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Photosynthetic energy conversion in the diatom *Phaeodactylum tricornutum*

Measuring by calorimetry, oxygen evolution and pulse-amplitude modulated fluorescence

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Abstract Isothermal microcalorimetry can be used to investigate the photosynthetic energy conversion of autotrophic organisms. In this study, for the first time a diatom alga was used to compare the calorimetrically measured heat flux with measurements of the photosynthetic performance by oxygen evolution and pulse-amplitude modulated fluorescence. The presented experimental setup proved suitable to compare calorimetric data with those of conventional methods of the determination of photosynthesis rates. Special attention was paid to the contribution of energy dissipation via non-photochemical quenching (NPQ) of chlorophyll fluorescence to the metabolic energy balance. This was achieved by a combination of different light conditions and the use of an inhibitor of NPQ. Although NPQ is an important photoprotective mechanism in diatoms, the inhibition of NPQ resulted in an activation of alternative, energy dissipating pathways for absorbed radiation which completely compensated for the fraction of energy dissipation by NPQ.

Keywords Calorimetry · Diatom · Fluorescence · Oxygen evolution · Photosynthesis

Abbreviations

Chl Chlorophyll
NPQ Non-photochemical quenching
PAR Photosynthetically active radiation
PSII Photosystem II
XC Xanthophyll cycle

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Introduction

The improvement of the efficiency of biomass production by photosynthetic organisms is an important issue in ecology, agriculture and biotechnology in the context of outrunning fossil energy resources. Photosynthesis is the only process which converts solar energy and CO_2 into organic matter. The photosynthetic activity is normally measured by gas exchange analysis based on rates of O_2 evolution or CO_2 uptake. Unfortunately, the photosynthetic rates are not always good predictors of growth rates. The reason is that photosynthesis reflects only the electron delivery for CO_2 assimilation but biomass production is linked with metabolic costs consuming a variable part of the photosynthetic energy [1]. This leads to the fact that the quantum efficiency of biomass production is a complex resultant of many biochemical reactions with energy losses on different levels of the cellular metabolism.

The first level is the primary photosynthetic reaction at photosystem II (PSII). Here, the quantum efficiency is influenced by the efficiency of light absorption and by energy dissipating processes. The latter comprise the emission of light with higher wavelength (fluorescence), the constitutive heat dissipation by chlorophylls (Chl), and the regulated dissipation of heat within the antenna complexes of the chloroplast membrane [2]. The regulated heat dissipation can be detected as the so-called non-photochemical quenching (NPQ) of fluorescence and is an important mechanism for photosynthetic organisms to

safely dissipate excessively absorbed light energy (reviewed in [3]).

The second level is determined by the fraction of electrons which have been released at PSII but are not used for the carboxylation reaction within the Calvin cycle. These 'alternative electrons' are consumed by light-dependent reactions, like the Mehler-peroxidase reaction (water–water cycle) or photorespiration, but also in the reduction of nitrate (recent review in [4]). In addition, photosynthetically produced ATP and NADPH might be used for carbon-concentrating mechanisms [4].

The third level is characterized by the macromolecular composition of the biomass and its degree of reduction. This relation is easily illustrated by the fact that the autotrophic synthesis of carbohydrates requires four electrons per carbon whereas the synthesis of lipids and proteins needs at least six electrons per carbon [5].

Thus, it is impossible to directly infer the quantum efficiency of growth from the photosynthesis rate without additional methods which provide the necessary information about NPQ, the amount of alternative electrons and the biomass composition [6–8].

An alternative approach to a direct measurement of the quantum efficiency of biomass production could be photocalorimetry [9]. Photocalorimetry measures the metabolic heat exchange of photosynthetic organisms in illuminated calorimetric chambers. The reaction heat measured by this method incorporates all processes mentioned above. Janssen et al. [10] even suggested the application of photocaloric measurements as an online monitor for industrial scale microalgae production. An overview about the historic and recent attempts to measure the heat related to photosynthetic processes by calorimetry is provided in [11]. To our knowledge, only two studies dealt with the influence of NPQ on the photosynthetic energy balance measured by calorimetry [12, 13]. There exist two methods which are conventionally used in plant physiology to measure the heat dissipation via the NPQ mechanism, namely photoacoustics [14] and, more important, the fluorometry method [15]. Photoacoustics delivers only a relative measure of heat dissipation [16, 17], whereas the fluorescence method measures heat dissipation indirectly as a quenching of the Chl fluorescence signal. In addition, indications were provided that the kinetics of changes in NPQ obtained from both methods, photoacoustics and fluorescence, do not correlate with a photocalorimetrically measured heat dissipation [12].

Under conditions of saturating illumination the NPQ mechanism is strongly coupled to the light-induced, transmembrane proton gradient and the de-epoxidation reaction of the xanthophyll cycle (XC) pigments in the chloroplast (e.g., [18, 19]). In vascular plants and algae of the green lineage, NPQ consists of different components and is partly independent of the XC (e.g., [20, 21]). In the diatom *Phaeodactylum tricornutum*, however, NPQ consists of virtually only one component, the energy-dependent quenching qE, which is closely correlated to the activity of the XC [3]. Thus, in this diatom the activation of NPQ under high irradiance can be effectively suppressed by the addition of dithiothreitol (DTT) which is an inhibitor of the de-epoxidase reaction of the XC [22]. Due to the direct correlation of NPQ and XC activity it should be much easier to study the effects of NPQ on heat dissipation measured by photocalorimetry in diatoms than in green algae. In addition, the maximum capacity of NPQ can be much higher in diatoms than in other photosynthetic organisms [23]. Wagner et al. [24] calculated that in *P. tricornutum* up to 35% of the absorbed quanta can be dissipated via the NPQ mechanism. This leads to the conclusion, that such an important mechanism in the regulation of energy dissipation should influence the metabolic energy balance in *P. tricornutum*.

Therefore, the following aims for this study were derived:

(i) A photocalorimetric measuring setup has to be developed which allows the illumination of algal samples with irradiances of up to 450 μmol photons m^{-2} s^{-1}. These high-light conditions are necessary to fully activate the NPQ mechanism.
(ii) The calorimetric signal has to be validated by measuring in parallel the net oxygen evolution rate. For estimation of the heat flux rate from the oxygen evolution, the oxycaloric equivalent ($\Delta_k H_{O_2}$ [25]) will be used.
(iii) The photocalorimetric signal has to be compared under conditions where the degree of heat dissipation via NPQ is triggered by irradiance and the use of DTT. Due to the easier characterization of the NPQ and the potentially higher heat yield the diatom *P. tricornutum* will be used instead of the conventionally applied green alga *Chlorella*.

Materials and methods

Algal cultivation

Batch cultures of *P. tricornutum* Böhlin obtained from the Culture Collection of Algae of Laboratoire Arago (Banyuls, France) were grown in airlift cultures at 20 °C with a light to dark cycle of 14:10 h. Cultures were illuminated with white light (FL40SS W/37, Sanyo) at an irradiance of 70 μmol photons m^{-2} s^{-1}. The algae were cultivated in modified f/2 medium according to [26] with the double concentration of sea salt, nitrate and phosphate. All cells

were harvested in the dark period and kept in the dark until the experiments. All measurements were performed with samples at Chl*a* concentration of 3.0 mg L^{-1}. All data were normalized to 1 mg Chl*a* by dividing the respective value through the actual concentration of Chl*a*.

All measurements (i.e., net oxygen evolution, heat flow and fluorescence) were performed under actinic illumination with two different irradiances (400–700 nm, photosynthetically active radiation (PAR)). At an irradiance of 160 μmol photons m^{-2} s^{-1} (PAR 160) it can be expected that the photosynthetic process is still close to its optimal performance with a low amount of heat dissipated via NPQ. In contrast, 450 μmol photons m^{-2} s^{-1} (PAR 450) were applied to saturate the photosynthesis rate and to fully activate the NPQ mechanism of heat dissipation.

Microcalorimetric measurement

The Thermo Activity Monitor (TAM) 2277 (Thermometric SB, Sweden) at 20 °C was used for the calorimetric measurements. A twin arrangement (two 20 mL identical stainless-steel ampoules for the reference and the test channel, respectively) was applied. The experimental setup is shown by Fig. 1. For each measurement 4 mL of algal suspension was placed in both ampoules, respectively. Actinic illumination of samples with spectral blue light was provided by a High Power LED light source (HPL-C, $\lambda_{max} = 464$ nm, Walz, Effeltrich, Germany) with the light source outside of the calorimeter. The samples were illuminated for 20 min. $KHCO_3$ (10 mM final concentration) was added to each sample as inorganic carbon source to avoid CO_2 limitation. In the present experimental setup stirring of the samples was not possible due to constructive restrains. Furthermore, the relatively small diameter of the fibreglass light guide resulted in a slightly inhomogeneous areal light distribution.

After stabilization of the base line the experimental procedure started with a control measurement of the heat flux with untreated samples of algal suspension in both ampoules. The heat flux under steady state conditions was averaged from the data points recorded during the last 80 s of the illumination period. The measurement frequency was 1 Hz. Different inhibitors were applied to the algal suspension in the test ampoule. Formaldehyde (CH_2O, 1% v/v) was used to completely block any metabolic activity without changes in the absorptivity of the cells. Thus, the difference in the heat flux rate between the control sample and the sample poisoned with formaldehyde (P_{heat}^{meas}; see Fig. 2) should correspond to the part of absorbed light which was used for photosynthesis. DTT (0.5 mM final concentration) was used to control NPQ. DTT inhibits the de-epoxidase reaction of the XC and thus NPQ. In diatoms, this leads to an almost complete prevention of NPQ.

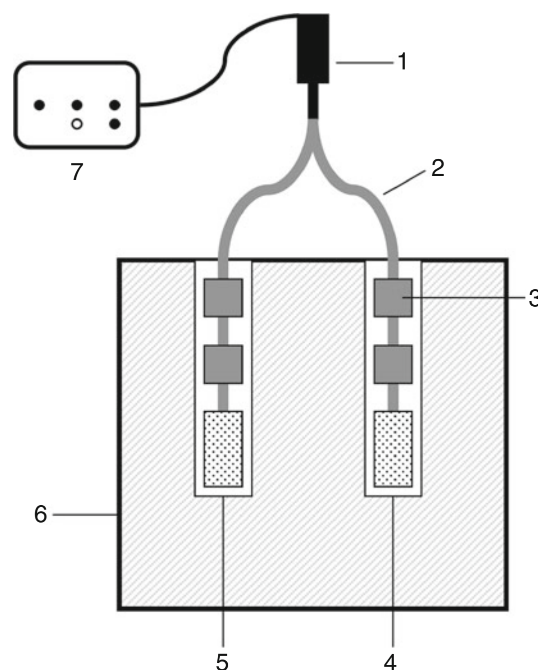

Fig. 1 Photocalorimetric setup: Illumination of the algal solution was provided by a two-arm fibreglass light guide (*2*) which connected the High Power LED unit (*1*) with the reference (*5*) and the test ampoule (*4*). The two arms of the light guide were fixed and thermal equilibrated in the channels of the TAM (*6*) by two additional aluminium rings (*3*). After connection with the light guide the ampoules were lowered in the thermal equilibration position, and stayed there for 35 min before they were further lowered to the measuring position. The 20 min period of actinic illumination was started after stabilization of the baseline of the heat signal. Irradiance was regulated by a control unit (*7*)

Oxygen measurement

The oxycaloric equivalent ($\Delta_k H_{O_2} = -470\,\mathrm{kJ\,mol^{-1}}$ [27]) provides a correlation between endothermic heat flow P_{heat}^{calc} (W L^{-1}) and photosynthetic Chl*a*-specific oxygen evolution rate P_{Onet}^{Chla}(mol O_2 mg^{-1} s^{-1}) according to Eq. 1.

$$P_{heat}^{calc} = \Delta_k H_{O_2} P_{Onet}^{Chla}[\mathrm{Chl}a] \quad (1)$$

where [Chl*a*] is the Chl*a* concentration (mg L^{-1}) of the algal suspension. Oxygen evolution measurements were thus considered for the evaluation of the calorimetric signal. The oxygen measurement conditions were chosen as similar as possible to the calorimetric experiments.

Oxygen evolution rates were measured as net photosynthesis rates (P_{Onet}; $\%O_2$ s^{-1}) by means of a Clark-type oxygen electrode (MI-730, Microelectrodes Inc., New Hampshire, USA) connected to a Photosynthetic Light Dispenser System (Illuminova, Uppsala, Sweden). This

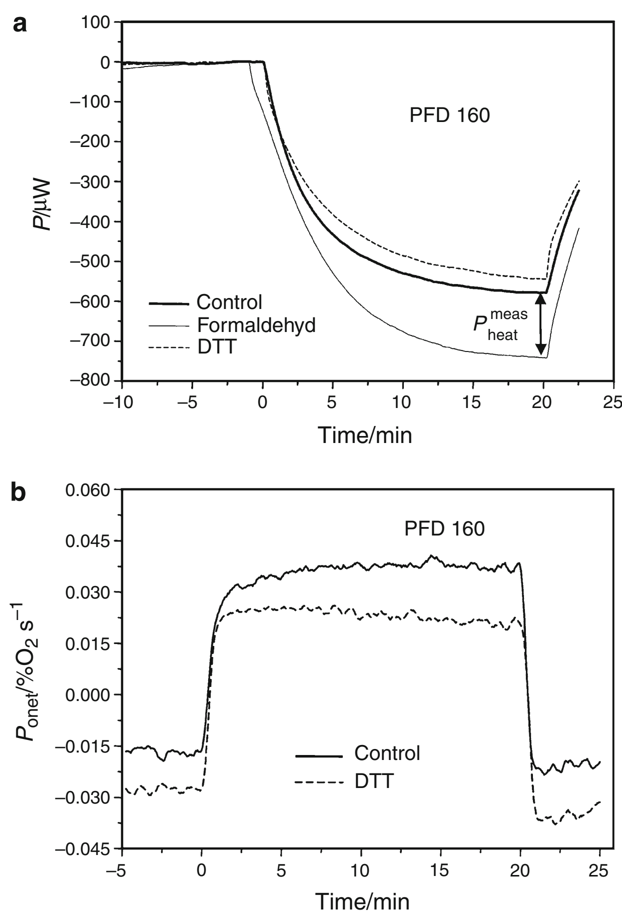

Fig. 2 Example of **a** heat flux (P) and **b** net oxygen evolution rate (P_{Onet}) in *Phaeodactylum tricornutum* at an irradiance of 160 μmol $\mathrm{m}^{-2}\ \mathrm{s}^{-1}$. After dark adaptation the illumination period (20 min) was started at time point zero. All measurements are shown in the comparison of untreated samples (*control*) and samples in the presence of DTT (0.5 mM). In addition, calorimetric measurements are presented with samples where cellular metabolism is completely inhibited by formaldehyde. The differences between the heat flux measured in samples treated with formaldehyde and samples under all other experimental conditions was used to calculate the light-induced heat flux rate $P_{\mathrm{heat}}^{\mathrm{meas}}$. The negative heat flux in control measurements was due to a slight imbalance in the illumination of the reference and the test ampoule, respectively

device was equipped with a special measuring chamber to allow the simultaneous measurement of oxygen evolution rates and Chl fluorescence parameters (NPQ, see below). Actinic illumination of samples with spectral blue light was provided by the HPL-C unit as described above.

Temperature, illumination and carbon source (i.e., $KHCO_3$) were identical to the calorimetric measurement. The oxygen concentration was recorded at a measuring frequency of approx. 0.3 Hz. For the evaluation of the steady state net photosynthesis rate oxygen concentrations of the last 2 min during the illumination period were averaged. $P_{\mathrm{Onet}}^{\mathrm{Chl}a}$ was calculated according to Eq. 2 from the raw signal P_{Onet}, the oxygen solubility $C_{\mathrm{O_2}}^{\mathrm{Sat}}$(mol O_2 L^{-1}) and the Chl*a* concentration [Chl*a*] (mg L^{-1}) of the algal suspension.

$$P_{\mathrm{Onet}}^{\mathrm{Chl}a} = P_{\mathrm{Onet}} C_{\mathrm{O_2}}^{\mathrm{Sat}} [\mathrm{Chl}a] \Big/ 100 \qquad (2)$$

To assure identical light conditions in the oxygen measuring device and the calorimeter, PAR was measured in aqueous solution with a spherical quantum sensor (US-SQS/L, Walz, Germany) connected to a light meter (LI-250A, LI-COR, USA). In case of the calorimeter, the spherical quantum sensor was inserted through a hole in a test calorimetric ampoule which was not used for routine measurements.

Chlorophyll fluorescence

To calculate the NPQ of Chl fluorescence and the relative electron transport rates (rETR) at PSII a pulse-amplitude modulated (PAM) fluorometer (unit 101/103, Walz, Effeltrich, Germany) was used. The fluorescence parameters were determined by the saturating pulse method according to [28]. Saturating light pulses (800 ms; 3,500 μmol photons $\mathrm{m}^{-2}\ \mathrm{s}^{-1}$) were delivered by a KL 1500 light source (Walz, Effeltrich, Germany). NPQ was calculated according to Eq. 3 [29].

$$\mathrm{NPQ} = \frac{F_{\mathrm{m}} - F_{\mathrm{m}}'}{F_{\mathrm{m}}'} \qquad (3)$$

Here F_{m} denominates the maximal fluorescence intensity in dark-adapted cells and F_{m}' is the maximal fluorescence intensity in illuminated cells. It has to be emphasized that the parameter NPQ describes relative changes of the maximal fluorescence and is not an efficiency term. Therefore, NPQ values are not expected to be linearly correlated with the respective amount of energy dissipation as heat.

rETR (μmol electrons $\mathrm{m}^{-2}\ \mathrm{s}^{-1}$) were calculated according to Eq. 4:

$$\mathrm{rETR} = \Phi_{\mathrm{PSII}} \mathrm{PAR}\ 0.5 \qquad (4)$$

where Φ_{PSII} is the fluorescence quantum yield of PSII (according to [28]), PAR is the amount of incident PAR in the measuring device. The complete photosynthetic electron transport requires the excitation of electrons at PSII and PSI. Thus, the factor 0.5 is based on the assumption that the photosynthetically absorbed radiation is equally distributed among both photosystems, PSII and PSI.

Results

Figure 2 presents examples of photocalorimetric and oxygen evolution measurements under actinic illumination

with PAR 160 in the diatom *P. tricornutum*. The reference side contained an ampoule with an untreated algae sample whereas the test side was filled with untreated algae (control) or algae treated with different agents. The calorimetric system required approx. 60 min for thermal equilibration and stabilization of the baseline (not shown). Afterwards, illumination was started (time point zero in Fig. 2). The light irradiance was highly stable over time as was confirmed by the analysis of the steady state signal during illumination and independent measurements with a light meter (results not shown). Therefore, the observed hyperbolically shaped heat flow signal could be due to different heating kinetics in both ampoules potentially caused by differences in the heat capacity of the ampoules. The system reached a steady state after approx. 20 min of illumination. In the course of experiments two different irradiances (160 and 450 µmol m^{-2} s^{-1}) were applied. It has to be emphasized that the shape of the heat signal was independent of the irradiance. Assuming a perfect equal distribution of the light into both ampoules no influence of illumination in the control measurement is expected. However, slight imbalances of the light distribution resulted in different heat flow rates under steady state conditions even with untreated algal samples in both ampoules.

The measurements of dissolved oxygen concentration revealed a respiratory activity during the dark adaptation period (negative values) and a significant net oxygen evolution during the illumination period (Fig. 2b). A stable oxygen production was achieved after approx. 5 min of illumination. Importantly, the signal measured as net oxygen evolution includes any respiratory losses and can be regarded as proportional to the amount of photosynthetically absorbed energy used for biomass formation.

Formaldehyde was used as an inhibitor of any cellular activity and was added to the test ampoule of the microcalorimeter. Thus, in the test ampoule incident irradiance will be converted completely into heat, whereas in the reference ampoule the endothermic photosynthetic reaction converts absorbed light into biochemical energy and finally into biomass. Thus, an increase of the heat flow due to the addition of formaldehyde was expected and experimentally proven (Fig. 2a). The difference between both signals (with and without formaldehyde—P_{heat}^{meas}; Fig. 2a) can be regarded as the amount of light energy conserved in the biomass. This amount of energy should be equivalent to the net oxygen evolution rate (Fig. 3). It should be noted, that the addition of formaldehyde fixed the oxygen concentration to a constant value (data not shown) hence confirming the complete inhibition of cellular activity. The increase of P_{heat}^{meas} from 177 to 249 µW in cells illuminated with PAR 160 and PAR 450, respectively, was comparable to the increase of P_{Onet} from 76 to 121 µmol O_2 mg^{-1} h^{-1} (Fig. 3). Net oxygen evolution rates can be converted into heat flux rates (P_{heat}^{calc}) using the oxycaloric equivalent. Surprisingly, the ratios of the measured to the calculated heat flux rates ($P_{heat}^{meas}/P_{heat}^{calc}$; Table 1) deviated significantly from the expected ratio of one.

For analyzing the effect of NPQ on photosynthetic energy distribution, the experiments were done with two different irradiances (low—inactivated NPQ; high—fully activated NPQ) and in the presence of DTT (inhibitor of the XC and, thus, of NPQ). Illumination with PAR 160 resulted in a very low NPQ value of 0.13, whereas the illumination with PAR 450 strongly increased NPQ values to about 1.2 (Fig. 4). The addition of the inhibitor DTT did not change the NPQ values in cells illuminated with low irradiances, but decreased NPQ values in cells illuminated with high irradiances, as expected (Fig. 4). The same NPQ was observed for samples with low irradiance independent of the presence of DTT as well as for samples with high irradiance in presence of DTT. This finding supports the assumption that the remaining low NPQ of 0.13 was caused by NPQ processes independent of the XC.

It was found in the measurements of heat flow and oxygen evolution that the addition of DTT provoked strong side effects. In comparison to control measurements these side effects caused a significant shift of the calorimetric baseline to negative values (data not shown) and increased oxygen consumption during the dark adaptation period (Fig. 2b). Further experiments were performed in the oxygen measuring device where DTT was added to culture medium without algae and significant oxygen consumption was observed (data not shown). Thus, it could be deduced that the measurements of oxygen evolution were affected by unspecific reactions of DTT, e.g., the reduction of

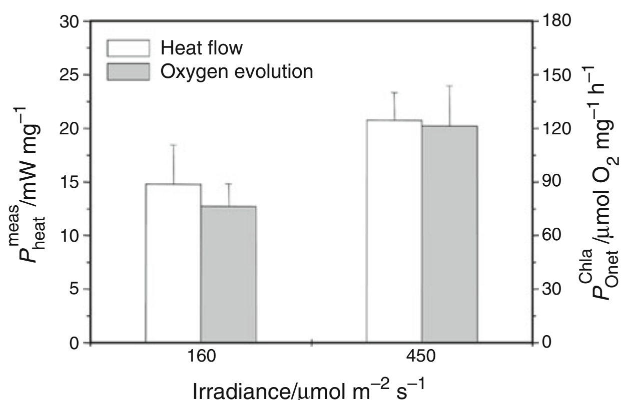

Fig. 3 Comparison of heat flow P_{heat}^{meas} and Chl-specific net oxygen evolution rate P_{Onet}^{Chla} in *Phaeodactylum tricornutum* during illumination with irradiances of 160 and 450 µmol m^{-2} s^{-1}. The data are given as mean values (±standard deviation; $n = 4$)

Table 1 Comparison of the ratio of the measured to the calculated heat flux ($P_{heat}^{meas}/P_{heat}^{calc}$) in *Phaeodactylum tricornutum* under different light conditions and in the absence (control) or presence of DTT (+DTT), respectively

	$P_{heat}^{meas}/P_{heat}^{calc}$	
	PAR 160	PAR 450
Control	1.5 ± 0.4	1.3 ± 0.3
+ DTT	1.8 ± 0.6	1.8 ± 0.7

The oxycaloric equivalent was used for the calculation. Data are shown as mean values (±standard deviation; $n = 4$). PAR 160 and PAR 450 refer to an illumination of the samples at an irradiance of 160 and 450 μmol m^{-2} s^{-1}, respectively

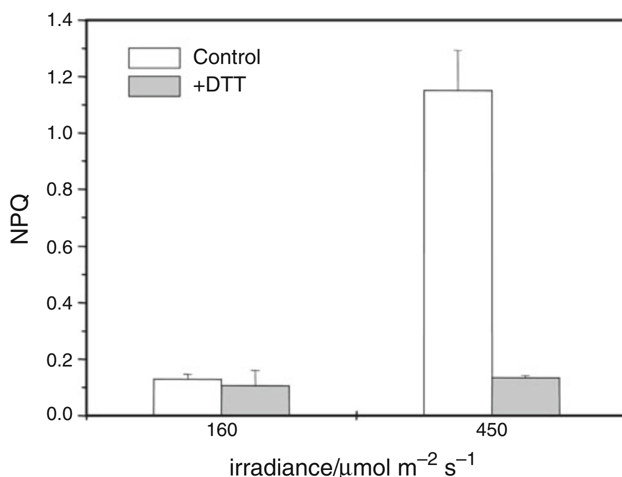

Fig. 4 Fluorimetrically analyzed (PAM) non-photochemical quenching (NPQ) in *Phaeodactylum tricornutum* during illumination with irradiances of 160 and 450 μmol m^{-2} s^{-1}. All data are given as mean values (±standard deviation; $n = 3$). Measurements were performed with untreated samples (*control*) and in samples pretreated with 0.5 mM DTT

oxygen to superoxide [30]. In experiments with algal suspension an interaction with the superoxide dismutase [31] might additionally contribute to the increased oxygen consumption. To eliminate this 'DTT effect' the measurements of oxygen evolution were corrected by the difference between dark oxygen consumption in DTT-treated and untreated samples.

Figure 5 shows the influence of DTT on the heat flow and oxygen evolution at low and high irradiance. In cells illuminated with PAR 160 the addition of DTT did not change P_{Onet}^{Chla} (Fig. 5) and rETR (data not shown), whereas the calorimetric measurements in the presence of DTT showed a trend of increased heat flux (Figs. 2a, 5). In cell suspensions illuminated with PAR 450 the addition of DTT resulted in a decrease of P_{Onet}^{Chla} (Fig. 5) and a significant increase in rETR (Fig. 6). Under conditions of high irradiance no significant change in the measured heat flux rate

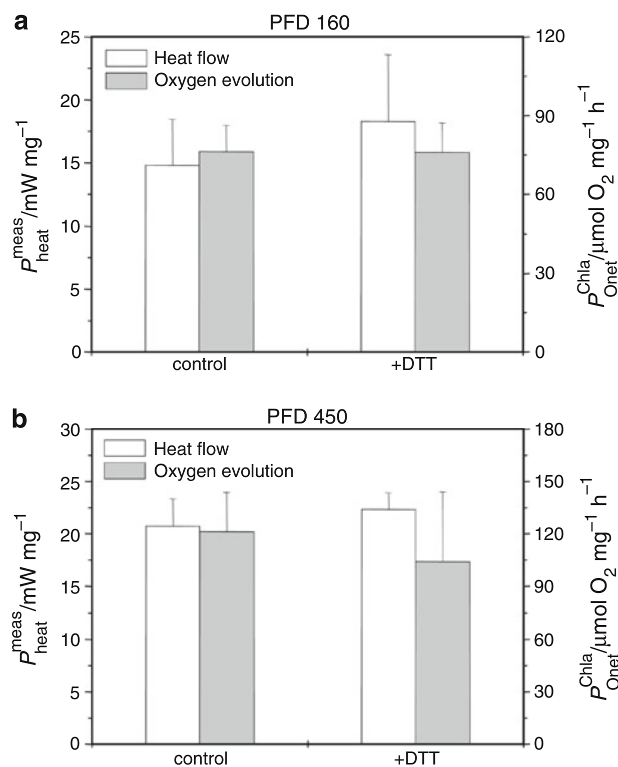

Fig. 5 Influence of 0.5 mM DTT on heat flow P_{heat}^{meas} and Chl-specific net oxygen evolution rate P_{Onet}^{Chla} in *Phaeodactylum tricornutum* during illumination with irradiances of 160 μmol m^{-2} s^{-1} (**a**) and 450 μmol m^{-2} s^{-1} (**b**). The data are given as mean values (±standard deviation; $n = 4$). Measurements were performed with untreated samples (*control*) and in samples treated with 0.5 mM DTT

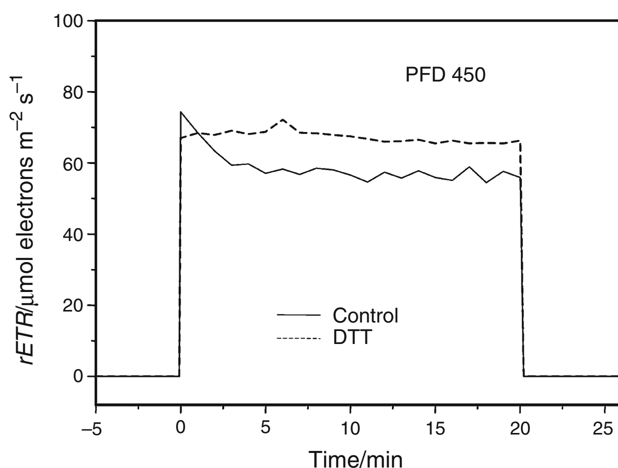

Fig. 6 Example of measurements of the relative electron transport rate (*rETR*) in *Phaeodactylum tricornutum* at an irradiance of 450 μmol m^{-2} s^{-1}. After dark adaptation a 20 min illumination period was started at time point zero. The measurements are shown in the comparison of an untreated sample (*control*) and a sample in the presence of DTT (0.5 mM)

was observed (Fig. 5). In the presence of DTT no significant changes in the ratio of the measured to the calculated heat flux were observed (Table 1).

Discussion

The aim of this study was to compare the photosynthetic energy conversion in the diatom *P. tricornutum* measured by photocalorimetry and by oxygen balance with the focus on the contribution of the energy dissipating NPQ process to the metabolic energy balance. This required the development of a photocalorimetric measuring system which allows the illumination of algal solutions at irradiances saturating photosynthesis. In this study a commercially available, blue light emitting LED unit was used to illuminate the samples within the ampoules of the calorimeter.

Basic experiments with this setup showed that the calorimetrically detected heat absorption rate correlated with the net oxygen evolution rate in the comparison of illumination with low and high irradiances. However, the conversion of the net oxygen evolution rates by the oxycaloric equivalent resulted in an underestimation of the calculated compared to the measured heat absorption rate. Although attention was paid to achieve comparable light conditions in both measuring systems, it could be assumed that the fraction of radiation absorbed by the algal suspension was higher in the ampoules used in the calorimeter than in the cuvette of the oxygen measuring device. This could be due to differences in the optical path length and in the degree of light scattering in both measuring systems. This assumption is supported by the fact that the ratio $P_{\text{heat}}^{\text{meas}}/P_{\text{heat}}^{\text{calc}}$ was higher in the measurements at PAR 160 in comparison to PAR 450. The photosynthetic activity is characterized by two distinct parts: (i) the light-limited part where the photosynthesis rate is directly correlated with irradiance and (ii) the light-saturated part where the increase of irradiance does not lead to a further increase of the photosynthesis rate [32]. According to our results, the impact of different light conditions should thus be more pronounced under light-limited conditions (PAR 160) than under light-saturated conditions (PAR 450). In addition, differences in the optical conditions between the calorimeter and the oxygen measuring device could be also due to the fact that stirring of the samples was not possible in the calorimeter. Furthermore, the formation of peroxides or superoxide as potential side effects of DTT let expect significant deviations from the oxycaloric equivalent [27] and thus strong deviations in the ratio of $P_{\text{heat}}^{\text{meas}}/P_{\text{heat}}^{\text{calc}}$ from unity. Indeed, the deviations of this ratio from unity are much higher for DTT-treated samples as for control measurements.

The influence of NPQ on the metabolic energy balance was measured under conditions where the activation of NPQ was triggered by light and by the use of DTT. Under conditions of low irradiance (PAR 160) only a very low NPQ value was measured and it was expected that the addition of DTT should have no effect on NPQ. This assumption was proven by the results of this study. Furthermore, it could be expected that the addition of DTT does not change the energy distribution within PSII and therefore, should not influence the net oxygen evolution rate and the heat flux rate at low irradiance. Again, the results corroborate the expectations in case of the net oxygen evolution rates. However, the heat absorption rate slightly increased in the presence of DTT which might be due to inhibitory side effects of DTT on cellular processes.

For the experiments at high irradiance (PAR 450) it could be assumed that the addition of DTT prevents the activation of the NPQ process and results in decreased heat dissipation. In consequence, this should be detectable as an increased heat absorption rate. Although the inhibitory effect of DTT on the activation of NPQ was clearly observed fluorometrically in the experiments under high irradiance, only a very small increase in the measured heat absorption was detected in the presence of DTT. Since the absorptivity of the cells was not changed by the addition of DTT it has to be concluded that other energy dissipating processes compensate for the inactivated NPQ mechanism. The energy dissipation by Chl within PSII is limited to the release of electrons into the electron transport chain and the emission of heat and fluorescence. Indeed, an increase in the fluorescence intensity, measured by the PAM fluorescence, was observed (data not shown). However, it is known that the maximal capacity of energy dissipation by Chl fluorescence is limited to few percent [33] and can only have compensated partly for the inhibited NPQ. Consequently, the suppression of energy dissipation by NPQ should have resulted in an increase of the PSII electron transport rate and the oxygen evolution rate. Whereas the measured increase in the electron transport rate is in line with this assumption, the rates of $P_{\text{Onet}}^{\text{Chl}a}$ tended to decrease in the presence of DTT. Such a result could be explained by an increased activity of alternative electron sinks like, e.g., cyclic electron transport around PSII, the Mehler reaction, or photorespiration. All these processes have in common that the electron transport rates are higher than the respective oxygen evolution rates since the electrons are transferred back to molecular oxygen. Furthermore, the known side effect of DTT to form superoxide would have a similar effect.

As mentioned above, it is not possible to quantify the fraction of energy dissipation via NPQ by the PAM fluorescence method. Therefore, it is not clear whether alternative electron pathways and fluorescence increase completely compensated the inhibited NPQ process. Thus, in addition increased energy dissipation by constitutive heat dissipation of Chl [2] has to be considered. It is, however, not known to which extent this process can vary.

In conclusion, in this study it was shown that the combined measurement of the photosynthetic energy conversion by calorimetry, oxygen balance and fluorescence is feasible and provides information about the metabolic flow of photosynthetically absorbed radiation. Interestingly, the inhibition of energy dissipation by the NPQ mechanism resulted in a re-distribution of photosynthetic electrons into alternative electron pathways. From the comparison of calorimetric, oxygen evolution and fluorimetric measurements it was concluded that under the experimental conditions of this study the energy dissipation by NPQ was completely compensated by these alternative processes. It remains to be elucidated whether such compensation is possible only on a short time scale. Another open question is found when comparing the measured with the calculated heat flux rate. The relatively high ratio of $P_{\text{heat}}^{\text{meas}} / P_{\text{heat}}^{\text{calc}}$ could be due to differences in the light conditions between the calorimetric setup and the oxygen measuring device. However, this aspects needs to be clarified by future experiments with an optimized illumination of the algal solution. An alternative approach could be a setup with an LED light source integrated within the ampoules of the calorimeter as suggested by [11] which would also allow stirring of the algal solution.

The contribution of alternative electron pathways during the adaptation of photosynthesis to changing environments is an important aspect in plant physiology. The approach presented in this study is a promising attempt to integrate calorimetric measurements into the conventional set of methods for measuring photosynthetic activity.

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