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A study on the kinetic parameters, Vmax and Km of algal alkaline phosphatase

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INTRODUCTION

Phosphorus availability is an important factor controlling phytoplankton productivity and species composition in lakes[1]. Phosphorus limitation for primary productivity has been of great interest[2]. The demand for phosphorus of phytoplankton often excesses the available amount of inorganic phosphate in lake water can support[3,4].

Alkaline phosphatase(AP_{ase}) provides orthophosphate cleaving from organic phosphomonoesters, a fraction of external dissolved organic phosphorus(DOP) pool, under phosphate depletion so algae can have an additional source of orthophosphate[5,6]. The phosphatase activity primarily depends on the type and concentration of substrate and enzyme. Other factors affecting phosphatase activity are temperature, ionic strength, pH and metal ions[7]. On the contrary to the fact that numerous biologically important enzymes are associated with cell membrane[8], AP_{ase} is either bound to the cell membrane or free in the water. The free extracellular enzyme activity is an important component in the dyamics of phosphorus cycling[2].

Enzyme kinetic parameters, V_{max} and K_m, of AP_{ase} have been used as indicators of the demand for phosphate and to compare the relative abilities of plankton to sequester phosphate deficiency under condition of PO_4^{3-} depletion[9]. The tendency for phosphatases to combine with and hydrolyze their substrates is given by the K_m-value(Michaelis-Menten constant). A low K_m means the enzyme has a high affinity to the substrate[10]. K_m varies with the substrate structure and pH[11]. K_m increases with the increase of dissolved inorganic phosphorus(DIP) concentration in the water. The fact that K_m of free enzyme in lake water is similar to K_m of algae indicated that free enzyme AP_{ase} activity was probably released by algae[12]. However, a study on the APase activity of algal species and enzyme source is unexploited field of research.

In this paper we compare K_m values for the pure cultures of 30 algal species and natural communities of phytoplankton.. The free extracellular enzyme activity was measured for natural community of phytoplankton. And in AP_{ase} induction time course for natural plankton samples under phosphorus-limited conditions change of K_m was measured.

MERHODS

Preparation of sample

The pure cultures of 11 freshwater algal species employed in this study were bought from UTEX(The Culture collection of Algae at the University of Texas at Austin) and maintained axenically in 250ml flasks of synthetic mineral medium in an illuminated incubator. Purified bovine AP_{ase} was from Sigma Chemical Co. Ltd. The 19 marine algal species were isolated from the coastal waters of the Yellow sea in Marine Phytoplankton Laboratory, Kunsan National University[13]. Because AP_{ase} activity is very low in normal phosphorus-rich culture medium, AP_{ase} was induced for the measurement of AP_{ase} by transfering 30ml full-grown culture to 200ml of phosphorus-free medium for rapid induction(Cells were centrifuged and rinsed the cells with phpsphorus-free medium before transfer). The kinetics of AP_{ase} activity by natural plankton community were studied in Lake Soyang , located at the upstream part of the Han River in Korea. The samples were collected with a 5 liter Van Dorn sampler at surface from Oct. 1993 to Apr. 1994. Samples were filtered through a 80 μm pore size net to remove zooplankton. To differentiate cell-bound from free enzyme, collected samples were centrifuged(6,000 rpm, 15min.) and the supernatants were filtered with Millipore filters of 0.22μm pore size. The filtrate was used as source of free enzyme. The settled cells were resuspended with the same amount of phosphorus-free medium and used as the source of cell-bound enzyme.

Kinetics of Alkaline Phosphatase

AP_{ase} activity was measured by methylumbelliferyl phosphate(MUFP) method(Pettersson & Jansson, 1978,[12]). For the measurement of K_m, a stock MUFP solution was diluted to various concentrations with Tris buffer of pH 8.0 and stored frozen. Fluorescence was measured with fluorometer(Perkin Elmer LS3 model) with the exitation at 365 nm and emission at 460 nm. 1.8 ml samples were added to 0.2 ml each of MUFP in a cuvette just before measurement. Fluorescence produced was measured at 30 minutes intervals for 1 hour. AP_{ase} activity(V) measured at 7 different substrate concentration(S)

was fitted to Michaelis-Menten kinetics model and V_{max} and K_m were calculated by nonlinear regression method as below..

$$V = \frac{V_{max} \times S}{(K_m + S)}$$

Chemical Analysis Method

DIP concentration was determined by the method of Ascorbic acid aftered filtration through an GF/C glass filter[14].(APHA, 1992). Chlorophyll a concentrations were measured according Lorenzen(1967,[15]).

RESULTS

The K_m showed large variation with species of algae. K_m of marine cryptomonads were 4.1-21.6 μM(mean 14.6, SE 3.0, n 5) and K_m of unidentified spherical algae were 4.1-18.6 μM(mean 7.4, SE 5.3, n 13). Pennate diatoms showed smaller K_m(2.5 μM) than other marine algae(Table 1). Some green algae showed large K_m(650 μM for *Selenastrum capricornutum*). *Chlorella* sp. and *Nitzschia palea* showed smaller K_m(1.7μM and 2.0μM, respectively) than other species examined.

Algal AP_{ase} examined generally exhibited larger K_m than the purified AP_{ase} from bovine intestine(1.7μM). The extracellular free enzyme in the filtrate of *Anabaena flos-aquae* culture showed smaller K_m(52μM) than cell-bound form(276μM). This result was simillar to that of surface plankton in Lake Okutama, Japan, when *Asterionella formosa* was dominant. K_m of the free enzyme and cell-bound enzyme were 1.1μM and 4.0μM, respectively. The K_m(12.0μM) of summer plankton in Lake Soyang, when *Anabaena* sp. was dominant species, was larger than that of spring plankton when *Asterionella* sp. was dominant(Table 2).

The dissolved AP_{ase} activity accounted for 36-97% of total activity from fall to spring turnover in Lake Soyang(Table 3). The K_m(1.1-3.5) of dissolved AP_{ase} activity in the filtrate of surface water was simillar to that(0.7-3.5μM) of total enzyme.

In time cource of APase induction for the natural plankton samples under phosphorus-limited conditions, V_{max} of cell-bound enzyme increased and K_m decreased with the decrease of DIP content in the medium(Fig. 1).

DISCUSSION

The K_m of AP_{ase} activity have been used as indicator of phosphorus deficit and a low K_m means that the enzyme have a high affinity to substrate. Therefore, if one species has a lower K_m, it is profitable for substrate availability under condition of phosphate limitation. In this study, examined species have a variable K_m with species. Diatoms generally have a higher affinity to substrate than green algae except for *Chlorella* sp.(Fig. 2).

The extracellular free enzyme in filtrate of *Anabaena flos-aquae* culture that showed smaller K_m may be easier to combine with substrate than cell bound enzyme did. The dissolved AP_{ase} activity was responsible for the significant portion of total activity from fall to spring in Lake Soyang. Thus, the dissolved AP_{ase} is an important component in dynamics of phosphorus cycling. This results are in agreement with Rai & Jacobsen(1993).

The K_m of dissolved activity in the filtrate of surface water in Lake Soyang was simillar to that of total enzyme. A point of view from the fact that K_m values of free enzyme is similar to K_m values of algae indicated that free enzyme AP_{ase} activity was probably released by algae(Chrost and Overbeck, 1987). However, a study on the AP_{ase} activity of algal species and enzyme source is unexplored field of research and need continuose research in the future.

In time course of AP_{ase} induction for the natural plankton samples under phosphorus-limited conditions, DIP was important factor affecting V_{max} and K_m. AP_{ase} activity increased with the decrease of DIP content. And was suppressed with the increased of DIP *vice versa*.

CONCLUSION

1. In this study the K_m of AP_{ase} activity showed large variation with species. Diatoms generally showed smaller K_m than other algae.

2. The dissolved AP_{ase} activity was responsible for the significant part of total activity in Lake Soyang. And the dissolved AP_{ase} showed similar K_m with total AP_{ase} activity.

3. In time course of AP_{ase} induction for natural plankton samples under phosphorus-limited condition, K_m showed large change impling of isozyme system. K_m became smaller with depletion of DIP and increase of V_{max}.

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Table 1. Comparison of kinetic parameters, K_m, for the pure culture of marine phytoplankton.

Specics	K_m(μM)
cryptomonoid(GSNU WH 0011)	4.1
cryptomonoid(GSNU WH 0016)	12.9
cryptomonoid(GSNU WH 0001)	16.0
cryptomonoid(GSNU WH 0028)	18.5
cryptomonoid(GSNU WH 0007)	21.6
spherical(GSNU WH 0023)	1.4
spherical(GSNU WH 0020)	2.6
spherical(GSNU WH 0024)	2.6
spherical(GSNU WH 0026)	3.4
spherical(GSNU WH 0025)	4.6
spherical(GSNU WH 0010)	5.9
spherical(GSNU WH 0017)	6.8
spherical(GSNU WH 0021)	6.8
spherical(GSNU WH 0004)	7.1
spherical(GSNU WH 0006)	8.3
spherical(GSNU WH 0014)	12.0
spherical(GSNU WH 0015)	16.8
spherical(GSNU WH 0013)	18.1
pennate diatom(GSNU WH 0005)	2.5

Table 2. Comparison of kinetic parameters, K_m, for the pure culture of freshwater phytoplankton.

Source of Enzyme		K_m (μM)
Nitschia palea		2.0
Pediastrum sp.		7.2
Chlorella ellipsoidea		1.7
Chlorella vulgaris		1.7
Scenedesmus sp.		11.6
Scenedesmus brasiliensis		13.4
Selenastrum capricornutum		650
Microcystis aeruginosa		6.4
Purified bovine AP_{ase}		1.7
Anabaena flos-aquae	cell-bound enzyme	276
	extracellular enzyme	52.0
Lake Soyang	summertime (*Anabaena flos-aquae* dominant)	12.0
	springtime (*Asterionella* sp. dominant)	1.5
Lake Okutama	total enzyme	18.0
	cell-bounded enzyme	4.0
	extracellular enzyme	1.1
Lake Tsukui	total enzyme	24.0

Table 3. Alkaline phosphatase activity and K_m of the total enzyme and dissolved extracellular form of lake surface water.

Site	Date	Dominant species	Classification	K_m (μM)	V_{max} (nM/min)	Chl.a (mg/l)	DIP (μg/l)
Soyang Dam	Oct. 1993	*Anabaena* sp.	Total Dissolved	12.0	63.0	7.2	2
	Nov. 1993	*Asterionella gracillima*	Total Dissolved	3.0 2.6	2.6 1.3	1.0	7
	Jan. 1994	*Melosira* spp.	Total Dissolved	3.5 3.5	2.8 1.0	1.1	11
	Mar. 1994	*Melosira* spp.	Total Dissolved	0.7 1.1	6.6 6.4	5.1	4
	Apr. 1994	*Asterionella gracillima*	Total Dissolved	1.5 1.6	117.5 97.8	4.8	2
Soyang Sanggulli	Oct. 1993	*Anabaena* sp.	Total Dissolved	2.7 2.2	15.0 7.0	4.5	7
	Nov. 1993	*Asterionella gracillima*	Total Dissolved	0.3 0.7	7.0 2.1	1.0	8
	Jan. 1994	*Melosira* spp.	Total Dissolved	12.6 1.6	1.2 0.5	0.9	12

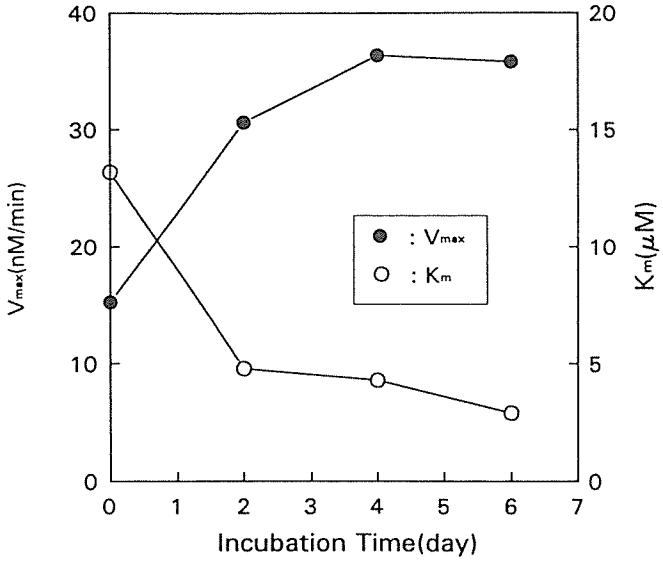

Fig. 1. The variation of V_{max} and K_m in time course of AP_{ase} induction for natural plankton samples in Lake Soyang.

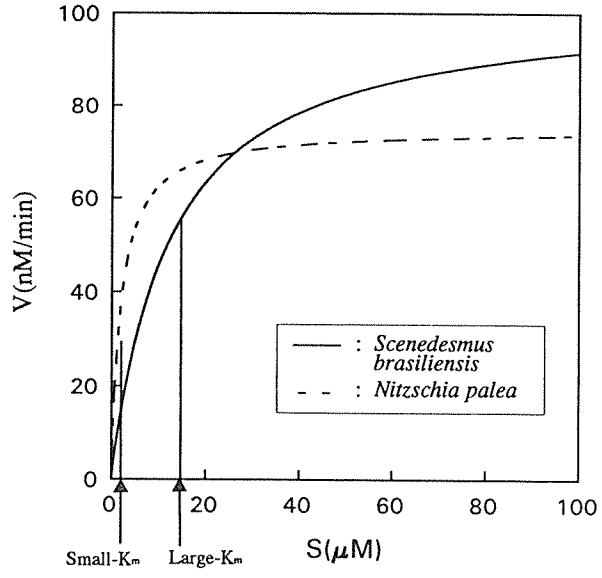

Fig. 2. Comparison of K_m for *Scenedesmus brasiliensis* and *Nitzschia palea*.