Relaxation of the non-photochemical chlorophyll fluorescence quenching in diatoms: kinetics, components and mechanisms

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Diatoms are especially important microorganisms because they constitute the largest group of microalgae. To survive the constant variations of the light environment, diatoms have developed mechanisms aiming at the dissipation of excess energy, such as the xanthophyll cycle and the non-photochemical chlorophyll (Chl) fluorescence quenching. This contribution is dedicated to the relaxation of the latter process when the adverse conditions cease. An original nonlinear regression analysis of the relaxation of non-photochemical Chl fluorescence quenching, qN, in diatoms is presented. It was used to obtain experimental evidence for the existence of three time-resolved components in the diatom Phaeodactylum tricornutum: qNf, qNi and qNs. qNf (s time-scale) and qNs (h time-scale) are exponential in shape. By contrast, qNi (min time-scale) is of sigmoidal nature and is dominant among the three components. The application of metabolic inhibitors (dithiothreitol, ammonium chloride, cadmium and diphenyleneiodonium chloride) allowed the identification of the mechanisms on which each component mostly relies. qNi is linked to the relaxation of the ΔpH gradient and the reversal of the xanthophyll cycle. qNs quantifies the stage of photoinhibition caused by the high light exposure, qNf seems to reflect fast conformational changes within thylakoid membranes in the vicinity of the photosystem II complexes.

1. Introduction

Diatoms constitute the most dominant group of eukaryotic organisms in marine waters [1]. In marine ecosystems, diatoms play crucial roles in several biogeochemical cycles, including that of carbon [2]. It is estimated that diatom photosynthesis is responsible for up to 20% of the global primary production [3] and up to 40% of the carbon sequestered in the oceans [2]. This makes diatoms a major feeding source for other living organisms [2]. Thus, diatoms render tremendous ecological services, any of which is efficiently performed only when diatom fitness is preserved.

In marine ecosystems, the intensity of the environmental constraints is constantly modified [4,5]. For instance, turbulent water movements regularly expose microalgae to stresses such as high light (HL) conditions [6]. Regardless of their origin, stress conditions usually trigger a change in the equilibrium between the absorbed light energy and energy utilization [7], which ultimately results in lowering of primary productivity [8–11]. To minimize HL effects, microalgae have developed short- and long-term mechanisms to tune the balance between energy...
Phaeodactylum tricornutum UTEX 646 was grown in a dynamic, light-intensity-dependent short-term process of dissipation of excessive excitation energy into heat in the LHC, the xanthophyll cycle [7,12–14]. The de-epoxidation step is catalysed by lumen-localized enzymes, the so-called Ddx de-epoxidases (DDEs) [13,14]. DDE binding to the thylakoid membranes is activated by the acidification of the thylakoid lumen, resulting from the establishment of the trans-thylakoidal pH gradient (ΔpH) under HL [15,16]. When the HL condition disappears, the stroma-located diatoxanthin (Dtx) epoxidase (DTE) catalyses Dtx expoxidation to Ddx [13,14]. Ascorbate and reduced nicotinamide adenine dinucleotide phosphate (NADPH) are the essential cofactors for de-epoxidase and epoxidase, respectively [13,14] (see electronic supplementary material, Data S1).

In this contribution, an original method of nonlinear regression analysis of NPQ of the maximum variable Chl fluorescence yield (qN) relaxation kinetics is presented and used to elucidate the mechanisms on which the qN relaxation process relies. Metabolic inhibitors, such as dithiothreitol (DTT), ammonium chloride (NH₄Cl), cadmium (Cd) and diphenylelondichloride (DPI) were used to resolve the number of components, their kinetics and the mechanism on which they depend in the diatom Phaeodactyllum tricornutum. A deep knowledge of the dissipation mechanisms of excess energy is of crucial importance for the understanding of diatom ecology and can certainly contribute to better exploitation of their capacity to produce high-value metabolites, the synthesis of which is enhanced under stress [23–25].

2. Material and methods
(a) Growth conditions
Phaeodactylum tricornutum UTEX 646 was grown in f/2 medium under a 16 L : 8 D regime and at 24 ± 1°C. Two other P. tricornutum strains: CCMP632 and NCC340, and Odontella aurita NCC86, Entomoneis paludosa NCC18.2, Skeletonema costatum NCC60, Thalassiosira pseudonana CCMP1335 and T. weissflogii NCC133 were cultured in artificial seawater [26] under the same light regime and at 16 ± 1°C. The different species were grown under cool-white fluorescent tubes (Philips TL-D 90, 36 W; photon flux density 300 μmol photons PAR m⁻² s⁻¹) until the cultures reached the exponential growth phase, i.e. 4 days [27]. The irradiance was measured with a 4r waterproof probe (Walz, Germany) connected to a Li-Cor 189 quantum meter. Cell density was estimated either using the absorbance at 750 nm or by direct numbering using a Malassez haemocytometer (microscope magnification 400×). Growth rate was calculated as \( r = \ln N_i - \ln N_0)/\Delta t \), where \( N_0 \) and \( N_i \) represent the cell density at time \( t = 0 \) and \( t = t \), respectively, and \( \Delta t \) is the age of the culture (days).

(b) Chlorophyll fluorescence yield measurements
Chl fluorescence yield was monitored at the growth condition after a dark-adaptation period (15 min). \( F_0 \) was recorded under a weak modulated light (less than 15 μmol PAR m⁻² s⁻¹, 800 Hz). NPQ was induced during a 7 min non-saturating white actinic radiation (photon flux density 800 μmol PAR m⁻² s⁻¹, KL 1500; H. Walz, Germany). At the end of the actinic illumination, the dark relaxation of the Chl fluorescence yield was recorded in order to allow quenching analysis. For each sample, the minimum (\( F_{0,0}; F_{0,0}' \), maximum (\( F_{M,0}; F_{M,0}' \) and maximum variable (\( F_v,F_v',F_v'' \)) Chl fluorescence yields in a dark-adapted state, in a light-adapted state and during the dark relaxation were measured, respectively [22] (see figure 1a for a representative recording). The slow Chl fluorescence induction kinetics were recorded using either a PAM 101–103 fluorometer (H. Walz, Germany) or an FMS1-modulated fluorometer (Hansatech Instruments, UK) [28].

DTT (final, 200 μM stock, 20 mM), NH₄Cl (5 mM/1 M) and DPI (0.1–5 μM/2 mM) were diluted in distilled water or DMSO (DPI). For assays, the algae were incubated with the metabolic inhibitors 15 min before Chl fluorescence measurements (i.e. during the dark incubation) except for Cd (20 mg l⁻¹) that was present during the foregoing 24 h, as indicated in [27].

The intracellular Cd amount was determined as explained in the electronic supplementary material, Data S2.

To avoid CO₂ shortage during measurements, the cultures were provided with NaHCO₃ (final, 4 mM stock, 0.2 M) [29]. Because the light intensity experienced by cells depends on cell density [30], the fluorescence measurements were performed using sample containing similar Chl amount as estimated by the absorbance at 665 nm (not shown).

(c) Pigment extraction and analysis
Pigment extraction and analysis by HPLC were performed as indicated in [31]. The de-epoxidation ratio (DER) was calculated as DER (%) = 100 × [Ddx]/([Ddx] + 0.5[Ddx]).

(d) Oxygen evolution
Oxygen evolution measurements were performed as in [28]. Briefly, the oxygen was determined using a thermostated chamber equipped with a Clark-type oxygen electrode (DW2, Hansatech Instruments, UK). Oxygen evolution was
measured under actinic irradiance ranging from 0 to 2200 μmol photons PAR m⁻² s⁻¹.

(e) Mathematical verification: statistics
The identical recording and processing of slow Chl fluorescence induction kinetics were assured by means of user-defined procedures. Data extracted from records were processed by the graphic software SIGMAPLOT 2000 for Windows (v. 6.10, SPSS, USA). Statistical verifications of calculations carried out by non-linear regression (fitting) procedures were evaluated by parameters $R^2$ and Norm. $R$, the coefficient of determination, measures how well a regression model describes the fitted data. ‘Norm’ stays for the square root of the sum of squares of the residuals. An $R^2$ value close to unity indicates that the relation between the independent and dependent variables is very well described by an entered regression equation. When the change in Norm value between two subsequent iterations is less than a given tolerance, the solution is considered to have been found.

Statistical significance of differences between corresponding pairs of data shown in graphs was found on the basis of the standard Student’s t-test applied to individual unpaired or paired column data. The t-test determines whether the mean values of two data columns are significantly different by testing the hypothesis that the corresponding means are equal. The statistical significance ($p < 0.05$) is labelled by an asterisk. Results for $p > 0.05$ are not marked in graphs and are considered as statistically non-significant.

3. Results and discussion

(a) *Phaeodactylum tricornutum* is able to fully relax non-photochemical quenching
Figure 1a displays a typical slow Chl fluorescence induction kinetics recorded using non-treated *P. tricornutum*. Values of the maximum quantum yield of photosystem II (PSII) photochemistry ($\Phi_{Po}$) are close [27,28,32–34] or below the values reported in earlier studies [35]. The reasons for this discrepancy are not clear at present: it could result from the presence of a small pool of Dtx measured in the dark-adapted samples (data not shown, max 5%).

The high but non-saturating irradiation activated non-photochemical quenching as indicated by the low maximum Chl fluorescence yield reached at the end of the light phase ($F_{M}$). As reported by Ruban et al. [36], the quenching intensity is three to five times larger (table 1) than that with higher plants [22]. As is frequently observed [27], $F_{m}^\prime$ reached its minimum after approx. 3–5 min of actinic light exposure (figure 1a). The $F_{0}$ value is lower than the $F_{M}$ level indicating that the processes involved in NPQ also affect the fluorescence yield of ‘open’ PSII reaction centres (figure 1a,b). Here, the real values of $F_{0}$ can be slightly lower than the indicated values because of the presence of some remaining $Q_{A}^{-}$ at the beginning of the relaxation phase [36]. After switching the actinic light off, NPQ relaxes (half-life more than 20 min; figure 1b). Similar values for the half-life of relaxation have been reported [33,37].

During this relaxation period, the fluorescence peaks result from a temporary closure of PSII reaction centres by short (0.8 s) saturation pulses applied to probe the maximum fluorescence yield ($F_{M}^\prime$) in the absence of photochemical fluorescence quenching. During the relaxation period, the actual quantum yield of PSII photochemistry ($\Phi_{Po}$) (for equations, see the electronic supplementary material, Data S3) increased regularly compared with the initial value of $\Phi_{Po}$ (figure 1b).

(b) qN relaxation kinetics in *Phaeodactylum tricornutum* are composed of three components
To characterize the components causing the dark-relaxation kinetics of qN, the nonlinear regression analysis proposed...
Table 1. Effect of xenobiotics on the basic set of Chl fluorescence parameters, components of NPQ of Chl fluorescence (qN, qN1) and DER. Statistical significance of resulted values found for given treatments compared with ‘control’ is *p < 0.05, **p < 0.01 and ***p < 0.001. Means and corresponding standard deviations are the results of three to five samplings. For definitions and explanation of parameters summarized in this table, see the electronic supplementary material, Data S3 and S4.

<table>
<thead>
<tr>
<th>parameter</th>
<th>control</th>
<th>+ DTT</th>
<th>+ NH4Cl</th>
<th>+ Cd</th>
<th>+ Cd+ DTT</th>
</tr>
</thead>
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<tr>
<td>Fv/Fm</td>
<td>0.57 ± 0.01</td>
<td>0.56 ± 0.03</td>
<td>0.52 ± 0.01**</td>
<td>0.58 ± 0.03</td>
<td>0.53 ± 0.02*</td>
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<tr>
<td>qN</td>
<td>0.39 ± 0.05</td>
<td>0.35 ± 0.05</td>
<td>0.25 ± 0.04**</td>
<td>0.37 ± 0.08</td>
<td>0.28 ± 0.03*</td>
</tr>
<tr>
<td>qN1</td>
<td>0.93 ± 0.02</td>
<td>0.89 ± 0.02*</td>
<td>0.75 ± 0.09**</td>
<td>0.93 ± 0.02</td>
<td>0.71 ± 0.07**</td>
</tr>
<tr>
<td>qNf</td>
<td>0.95 ± 0.01*</td>
<td>0.91 ± 0.02*</td>
<td>0.95 ± 0.02</td>
<td>0.96 ± 0.01</td>
<td>0.85 ± 0.04**</td>
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<td>q0</td>
<td>0.68 ± 0.04</td>
<td>0.58 ± 0.04*</td>
<td>-0.15 ± 0.04***</td>
<td>0.65 ± 0.09</td>
<td>-0.0 ± 0.1***</td>
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<tr>
<td>qN1</td>
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<td>0.09 ± 0.01</td>
<td>0.05 ± 0.02*</td>
<td>0.07 ± 0.01*</td>
<td>0.07 ± 0.001**</td>
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<td>NPQ</td>
<td>4.63 ± 0.73</td>
<td>3.12 ± 0.67*</td>
<td>0.47 ± 1.27***</td>
<td>4.67 ± 1.60</td>
<td>0.62 ± 2.83**</td>
</tr>
<tr>
<td>qNF</td>
<td>0.02 ± 0.01</td>
<td>0.04 ± 0.01*</td>
<td>0.30 ± 0.08***</td>
<td>0.04 ± 0.02</td>
<td>0.31 ± 0.07***</td>
</tr>
<tr>
<td>t1/2 (s)</td>
<td>8.1 ± 3.1</td>
<td>23 ± 21</td>
<td>65 ± 38**</td>
<td>14.8 ± 2.6**</td>
<td>20.0 ± 4.5**</td>
</tr>
<tr>
<td>qNi</td>
<td>0.75 ± 0.09</td>
<td>0.67 ± 0.04</td>
<td>—</td>
<td>0.47 ± 0.09**</td>
<td>0.34 ± 0.20*</td>
</tr>
<tr>
<td>xII (min)</td>
<td>8.9 ± 2.1</td>
<td>6.61 ± 0.35</td>
<td>—</td>
<td>17.0 ± 3.77**</td>
<td>4.25 ± 0.32*</td>
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<tr>
<td>qNs</td>
<td>0.18 ± 0.09</td>
<td>0.20 ± 0.06</td>
<td>0.18 ± 0.03</td>
<td>—</td>
<td>0.20 ± 0.11</td>
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<tr>
<td>t1/2 (min)</td>
<td>—</td>
<td>—</td>
<td>0.47 ± 0.07</td>
<td>0.45 ± 0.10</td>
<td>390 ± 490</td>
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<td>DER</td>
<td>62%</td>
<td>4.6%</td>
<td>5.1%</td>
<td>58%</td>
<td>52%</td>
</tr>
</tbody>
</table>

*aqN1 = qNF + qN1 + qNs.
*bThe relaxing part of qNs.
*cThe non-relaxing (‘permanent’) part of qNs.
*dA too large spread in values of t1/2.

by Roháček [22] for higher plants was adapted to the case of diatoms. Owing to space limitations in this contribution, the mathematical reasoning is presented in the electronic supplementary material, Data S4. The procedure allows the fitting of the qN relaxation kinetics with three components (figure 2). This finding agrees with Grouneva et al. [20,38] who found that establishment of non-photochemical quenching consists of three components. The three components described here differ in their shape, half-life of relaxation and amplitude (table 1). The slow (qNs) and fast (qNf) components have exponential shapes while the intermediate and major component (qNi) is of sigmoidal shape (figure 2).

This shape can be explained by the difference in the rate of Dtx epoxidation and NPQ relaxation kinetics during the relaxation period (C. meneghiniana [39]; P. tricornutum [40]). Indeed, a sigmoidal relationship was found between the two parameters only in P. tricornutum [40]. The sigmoidal shape of the qNi component is not a peculiarity of P. tricornutum but has been found in each diatom species tested so far (see electronic supplementary material, Data S5).

(c) qNi relies on dissipation of the proton gradient and reversal of the xanthophyll cycle

The three components described in §3b reflect the pathways used by the photosynthetic apparatus to relax non-photochemical quenching after HL treatment. Grouneva et al. [20,38] found that the three components contribute to the establishment of NPQ. Therefore, we hypothesize that the three components described in this paper could be the counterparts of the pathways through which NPQ is established during actinic illumination. To test this hypothesis, a pharmacological approach was used to inhibit those pathways in the hope that the inhibitors would selectively affect either component. To this end, NH4Cl a dissipator of ΔpH [41], was used. Representative kinetics obtained with each metabolic inhibitor are presented in the electronic supplementary material, Data S6.

In the presence of NH4Cl, the Fv/Fm ratio (Fv/Fm) was significantly reduced (table 1), confirming severe alterations of PSII functioning were induced by NH4Cl [38], e.g. by inhibiting the oxygen evolving centre [42]. NH4Cl slowed down the establishment of qN and NPQ (see electronic supplementary material, figure S6–1; table 1), but not that of qN1, in which case the amplification of qNF compensated this effect, followed concurrently by the lowering of q0 and the actual photochemical capacity of PSII (qP). NH4Cl gave a very clear answer, as this uncoupler fully inhibited the intermediate and major component qNi as well as of Dtx formation (table 1). The Dtx found at the end of the light phase was already present at the end of the dark phase (data not shown). The absence of de novo Dtx formation was expected because Ddx de-epoxidation requires acidification of the thylakoid lumen, a phenomenon that NH4Cl abolishes [41].

It was well established that Dtx formed through the operation of the xanthophyll cycle (see electronic supplementary material, Data S1) participates in process of non-photochemical quenching. To determine the contribution of the xanthophyll cycle to NPQ relaxation, the intensity of the different qN components was estimated in DTT-treated cells, DTT being an inhibitor of the Ddx de-epoxidase (see...
Figure 2. The relaxation kinetics of qN are composed of three components. Resolution of three qN components in the control sample (cf. figure 1) of *P. tricornutum* by the method of nonlinear regression analysis of experimental data is presented. Time courses of the maximum variable Chl fluorescence yield \( F_{v}^{0} \) and actual magnitudes of three qN components are results of the fit according to equation 5-7 within the experimental data (black symbols). The input values of \( F_{v0} \), \( F_{v1} \) and \( F_{v}^{0}(1)\)-level (black dotted line) applied to the fit procedure are highlighted together with the resulting numerical values for magnitudes of the fast (qNf), intermediate (qNi) and slow (qNs) components of the actual maximum NPQ (qN1). The fit quality is demonstrated by squared value of the coefficient of determination \( R^{2} \). For experimental conditions, stated symbols and more details on the regression analysis, see the electronic supplementary material, Data S4. (Online version in colour.)

**Figure 2:** The relaxation kinetics of qN are composed of three components. Resolution of three qN components in the control sample (cf. figure 1) of *P. tricornutum* by the method of nonlinear regression analysis of experimental data is presented. Time courses of the maximum variable Chl fluorescence yield \( F_{v}^{0} \) and actual magnitudes of three qN components are results of the fit according to equation 5-7 within the experimental data (black symbols). The input values of \( F_{v0} \), \( F_{v1} \) and \( F_{v}^{0}(1)\)-level (black dotted line) applied to the fit procedure are highlighted together with the resulting numerical values for magnitudes of the fast (qNf), intermediate (qNi) and slow (qNs) components of the actual maximum NPQ (qN1). The fit quality is demonstrated by squared value of the coefficient of determination \( R^{2} \). For experimental conditions, stated symbols and more details on the regression analysis, see the electronic supplementary material, Data S4. (Online version in colour.)

Electronic supplementary material, Data S1). In the presence of DTT, the amplitude of qNi was ca 90% of the control value (table 1), demonstrating that the qNi relaxation component relies on dissipation of ΔpH and the reversal of the xanthophyll cycle. Consequently, qNi should be considered as the counterpart to the steady-state quenching component found in *C. meneghiniana* [37] and of qE in green algae and higher plants [43].

If the reasoning presented above is sound, the inhibition of the reversal of the xanthophyll cycle, i.e. the conversion of Dtx to Ddx, will affect the qN relaxation kinetics, especially qNi. To test this hypothesis, qN relaxation kinetics were recorded in the presence of Cd or DPI, two compounds reported to interact negatively with the reversal of the xanthophyll cycle (Cd [27]; DPI [44]).

Cd effects were similar to those reported in [27], i.e. (i) the DER reached in Cd-treated cells was 55% higher than that in control cells at the end of qN induction period (table 1) and (ii) qN relaxation was slower in the presence of Cd (table 1; electronic supplementary material, Data S6). In our conditions, the intracellular Cd concentration was around 3 fg cell\(^{-1}\) (see electronic supplementary material, Data S7). Other works on *P. tricornutum* reported 10–30 fg cell\(^{-1}\) [45–47]; for a review, see [4].

In the presence of Cd, the intensity of qNi was reduced to the same extent as the DER (table 1) and was slowed down, suggesting that Cd could directly interact with DTE (see electronic supplementary material, Data S1). An indirect effect of Cd could not be completely excluded, however, as Cd has been reported to interact with biochemical reactions generating NADPH, the cofactor of DTE [48–49] (see electronic supplementary material, Data S1). To obtain more information about the putative other target(s) of Cd, relaxation of NPQ was studied in Cd-treated cells incubated with DTT, an inhibitor of the forward reaction of the xanthophyll cycle and a chelator of Cd [43]. A synergistic effect of Cd and DTT resulted in a very similar impact on values of basic fluorescence parameters (see the electronic supplementary material, Data S3), as found for the NH\(_4\)Cl treatment.

DPI has been described as an inhibitor of the zeaxanthin epoxidase [44]. For very low DPI concentrations (less than 0.5 mM), qN remains unaffected (figure 3a-c). The progressive inhibition of the reversal of the xanthophyll cycle blocked the relaxation of qP, indicating that PSII reaction centres remained mostly closed when the xanthophyll cycle could not be reversed (data not shown). The amplitude of qNi progressively decreased while the DPI dose increased, and for the highest DPI concentration (5 mM) tested (figure 3b), the amplitude of qNi reached the minimum level close to zero, demonstrating the participation of the reversal of the xanthophyll cycle in qN relaxation (figure 3c).

Regardless of DPI concentration, qNf remained unaffected (figure 3c). As in Grouneva et al. [20,38], a small Dtx pool was detected before HL treatment. If such a pool is involved in the relaxation of qN, it is likely not available to Dtx epoxidase, as the DER calculated before actinic illumination and after the relaxation period were similar (data not shown). The increasing amount of DPI triggered a complementary increase in qNs to that of qNi; for a DPI concentration of 5 mM, qNs reached the maximum (figure 3c). The mechanism on which qNs relies remains obscure. qNs could reflect ΔpH dissipation as it has been shown that after Dtx has been activated, ΔpH is no longer needed for efficient NPQ [50]. It is also possible that in our conditions, diatoms may have experienced moderate photoinhibition because the value for the irradiance corresponding to the light saturating photosynthesis, \( E_{\text{c}} \), was around 250 \( \mu \text{mol photons m}^{-2} \text{s}^{-1} \) (see electronic supplementary material, Data S8). This value, however, is far from the HL level determined by Ting & Owens [30] as being fully photoinhibitory. More experiments have to be performed to clarify this point because in the presence of NH\(_4\)Cl, qNs was as intense as in the control but significantly accelerated (table 1). An interesting feature evidenced by the qN analyses presented in this contribution is the possibility that qNs becomes permanent (table 1 and figure 3c) i.e. its half-life cannot be measured. This was observed when the xanthophyll cycle was blocked by DTT and in the presence of Cd. The reasons for such a phenomenon remain unclear at present and may result from additional target(s) of DTT and Cd.

(d) The sigmoidicity of the qNi component is an intrinsic characteristic of ΔpH gradient relaxation in diatoms and reflects cooperative mechanisms. The results presented in this article establish that the component qNi is of sigmoidal shape (figure 2; electronic supplementary material, Data S5). In the presence of DTT, the kinetics of qNi remained sigmoidal, suggesting that this shape is linked to the establishment of ΔpH in diatoms. This reasoning fits with NPQ kinetics obtained from cells treated with *N,N*-dicyclohexylcarbodiimide (DCCD), an
inhibitor of ATP synthase [51,52]. In the presence of this chemical, protons were accumulated in the thylakoid lumen, triggering an intense pH gradient even under low-light illumination, which resulted in the development of NPQ. The kinetics of NPQ development recorded in these conditions are of sigmoidal shape [29]. Such a shape was not observed in the DCCD-treated green alga *Chlamydomonas* sp. ICE-L [53]. It has also been suggested that the activation of the quenching capacity of Dtx requires the protonation of special amino acid residues of the LHCII resulting of qNi is sigmoidal in shape. This feature seems unique to diatoms. From the mechanism point of view, qNi relies on ΔpH relaxation and Dtx epoxidation. More experiments have to be performed to clarify the nature of the events involved in qNi and qNs. qNf could be related to a fast conformational change occurring within the thylakoid membranes at the start of the relaxation process. The different (very fast in their creation) qN components could be localized on PSII attached or detached LHCS [58]. The former would be related to the qN enhancing mechanism(s), whereas qNs could be heterogeneous from the mechanistic point of view and related to photoninhibition and/or partial dissipation of the pH gradient.

**4. Conclusion and perspectives**

The kinetics of the relaxation of NPQ maximum variable fluorescence in diatoms result from the development of three individual components, qNf, qNi and qNs (figure 2). The kinetics of qNf and qNs follow an exponential curve, whereas that of qNi is sigmoidal in shape. This feature seems unique to diatoms. From the mechanism point of view, qNi relies on ΔpH relaxation and Dtx epoxidation. More experiments have to be performed to clarify the nature of the events involved in qNf and qNs. qNf could be related to a fast conformational change occurring within the thylakoid membranes at the start of the relaxation process. The different (very fast in their creation) qN components could be localized on PSII attached or detached LHCS [58]. The former would be related to the qN enhancing mechanism(s), whereas qNs could be heterogeneous from the mechanistic point of view and related to photoninhibition and/or partial dissipation of the pH gradient.

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**Figure 3.** Effect of DPI on *P. tricornutum*. Influence of different concentrations of DPI (0 μM—control, 0.1, 0.5, 1, 3, 5 μM) on slow Chl fluorescence induction kinetics (a–c) and individual components of NPQ (c–e) of *P. tricornutum*. In comparison to the ‘Control’ (figure 1), a marked influence of DPI on the shape of induction kinetics was observed starting from 0.5 μM DPI (a) leading consecutively to activation of the strong inhibitory fluorescence quenching as seen for 5 μM DPI (b). This effect is reflected in the shape of qN components of NPQ (c) and their relaxation kinetics parameters (d,e) which are polynomial fits (n = 3) within experimental data (means of three repetitions and s.d. error bars are shown, relaxation parameters are expressed in seconds (s) or minutes (min), cf. table 1). For explanation of given parameters, see the electronic supplementary material, Data S4. (Online version in colour.)
References


