore, carbohydrate composition data indicate that ultrafiltered neutral sugars (UNS) are potentially useful to assess the lability and degradation state of organic matter\textsuperscript{15,16}. Therefore, there is a need to obtain more information on the distribution and molecular characterization of carbohydrates in various size fractions of organic matter, so as to assess their role in the dynamics of organic matter in marine waters.
In the present study, HMW fractions of DOM were isolated by ultrafiltration from one location in the Bay of Bengal. These samples were then used for the analysis of UDOC, UTCHO and the UNS concentration and composition so as to investigate the relationship between size and diagenetic state of organic matter.

Materials and Methods

Seawater samples were collected on 12th August 2001 from four discrete depths (2 m, 100 m, 500 m, and 1000 m) using Niskin water samplers (30 liter) attached to the CTD rosette system during 166th cruise of ORV Sagar Kanya at a station 09° 08’ 48.9” N, 88° 32’ 18.5” E in the southern region of the Bay of Bengal was sampled. Immediately after collection, water samples were passed through a 200 µm screen to remove different macro zooplankton and preserved with 3 mg/l of HgCl$_2$. Samples were filtered (8 to 10 liter) through pre-ashed (450°C) 142 mm GF/F (0.7 µm pore size) filters followed by 142 mm Nucleopore (0.2 µm) filters using in situ Water filtration equipment (Sea Star, Canada). Very high molecular weight (VHMW) (30 kDa to 0.2 µm) fraction was collected using an Amicon stirred cell assembled with a 30 kDa cut off cellulose membrane filter (# YM 30). The filtrate (<30 kDa) was then passed through 10 kDa filter (# YM 10) to collect HMW (10 kDa to 30 kDa cut off) fraction of the DOM. In general, the working pressure in the cell was kept at ~30 psi using AR grade nitrogen. Before running the sample, the membrane was cleaned and rinsed three times with the sample water.

In general, 8 to 10 liter water sample was concentrated to 50 ml, yielding a concentration factor of 160 to 200. The concentrated ultrafiltered water sample was then dialyzed using a dialysis bag of 8 kDa cut off to remove salts and made to a known volume. Samples were then frozen at −20°C until analysis. DOC was determined by catalytic high temperature method (Shimadzu TOC analyzer). Ultrafiltered total carbohydrates (UTCHO) were determined by the phenol - sulphuric acid method\textsuperscript{9}. When a standard containing 1 mg/l of 50 kDa and 25 kDa dextran was prepared in artificial seawater and processed through the entire protocol described above gave a recovery of ~60%. A sample containing a known amount of carbohydrates was used to assess the mass balance. Carbon mass balance indicated that carbohydrates recovered in the concentrate and permeate accounted for 83% of the initial carbohydrates, showing that carbohydrate contamination or losses were minor during the ultrafiltration process.

The monosaccharide composition of the ultrafiltered samples was determined by the capillary gas chromatographic technique described earlier\textsuperscript{5}. Briefly, a known volume of the ultrafiltered sample was concentrated to dryness using rotary evaporator and treated with cold 12 M H$_2$SO$_4$ for 2 h at room temperature (28 ± 2°C). The sample was diluted to 1.2 M H$_2$SO$_4$ using cold distilled water, flushed with nitrogen gas, sealed, and then hydrolyzed for 3 h at 100°C. After cooling, an internal standard (inositol) was added to the hydrolysate. Subsequently, the solution was neutralized, treated with an excess of NaBH$_4$, acetylated and analysed using a capillary gas chromatograph (Chrompack GC model CP-9002). The GC was equipped with a fused silica capillary column coated with CPSil-88 (25 m, id = 0.32 mm), a flame ionization detector (FID) and a programmable on-column injector. At the time of injection the on-column injector was maintained at 70°C and then the temperature was rapidly raised to 300°C at 30°C min\textsuperscript{−1} and then maintained at this temperature until the end of the sample run. The detector was maintained at 300°C. A sample (0.4 µl) was injected when the initial oven temperature was 70°C. The oven temperature was rapidly raised to 150°C at 30°C min\textsuperscript{−1}, and thereafter at 3°C min\textsuperscript{−1} to 230°C, and maintained at this temperature for 10 min. Quantification of the components was achieved by peak area integration of the GC results using Maestro Chromatographic data Handling System installed in the instrument. In this study the sum of all the identified aldoses has been defined as neutral sugars. The contribution of individual monosaccharides to the total neutral sugars is expressed as wt %.

Results and Discussion

The concentrations of UDOC in both VHMW and HMW were higher in the surface waters that decreased with depth, especially in the upper 100 m of the water column (Fig. 1A). Below 100 m, UDOC concentrations in both these fractions of the DOM showed small variations.
As observed for UDOC, the concentrations of UTCHO in VHMW DOM were relatively higher in the surface waters and decreased with increasing water column depth. The HMW DOM showed a little different pattern at this location (Fig. 1B). The UTCHO concentration in HMW was low in surface waters and showed an increase at 100 m followed by a decrease for the subsequent sampling depths of the water column. A sub-surface chlorophyll a maximum at 60 m at this station may be responsible for the observed increase in HMW DOM at this depth (Ramaiah N, personal communication). The concentrations of UTCHO showed significant positive relationships with the concentrations of UDOC of VHMW DOM ($R^2 = 0.9323; N = 4, P > 0.001$) and relatively less significant positive relation with HMW DOM ($R^2 = 0.4234; N = 4, P > 0.1$).

UNS concentrations in both size fractions of DOM were higher at surface water (Fig. 1C) as compared to those observed at greater depths (>100 m). Concentrations of UNS in VHMW and HMW decreased about two-fold with depth (Fig. 2B). The concentrations of UNS in VHMW showed significant relationship ($R^2 = 0.9209, N = 4, P > 0.001$) with UDOC of VHMW. UNS did not show significant positive relationship with UDOC in HMW size fraction ($R^2 = 0.2388$).

The concentration and the composition of carbohydrates may be influenced by the nutrient status, phytoplankton species composition and/or growth phase of organisms. During the cruise, nitrate and inorganic phosphate-phosphorus were almost undetectable in the upper 20 m water column at this station (Sardessai S, personal communication). Therefore, it is likely that the nutrient limitation may have influenced the UNS distribution in different size fractions at this station. Differences in the concentration of UNS with depth in various size
fractions may also reflect the variability in the biochemical composition of plankton community, growth phase of plankton or process resulting in the formation and removal of carbohydrates at this study site. The UTCHO- C% accounted for 9.6 to 42% and 6.3 to 39% of UDOC in VHMW and HMW size fractions of the DOM, respectively (Fig. 2A). UNS yield varied from 2.2 to 6.2%. The UNS yields of VHMW decreased with depth (Fig. 2B). VHMW material of the DOM had a higher content of UNS. Moreover, the yields were always higher for VHMW compared to HMW organic matter collected at various depths.

UNS yields for the freshly produced UDOM from different marine phytoplankton reported to vary from 20 to 40%, and it generally decreased with increasing decomposition of organic matter and depth. Therefore, UNS yield is useful tool to assess the diagenetic alteration of organic matter. The decrease seen in VHMW fraction with the depth indicates that the UNS are relatively reactive components that are selectively removed from organic matter as it ages in the ocean. The higher yield observed in the surface samples, which decreases with the increase in the depth suggests the presence of diagenetically altered organic matter at greater depths of the Bay of Bengal. The higher yield observed in the VHMW compared to HMW agrees well with the suggested size continuum model that the reactivity of the molecule decreases with the size. It is important, however to know that HMW DOM is not completely un-reactive in surface waters. This was evident from the decrease in UNS with depth observed for the HMW fraction at this station.

The UNS composition (as wt%) of the VHMW and HMW size fractions is shown in Fig. 3. The composition of UNS in size fractionated DOM changed substantially between surface waters and 1000 m. Glucose, galactose, mannose, xylose, arabinose, ribose, fucose and rhamnose were detected in both VHMW and HMW size fractions collected from various depths at this station. With the exception of glucose, the relative abundance of most of the other
monosaccharides decreased with depth for both the fractions of the organic matter. In addition, glucose was the most abundant monosaccharide followed by xylose, mannose and galactose in HMW DOM. It was interesting to note the increasing abundance of glucose with depth in HMW size fraction of the DOM. At greater depths (>100 m) glucose accounted for 50 to 61% of the total monosaccharides of HMW fraction of the organic matter. In addition, glucose monosaccharides decreased with depth for both the DOM. At greater depths (>100 m) glucose accounted for 50 to 61% of the total monosaccharides of HMW DOM. In addition, glucose monosaccharides decreased with depth for both the DOM.

These UNS are of common occurrence in the UDOM collected from various seas and oceans. Of these, galactose, rhamnose and fucose were most abundant in VHWM fraction of the DOM whereas, glucose was most abundant in HMW DOM at this station. Abundance of deoxy sugars (rhamnose and fucose), galactose and fucose in HMW fraction (>1 kDa) of DOM has been reported from other seas and oceans. The observed UNS composition may be due to the abundance of these surface-active monosaccharides in phytoplankton exudates. The decrease in the other monosaccharides except glucose suggests rapid utilization of these monosaccharides. Furthermore, increasing wt % glucose with depth indicates selective preservation of glucose-rich structural polysaccharides in degraded organic matter. The presence of all these sugars indicates that the UDOM is mostly made up of heteropolysaccharides. Furthermore, the abundance of similar sugars in the UDOM isolated from terrestrial and marine origins indicates that degradational processes rather than sources determine the UNS composition.

Dissolved carbohydrate is an important fraction of DOM in the Bay of Bengal. In summary, DOC was relatively more abundant in HMW DOM, whereas UTCHO and UNS were more abundant in VHWM size fraction. UNS concentrations and yields generally decreased with depth suggesting bioreactive or labile nature of these compounds. Monosaccharide data indicates that rhamnose, fucose, galactose and glucose were abundant in VHWM and HMW size fractions of DOM. Concentration of monosaccharides other than glucose generally decreased with depth. UNS were relatively more abundant in VHWM suggesting that this fraction was relatively more labile.

Acknowledgement
Authors thank Dr Satish R Shetye, Director for encouragement and facilities. We also appreciate the help rendered by Dr Prasanna Kumar and other scientists on board the ship during the sample collection and processing, and Mr A P Selvam his help in running gas chromatography. We also thank the Department of Ocean Development for financial support to carry out the studies. This is NIO contribution No. 4191.

References


