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Adaptations of Estuarine and Freshwater Phytoplankton to Urea Decomposition

MYUNG GIL PARK, JAE HYUNG SHIM AND BYUNG CHEOL CHO*
Department of Oceanography, Seoul National University, Seoul 151-742, Korea

기수 및 담수 식물플랑크톤의 요소 분해에 대한 적응

박명길 · 심재형 · 조병철
서울대학교 해양학과

The concentration-dependence of and the effect of light on urea decomposition, and the suppression of urea decomposition by ammonium were studied to understand adaptations in phytoplankton to utilization of urea in the estuarine system of the Mankyung and Dongjin rivers and a hypertrophic pond. Results of size-fractionation showed that bacterial fraction played a minor role (14%) in urea decomposition in the estuary. However, the role of bacteria in urea decomposition seemed to increase in a hypertrophic pond. Natural phytoplankton communities exhibited a monophasic or biphasic kinetics of urea decomposition over a wide range of concentration (upto 7.7 mM). The addition of high concentration of ammonium and incubation of the euphotic samples in the dark caused reductions in the urea decomposition rates. It is suggested that understanding of adaptations in phytoplankton to urea decomposition would help to study the temporal and spatial variabilities of urea decomposition rates in the field and the significance of urea in nitrogen cycle.

만경 · 동진강 하구와 과부영양의 연못에서 식물플랑크톤이 요소의 분해시 갖고 있는 적응 기작을 연구하기 위해, 요소 분해에 있어서 요소 농도의 의존성, 빛의 영향, 그리고 암모늄 이온 농도의 증가에 의한 억제 효과를 연구하였다. 하구에서 크기 구배의 실험 결과는 박테리아가 요소 분해에 있어서 작은 (14%) 역할을 함을 나타냈다. 그러나 과부영양의 연못에서는 박테리아에 의한 요소 분해의 역할이 증가하는 것으로 보였다. 자연산 식물플랑크톤 군집에 의한 요소의 분해는 넓은 범위의 요소 농도(7.7 mM까지)에 있어서 monophasic 또는 biphasic kinetics를 나타냈다. 요소의 분해 속도는 높은 암모늄 이온이 존재시와 빛이 없는 상태에서 감소하였다. 이러한 연구 결과들은 식물플랑크톤에 의한 요소 분해의 적응에 대한 이해가 현장에서 요소 분해의 시공간적 변이와 질소 순환에서 요소의 중요성을 이해할 때 도움이 될 것으로 제시하였다.

INTRODUCTION

The distribution and utilization of urea in natural waters are well studied (Remsen, 1971; McCarthy, 1972; Remsen *et al.*, 1972; Eppley *et al.*, 1973; Mitamura and Saijo, 1980 ; Kristiansen, 1983; Harrison *et al.*, 1985; Turley, 1985; Cho, 1988). It is now concluded that urea comprises a significant nitrogen pool in natural waters, and its decomposition and utilization in the euphotic zone are mainly due to phytoplankton.

We were interested in dynamics of urea decom-

*Corresponding author
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position in two unique aquatic environments, a shallow and persistently mixed estuarine system of the Mankyung and Dongjin rivers (Shim *et al.*, 1991) and a hypertrophic pond with high ammonium concentration (usu. greater than 40 μM, Park, 1993). Thus, we considered major environmental factors which would affect urea decomposition rates in two environments as follows: In the estuary with strong tidal mixing, phytoplankton would experience diel light-dark cycles overlapped with light-dark conditions exerted by the tidal mixing. Some field studies showed that urea decomposition was light-dependent (Mitamura and Saijo, 1975, 1980; Webb and Haas, 1976). Thus, it seems important to measure how rapidly phytoplankton respond to the fluctuating light conditions in the estuary. Next, urea decomposition can be affected by ammonium. For phytoplankton, it is recently reported that even 1 μM ammonium effectively repressed the urease activity (Mitamura, 1986b). In the estuary, greater than 10 μM ammonium is often found (Park, 1993). Thus, the degree of repression in urea decomposition needs to be measured. In a hypertrophic pond with high ammonium concentration (greater than 40 μM), urease might be totally suppressed by ammonium. Thus, it seems interesting to know if phytoplankton have substantial urease activity even at such a high ammonium concentration. Finally, concentration of urea would be expected to change widely in estuaries and freshwaters (Berman, 1974; Savidge and Johnston, 1987). It is useful to know how phytoplankton adapt over a wide range of urea concentration. The effect of salinity on urea decomposition was not considered here because salinity effect on urea decomposition was reported to be minimal in a temperate estuary (Savidge and Johnston, 1987). In this report, we investigated the concentration-dependence of and the effect of light on urea decomposition, and the suppression of urea decomposition by ammonium to understand adaptations in phytoplankton to utilization of urea.

MATERIALS AND METHODS

Study areas and sample collection

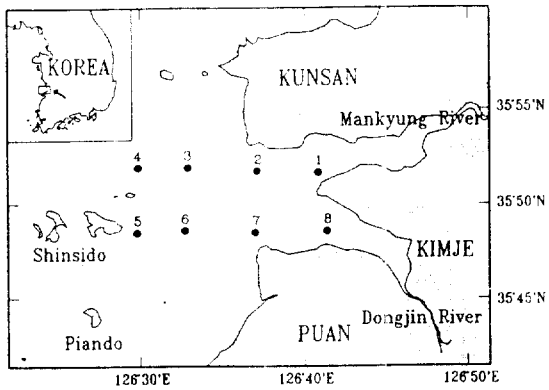

Fig. 1. A map showing sampling stations during a study period of October, 1992-October, 1993.

The estuarine system of the Mankyung and Dongjin rivers is located at the midwest coast of Korea (126° 30'E – 126° 45'E, 35°45'N – 35° 55'N, Fig. 1). The study area is a shallow (usually <10 m deep), well-mixed estuary with a large tidal-range of 4.3 m at its mouth (Lee and Kim, 1987; Shim *et al.*, 1991). The other study area, a hypertrophic pond, is located in Seoul National University in Seoul, Korea. To measure the concentration-dependence of urea decomposition, surface samples were collected by a Niskin bottle (5 *l*) at Stn 3 in the estuary (October 31, 1992) and in the middle of the pond (June 1, and November 5, 1992). To measure the effects of light on urea decomposition, samples were collected from 0 m and 3 m at Stn 7 (October 9, 1993) and from 0 m only at Stn 7 (February 27, 1993) in the estuary. The sample from 3 m depth on October 9, 1993 was from the bottom of the euhotic zone; Secchi depth was only 1.2 m. To measure the effects of ammonium concentration on urea decomposition, samples were collected at Stn 5 from 0 m and 10 m in the estuary (October 9, 1993) and from the surface in the pond (November 4, 1993).

Determination of in situ urea decomposition rates

For the determination of urea decomposition rates we followed basically the method of Cho (1988). The radio-respirometry was used to measure urea decomposition rates; 50 m*l* aliquots were

dispensed into sterilized, 300 ml BOD bottles and then 0.2 μm filter-sterilized ^{14}C-urea (sp. act. 55.3 mCi mmol^{-1}, Amersham) was inoculated to attain 50 nCi per 50 ml (final concentration of added urea of 46.5 nM). The bottles were sealed with a silicon stopper holding a plastic center well (Kontes Glass Co.). Inside the plastic center well, an accordian-folded piece of chromatography paper (2 cm×8 cm; Toyo filter papers, No 2) moistened with 0.2 ml of monoethanolamine was contained to trap $^{14}CO_2$ produced from ^{14}C-urea decomposition. Samples were incubated under simulated *in situ* conditions for 0.5−4 h. After incubation, urea decomposition was terminated by adding 1 ml of 2N H_2SO_4 to liberate $^{14}CO_2$ in solution. To correct for abiotic decomposition of urea, buffered formalin-added controls were run with each set of samples. After 24 h, wells and accordian-folded pieces were removed from the BOD bottles and placed in scintillation vials containing 10 ml of Lumagel. The radioactivity was measured by a liquid scintillation counter (Packard Tri-Cab, Model 2550) using the external standard ratio method. All measurements were done in at least duplicates. To determine whether dissolved enzymes, bacteria, or phytoplankton are important in urea decomposition, whole water and size-fractionated water samples through 0.2 μm Nuclepore filters and Whatman GF/C filters were incubated with addition of ^{14}C-urea as above. Since some portion (<30%) of bacteria might be retained on GF/C filters in the estuary (Lee *et al.*, 1991), the urea decomposition activity in bacterial fraction would be conservative estimate (see Discussion). Urea decomposition rates (V) were calculated as follows: $V=(V_L \times S)/(A_d \times t)$ where V_L is the activity of the liberated $^{14}CO_2$; S, *in situ* concentration of urea; A_d, the radioactivity of added urea; and t, duration of incubation (Turley, 1985). Effects of ammonium on urea decomposition were measured by adding various concentrations of ammonium (up to 2.5 mM) to the samples and comparing the urea decomposition rates with raw control.

Kinetic analysis of urea decomposition

The kinetics of urea decomposition was carried by adding ^{14}C-urea and non-labelled urea to whole or size-fractionated water samples to add concentrations in the range of 1−2600 μM for estuarine samples (stn 3; Oct. 31, 1992) and 1−7700 μM for freshwater samples. Formalin-added controls were run simultaneously. The data followed Michaelis-Menten kinetics, which can be described by the following equation: $V=(V_{max} \times S)/(S+K_m)$ where V is the velocity of urea decomposition at a given concentration of urea (S), V_{max} is the maximum decomposition rate at saturated concentration of urea, and K_m is the concentration of urea at which the decomposition rate is $V_{max}/2$. The Lineweaver-Burk transformation was used to estimate V_{max} and K_m as follows: $1/V=(K_m/V_{max})\times(1/S)+1/V_{max}$.

Other analyses

Urea concentrations were determined by the diacetyl monoxime thiosemicarbazide method described by Price and Harrison (1987). Samples for the analysis of urea were filtered through GF/C glass fibre filters and stored at −20℃ for the subsequent analysis. Ammonium concentrations were measured according to Grasshoff *et al.* (1983). Water temperature and salinity were measured with a T-S Bridge (Hydro-Bios type MC5). Regression analysis was done for kinetics data using a SAS statistical software package.

RESULTS

Physico-chemical characteristics of the environments

Physical and chemical parameters for the examined samples in this study are shown in Table 1. In the estuary, temperature ranged from 3.0 to 20.8℃. Salinity showed a small variation from 29.3 to 33.7‰. Ammonium concentrations ranged from undetectable to 11.0 μM. Urea concentrations ranged from 0.1 to 2.2 μM. In a hypertrophic pond temperature ranged from 15.6 to 25.5℃ and urea concentration from 2.3 to 3.5 μM.

Kinetics of urea decomposition

Urea decomposition in estuarine samples (Fig. 2

Table 1. Sampling date, station (stn), temperature, salinity, urea, and ammonium concentrations in the estuarine system of the Mankyung and Dongjin rivers and a hypertrophic pond during a study period of June 1992 to November 1993

Sampling date	Stn	Depth (m)	Temperature (℃)	Salinity (‰)	Urea (μM)	NH_4^+ (μM)
Estuary						
Oct.31, 1992	3	0	16.5	33.7	1.5	11.0
Feb.27, 1993	7	0	3.0	29.8	0.1	6.0
Oct. 9, 1993	5	0	20.8	30.2	1.0	N.D.*
		10	19.8	29.3	2.0	N.D.
	7	0	20.0	29.8	1.2	7.1
		3	20.0	31.0	2.2	7.0
Hypertrophic pond						
Jun. 1, 1992		0	25.5	-	3.5	—
Nov. 5, 1992		0	15.6	—	2.4	—
Nov. 4, 1993		0	17.5	-	2.3	46.8

* N.D.: not detected

a) showed biphasic kinetics over a wide range of concentration (1.5–2602 μM). The Lineweaver-Burk plot generated two lines: one with V_{max} of 1.6 nMh^{-1} and K_m of 0.4 μM, the other V_{max} of 7.9 nMh^{-1} and K_m of 101.1 μM. In this sample, GF/C filtrates including most of bacterial fraction comprised a minor portion (5.7%) of total activity. Thus, phytoplankton seemed to be responsible for the observed kinetics. In a hypertrophic pond kinetic analyses of urea decomposition showed biphasic or monophasic kinetics (Fig. 2b & c). When biphasic kinetics appeared, one line had V_{max} of 0.04 μMh^{-1} and K_m of 1.1 μM, and the other had V_{max} of 0.62 μMh^{-1} and K_m of 208.8 μM (Fig. 2b). When monophasic kinetics was observed, value of V_{max} was 0.05 μMh^{-1} and that of K_m 76.9 μM (Fig. 2c). Interestingly, in contrast to the raw water sample with monophasic kinetics, dissolved enzyme fraction showed biphasic kinetics with values of K_m of 0.04 μM and 202.0 μM (Fig. 2c). Also, the GF/C filtrate including some portion of bacterial assemblages and dissolved urease comprised a small portion (16.6%) of total activity.

Effect of light on urea decomposition

Two time-course experiments in the estuary showed that surface samples incubated in the dark showed gradual decrease within 4 h (Fig. 3a & b) whereas surface samples incubated under the *in situ* light remained relatively unchanged or increased. A similar trend was found for the bottom sample (near the bottom of the euphotic zone, Fig. 3c). Interestingly, urea decomposition activity was higher in bottom sample than at the surface. During the incubation any substantial changes in urea concentration were not found (not shown).

Effect of ammonium on urea decomposition

Addition of ammonium always caused decreases in urea decomposition rates (Fig. 4a-c). Interestingly, in estuarine samples from the aphotic zone addition of 40 μM ammonium did not cause substantial decrease in urea decomposition (Fig. 4b). However, for the surface sample, elimination of urea decomposition activity was observed at 40 μM ammonium added (Fig. 4a). Urea concentration was 1.0 μM at the surface and 2.0 μM at the bottom, but ammonium was undetectable in both samples (Table 1). In a hypertrophic pond where high concentration of ammoium were found (47 μM), substantial rates (1.6–28.9 nMh^{-1}) of urea decomposition were observed in the examined samples. Addition of 0.5 mM of ammonium caused suppression of urea decomposition activity to half (Fig. 4c). In one set of experiment, addition of ammonium greater than 5 mM completely inhibited urea decomposition (not shown).

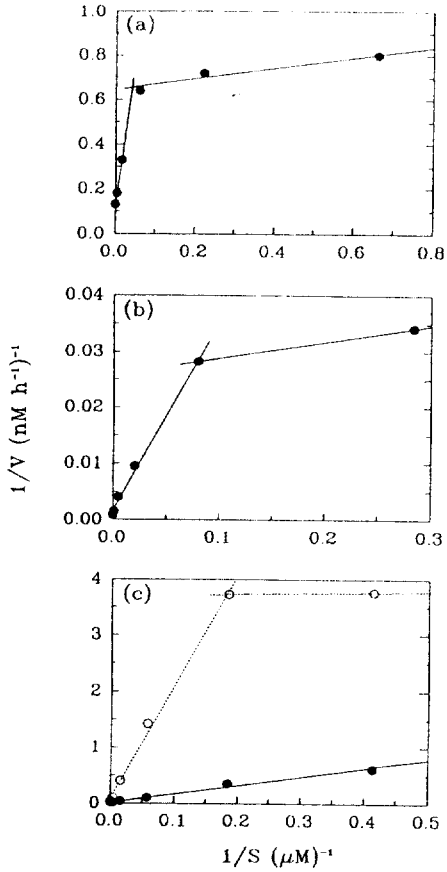

Fig. 2. Lineweaver-Burk plots of urea decomposition. (a) Surface samples from the estuarine system (station 3) of the Mankyung and Donjin rivers on October 31, 1992. Two curves are $y=0.65+0.24x$ ($V_{max}=1.6$ nMh^{-1}, $K_m=0.4$ μM) and $y=0.13+12.88x$ ($V_{max}=7.9$ nMh^{-1}, $K_m=101.1$ μM). (b) & (c) Samples from the surface of a hypertrophic pond collected on June 1 and November 5, 1992, respectively. For samples of June, two curves are $y=0.0259+0.029x$ ($V_{max}=0.04$ μMh^{-1}, $K_m=1.1$ μM), and $y=0.0016+0.336x$ ($V_{max}=0.62$ μMh^{-1}, $K_m=208.8$ μM). For samples of November, for raw freshwater (●) $y=0.02+1.52x$ ($V_{max}=50.6$ nMh^{-1}, $K_m=76.9$ μM); for <0.2 μm fraction (○) $y=3.7+0.1x$ ($V_{max}=0.27$ nMh^{-1}, $K_m=0.04$ μM) and $y=0.1+19.98x$ ($V_{max}=10.11$ nMh^{-1}, $K_m=202.0$ μM). Here, y denotes 1/V and x denotes 1/S, where V represents urea decomposition rate (nMh^{-1}) and S urea concentration (μM).

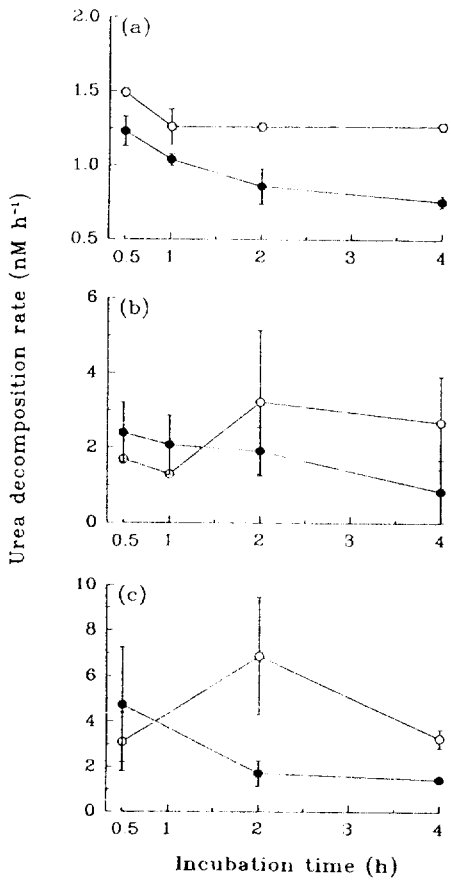

Fig. 3. Effect of light on urea decomposition in the estuarine system (station 7) of the Mankyung and Dongjin rivers. (a) Samples collected from the surface in February 27, 1993. (b) Samples collected from the surface, and (c) those from 3 m in October 9, 1993. Open symbols represent samples incubated in simulated *in situ* light; closed ones in the dark.

DISCUSSION

Phytoplankton seem to be the major agents of urea decomposition in the estuary on the basis of size-fractionation data. However, it must be considered that in the estuary ca <30% of bacteria could be retained on GF/C filters (Lee *et al.*, 1991). Thus, some bacterial urease activity might have been included as a part of phytoplankton ureolytic activity. Since the maximum value of per-bacterium urea decomposition rates in marine environments are reported to be 0.32 amol cell^{-1}h^{-1} (Cho *et al.*, unpublished data), the contribution of large bacteria would be ca 0.1 nMh^{-1} if we used 1×10^9 cells l^{-1} (Cho and Shim, 1992). This would be about 8% of the total urea decomposition activity

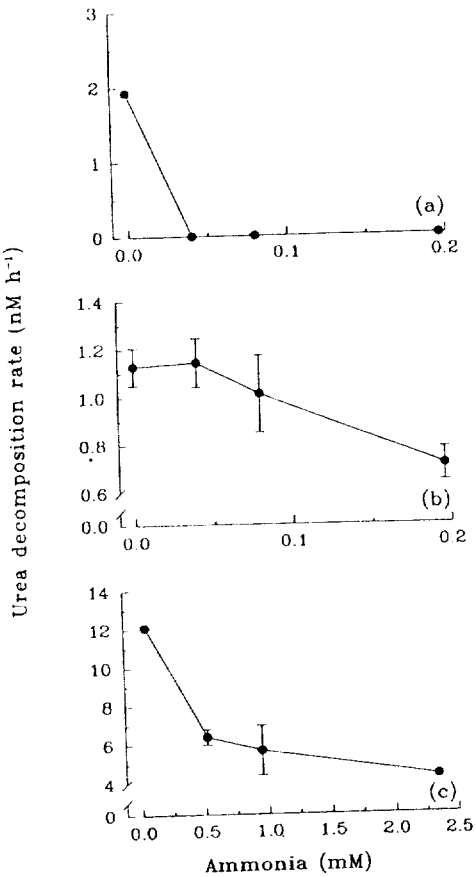

Fig. 4. Effect of ammonium concentration on urea decomposition. (a) Samples from the surface of the estuarine system of the Mankyung and Dongjin rivers (station 5: Oct. 9, 1993) were incubated in simulated *in situ* light, and (b) those from 10 m in the dark. (c) Surface samples from the surface of a hypertrophic pond (Nov. 4, 1993) were incubated under *in situ* light.

(1.2 nMh^{-1}). Thus, bacteria including dissolved free urease seem to be minor (14%) contributors to urea decomposition in the estuary. This small contribution of bacteria to urea decomposition in the estuary are consistent with other observations (Turley, 1985; Cho, 1988). For the hypertrophic pond, per-bacterium urea decomposition rates have not been reported. But, examination of data of Park (1993) indicates that values of per-bacterium urea decomposition rates in the hypertrophic pond would be similar to those in marine environments. If we use the above value of 0.32 amol cell $^{-1}h^{-1}$ for the pond and bacteria caught on GF/C filter of $4-10\times10^9$ cells l^{-1}, then, the contribution of the large bacteria would be ca 1.3–3.2 nMh^{-1}. This would be a substantial contribution to the total urea decomposition. Thus, contribution of phytoplankton to urea decomposition in the pond, measured by size-fractionation using GF/C filters, could be substantially over-estimated. This would require careful interpretation on the results of the pond.

The kinetics of urea decomposition from our study suggests that natural communities of estuarine phytoplankton can utilize urea over a wide range of concentration (upto 2.6 mM). A biphasic kinetics for urea decomposition, consisted of one linear curve with an apparent K_m value of 0.4 μM and another with K_m of 101.1 μM, was observed (Fig. 2a). Our values of V_{max} and K_m of the estuarine samples were close to the values (range of V_{max}, 0.23–6.18 nMh^{-1}; range of K_m, 45–685 nM; Savidge and Hutley, 1977) reported for a temperate estuary. However, one K_m value of 101.1 μM is much larger than the reported values. Savidge and Hutley (1977) reported complex kinetics of urea decomposition in small size-fraction of coastal waters, indicating superposition of several kinetics by a range of phytoplankton in the sample. Our result also might suggest that two communities of phytoplankton with different affinities to urea existed together in the water sample. One subgroup of phytoplankton seemed to be exposed to near saturation and well adapted to the environmental concentration of urea (1.5 μM). However, the presence of K_m of 101.1 μM was intriguing. Since such a high concentration of urea has not been reported in the pelagic environments, it might be found in sediment environments (Nakas and Litchfield, 1977). Further, since majority of phytoplankton in the estuary are tychopelagic, the high K_m might be found in benthic diatoms living in sediment with high urea concentration. The multiphasic decomposition kinetics would provide some metabolic flexibility to phytoplankton over widely changing conditions of urea concentration.

In a hypertrophic pond, biphasic kinetics was also observed with similar magnitudes of K_m va-

lues found in the estuary. We are not aware of literature reporting K_m values of urea decomposition for freshwater phytoplankton. However, K_m values for ^{15}N-urea uptake are available (Mitamura, 1986a). Mitamura (1986a) reported a range of K_m from 0.05 to 2.2 μM. If we assume our K_m values are solely derived from phytoplankton and compare them with the reported values, our K_m values were generally on the large side of the range or larger than the reported ones. However, it should be determined if bacteria are also responsible for the observed biphasic kinetics in the pond. Interestingly, in the dissolved fraction, biphasic kinetics was found. We think this is the first report on kinetic analysis on dissolved urease enzymes. Thus, we can not compare our results with other studies. It seemed that one K_m value of 202.0 μM had phytoplankton origin. However, other K_m value was 0.04 μM. Since such a high affinity K_m was found for marine bacteria (Cho, 1988; manuscript in prep.), we think that both bacterial and phytoplankton urease enzymes existed in 0.2 μm filtrate. The high K_m value in the filtrate was greater than K_m value found in raw water (76.9 μM). Thus, the dissolved urease might be derived from previously existed community of phytoplankton.

One noteworthy observation from our results was that substantial activities of urea decomposition (1.6–28.9 nMh^{-1}) occurred in spite of high concentration of ammonium (>40 μM) in a hypertrophic pond. Since phytoplankton urea decomposition have been reported to be suppressed by addition of μM level of ammonium (McCarthy and Eppley, 1972; Mitamura, 1986b), thus, we were interested in how much more ammonium concentration would be required to completely suppress urea decomposition activities in the hypertrophic pond. Surprisingly, to reduce the *in situ* urea decomposition activity to half, addition of high ammonium concentration (>500 μM) was required, indicating that phytoplankton adapted to such high concentrations of ammonium. In the estuary, addition of high concentration of ammoium (200 μM) also caused suppression of urease activity. However, we suspect that inhibition of urease activity in the estuary was rather complicatedly regulated. When bottom samples were incubated in the dark, *moderately high* concentration (40 μM, final conc.) of ammonium was not effective to suppress urea decomposition. This can not be interpreted as bacterial decomposition of urea because urea decomposition by natural marine bacteria are reported to be completely repressed by 50 μM of ammonium (Cho, 1988). On the contrary, under light, addition of 40 μM ammonium (final conc.) was effective to repress urea decomposition activity of the surface samples. Thus, light seemed to cause repression of urea decomposition at the *moderately high* concentration of ammonium. However, when bottom samples (from 3 m) with relatively lower ammonium concentration (final concentration of 7 μM) were exposed to light, urea decomposition rate was not inhibited and actually increased (Fig. 3c). Thus, interaction of light and *moderately high* concentration (e.g. 40 μM) of ammonium seemed to suppress urea decomposition activity in the estuary. Although further investigations are needed to confirm this interpretation, it might be assumed that light-stimulated phytoplankton growth would prefer ammonium to urea and thereby suppressing urea decomposition activity. However, dark adapted phytoplankton would retain urea decomposition activity even under moderately high concentration of ammonium during a short exposure (e.g. 4 h incubation in our experiment).

Our findings of biochemical characteristics of urea decomposition and light-dependence of urea decomposition would aid to understand the variability of vertical distribution of urea decomposition in water column in the estuary and to understand short-term temporal variability in urea decomposition activities in a hypertrophic pond. In a tidally well-mixed estuarine system of the Mankyung and Dongjin rivers, phytoplankton would experience frequent fluctuations of light and dark conditions due to vertical mixing. Mitamura and Saijo (1975) reported the 7.3 fold difference in urea decomposition activity between light and dark incubated samples in Mikawa Bay, but Silva (1985) reported the small difference (usu. <2 fold) similar to our results in the tidally well-mixed Menai Straits. This

weak dependence of light on urea decomposition activities may be due to strong tidal mixing.

Phytoplankton below the euphotic zone might still keep urea decomposition activity until surfacing in the next tidal cycle. When exposed to light, dark-adapted phytoplankton would increase activity within a short time (ca 2 h), enabling them to be ready for the use of urea. However, in this situation high ammonium concentration (>40 μM) could be critical to the utilization of urea and cause suppression of urea decomposition. Such high concentrations of ammonium could be often found in the estuary (Park, 1993). Thus, light-dark cycles due to tidal mixing overlapped with high ammonium concentration (>40 μM) could largely influence vertical distribution of urea decomposing activities in the estuary. Similar discussion could be applied to a hypertrophic pond; the short-term temporal variability of urea decomposition might be explained by changes in urea concentration, adaptation to light-dark cycles, and inhibition of high ammonium concentration.

In conclusion, understanding of biochemical adaptations in phytoplankton to urea decomposition would be prerequisite to study the temporal and spatial variabilities of urea decomposition rates in the field.

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