Photoprotective acclimation of xanthophyll pigments to high light in marine diatoms

海産珪藻の強光に対するキサントフィル色素の光保護適応

12D5702　片山　智代　　指導教員　田口 哲

SYNOPSIS

海産珪藻は海洋の基礎生産量の約半分を占める重要な藻類であり、沿岸域に多く優占している。海産珪藻は大きな光環境変動に直面することがあり、非常に制限された光環境から過剰な光環境にさらされる。珪藻は、強光に対して過剰なエネルギーを熱として放散する光保護機構をもつ。珪藻類の熱放散にはキサントフィルサイクル色素であるダイアトキサンチン(Diatoxanthin; DT)が関係することが知られている。熱放散を行う DT は光化学系 II(Photosystem II; PSII)に直結しており、PSII のクロロフィル *a*(Chl *a*)蛍光の測定から求められる非光化学消光(Non-photochemical quenching; NPQ)は熱放散の大きさを表す指標として用いられている。光保護適応を明らかにするための DT と NPQ は時間規模によって異なる応答を示す可能性が考えられる。そこで本研究では、強光照射下での数日および、数分から数時間における DT と NPQ の応答を調べることにより、海産珪藻の光保護適応を明らかにすることとした。まず、温帯の沿岸域に普遍的に存在する海産珪藻を実験対象種として、強光環境における数日間および、数分から数時間における光保護機構の応答を調べた。さらに北海道サロマ湖にて、珪藻が優占するアイスアルジー群集の光保護機構の応答を数分から数時間の短時間の規模で調べた。暗所適応した温帯性珪藻 *Thalassiosira weissflogii* を光飽和の明暗周期下に移行すると、最大光合成量子収率(F_v/F_m)と細胞数が増加し、それらが最大値を示した 2 日目以降に DT と NPQ は増加した。さらに、弱光適応した *T. weissflogii* を始めとする温帯性珪藻 3 種およびアイスアルジー群集の DT と NPQ は、強光照射して 1 分から 120 分の間にも増加した。120 分間強光照射し続けると、キサントフィルサイクル由来ではない DT の新たな合成が見られた温帯性珪藻 3 種は、新たな DT の合成が起こらなかったアイスアルジー群集よりも F_v/F_m の減少は小さく、過剰な光エネルギーによる損傷が比較的小さかった。温帯性珪藻 3 種において新たに合成された DT は、NPQ との間に直接的な関係性を示さなかった。新たな DT の合成が起こらなかったアイスアルジー群集は DT と NPQ の間に直線関係を示したが、NPQ 全量に対して光損傷によって誘発される NPQ(NPQ_{Slow})の寄与が温帯性珪藻に比べて大きかった。本研究は、弱光および暗所の環境から強光環境に変動した時、成長が飽和した数日後においても、数分から数時間の短時間においても、海産珪藻はキサントフィルサイクルを活性化させて熱放散を行うことを明らかにした。さらに、温帯性珪藻は光照射に対し、キサントフィルサイクルを介した熱放散による光保護を行うとともに、キサントフィルサイクル由来でない DT を新たに合成することによって過剰な光エネルギーによる損傷を抑える、という適応戦略を有する可能性が示唆された。

Keywords: ballast water, diatoxanthin, ice algae, melting ice, mesophilic diatom, non-photochemical quenching, photoinhibition, photoprotection, xanthopohyll cycle pigments

Introduction

Diatoms are one of the major components of natural assemblages microalgae from polar to tropical marine ecosystem and account for approximately 40% of marine primary producers. They inhabit in highly mixed surface water column in coastal waters. Diatoms are also dominant members of the sea ice algal community. Microalgal communities found in sea ice, known as ice algae, play also an important role in primary production in the sea ice ecosystem.

Diatoms utilize light as an energy source via photosynthesis. The availability of light is one of the major factors controlling the growth of diatoms. Light intensity varies from extremely low or dark to relatively high in the ocean environment. For instance, ice algae, predominated by diatoms, is forced to acclimate to low light conditions due to the thick surface snow cover and ice. When cells are released from retreating sea ice, some ice algal cells might be exposed suddenly to high light conditions. Releasing of ice algae from sea ice could be accelerated by increase in melting sea ice due to recent global warming, particularly in the Northern Hemisphere. The similar situation in which diatoms have to survive in such highly variable light environments can be also induced by anthropogenic causes such as releasing to surface water after transportation in the ballast water of ships.

Although light is an essential resource for the survival of diatoms, light can be also harmful at supra-optimal irradiance leading to a damage of photosystem II (PSII) and reduction in the photosynthetic electron transport capacity. Marine diatoms evolved to adapt to high light to avoid harmful damage. One mechanism of the most important protective acclimation against high light intensity for diatoms is thermal dissipation of excess energy by xanthophyll cycle pigments in their de-epoxidated state. In diatoms, xanthophyll cycle pigments consist predominantly of diadinoxanthin (DD) and diatoxanthin (DT). In general, DD transfers excitation energy to chlorophyll *a* (Chl *a*) and plays a role in acquisition of light energy as a photosynthetic light-harvesting pigment, whereas DT absorbs excitation energy from Chl *a* and plays a role in thermal dissipation as a photoprotective pigment. Thermal dissipation of excess energy absorbed in PSII can be quantified by estimating non-photochemical quenching (NPQ) of Chl fluorescence.

Relationship between the ratio of DT to Chl *a* and NPQ is known to be linear in marine planktonic diatom species, which

indicate NPQ is dependent on the presence of DT. However, Lavaud et al. (2004) observed the non-linear relationship between the ratio of DT to Chl *a* and NPQ for mesophilic marine diatom *Thalassiosira weissflogii*. In the recent studies, the non-linear relationship has been also observed for a few species of marine diatoms, which had been previously reported to have linear relationship. The non-linear relationship between DT and NPQ occurred mostly with *de novo* synthesis of DT molecules. Because the DT molecules produced through *de novo* synthesis do not bind to the light-harvesting complexes that are involved in the NPQ process, the *de novo* synthesized DT may be not directly related to NPQ (Schumann et al. 2007). NPQ is induced by DT synthesis through xanthophyll cycle, which could lead to thermal dissipation, but also decrease in fluorescence yield due to damage of PSII reaction centers (Maxwell and Johnson 2000). The NPQ refers to both photoprotective processes and damage to the reaction centers of PSII, which might result in the increase with quenching under high light condition. However, most of previous studies reporting relationship between DT and NPQ after exposure to high light have examined total NPQ, but not distinguished the two NPQ components: thermal dissipation (NPQ_{Fast}) and photoinhibitory (NPQ_{Slow}) components of NPQ.

Energy-dependent quenching and xanthophyll cycle are key mechanisms of photoprotective acclimation in the avoidance of high light stress in photosynthesis. The amount of xanthophyll cycle pigments and NPQ can determine the ecological success of a species or group in a particular light climate, as well as can be indicator of the species successions. Xanthophyll cycle in photoprotective mechanism has been demonstrated in the laboratory, most often in monocultures but only a few studies have investigated its development on the natural microalgal community *in situ*. There are fewer studies of photoprotection on natural diatoms to natural irradiance based on the examination of NPQ in addition to xanthophyll cycle.

The present study investigated the photoprotective acclimation of marine diatoms to high light based on the xanthophyll cycle pigments and Chl *a* fluorescence of PSII in both controlled laboratory and natural environments. The objectives of the present study were (1) to examine the photoprotective responses to high light condition of mesophilic marine diatom species in the laboratory on a timescale of days, and (2) on a short time scale of minutes to hour in mesophilic marine diatom species in the laboratory and ice algal community in the natural environment. Finally, the present study provides us with the basis for further research on the photoprotective acclimation of natural assemblages of microalgae.

Materials and Methods

Study 1. Dark-light transition experiments in mesophilic marine diatom species

Bacillariophyceae *Thalassiosira weissflogii* (CCMP1336) was maintained in semicontinuous cultures at 20ºC and a salinity of 35 under 300 µmol photons m^{-2} s^{-1} on a 12h:12h light dark cycle (L:D cycle). Experiment was started by transferring to a fresh, modified f/2 medium sea water in dark bottles. Cells were stored in the dark for 14 days. Samples from days 3, 8, and 14 were returned to a L:D cycle with silicate addition of 0 µM, 10 µM, and 130 µM, respectively and monitored for 4 days. Subsamples were taken for the measurements of cell density, nutrient concentration, pigment concentration, and Chl fluorescence to study on maximum quantum yields of PSII (F_v/F_m) and NPQ.

Study 2. High light exposure experiments in mesophilic marine diatom species and natural ice algal community

Bacillariophyceae *T. weissflogii* (CCMP1336), *Chaetoceros muelleri* (CS-176), and *T. pseudonana* (CS-173) were maintained in semicontinuous cultures at 20ºC and a salinity of 32 under 40 µmol photons m^{-2} s^{-1} on a 12h:12h L:D cycle. The experiments were started by transferring the cells in exponential growth to a fresh, modified f/2 medium. Cells were exposed to 900 µmol photons m^{-2} s^{-1} for 120 min.

Samples of ice algal communities were collected in 2012 to 2014 at the station (44ºN, 143ºE) off the east coast of Saroma-Ko Lagoon, Hokkaido. Whole ice cores were taken and cut 3 cm from the bottom of ice core. The bottom portions of ice-core were melted in the filtered seawater under dark condition. Experiments were conducted four times during three years at the Saroma Research Center of Aquaculture, in simulated *in situ* conditions. The ice algal communities were dominated by diatoms particularly the pennate *Pinnularia quadratarea* var. *constricta* in Exp. 1 and the centric *Detonula confervacea* in the other experiments. Each ice algal suspensions (Exp. 1, 2, 3, and 4) were exposed to sunlight of midday for 120 min. The weight average of photosynthetically active radiation (PAR) of sunlight was ranged from approximately 600 to 1400 µmol photons m^{-2} s^{-1}.

Subsamples were collected during the exposure period from 1 to 120 min to measure pigment concentration. Initial rate constants (k) of temporal changes in the ratio of DT to Chl *a* were calculated for de-epoxidation of DD to DT in light conditions by fitting to a first-order kinetic model (Olaizola and Yamamoto 1994):

$$DT_t = DT_\infty + (DT_0 - DT_\infty)\, e^{-k\,t} \quad (1)$$

where DT_0 and DT_∞ are the values measured immediately before the light exposure experiment and after the complete de-epoxidation of DD to DT in light condition, respectively. Chl fluorescence was also determined to study on F_v/F_m and NPQ. NPQ was distinguished on the basis of their relaxing quenching kinetics: fast (NPQ_{Fast}) and slow (NPQ_{Slow}) relaxing NPQ components, which were estimated according to Maxwell and Johnson (2000). Total NPQ was equal to sum of NPQ_{Fast} and NPQ_{Slow}.

Results and Discussions

Study 1. Dark-light transition experiments in mesophilic marine diatom species

When the dark-acclimated *T. weissflogii* were transferred to L:D cycle, the cell density immediately increased to the maximum and was saturated after 2 days (Fig. 1A). Maximum quantum yields of PSII (F_v/F_m) reached its maximum value on either day 1 or day 2 (Fig. 1B). Similar levels were observed on both days, regardless of the silicate availability but the dark storage length (Two-way ANOVA, $p < 0.01$). The increases in cell density and F_v/F_m suggested that the ability to resume photosynthesis upon exposure to the high light is maintained regardless of the length of continuous darkness, and cells accli-

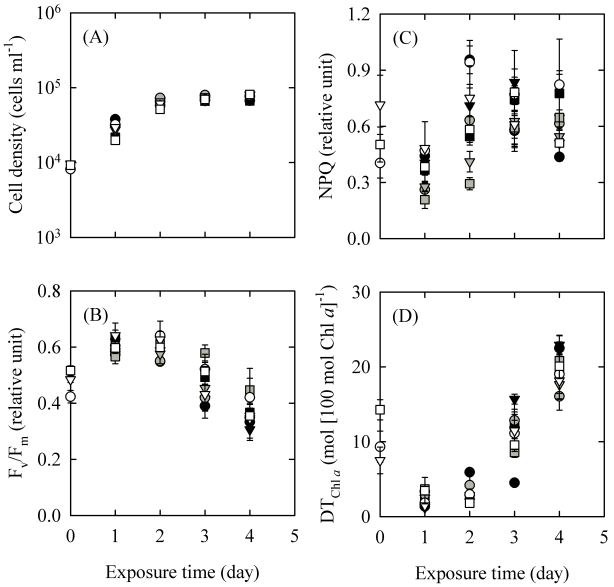

Figure 1. Temporal changes in cell density (A), F_v/F_m (B), NPQ (C), and the ratio of DT to Chl *a* (D) after the exposure to L:D cycle with silicate addition of 0 μM (black), 10 μM (gray), and 130 μM (white) on day 3 (circle), day 8 (reversed triangle), and day 14 (square) of the dark storage.

mate to high light condition within 2 days (Katayama et al. 2011).

NPQ reached the lowest level on day 1 following exposure to L:D cycle (Fig. 1C) with increase in F_v/F_m. After reaching the low levels, NPQ increased and stayed at relatively constant levels on day 3 and 4. The ratio of DT to Chl *a* ($DT_{Chl\ a}$) decreased on either day 1 or day 2 (Fig. 1D), which could be caused by rapid cell division at the beginning of the L:D cycle. Afterward $DT_{Chl\ a}$ increased until day 4. The longer exposure to the high light may enforce the cells going through photoprotection process, such as activation of xanthophyll cycle and non-photochemical quenching.

Study 2. High light exposure experiments in mesophilic marine diatom species and natural ice algal community

Before high light exposure, the ratio of DD and DT to Chl *a* ($[DD+DT]_{Chl\ a}$) in the three species of mesophilic marine diatoms were similar to ice algal communities (Fig. 2). After the exposure to high light, de-epoxidation of DD to DT occurred rapidly in both the mesophilic diatom species and ice

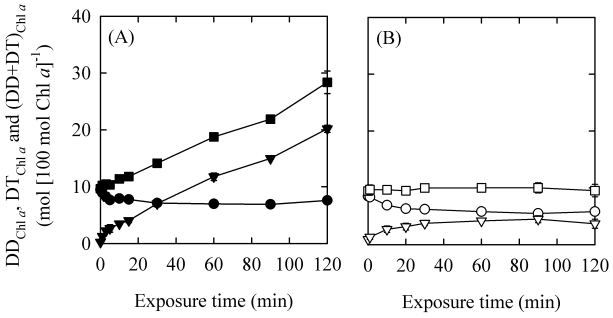

Figure 2. Temporal changes in the ratio of DD (circle), DT (reversed triangle), and DD+DT to Chl *a* (square) during the exposure to the high light in *T. weissflogii* (A) and Exp. 3 in the ice algal community (B).

algal communities. The initial rate constants (*k*) of de-epoxidation of DD to DT for the first 10 minutes in the mesophilic diatom species (0.15–0.23 min^{-1}) were similar values to those of ice algal communities (0.17–0.30 min^{-1}) (Table 1). The values of $DT_{Chl\ a}$ (11–20 mol DT [100 mol Chl a]$^{-1}$) at the end of light exposure experiments in the mesophilic diatom species were higher than the range of ice algal communities (1.0–3.7 mol DT [100 mol Chl a]$^{-1}$) (Table 1).

Increase in $DT_{Chl\ a}$ with the increase in $(DD+DT)_{Chl\ a}$ in the mesophilic diatom species indicates the occurrence of the *de novo* synthesis of $DT_{Chl\ a}$. In contrast, the increase in $DT_{Chl\ a}$ without significant changes of $(DD+DT)_{Chl\ a}$ observed for the ice algal communities suggests that the dynamics of these xanthophyll pigments are controlled by the xanthophyll cycle (Katayama and Taguchi 2013). After light exposure for 120 min the degree of de-epoxidation, calculated as the ratio of DT to DD+DT, ranged from 21 to 39% in the ice algal communities, which was lower than the range of the mesophilic diatom species (48–72%) (Table 1) including the *de novo* synthesized $DT_{Chl\ a}$.

Before high light exposure, the NPQ in mesophilic diatom species and ice algal communities was lower than 0.3. After the exposure to high light, the NPQ showed an exponential increase in both the mesophilic diatom species and ice algal communities. The NPQ values (1.5–2.2) after 120 min in the mesophilic diatom species were similar to the range of ice algal communities (1.6–2.9) (Table 1).

The NPQ saturated with the $DT_{Chl\ a}$ in the mesophilic diatom species, whereas significantly linear relationships between them was observed in the ice algal communities in the

Table 1. Initial rate constant (*k*) of de-epoxidation of DD to DT (min^{-1}), the ratio of DT to Chl *a* (mol DT [100 mol Chl *a*]$^{-1}$), the ratio of DD+DT to Chl *a* (%), and NPQ (relative unit) after light exposure for 120 min. Slope between $DT_{Chl\ a}$ and NPQ (100 mol Chl *a* [mol DT]$^{-1}$), the decrease in F_v/F_m compared to initial value (%), and slope between DT/(DD+DT) and relative F_v/F_m to initial value (relative unit) during high light exposure experiments in the mesophilic diatom species and the ice algal community.

	k	$DT_{Chl\ a}$	DT/(DD+DT)	NPQ	Slope between $DT_{Chl\ a}$ and NPQ	Decrease in F_v/F_m	Slope between DT/(DD+DT) and F_v/F_m
Mesophilic diatoms							
T. weissflogii	0.23	20	72	1.5	0.63	31	−0.47
C. muelleri	0.15	11	48	2.1	0.61	21	−0.41
T. pseudonana	0.23	14	72	2.2	0.16	62	−1.0
Ice algal community							
Exp. 1	0.17	1.9	25	2.9	2.0	87	−4.4
Exp. 2	0.20	1.0	21	1.6	1.4	97	−4.6
Exp. 3	0.30	3.7	39	1.9	0.42	75	−2.1
Exp. 4	0.21	1.5	29	2.5	1.6	93	−3.8

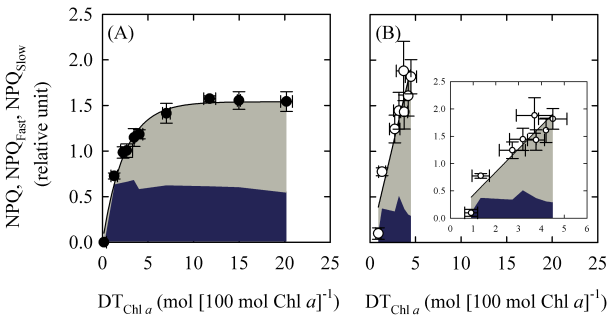

Figure 3. Relationship between the ratio of DT to Chl *a* and NPQ during the exposure to the high light in *T. weissflogii* (A) and Exp. 3 in the ice algal community (B, shown enlarged in the inset). Areas of dark gray and light gray indicate NPQ_{Fast} and NPQ_{Slow}, respectively.

similar experimental durations ($p < 0.01$, Fig. 3). The saturated relationship might be influenced by *de novo* DT synthesis. When the contribution of the *de novo* synthesized $DT_{Chl\ a}$ to total $DT_{Chl\ a}$ is significantly sufficient, the relationship between $DT_{Chl\ a}$ and NPQ might become saturated.

The slopes of the relationship between $DT_{Chl\ a}$ and NPQ observed for the mesophilic diatom species were lower than 1, whereas for ice algal communities, the slope reached to 2 (Table 1). The slope of the relationship between $DT_{Chl\ a}$ and NPQ was shown to reflect the quenching efficiency of DT (Goss and Jakob 2010). The significant difference in the slope between mesophilic diatom species and ice algal communities ($p < 0.01$) could be explained by dividing NPQ to two NPQ components: thermal dissipation (NPQ_{Fast}) and photoinhibitory (NPQ_{Slow}) components of NPQ. When the amounts of $DT_{Chl\ a}$ were lower than approximately 3 mol DT (100 mol Chl a)$^{-1}$, the proportions of NPQ_{Fast} were larger in the mesophilic diatoms than the ice algal communities with DT synthesis, whereas those of NPQ_{Slow} were larger in the ice algal communities (Fig. 3). The apparent initial increase in NPQ by the quenching due to photoinbition may therefore contribute to the higher quenching efficiency of DT in the ice algal communities compared to mesophilic diatoms.

The decreases in F_v/F_m compared to the initial values (75–97%) during the light exposure experiments of the ice algal communities were larger than those of mesophilic diatom species (21–62%) (Table 1). The significant negative linear relationships between the relative F_v/F_m and DT/(DD+DT) indicated that the accumulation of $DT_{Chl\ a}$ reduces potential maximum PSII efficiency in the mesophilic diatom species and ice algal communities ($p < 0.01$, Fig. 4). The steeper slopes in the ice algal communities (< −2.0) than those in the mesophilic

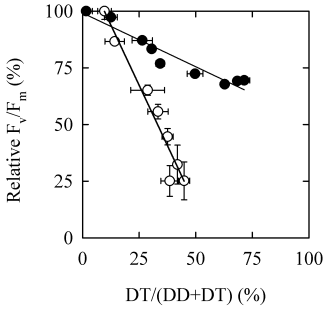

Figure 4. Relationship between the ratio of DT to DD+DT and relative F_v/F_m to initial value during the exposure to the high light in *T. weissflogii* (black) and Exp. 3 in the ice algal community (white).

diatom species (> −1.0) ($p < 0.01$, Table 1) could be related to the *de novo* DT synthesis. The *de novo* synthesized DT molecules are assumed to have an antioxidant function (Lepetit et al. 2010), serving as another photoprotective mechanism not related to thermal dissipation. The occurrence of *de novo* synthesis of DT in the mesophilic diatom species might assist to resist photodamage, resulting the gentler slope between F_v/F_m and DT/(DD+DT) in the mesophilic diatom species than those in the ice algal communities.

Conclusions

Photoprotective acclimations of low- or dark-acclimated mesophilic diatoms to high light are appeared to enhance thermal dissipation through xanthophyll cycle after not only a period of days under L:D cycle but also within minutes to hours. Similar initial rate constant (k) of de-epoxidation between mesophilic diatom species and ice algal communities suggest that the potential activity of de-epoxidation is regarded as similar regardless of difference in the ecological groups. However, the presence of *de novo* DT synthesis in mesophilic diatoms causes the enhancement of $DT_{Chl\ a}$ and DT/(DD+DT). Consequently, it plays a role in the photoprotection by reducing photoinhibition as an antioxidant function. Although the lack of response of *de novo* DT synthesis to light exposure in ice algal communities suggests that *de novo* DT synthesis might be minimal at low temperatures, further work must be performed to characterize how physiological and environmental conditions influence the process of *de novo* DT synthesis in marine diatoms and quantify the photoprotective capacity of *de novo* synthesized DT.

Implications

The present study confirms that marine diatom species have a capability to protect from the high light and simultaneously recover from any damage in the PSII reaction centers caused by the high light. The protection processes is enhanced by the presence of *de novo* DT synthesis. The recovery processes dominate under low light or dark conditions. In natural environments, the ice algal community are adapted to stable, low light conditions and then the transition to high light could damage severely when they are released into surface water of water column in spring because they do not processes the *de novo* DT synthesis. Instead, ice algal cells have been evolved to aggregate and sink out of the surface layer rapidly. Therefore, the possibility in survive of the damaged ice algal cells could be predicted within the water column in the shallow coastal waters or stratified waters under near- or complete darkness.

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