

DOP utilization by the two toxic dinoflagellates, *Alexandrium tamarense* and *Gymnodinium catenatum*, and its advantage in species competition

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Utilization of dissolved organic phosphorus (DOP) by the two toxic dinoflagellates *Alexandrium tamarense* (Lebour) and *Gymnodinium catenatum* (Graham) isolated from Hiroshima Bay of Japan was studied. *A. tamarense* grew poorly on fructose-6-phosphate (F6P), glucose-1-phosphate (G1P), glycerophosphate (Glycero-P), ribose-5-phosphate (R5P) with a phosphomonoester bond, although it grew well on nucleotides (adenosine 5-diphosphate; ADP and adenosine triphosphate; ATP) as well as on dissolved inorganic phosphorus (DIP metaphosphate; Meta-p, pyrophosphate; Pyro-p, tripolyphosphate; Tripoly-p and orthophosphate; Ortho-P). This implies that *A. tamarense* is able to utilize DOP as well as DIP from ambient water using nucleotidase, pyrophosphatase and polyphosphatase, which hydrolyze phosphodiester. In contrast, *G. catenatum* was able to utilize DOP compounds of various molecular weights and structures as well as DIP.

In the time-course experiments, alkaline phosphatase (AP) activity was induced at ambient orthophosphate concentration of 0.43 μM and 3.3 μM for *A. tamarense* and *G. catenatum*, respectively, and it increased with orthophosphate depletion. The AP activity was the maximum at the optimum temperature for the growth of *A. tamarense* and *G. catenatum*, i.e. 15°C and 25°C, respectively. These results suggest that DIP depleted conditions in Hiroshima Bay might have led to the outbreaks of noxious dinoflagellates in recent years.

The spatio-temporal variability of alkaline phosphatase hydrolyzable phosphorus (APHP) in the Hiroshima Bay water was investigated. The AP used was a commercial AP and those extracted from *A. tamarense* and *G. catenatum*. Concentration of DIP in the natural seawater of Hiroshima Bay was lower than 1 μM in all year round with the lowest value in spring. The DIP concentration showed no significant correlation with the AP activity that was measured with standard colorimetric method, suggesting that induction of AP may occur throughout the year due to chronic DIP depletion. In Hiroshima Bay, dinoflagellates generally form blooms during stratified season (spring, summer and autumn), while diatoms predominate in mixed season (winter). The concentration of APHP was high at all depths of all stations in spring, but low in summer and autumn. The APHP produced and accumulated in the water column in spring could support bloom formations of dinoflagellates during spring to early summer. However, the low DIP concentration in spite of detectable AP activity in summer and autumn indicates that dinoflagellates appearing in these seasons should be supported by fast recycling of phosphorus, i.e., the APHP regenerated from DOP by AP they produced. APHP is considered play an important role for the bloom formation of noxious dinoflagellates in stratified season in Hiroshima Bay.

Experiments on uptake and excretion of DOP were carried out for the two toxic

dinoflagellates, *A. tamarense* and *G. catenatum*. ATP, UMP (uridine-5-monophosphate), G-6-P (glucose-6-phosphate) and Glycero-P were used as the sources of DOP in the preliminary uptake experiments. Short-term uptake experiment was conducted using ATP because its vital utilization was proven for both species. Maximum specific uptake rate (V_{\max}) of ATP (in terms of P) by *A. tamarense* and *G. catenatum* was estimated to be 216 day⁻¹ and 175 day⁻¹, respectively. Affinity index ($\alpha=V_{\max}/K_s$) with regard to ATP was evaluated to be 38.4 for *A. tamarense* and 23.0 for *G. catenatum*, respectively. Excretion rate of DOP obtained by batch culture experiments was estimated to be 0.084 pmol cell⁻¹ hr⁻¹ and 0.012 pmol cell⁻¹ hr⁻¹ for *A. tamarense* and *G. catenatum*, respectively, accounting for ca. 30% and 25% of the assimilated phosphorus. The results imply that these two species have an advantageous position under DIP-depleted condition due to their utilization ability of DOP.

The population dynamics of *A. tamarense* and *G. catenatum* along with *Skeletonema costatum*, which is the dominant species among diatoms in Hiroshima Bay, were simulated using mathematical model. Prior to the model simulation, competition experiments using these three species were carried out to obtain data by which the simulation model will be validated. *S. costatum* dominated attaining the order of 10⁴ cells ml⁻¹ over those of the other two species in the medium with DIP. The growth of *S. costatum* was also stimulated with the addition of DOP such as UMP or Glycero-P whereas *S. costatum* is unable to uptake DOP. This implies that growth of *S. costatum* could be supported by DIP, which was transformed from DOP by the AP produced by *A. tamarense* and *G. catenatum*. The hydrolysis rate of DOP was estimated to be 30% hr⁻¹ for UMP and 15% hr⁻¹ for Glycero-P, respectively, using the numerical model.

Finally, the simulation model was run assuming the environmental conditions of spring in Hiroshima Bay. *S. costatum* dominated over the other two species in the initial stage of the computation. However, *G. catenatum* was getting higher in the cell density and close to the level of *S. costatum* at the end of the calculation (ca. 330 hr). On the other hand, the cell density of *A. tamarense* was lower than the peak density that is usually observed in spring of Hiroshima Bay. This was considered to be due to no consideration of cell recruitment from cyst germination in the model.

In the sensitivity analyses by means of doubling and halving of parameters, DIP depletion had little effect on the cell density of *G. catenatum*. In contrast to *G. catenatum*, growth of *A. tamarense* and *S. costatum* was much affected by the change of the parameter values. These results indicated that if DIP depletion will be continuing, species that have a large phosphate pool in the cell like *G. catenatum* would predominate in the community. In conclusion, the utilization ability of DOP by *A. tamarense* and *G. catenatum*, which is mediated by alkaline phosphatase, should ensure their survival in DIP-depleted waters of Hiroshima Bay. Particularly, *G. catenatum* showed higher affinity to various DOP compounds than to DIP. The results of the present study suggest that if the existing DIP-depleted condition in Hiroshima Bay continues for long time, species such as *G. catenatum*, which has high affinity to DOP, will dominate.

Key words: dissolved organic phosphorus (DOP), dissolved inorganic phosphorus (DIP), *Alexandrium tamarense*, *Gymnodinium catenatum*, Hiroshima Bay