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Kinetic Study of Vitamin B_{12} Production and Consumption in Natural Seawater

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Kinetics for the production and consumption of vitamin B_{12} by the natural microbial community in a coastal seawater were analyzed, and their contribution to production and consumption is discussed.

The total consumption rate for vitamin B_{12} of natural bacterial and phytoplankton communities was 4.85 ng/*l*/day in the dark and ranged from 6.10 to 6.41 ng/*l*/day in the light. The contribution of the bacterial community to the total light vitamin B_{12} consumption was about 50–59% and the remains were due to the phytoplankton community. The half-saturation constant for vitamin B_{12} uptake of phytoplankton (larger than 5 μm) were 0.2–0.3 ng/*l* in the light, and those of bacteria were 0.17–0.48 ng/*l*. The *in situ* vitamin B_{12} uptake rate of both communities were found to be almost saturated at the naturally occurring concentration of vitamin B_{12}.

The gross production rate for vitamin B_{12} of both communities totaled 5.32 ng/*l*/day in the dark and 6.04–6.45 ng/*l*/day in the light, and the bacterial community contributed about 73% to the total in the dark and about 63% in the light. Thus, the total vitamin B_{12} gross production rate of both communities was almost equal to their total consumption rate. The bacterial community produced vitamin B_{12} in excess to the amount they consumed, but the phytoplankton community only produced vitamin B_{12} in quantities less than the amount they consumed.

Consequently, both bacterial nad phytoplankton communities were found to contribute greatly to both production and consumption of dissolved vitamin B_{12} in eutrophic coastal seawaters.

Many marine phytoplankters have been found to be auxotrophic. They require most commonly vitamin B_{12} as a growth factor. The growth rate, maximum cell yields, photosynthetic rate, and vitamin uptake rate of the vitamin-requirers depend on the ambient concentration of the dissolved essential vitamin. Thus the concentration of dissolved vitamin B_{12} is estimated to control not only the growth of vitamin-requirer but also the succession of phytoplankton species in natural seawater.[1-4]

The concentration of dissolved vitamin B_{12} in natural seawater results from the balance of rates of supply and removal of it. Though the consumption of vitamin B_{12} as well as its production have been thought to be mainly both the phytoplankton and the bacterial communities,[5-7] its supplying and removing rates by those communities have not yet been fully understood.

The present paper describes the kinetics for vitamin B_{12} production and consumption in natural coastal seawater and the contribution of microorganisms to them.

Materials and Methods

Natural Seawater Samples

Seawater samples were collected from the surface water in Uranouchi Inlet on May 13, 1983. All samples were passed through a 200 μm-nylon net to remove zooplankton, and the dissolved vitamin B_{12} contents, and the number of heterotrophic bacteria and phytoplankters were determined.

Vitamin B_{12} Consumption Rate

The rate of vitamin B_{12} consumption was computed from ^{57}Co-vitamin B_{12} uptake kinetics. Vitamin B_{12} (0, 0.25, 0.5, 1.25, 2.5, and 5.0 ng) and ^{57}Co-vitamin B_{12} (0.01 μCi; RCC Amersham, specific activity: 0.22 μCi/ng) were added to two sets of 50 m*l* of duplicate samples in three series. The first series were incubated as such, the second ones with a photosynthetic inhibitor, 3-(3,4-dichlorophenyl)-1, 1-dimethylurea (DCMU, 0.05 mM), and the others with a oxidative- and photophosphorylation inhibitor, *m*-chlorocarbonyl-cyanide phenylhydrazone (CCCP, 0.05 mM). One set

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of each series was incubated for 4 h at 20°C in the dark and the other set in the light (3,000–18,000 lx). After the incubation, the mixtures were sequentially filtered through 5.0 and 0.2 μm Nuclepore filters. The radioactivity on the filter was measured by a gamma scintillation counter. The kinetic parameters were calculated by the method of Wright and Hobbie.[8)]

Net and Gross Production of Vitamin B_{12}

The net production rates were determined from the increased concentration of dissolved vitamin B_{12} in both whole sample waters and in their filtrates passed through a 5 μm Nuclepore filter, during the 26 h-incubation under the same conditions as above. The gross production rates of vitamin B_{12} were obtained from summing up the net production rates and the consumption rates. Concentration of dissolved vitamin B_{12} were determined by the *Euglena* method described in a previous paper.[9)]

Results

Effects of Metabolic Inhibitors on Vitamin B_{12} Uptake

Effects of DCMU and CCCP on vitamin B_{12} uptake were confirmed with pure phytoplankton and bacterial cultures (*Chattonella antiqua* and unknown marine bacterium) in advance of natural seawater uptake experiments (Fig. 1). From the results, DCMU (0.05 mM) was found to inhibit completely phytoplankton uptakes of vitamin B_{12} but not to inhibit at all bacterial ones. On the other hand, CCCP (0.05 mM) inhibited the vitamin uptake of both organisms. Thus the uptake during the incubation with DCMU obviously represents the bacterial uptake, and the uptake without DCMU is due to both organisms. Then the values of CCCP-treated samples were used as controls for the abiotic absorption of vitamin B_{12}.

Vitamin B_{12} Consumption

Kinetic parameters for vitamin B_{12} uptake of natural seawater are shown in Table 1. Larger fractions than 5.0 μm were found to take up the vitamin B_{12} during the incubation without a photosynthesis inhibitor, DCMU, but not to take it up at all with the inhibitor. Thus, it is apparent that the vitamin B_{12} uptake into these fractions was mainly due to the larger phytoplankton community, but not to any bacterial community. In 0.2–5.0 μm fractions, the vitamin B_{12} uptake was observed under both incubation with and without DCMU; therefore the uptake into these fractions incubated without DCMU was due to both bacterial and phytoplankton (smaller than 5.0 μm: ultraplankton) communities, but the uptake with the inhibitor was due to bacterial community alone.

The maximum vitamin B_{12} uptake rates of the larger phytoplankton community than 5.0 μm were calculated to be 2.57–2.87 ng/*l*/day during the light incubation. In the dark, however, the maximum uptake rates of these phytoplankton community were depressed to about 60% of those in the light. On the other hand, the maximum uptake rates of the bacterial community were found to be fairly large and independent of the light conditions; the rates ranged from 3.60 to 3.72 ng/*l*/day. The half-saturation contsants for vitamin B_{12} uptake of the larger phytoplankters were 0.20–0.30 ng/*l* in the light, and those of bacteria ranged from 0.17 to 0.48 ng/*l*. These values were much smaller than the vitamin B_{12} level found in the same sample, 2.45 ng/*l*. The uptake rates at the naturally occurring concentra-

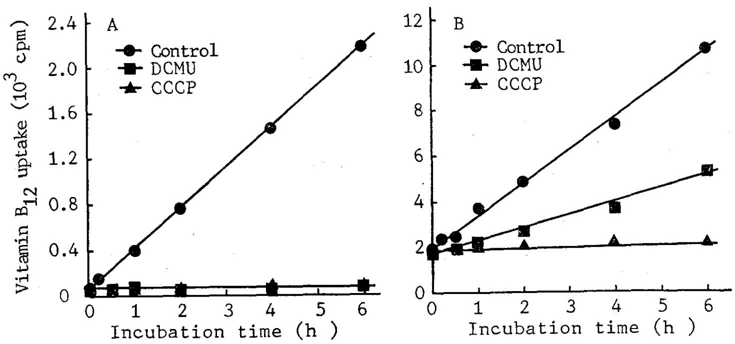

Fig. 1. Effects of metabolic inhibitors on the vitamin B_{12} uptake by axenic culture of *Chattonella antiqua* (A) and by mixed culture with *C. antiqua* and an unknown marine bacterium (B).

Table 1. Parameters of vitamin B_{12} uptake by microorganisms in natural seawater

Farctions and inhibitor	Parameters*1	Incubation condition			
		Dark	Light (3000 lx)	Light (9000 lx)	Light (18000 lx)
	K_t+S_n	2.36	2.65	2.70	2.75
Larger than	K_t		0.20	0.25	0.30
5 μm+None	V	1.61	2.57	2.59	2.87
	V_t	1.61	2.37	2.35	2.55
	T_t	35.8	24.8	25.0	23.0
	K_t+S_n	3.06	5.00	5.50	5.00
0.2–5 μm	K_t	0.61	2.55	3.05	2.25
+None	V	4.20	7.99	7.78	7.99
	V_t	3.36	3.92	3.45	3.92
	T_t	17.5	15.0	17.0	15.0
Larger than 5 μm+DCMU	—*2	—*2	—*2	—*2	—*2
	K_t+S_n	2.80	2.62	2.33	2.93
0.2–5 μm	K_t	0.35	0.17		0.48
+DCMU	V	3.62	3.62	3.60	3.72
	V_t	3.18	3.42	3.60	3.14
	T_t	18.5	17.2	15.3	18.7

*1 K_t: Half-saturation constant ;ng/*l*), S_n: Concentration of vitamin B_{12} in the natural seawater (2.45 ng/*l*), V: Maximum uptake rate (ng/*l*/day), V_t: Uptake rate in the natural seawater (ng/*l*/day), T_t: Turnover time (h)
*2 No vitamin B_{12} was taken up.

Table 2. Uptake rates of vitamin B_{12} by microorganisms in natural seawater*

Microorganisms	Incubation condition			
	Dark	Light (3000 lx)	Light (9000 lx)	Light (18000 lx)
Phytoplankters				
0.2–5 μm (a)	0.06	0.62	0.15	0.62
Larger than 5 μm (b)	1.61	2.37	2.35	2.55
Total (a+b)	1.67	2.99	2.50	3.17
Bacteria	3.18	3.42	3.60	3.14
Total microorgnisms	4.85	6.41	6.10	6.31

* Uptake rate: ng/*l*/day.

tion of vitamin B_{12} were computed to be 2.35–2.55 ng/*l* for the larger phytoplankton community in the light and 3.14–3.60 ng/*l* for the bacterial community in both light conditions; the *in situ* vitamin B_{12} uptake rates of these communities were estimated to be almost saturated at the naturally occurring concentration of vitamin B_{12}. On the other hand, the half-saturation constants of ultraplankters (smaller than 5.0 μm) including the bacterial community ranging from 2.25 to 3.05 ng/*l* were much larger than the constant of the bacterial community or of the larger phytoplankters.

From the above kinetic analysis, the vitamin B_{12} uptake rates for each type of natural community are summarized in Table 2. The total uptake rate of all of the bacterial and phytoplankton communities were 4.85 ng/*l*/day in the dark, and ranged from 6.10 to 6.41 ng/*l*/day in the light. In the light, the contribution of the bacterial community to the total uptake rates was about 50–59% and the remains were due to the phytoplankton community. Among the phytoplankton community, the contribution of ultraplankters to the total uptake rates was negligible.

The vitamin B_{12} uptake rates per cell basis for both microorganisms were calculated from their initial number of the experiment (total aerobic heterotrophic bacteria: 1.7×10^5 MPN/m*l*, total phytoplankters: 3.04×10^3 cells/m*l*). The bacterial

Table 3. Net production, consumption, and gross production of vitamin B_{12} by microorganisms in natural seawater

Microorganisms	Incubation (lx)	Net production (ng/*l*/day)	Consumption (ng/*l*/day)	Gross production (ng/*l*/day)
Phytoplankters larger than 5 μm	Dark	−0.15	1.61	1.46
	Light (3000)	−0.19	2.37	2.18
	Light (9000)	−0.12	2.35	2.23
	Light (18000)	−0.04	2.55	2.51
Bacteria (+ultraplankters)	Dark	0.62	3.24	3.86
	Light (3000)	0.32	4.04	4.36
	Light (9000)	0.06	3.75	3.81
	Light (18000)	0.10	3.76	3.86
Total microorganisms	Dark	0.47	4.85	5.32
	Light (3000)	0.13	6.41	6.54
	Light (9000)	−0.06	6.10	6.04
	Light (18000)	0.06	6.31	6.37

uptake rate averaged 1.94×10^{-5} pg/cell/day, and the phytoplankton uptake rate ranged from 9.48×10^{-4} to 1.04×10^{-3} pg/cell/day in the light and was 5.49×10^{-4} pg/cell/day in the dark.

Net and Gross Production of Vitamin B_{12}

The net and gross production rates for vitamin B_{12} of the natural communities are shown in Table 3, along with the consumption rates. In this table, the production by ultraplankters is included in the production by bacteria because it was impossible for the present methods to separate the ultraplankters from the bacterial fraction during incubation.

As a result of the balance between production and consumption of vitamin B_{12}, the net production rates of the phytoplankton community were negative in the light as well as in the dark; they consumed more vitamin B_{12} than they produced by themselves, independent of the light condition. On the other hand, the net production rates of bacterial community were positive: 0.62 ng/*l*/day in the dark and 0.06–0.32 ng/*l*/day in the light. Consequently, the total net production rates by the combined bacterial and phytoplankton communities were 0.47 ng/*l*/day in the dark and −0.06–0.13 (mean: 0.04) ng/*l*/day in the light.

The gross production rates by the phytoplankton community ranged from 2.18 to 2.51 ng/*l*/day in the light, though the rate was smaller in the dark (about 60% of the light production). The gross production rates of the bacterial community, however, were much the same in the dark as in the light; they ranged from 3.81 to 4.36 ng/*l*/day. The gross production rates by both these communities totaled 6.04–6.54 ng/*l*/day in the light. The contribution of the bacterial community to the total gross production of vitamin B_{12} was about 73% in the dark and about 63% in the light. Thus, the total gross production rate by both communities was almost equal to their total consmuption rate in the light.

The mean vitamin B_{12} production rates per cell were calculated to be 2.34×10^{-5} pg/cell/day for the bacterial community, and 7.59×10^{-4} pg/cell/day for the phytoplankton community in the light and 4.80×10^{-4} pg/cell/day for them in the dark.

Discussion

The natural seawater which was used for the experiment contained 2.45 ng/*l* of dissolved vitamin B_{12}, 1.7×10^5 MPN/m*l* of total aerobic heterotrophic bacteria, and 3.04×10^3 cells/m*l* of total phytoplankters. *Chaetoceros* spp., *Nitzchia* spp., and unknown small flagellates dominated in the water. These values are consistent with the ranges of ones which are generally found in eutrophic seawaters. Thus, the present results represent a general conception of vitamin B_{12} dynamics in eutrophied coastal seawaters in warm seasons, where diatoms are dominating.

Parker[10] demonstrated that the *in situ* vitamin B_{12} uptake rate of microorganisms (larger than 0.45 μm) in the water of Lake Washington ranged from 0.16 to 4.5 ng/*l*/day. The maximum uptake rate and the half-saturation constant were reported to range from 2.2 to 6.5 ng/*l*/day and from 5.4 to 110 ng/*l*, respectively. Though there might be the discrepancy in the microbial flora and water quality, the uptake rate and maximum uptake rate found then were similar to those reported in this study. But the half-saturation constant of coastal microorganisms were found to be much lower than that of microorganisms in the Lake.

The present results clarify that both bacterial and phytoplankton communities contribute greatly to the production and the consumption of dissolved vitamin B_{12} in eutrophic coastal seawaters. The rate of vitamin B_{12} consumption by both communities is fast as much as they use up the available vitamin B_{12} within half a day if there is no supply of the vitamin. Furthermore, both communities produce vitamin B_{12} by themselves as fast as they consume it. The bacterial community contributes more to both the consumption and the production of vitamin B_{12} than the phytoplankton community does, not only in the dark but also in the light. The bacterial community produces vitamin B_{12} in excess of the amount consumed by themselves. On the contrary, the phytoplankton community only produce vitamin B_{12} in quantities less than the amount consumed by themselves. This conception is consistent with the observations that dissolved vitamin B_{12} in seawater decreased during the bloom of phytoplankton,[11] and that the occurrence of vitamin B_{12}-producing bacteria in seawater is much higher than that of vitamin B_{12}-consuming ones.[7]

The half-saturation constants of both bacterial and phytoplankton communities (excluding ultraphytoplankton) for vitamin B_{12} uptake were about 0.2 to 0.5 ng/*l*. From the Monod's equation, the substrate concentration of 10 times as much as the half-saturation constant could give the uptake rate of 91% as much as the maximum uptake rate. Thus, the vitamin B_{12} uptake rates of both microorganisms which appeared in the natural seawater could be almost saturated at a vitamin B_{12} concentration around 5 ng/*l*. As the vitamin B_{12} concentration in coastal seawaters in Japan averaged around 4 to 10 ng/*l* as mentioned previous papers,[9,11-14] the vitamin B_{12} uptake rates of the bacterial and phytoplankton communities would be almost saturated at the *in situ* concentration in the coastal seawaters.

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