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An explanation for the inter-species variability of the photoprotective non-photochemical chlorophyll fluorescence quenching in diatoms

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ABSTRACT

Diatoms are a major group of microalgae whose photosynthetic productivity supports a substantial part of the aquatic primary production. In their natural environment they have to cope with strong fluctuations of the light climate which can be harmful for photosynthesis. In order to prevent the damage of their photosynthetic machinery, diatoms use fast regulatory processes among which the non-photochemical quenching of chlorophyll *a* fluorescence (NPQ) is one of the most important. In a previous work, we highlighted differences in the kinetics and extent of NPQ between diatom species/strains originating from different aquatic habitats. We proposed that the NPQ differences observed between strains/species could potentially participate to their ecophysiological adaptation to the light environment of their respective natural habitat. In order to better understand the molecular bases of such differences, we compared the NPQ features of four strains/species of diatoms known for their NPQ discrepancy. We could identify new spectroscopic fingerprints concomitant to NPQ and the related xanthophyll cycle. These fingerprints helped us propose a molecular explanation for the NPQ differences observed between the diatom species/strains examined. The present work further strengthens the potential role of NPQ in the ecophysiology of diatoms.

1. Introduction

Diatoms are a major group of microalgae ubiquitous in all marine and freshwater ecosystems. They are among the most significant photosynthetic organisms contributing to about 40% of the aquatic primary production, especially in coastal ecosystems [1,2]. Their biological characteristics have largely shaped the structure of contemporary aquatic ecosystems [1,3]. As for most microalgae, the photosynthetic productivity of diatoms strongly depends on the aquatic light climate [4–6]. Planktonic as well as benthic diatoms tend to dominate ecosystems (coasts and estuaries) where they have to cope with a light climate with high-frequency irradiance fluctuations coupled with large amplitudes. Depending on the rate of water mixing, on the tidal cycle, on the daily/seasonal changes of solar irradiation, diatoms can be exposed to punctual or chronic excess light, possibly generating stressful conditions that impair their photosynthetic efficiency and their productivity (i.e. photoinhibition/photoinactivation) [7–10]. In order to prevent such situation, diatoms [11,12], and to a larger extent microalgae and other photosynthetic organisms [13–17], have evolved fast regulatory physiological processes which compose a 'photoprotective network'. They help to safely dissipate the excess of absorbed light energy as heat and/or to balance the excitation energy within the photosynthetic apparatus thus preventing/lowering the potential oxidative damages to the photosynthetic machinery. Additional mechanisms help repair the unavoidable photodamages [10,18]. In diatoms, two fast processes are believed to be essential for the photoprotection: the electron cycle of the photosystem II reaction center (PS II CET) and the non-photochemical quenching of chlorophyll *a* (Chl *a*) fluorescence (NPQ) [11,12,14,19]. NPQ is composed of three components, namely the high-energy state (qE), the state-transition (qT) and the 'photoinhibitory' (qI) quenchings [13,17]. In diatoms, qT does not exist and the origin of qI remains unclear [11,12]. Therefore, the diatom NPQ mainly relies on qE, a quenching mechanism which is controlled by i) the build-up of the transthylakoidal proton gradient (ΔpH), ii) the conversion of the xanthophylls diadinoxanthin (DD) into diatoxanthin (DT) called 'xanthophyll cycle' (XC), and iii) the presence of specific polypeptides of the light-harvesting complex (LHC) antenna named Lhcx [11,12,14,20–23]. Additionally, there exists a PS II reaction center quenching which might be related to the PS II CET [24,25]. These fast regulatory mechanisms proved to be important in field situation for maintaining the photosynthetic efficiency and productivity of diatoms [15,21]. NPQ was shown to significantly participate in the cellular response under fluctuating light conditions [26–28]. Recently, an

Abbreviations: Chl *a*, Chlorophyll *a*; Chl *a* FIKs, Chl *a* fluorescence induction kinetics; Chl a_{711}, Chl *a* fluorescence emission band at 711 nm; DD, diadinoxanthin; DES, de-epoxidation state; DT, diatoxanthin; FCP, fucoxanthin-chlorophyll protein; LHC, light-harvesting complex; NPQ, non-photochemical Chl *a* fluorescence quenching; PAM, Pulse-amplified modulation; PS II, Photosystem II; PS II CET, PS II cyclic electron cycle; ΔpH, transthylakoidal proton gradient; Δ522 nm, absorption change at 522 nm

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increasing number of reports suggested that the diatom/microalgae inter-species differences in NPQ are involved in the differential colonization of aquatic habitats depending on the rate of light fluctuations of the light climate [9,27–33].

While the most recent advances in our knowledge of the physiological regulation of diatom photosynthesis, and especially of NPQ and the XC, were generated by the use of genetically modified cells of the model diatom *Phaeodactylum tricornutum* [20,23,34,35], the comparison of different species/strains [9,18,20,27,30,36,37] together with the use of different growth conditions [18,30,37,38] has also proven to be useful. Indeed, the XC and NPQ extent and kinetics can differ with regards to the species [9,18,27,30], and to the light [30,38,39] and the temperature [18] acclimations. In a previous study, we compared the photoprotective ability of *P. triconutum* and *Skeletonema costatum* [27]. One of the major differences concerning NPQ: its extent in *S. costatum* was approximately half the one in *P. tricornutum*. It was not due to a lower de-epoxidation rate of DD to DT, as reported before for diatoms and other related organisms [18,40,41]. We suggested two explanations for such discrepancy [27]: 1) a different organization and FCP (fucoxanthin-chlorophyll protein) composition of the LHC antenna of PS II, including both Lhcf and Lhcx proteins, 2) a lower amount of DT molecules involved in the NPQ process; the two hypotheses not being exclusive from each other [18]. The present work aimed at going further deep in the understanding of NPQ differences between diatom species/strains. For that purpose, we examined four species/strains currently used as models for NPQ investigations and known to show different NPQ amplitude and regulation [20,27,36,42–44]: a low and a high NPQ strains of *P. tricornutum* (P.t.2 and P.t.4, respectively), *S. costatum* and *Cyclotella meneghiniana*. The three species are also representative of the two major groups of diatoms, the pennates (*P. tricornutum*) and the centrics (*S. costatum* and *C. meneghiniana*); the genome of *P. tricornutum* is available and *C. meneghiniana* is a close relative of *Thalassiosira pseudonana* whose genome is also published [45]. Finally, the ecological niches where P.t.2, P.t.4 and *S. costatum* were isolated (temperate estuary, high latitude shore, Mediterranean semi-enclosed bay, respectively) show very different underwater light climates [20,27].

2. Materials and methods

2.1. Cultures

Phaeodactylum tricornutum Böhlin CCAP1052–CCAP1055/1 ('P.t.2 strain', Culture Collection of Algae and Protozoa, Oban, Scotland) and CCAP 1052/6 ('P.t.4 strain', Sammlung Algenkulturen Göttingen (SAG), Germany), *Skeletonema costatum* (Greville) Cleve (Laboratoire Arago algal collection, Banyuls-sur-Mer, France) and *Cyclotella meneghiniana* Kützing SAG 1020_1a (SAG, Germany) cells were grown photoautotrophically in sterile natural seawater F/2 medium [46]. Non-axenic cultures of 300 mL were incubated at 18 °C in airlifts continuously flushed with sterile air. They were illuminated at a light intensity of 40 μmol photons m^{-2} s^{-1} with white fluorescent tubes (Claude, Blanc Industrie, France) with a 16 h light/8 h dark photoperiod ('continuous light', CL cells, see [47]). Intermittent light (IL) cells of *P. tricornutum* were grown in the same conditions except the light regime which was 5 min light/55 min dark cycle (see [47]). Cells were harvested during the exponential or stationary phase of growth as specified in Table 1, centrifuged at 3000*g* for 10 min and resuspended in their culture medium to a final concentration of 10 μg chlorophyll *a* (Chl *a*) mL^{-1}. The concentrated suspension was then continuously stirred at 18 °C under low light before use.

2.2. Pigment content

Pigment analyses were performed by HPLC as previously described [47]. Cells collected from the Clark electrode adapted to the PAM-fluorometer (see below) were deposited on a filter and frozen in liquid nitrogen. Pigments were extracted with a methanol:acetone (70:30, v/v) solution. The xanthophyll de-epoxidation state (%) was calculated as $(DT/(DD+DT))\times 100$ where DD is the diadinoxanthin, the epoxidized form, and DT is the diatoxanthin, the de-epoxidized form.

2.3. Spectroscopy

Ambient temperature absorption spectra were performed with a DW-2 Aminco spectrophotometer. For measuring the 522 nm absorption change (Δ522 nm) which reflects the 'activation of DT' (see [48]), spectra were performed on dark-acclimated cells (for 20 min) and cells exposed to light (5 min, 2000 μmol photons m^{-2} s^{-1}) as previously described [48]; the half-bandwidth was 0.5 nm. For partial inhibition of NPQ, cells were incubated prior to illumination (20 min, dark) with dithiothreitol (DTT) or NH_4Cl with a final concentration of 25 μM and 2 mM, respectively (see [49]). 77 K fluorescence emission spectra were measured with an F-3000 and F-4500 Hitachi spectrophotometer. Half-bandwidth for emission was 2.5 (F-4500) and 3 (F-3000) nm, respectively. Samples were dark-acclimated (20 min) and then concentrated at 1 μg Chl *a* on a Millipore AP-20 prefilter that was immediately frozen in liquid nitrogen before measurement. The same procedure was used for illuminated cells (2000 μmol m^{-2} s^{-1} for 5 min) albeit a Chl *a* amount of 3 μg was immediately frozen after illumination. The angle between the filter and the light source/detector was 45°. For complete inhibition of NPQ, cells were incubated prior to illumination (20 min, dark) with 0.2 mM DTT or 5 mM NH_4Cl [49].

Table 1
Photophysiological properties of the species/strains and of the NPQ type of cells used in this study.

Species and type of cells	Growth status	F_v/F_m	DD + DT	F711/F687	DES	DT	Δ 522 nm × 10^2	NPQ
P. tricornutum (P.t.2) 'low NPQ'	'CL', exp.	0.65 ± 0.02	8.8 ± 0.4	0.35 ± 0.06	27	2.38 ± 0.1	1.3 ± 0.10	2.38 ± 0.24
P. tricornutum (P.t.2) 'medium NPQ'	'CL', stat.	0.61 ± 0.03	13.6 ± 0.9	0.50 ± 0.07	36	4.9 ± 0.3	2.1 ± 0.10	3.50 ± 0.25
P.t.2, 'high NPQ'	'IL', exp.	0.68 ± 0.02	17.4 ± 2.3	1.22 ± 0.26	46	8.0 ± 1.1	2.5 ± 0.20	9.40 ± 0.61
P. tricornutum (P.t.4) 'low NPQ'	'CL', exp.	0.67 ± 0.02	12.7 ± 0.2	0.48 ± 0.01	38	4.8 ± 0.1	n.d.	1.58 ± 0.11
P.t.4, 'medium NPQ'	'CL', stat.	0.60 ± 0.03	23.8 ± 0.2	0.62 ± 0.09	43	10.2 ± 0.1	n.d.	2.63 ± 0.19
P.t.4, 'high NPQ'	'IL', exp.	0.64 ± 0.02	30.9 ± 0.7	1.00 ± 0.19	61	18.9 ± 0.4	n.d.	4.08 ± 0.21
S. costatum (S.c.) 'low NPQ'	'IL', exp.	0.64 ± 0.01	8.3 ± 2.1	0.19 ± 0.02	33	2.7 ± 0.7	0.33 ± 0.15	0.51 ± 0.07
S.c. 'medium NPQ'	'CL', exp.	0.59 ± 0.07	18.1 ± 0.1	0.29 ± 0.01	40	7.2 ± 0.05	1.00 ± 0.15	1.35 ± 0.29
S.c. 'high NPQ'	'CL', stat.	0.69 ± 0.02	26.9 ± 0.7	0.32 ± 0.01	59	15.9 ± 0.4	1.73 ± 0.17	2.5 ± 0.30
C. meneghiniana (C.m.) 'low NPQ'	'CL', exp.	0.63 ± 0.03	24.1 ± 0.3	0.29 ± 0.01	52	12.5 ± 0.2	0.86 ± 0.28	0.77 ± 0.09
C.m. 'medium NPQ'	'IL', exp.	0.58 ± 0.03	23.6 ± 0.2	0.29 ± 0.02	23	5.4 ± 0.05	0.70 ± 0.13	0.90 ± 0.09
C.m. 'high NPQ'	'CL', stat.	0.69 ± 0.02	57.9 ± 1.2	0.25 ± 0.01	64	37.1 ± 0.8	1.44 ± 0.26	1.94 ± 0.22

'CL', 'continuous light': 16 h:8 h Light:Dark cycle; 'IL', 'intermittent light': 5 min/55 min L:D cycle (see Material and methods section for details); exp. growth, exponential phase of growth; stat. growth, stationary phase of growth; the fluorescence ratios F_v/F_m (the maximum photosynthetic efficiency of PS II) and F711/F687 were measured on dark-acclimated cells (see also Fig. 4); Δ 522 nm × 10^2 (the absorption change at 522 nm), NPQ and DES (de-epoxidation state of diadinoxanthin (DD) into diatoxanthin (DT) in %: $(DT/(DD+DT)\times 100)$, DD and DT in mol. 100 mol Chl a^{-1}) were measured after 5 min at 2000 μmol s^{-1} m^{-2} (see also Fig. 1 and Fig. 3).

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