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The physiological role of CO_2-concentrating mechanism in marine diatoms under changing global environment

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[Background] Marine diatoms contribute for about 20% of the global biological carbon fixation. In seawater, photosynthesis is CO_2-limited because dissolved-inorganic carbon (DIC) is mainly in the form of HCO_3^- at alkaline pH, when the system is in equilibrium with atmospheric CO_2. HCO_3^- transporter is one of important components of the CO_2-concentrating mechanism (CCM) in cyanobacterium and green alga for CO_2 acquisition to overcome CO_2-limited in seawater. Like other photosynthetic aquatic organisms, diatoms possess CCM to maintain an efficient photosynthesis. During the diatom evolution, two major subtypes, pennate and centric were established; their genome and perhaps also CCM are diverse. Recently, it was demonstrated that mammalian type solute-carrier (SLC) 4 from marine pennate diatom *Phaeodactylum tricornutum* (PtSLC4-2), was identified as a plasma membrane type HCO_3^- transporter. Mammalian type SLC4 HCO_3^- transporters typically are sensitive to 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS). Distribution and function of HCO_3^- transporter among marine diatoms is yet much studied. In this study, two centrics, *Thalassiosira pseudonana*, *Chaetoceros muelleri* and two pennates, *Cylindrotheca fusiformis*, *P. tricornutum* were used.

[Experimental Method] Diatoms were grown in artificial seawater, which was supplemented with half-strength Guillard's f solution (F/2ASW) under continuous illumination (20-50 µmol m^{-2} s^{-1}) at 20°C under constant aeration of atmospheric air (0.039% CO_2). Cells were harvested at the log phase of growth by centrifugation, cell were washed with DIC-free F/2ASW buffered pH 8.2 and suspended in the same buffer at a chlorophyll *a* concentration of 10 µg mL^{-1}. The rate of photosynthetic O_2 evolution at various concentrations of DIC was measured with a Clark-type oxygen electrode, DIC at CO_2 compensation point was measured by gas chromatography. $K_{0.5}$ and P_{max} values were determined by the least-squares method. High light treatment was measured in the case of photosynthetic parameters with various light intensity 120, 380 and 1000 µmol m^{-2} s^{-1} during CO_2 compensation point. DIC flux parameter was measured by gas chromatography. DIDS, 3'(3,4-Dichlorophenyl)-1',1'-dimethylurea (DCMU), Carbonyl cyanide m-chlorophenyl hydrazone (CCCP) and), *N*-(5-sulfamoyl-1,3,4-thiadiazol-2-yl)acetamide (AZA) were used in this experiment.

[Result and Discussion] In the presence of DIDS, photosynthetic affinity in *P. tricornutum* and *C. muelleri* was decreased while there is no effect on those of *T. pseudonana* and *C. fusiformis*. It was suggested that CO_2 acquisition in *C. muelleri* was supported by the SLC4 and/or SLC 26 type HCO_3^- transporter. HCO_3^- transport is thought to be an energy dependent process. Under high light condition, cell would produce energy exceeding the capacity required. Diatoms which possess HCO_3^- transporter are believed to be more tolerant under high light condition by having such light energy sink. P_{max} in *P. tricornutum* and *C. muelleri* decreased with increasing light intensity during CO_2 compensation point. On the other hand photosynthetic rate in *C.fusiformis* and *T. pseudonana* was not sensitive to high light treatment under CO_2 compensation point. Whereas P_{max} in ptSLC4-2G, which over express of HCO_3^- transporter was found to be more tolerate to high light compared to wild type *P. tricornutum*, strongly suggesting that enhanced HCO_3^- transporter at the plasma membrane confer on the cells a high capacity of excess light energy dissipation. Interestingly, in the presence of DCMU, an inhibitor of the linear electron transport in the photosystem, *P. tricornutum* and *C. muelleri* were unable to take up DIC from the bulk medium, while *C. fusiformis* and *T. pseudonana* were actively took up DIC, indicating the fundamental divergence of the energy source for the operation of CCMs among diatoms, which does not relate to the evolutionary lineage. Physiological analysis in this study clearly indicates the difference in the mode of CCM among four marine diatoms with strong suggestions of potential diversity of their functions in adaptation machinery to light.