腐植栄養湖白駒池における溶存有機物の微生物分解

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Microbial decomposition of dissolved organic matter in a humic mountain lake

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Abstract

In Lake Shirakoma-ike, a small humic lake in a subalpine area, high-molecular-weight (HMW: >1 kDa) fractions predominated the total dissolved organic matter (DOM, filtrate through Whatman GF/F filter) from April to November, 1999. The annual average proportion in DOM size fraction in the surface water were as follows; 100 kDa<: 11 ± 7%, 10-100 kDa: 41 ± 11%, 1-10 kDa: 23 ± 9%, and <1 kDa: 25 ± 6%. In Lake Shirakoma-ike, the concentration of DOM increased dramatically during the snowmelt and rainy season (mid-May–early July), following the increase in the inflow stream by one week. The incubation experiments using each filtrate under a laboratory condition showed higher decomposition ratios of DOM obtained during the spring inflow event in the lake surface. The annual average decomposition ratios of low-molecular-weight (LMW: < 1kDa) DOM were significantly higher than those of HMW DOM both in the lake surface and the bottom waters throughout the study period; < 1kDa: 18.2 ± 9.5%, n = 18, 1-100 kDa: 8.4 ± 8.3%, n = 18, *t*-test, $P < 0.01$. The amount of DOM decomposed, however, was larger in the HMW (10-100 kDa) fraction than in the LMW (<1 kDa) fraction both in the lake and in the inflow waters.

キーワード：溶存有機物，分子量，微生物分解，硝酸，腐植栄養湖
Keywords: dissolved organic matter, molecular size, bacterial consumption, nitrate, humic lake

Introduction

Various characteristics of natural dissolved organic matter (DOM), such as chemical composition, molecular size, origin, and photochemical properties, affect bacterial decomposition of DOM in aquatic environments (Imai et al. 1998; Sun et al. 1997; Volk et al. 1997). In addition to those inherent characteristics, environmental factors such as inorganic nutrients and sunlight (photodegradation) also control bacterial utilization of DOM in humic waters (Tulonen et al. 1992; Bertilsson et al. 1998). Amon and Benner (1996) reported that HMW DOM (>1 kDa) was biologically more reactive than LMW DOM (<1 kDa), because that HMW components tend to be more fresh and less diagenetically altered than LMW components in oceanic and freshwater environments. Seasonal change in quantity and quality is quite remarkable in DOM in freshwater environments (Nishii et al. 2001; Stepanauskas et al. 1999). Studies on seasonal change in bacterial utilization of DOM, however, are still limited in freshwater environments. DOM drained from a watershed might also greatly affect bacterial decomposition of DOM in freshwater environments. Seasonal change in DOM in lake (or pond) and drainage waters may be marked in headwaters. It is expected to be easier to observe seasonal change in bacterial decomposition of DOM in such headwaters. Our study site, Lake Shirakoma-ike, is a small humic lake in a subalpine area in the temperate zone. The biomass of phytoplankton in this lake is lower than that of zooplankton, and DOM, a potentially important energy source in this humic lake ecosystem, might be transferred to higher trophic levels via bacteria through the microbial food chain. In this lake, we studied seasonal fluctuation in the bioavailability of DOM, with special attention to their molecular size. On a weekly basis, we measured the concentration, size composition, and bacterial decomposition of DOM using an ultrafiltration technique and a batch culture experiment under seminatural conditions during the ice-free season.

Materials and Methods

Lake Shirakoma-ike is a small humic mountain lake in Nagano Prefecture, central Japan (36°03' N, 138°22' E; 2115 m above sea level; surface area, 0.11 km^2; maximum depth, 8.6 m). This lake is surrounded by coniferous primeval forests composed of *Abies veitchii* and *Tsuga diversifolia*. The west coast is sandy with aquatic plants (*Sparganium glomeratum* and *Potamogeton fryeri*), and the other coasts of the lake are rocky. The lake water is brown due to high allochthonous input of DOM from the catchment, and inflow streams are observed only during the snowmelt and rainy seasons (usually May–July), and during heavy rains. Crustacean zooplankton (*Daphnia longispina* and *Acanthodiaptomus pacificus*) are abundant, but no fish occur in the lake. The biomass of zooplankton is much higher than that of phytoplankton (M. Yoshida, personal communication).

Sampling was conducted every week at the lake center (8.5 m depth) from 23 April to 18 November 1999. Water temperature, pH, electric conductivity (EC), transparency, and dissolved oxygen (DO) were measured using electronic meters (YSI Model 55 DO meter and YOKOGAWA Model pH 81 personal pH meter). Water samples were taken with a 6L Van Dorn sampler from five depths (0, 2, 4, and 6 m below the water surface and 1m above the lake bottom (hereafter referred to as b-1m)), and transferred to a laboratory within 2 h in acid-washed polyethylene 5 L bottles kept cool in an insulated box. Inflow water was collected when an inflow stream was observed. Each sample was filtered with a glass fiber filter (Whatman GF/F filters precombusted at 480°C for 2 h). The filtrate was then placed in ultrafiltration equipment (Amicon model 8050 with 50 ml stirred cell) and filtered consecutively with membrane filters (Amicon YM100, YM10, and YM1) to collect molecular size fractions of <100, <10, and <1 kDa. All equipment and membrane filters were rinsed extensively with Milli-Q water before use to avoid contamination. The filtrates were kept frozen (–20°C) in glass vials (precombusted at 480°C for 2 h) with Teflon-lined caps until analysis. All samples were kept cool with ice until incubation experiments.

We investigated the decomposition of various DOM size fractions by bacteria using incubation experiments primarily following Servais et al. (1987, 1989). We estimated the bacterial decomposition of DOM as its decrease under a laboratory condition using the filtrates of lake and inflow water. Since inorganic nutrients (N and P) were not added in the incubation experiments, mineralized organic carbon was not equal to the potential biodegradable DOM but to biodegraded DOM under *in situ* nutrient conditions. Water samples taken from the lake center (2 m and b-1 m) and the inflow stream were separated into four fractions (<GF/F, <100 kDa, <10 kDa, and <1 kDa) by filtering as described above. Samples filtered with a GF/A filter (precombusted, equivalent to 1.6µm pore size) was added (1% of the sample volume) to these size-fractionated samples as an inoculum. Although we did not test the efficiency of bacteriovore removal from the inoculum, heterotrophic protists in the 2-20 µm size class were assumed to be the principal predators of bacteria (Gasol et al. 1995), and thus the GF/A filter should have removed bacteriovores. These samples were shaken in advance, and incubated in glass bottles (one 1L bottle for the <GF/F sample and duplicate 50 ml vials for the fractionated samples) at 20°C in the dark for 60 days without shaking. All glassware was washed with 5% HCl acid solution, rinsed with distilled water, and combusted at 480°C for 2 h before use. Water samples (10 ml) were taken from each bottle before and after the incubation, and kept frozen (–20°C) in glass vials with Teflon-lined caps until analysis for DOC. Before DOC determination, the frozen samples were thawed and then inorganic carbon was purged by bubbling with N_2 gas (CO_2-free) after acidification to pH 2 with 6 N HCl. The DOC (non–volatile DOC) concentration was measured with a total organic carbon analyzer (Shimadzu TOC-5000). DOC analyses were continued until an average coefficient of variation (CV) of 2% or less was reached.

The amount of biodegraded carbon in each incubation bottle was calculated as the difference in the DOC concentration of each size fraction between the beginning and end of the experiment. Decomposition ratio of DOM (%) is defined as amount of DOC decreased divided by amount of DOC initially present in each fraction). Although the DOC concentration of all fractionated filtrates decreased due to bacterial decomposition in the incubation experiments, decomposition ratios of the largest size fraction (100 kDa<) often showed negative values due to its small amount in the filtrates. We showed the decomposition ratios only for the other size fractions (<1, 1-10 and 10-100 kDa) in this paper. A negative decomposition ratio was also observed for the other size fractions, when we treated it as 0%.

In order to check bacteria population during incubation experiments, bacterial numbers and frequency of dividing cells (FDC) were determined by the acridine orange direct count method (Hobbie et al. 1977; Hagström et al. 1979) for some incubation bottles. The increase in bacterial number and frequency of dividing cells was observed in all the bottles inspected. Although any sign of periphyton, precipitation and adhesion was not noticed in the incubation bottles in our experiments, a slight decrease in DOC concentration (about 0.4%) was observed during the incubation period in autoclaved GF/F filtrates in a preliminary experiment.

The concentrations of ammonium and reactive phosphorous (PO_4-P) were determined according to Solorzano (1969) and Murphy and Riley (1962). Total dissolved nitrogen (inorganic + organic nitrogen) was measured with a modified alkaline persulfate oxidation technique (Solorzano and Sharp 1980), and nitrate and nitrite were measured using ion chromatography (DIONEX DX-120). We calculated the dissolved organic nitrogen (DON) by subtraction (total nitrogen – inorganic dissolved N), and determined C:N ratio for DOM.

The concentrations of particulate organic carbon (POC) and particulate organic nitrogen (PON) were measured for particulate matter (PM) retained by GF/F filter with a CHN analyzer (Yanaco MT-5), and C:N ratio was also calculated for PM.

Absorbance at 260 nm were measured for fractionated samples before and after each incubation experiment, using a spectrophotometer with a 1 cm quartz glass cuvette. The absorbance at 260 nm is considered to be an index for the amount of humus-, lignin- and tannin-like compounds in water (Tambo and Kamei 1987; Fukushima et al. 1996, 1997; Imai et al. 1998).

Results

The water temperature in the lake surface (2 m) increased from 5°C in May to 21°C in early August, and then decreased to 4°C in late November. A gentle thermal stratification was observed between June and mid-September, with complete mixing beginning in October. The average temperature was 14°C in the lake surface and 10°C in the lake bottom (b-1 m) during the ice-free season.

The DO concentration was low (0.5-6.4 mg O_2 L^{-1}) in the hypolimnion from mid-June to mid-October. The pH varied between 4.0 and 5.8, with a slight vertical gradient, and the chlorophyll-*a* concentration in the surface layer was between 0.4 and 1.3 μg L^{-1} (M. Yoshida, personal communication).

Inflow was mainly observed during the snowmelt season (April–June) and the rainy season (June–July), and irregularly on heavy rain days in summer. The nitrate concentration varied between 0.01 and 0.15 mg N L^{-1} in the inflow stream, and between 0.01 and 0.07 mg N L^{-1} in the lake. High nitrate concentrations were observed during the snowmelt and rainy season (from mid-June to mid-July) in the inflow stream and in the lake. The nitrite concentration was below the detection limit in almost all samples in the inflow stream and in the lake. The ammonium concentration ranged from 2 to 10 μg N L^{-1} in the inflow stream, from 4 to 17 μg N L^{-1} in the lake surface, from 5 to 80 μg N L^{-1} in the lake bottom. The concentration of reactive P (PO_4-P) was consistently low (~12 μg P L^{-1}) in the inflow stream and in the lake.

The DOM concentration varied between 1.5 and 7.3 mg C L^{-1} in the inflow stream, and between 2.4 and 6.3 mg C L^{-1} in the lake (Fig. 1).

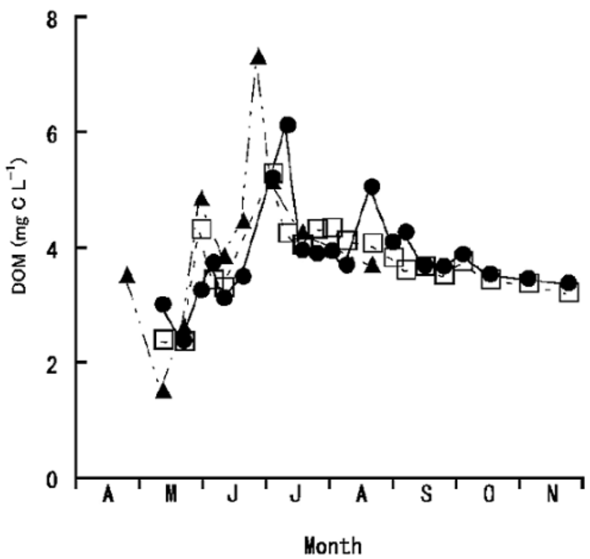

Fig. 1. Seasonal change of dissolved organic matter (DOM) in Lake Shirakoma-ike from April to November, 1999.
▲: at inflow, ●: 2m below lake surface, □: 1m above lake bottom (b-1m)

A large increase in DOM concentration was observed in the inflow stream and in the lake during snowmelt and the rainy season. The maximum DOM concentration in the inflow stream preceded that in the lake by 1 week.

The size composition of DOM was rather stable in the lake during the study period, with the 10-100 kDa fraction predominating. The annual average size composition of DOM was 100 kDa<: 11 ± 7%, 10-100 kDa: 41 ± 11%, 1-10 kDa: 23 ± 9%, and <1 kDa: 25 ± 6% (n = 12) in the lake surface, and 100 kDa<: 5 ± 3%, 10-100 kDa: 43 ± 8%, 1-10 kDa: 28 ± 10%, and <1 kDa: 23 ± 6% (n = 8) in the lake bottom.

The decomposition ratio of DOM showed a remarkable seasonal variation in the lake surface (Fig. 2b).

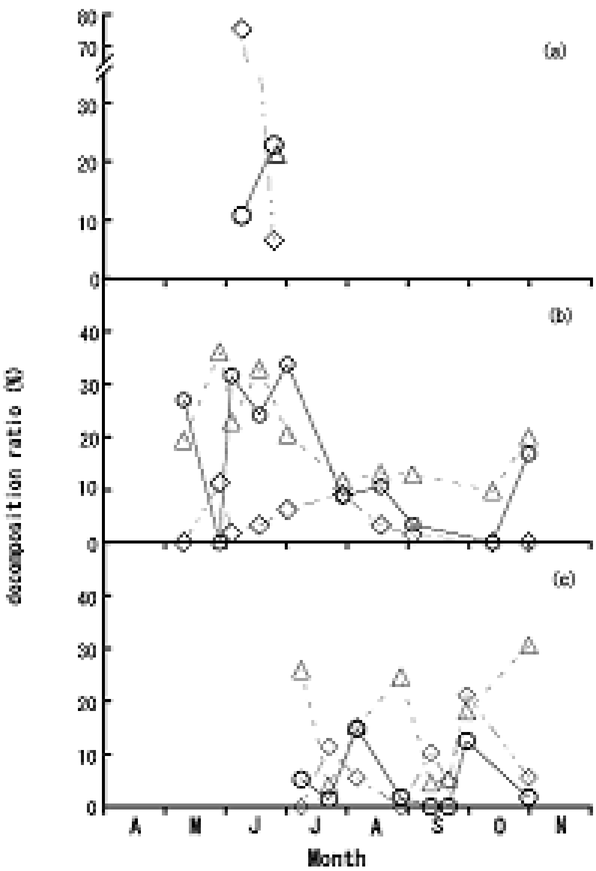

Fig. 2. Seasonal change in decomposition ratio of DOM in Lake Shirakoma-ike from April to November, 1999.
(a) at inflow, (b) 2m below lake surface, (c) 1m above lake bottom ○: 10-100kDa, ◇: 1-10kDa, △: <1kDa

The higher decomposition ratios were observed in the smallest size fraction (<1 kDa) and the largest size fraction (10-100 kDa) during spring inflow event in the lake surface. The ratios decreased thereafter and remained low during the summer stratification period at in the lake surface. The decomposition ratios of each size fraction were relatively low in the lake bottom, except for some high values of the smallest size fraction (Fig. 2c). The annual average decomposition ratio of the smallest size fraction (LMW fraction: <1 kDa) was significantly higher than the larger size fraction (HMW fraction: 1-100 kDa) in the lake (LMW: 18.2 ± 9.5%, HMW: 8.4 ± 8.3%, n = 18, t-test, P < 0.01). There was, however, no negative relationship between decomposition ratio and molecular size among the three fractions (<1 kDa, 1-10 kDa, and 10-100 kDa). A positive relationship was noticed between the decomposition ratio of HMW DOM fractions and nitrate concentration in the lake surface during the summer stratification period (10-100 kDa: r = 0.83, P < 0.01, n = 6), but the relationship was weak in the lake bottom. The concentrations of ammonium and phosphate were not correlated with DOM decomposition ratio except for <1kDa in the lake surface (<1 kDa: r = -0.79, P < 0.01, n = 10).

A large amount of DOM was decomposed in the inflow and the lake surface waters during the spring snowmelt season (Fig. 3a, b). The amount of decomposed DOM remained low during the summer stratification period both in the lake surface and bottom waters (Fig. 3b, c). Although the decomposition ratios were higher in LMW (<1 kDa) fraction as mentioned above, the amount of DOM decomposed was greatly larger in HMW (1-100 kDa) fraction than in LMW fraction both in the inflow and the lake surface waters, due to the predominant proportion of HMW fraction in those waters. The C:N ratios of DOM and PM in the lake bottom were higher than those in the surface and in the inflow: for DOM, lake bottom: 30.4 ± 9.9 (n = 19), lake surface: 24.9 ± 6.3 (n = 21), inflow: 23.8 ± 10.3 (n = 10), and for PM, lake bottom: 14.8 ± 3.4 (n = 19), lake surface: 10.3 ± 2.1 (n = 20), inflow: 11.5 ± 2.0 (n = 9). There was no negative relationship between decomposition ratio and C:N ratio in GFF filtrate and all molecular size fractions (<1 kDa, 1-10 kDa, and 10-100 kDa).

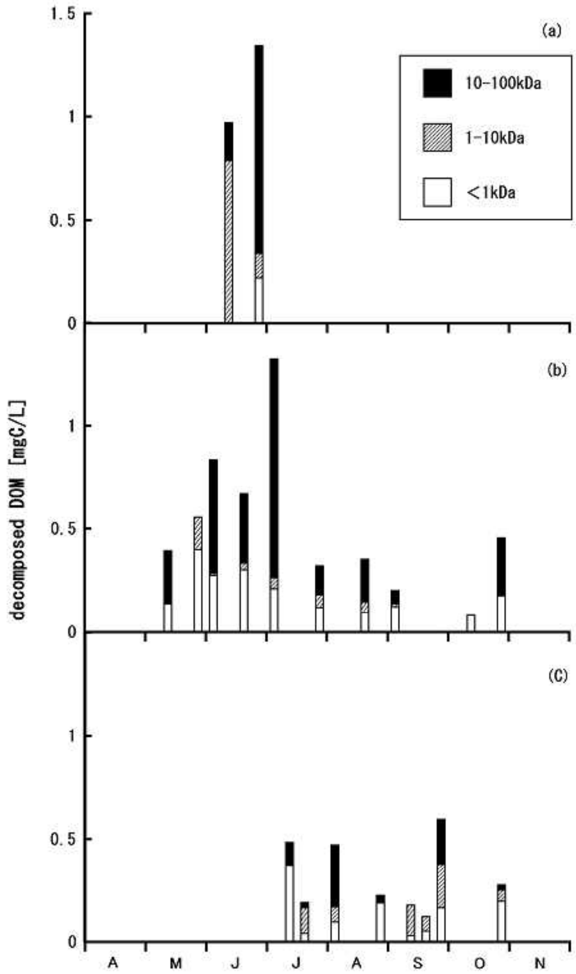

Fig.3 : Seasonal change of in amount of DOM decomposed in Lake Shirakoma-ike from April to October, 1999.
(a) at inflow, (b) 2m below lake surface, (c) 1m above lake bottom

UV-absorbance: DOC ratios of GFF filtrate were 41.1 ± 1.3 (n = 3) in inflow water, 34.4 ± 3.3 (n = 10) in the lake surface water, and 39.1 ± 3.0 (n = 8) in lake bottom water. The ratios were slightly lower in the surface water than in the bottom water during the study period (t-test, P < 0.01). A significant negative correlation was observed between the decomposition ratios and UV-absorbance: DOC ratios of GFF filtrate in the lake surface and bottom samples (r^2 = 0.61, P < 0.001). UV-absorbance: DOC ratios of GFF filtrate increased after 60 days-incubation experiments both in lake surface and bottom waters, and they reached the similar values after the incubation (39.0 ± 3.3 in lake surface and

40.5 ± 1.7 in lake bottom).

Discussion

In Lake Shirakoma-ike, the DOM concentration increased drastically due to inflow from the forest catchment during the snowmelt season. A large portion of runoff and associated hydrological transport in boreal regions occurs during spring floods (Bishop and Petersson 1996), and in boreal regions, such events carry up to 50% of annual runoff within a few weeks (Stepanauskas et al. 2000). Bishop and Petersson (1996) suggested that large snowmelt volumes flowing over frozen soils with reduced permeability could result in runoff from the organic-rich upper-soil horizon (i.e., the forest mor layer) directly to streams, thus bypassing degradation processes in mineral soils. This phenomenon could occur in the subalpine zone of the study site. The catchment of Lake Shirakoma-ike is covered with coniferous primeval forests and a high DOM load is brought by inflow during the snowmelt and rainy seasons. Organic matter and nitrate in the forest surface soil might be taken in by melting snow and carried into the lake by this process. A momentary and sudden high DOM concentration with high percentage of HMW fraction, and an increase in nutrient concentrations were observed in the lake in the snow melting period. Fresh organic matter and nutrients in inflow water would decrease during base flow when the soil is completely thawed and snowmelt reaches the inorganic layer. The DOM concentration in lake water decreased gradually when the inflow subsided and the supply from the catchment stopped. The concentration and size composition of DOM were relatively stable during and after the summer stratification period in Lake Shirakoma-ike.

In Lake Shirakoma-ike and its inflow, when the DOM concentration increased from the end of May to early July, the decomposition ratio and amount of DOM decomposed by bacteria increased simultaneously. HMW DOM represents the bulk of DOM and contributes more to bacterial consumption than LMW DOM, in spite of its low bioavailability. Fresh DOM was supplied with nutrients such as nitrate from its watershed during the snowmelt season in Lake Shirakomaike. A significant positive correlation was observed between the HMW DOM decomposition ratio and nitrate concentration in the lake surface. The supply of nitrate during the snowmelt could accelerate the consumption of fresh DOM in the lake. Nutrient limitations on DOM degradation have been reported in some humic waters. Satoh and Abe (1987) and Tulonen et al. (1992, 2000) found that the nutrient availability stimulates DOM decomposition and increases the growth efficiency of bacteria in mountain bogs and in humic lakes.

High UV-absorbance: DOC ratios of GFF filtrate in the inflow water might reflect rich humic substances derived from lake watershed. A decrease in the ratios in the lake surface water might be due to both autochthonous DOM excreted by primary production (Fukusima et al. 1996, 1997; Imai et al. 1998), and photochemical degradation of HMW DOM by UV-B (Kieber et al. 1990, Yoshioka et al. 2007).

A negative relationship between UV-absorbance:DOC ratios and decomposition ratios suggests selective microbial degradation of those DOM of low UV-absorbance: DOC ratio.

In Lake Shirakoma-ike, the decomposition ratio and the amount of DOM decomposed remained low both in the lake surface and bottom waters during the summer stratification period. The C:N ratios of DOM and POM were higher in the hypolimnion than in the epilimnion. The supply of DOM from the catchment into the lake is limited during the summer stratification period, and the DOM in the lake during the stratification period might be the remains or intermediates of DOM supplied in the spring. Moreover, some nitrogen-rich DOM might have already been consumed by bacteria.

Although LMW DOM (<1 kDa) was more labile than HMW DOM (1-100 kDa) in Lake Shirakoma-ike, there was no negative relationship between decomposition ratio and molecular size among the three fractions (<1 kDa, 1-10 kDa, and 10-100 kDa). Meyer et al. (1987) observed that bacterial growth and utilized DOM were highest with LMW DOM (<1 kDa) enrichment and lowest with intermediate molecular weight (IMW: 1-10 kDa) enrichment. The HMW fraction (10 kDa-0.22 µm) supports more growth than the IMW fraction, apparently because of lower molecular weight compounds complexed with HMW DOM in the refractory IMW DOM (Meyer et al. 1987). Low decomposition ratio in 1-10kDa DOM fraction observed in this study was in accordance with the results reported by Meyer et al. (1987).

The annual average decomposition ratios of LMW DOM (<1 kDa) were significantly higher than those of HMW DOM (1-100 kDa) both in the lake surface and the bottom waters throughout the study period. The amount of DOM decomposed, however, was larger in the HMW fraction (especially in 10-100 kDa in spring snowmelt season) than in the LMW fraction both in the lake and in the inflow waters, due to its relative abundance.

A simultaneous supply of both fresh HMW DOM and nitrate from the watershed during the snowmelt period might accelerate the bacterial consumption of DOM in the lake and influence the lake ecosystem through a microbial loop.

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