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Peter HARTIG; Sebastian LIPPEMEIER

Research and Technology Center Westcoast, Büsum

Comparison of photosynthetic measurements based on active fluorescence (PAM)- , O_2- and ^{14}C – methods

Traditionally measurements of phytoplankton photosynthesis are performed based on the detection of released oxygen (O_2) or fixed carbon (C). The latter is based on the time dependent incorporation of radiocarbon into particulate matter and has been used excessively since the work of Steemann-Nielsen (1952) in phytoplankton ecology. This method can be regarded as a highly sensitive classical standard method for the determination of phytoplankton photosynthesis. Unlike to the radiocarbon measurements the detection of the released O_2 is limited due to the unsatisfactory sensitivity of this method and therefore has not reached the same acceptance as the radiocarbon method in phytoplankton photosynthesis research. In addition, both methods are limited by several other methodological problems: e.g. long incubation times (0.5-24h), bottle effects, differentiation between net and gross photosynthesis and changes in phytoplankton assemblages due to concentration procedures.

In order to overcome these practical and methodological problems and to measure phytoplankton phytosynthesis *in situ* with a high frequency and/or on a relevant spatial scale a strong need to introduce new methods exists. One promising approach is the detection of the fast active chlorophyll fluorescence with the PAM-method developed by Schreiber which is described by Dau et al. in this volume. It has to be mentioned that another active fluorescence method (pump and probe, PNP) has been in use for several years by Falkowski and coworkers for phytoplankton research. Because we only used several PAM-equipments (see Domin et al. this volume) during the workshop in Zingst, we focus the following article on the PAM-method. To clarify to which extend this method can be applied for the estimation of

phytoplankton photosynthesis a comparision with the above mentioned classical methods (^{14}C, O_2) is strongly needed.

In Fig. 1 it is shown at which steps of the photosynthetic process the information for the assessment of phytoplankton photosynthesis is gathered by the different methods.

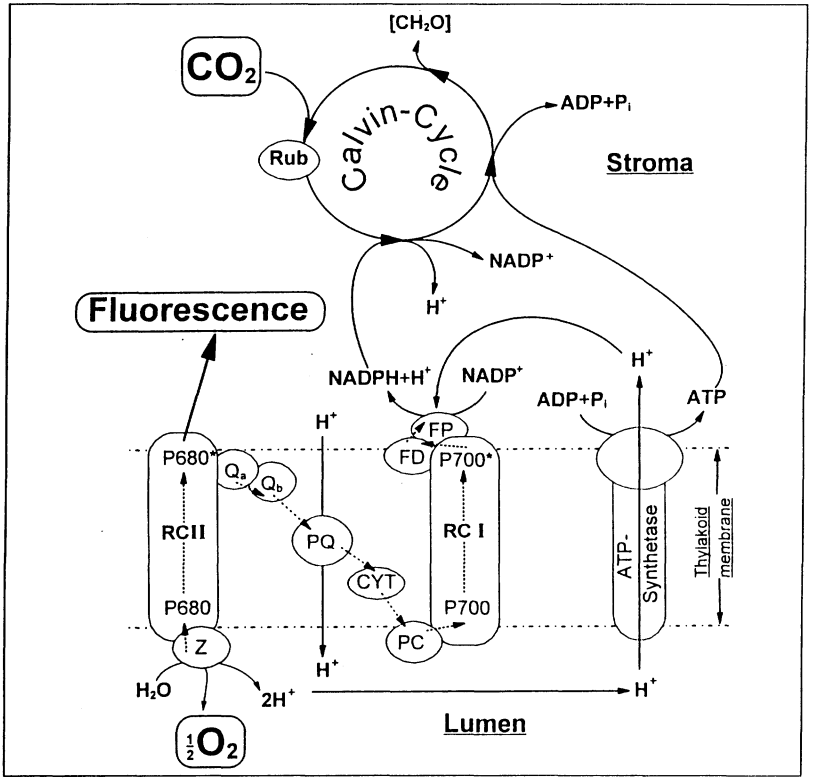

Fig. 1 Simplified, schematic presentation of the photosynthetic processes at which the information for photosynthetic measurements is gathered by the different techniques. The dotted arrows show the formal pathways of the electrons generated during the watersplitting process. Z: watersplitting complex; P680/P700: special chlorophyll molecules that can be excited to P680*/P700* due to light absorption; RCII/RCI: reaction centers of photosystem I/II; Q_a/Q_b: primary and secondary electron acceptor of RCII; PQ: plastoquinone; CYT: cytochrome b6f complex; PC plastocyanine; FD: ferredoxin; FP: ferredoxin-NADP-oxidoreductase; Rub: ribulose-1,5-bis-phosphate carboxylase; $[CH_2O]$: glucose (formal)

It becomes clear that fluorescence-emission and O_2-release are closely connected at the reaction center of photosystem II (RCII), whereas the CO_2-fixation takes place at a subsequent step in the Calvin cycle. This already implies that a stonger correlation of O_2 than of ^{14}C with the fluorescence-measurements can be expected. The most important differences between O_2-release and carbon-fixation arise from the fact that not all electrons evolved in the watersplitting process at RCII are strictly used for C-fixation. Alternative electron sinks are e.g. NO_3^- - reduction, photorespiration, Mehler reaction and reactions to repair damages caused by high irradiances. Since the PAM-method principally detects electron flow rates in RCII (Hofstraat et al. 1994, Hartig and Colijn 1996, Hartig et al. 1998) one can only expect a close correlation between the fluorescence- and the ^{14}C- based measurements if all electrons are used for C-fixation and not for other processes.

However fluorescence itself is highly variable due to different quenching mechanisms, which are not strictly connected with C-fixation or O_2-release. Without going into detail quenching includes all processes which lead to a reduction of fluorescence yields resulting in an inequality between fluorescence signals and O_2-release or C-fixation.

Relationships between classical- and active fluorescence-based methods of photosynthetic measurements conducted with higher plants and algae in different studies are presented in Tab. 1. It should be noted that this overview does not claim to be complete. Most of the studies that are intended to provide a comparison between the different methods of photosynthetic measurements have been carried out on higher plant leaves or on isolated chloroplasts. Only a few researchers have applied PAM fluorescence techniques on unicellular algae and phytoplankton for this purpose (Kroon et al. 1996, Hartig and Colijn 1996, Geel et al. 1997, Hartig et al. 1998). Because PNP has found a wide application in phytoplankton research we also present the relationship to the classical methods obtained with this technique. The results of the different studies on the relationship between PSII electron transport rates and carbon fixation respectively oxygen release in higher plants and algae showed different results (Tab. 1). Even when conducted under a variety of experimental conditions, most comparisons show a linear correlation under moderate irradiances, whereas a non-linear correlation was found under low light intensities and in some cases under high light intensities. Therefore irradiance seems to be an important factor which influences the assessment of photosynthesis based on fluorescence measurements.

Tab.1 Relationships between classical- and active fluorescence-based methods of photosynthetic measurements conducted with higher plants and algae in different studies. Abbreviations: HL: high light; LL: low light; IL: intermediate light, AL: actinic light, SD: spectral differences.

Author (Year)	Methods	Organisms	Relationships	Reasons for non-linearity
Boyd et al. (1997)	PNP, ^{14}C	Phytoplankton	Linear (for I_k, P_m); Non-linear (for ??	SD of AL sources
Falkowski et al. (1986)	PNP, O_2	Algae (cultures)	Linear (IL); Non-linear (LL and HL)	HL: cyclic electron flow around PSII, LL: quenching processes
Geel et al. (1997)	PAM 101, Xenon-PAM, O_2	Algae (cultures)	Linear (LL and IL); Non-linear (HL)	Oxygen consumption (not photorespiration)
Genty et al. (1989)	PAM 101, CO_2 IRGA	Higher plants (C3, C4)	Linear	
Genty et al. (1992)	PAM 101, $^{16}O_2/^{18}O2$	Higher plants (C3)	Linear	
Harbinson et al. (1990)	PAM 101, O_2	Higher plants (C3)	Linear; Non-linear (at 20% O_2)	Photorespiration
Hartig & Colijn (1996)	PAM 101 (PM), ^{14}C	Algae (cultures and microphytobenthos)	Linear	
Hartig et al. (1998)	PAM 101 (PM), ^{14}C	Algae (microphytobenthos)	Linear (IL); Non-linear (LL and HL)	LL: SD od AL sources, HL: electron sinks
Hofstraat et al. (1994)	PAM 101, μ (growth)	Algae (cultures)	Linear	
Horton (1989)		Higher plants	Linear	
Keiller & Walker (1990)		Higher plants	Linear	
Kolber & Falkowski (1993)	PNP, ^{14}C	Phytoplankton	Linear (r 0.86)	
Krall & Edwards (1991)	PAM 101, O_2	Higher plants (C4)	Linear	
Kroon et al. (1996)	PAM 101, O_2	Algae	Linear ; Non-linear (HL)	Adapt. Phenom. (Baumert 1996)
Oberhuber et al. (1993)	PAM 101, CO_2 (IRGA)	Higher plants (C3,C4)	Linear; Non-linear (LL)	Respirat. or presence of inactive PSII centers
Rees et al. (1992)	PAM 101, O_2	Algae (cultures)	Non-linear	Non-assimilatory electron flow
Seaton & Walker (1990)	PAM 101, O_2	Higher plants (C3,C4)	Linear; Non-linear (LL)	

For the observed deviations under low and high light conditions the authors offered possible explanations similar to those we already mentioned above and some additional reasons for non-linearity like e.g. spectral differences of light sources, oxygen consumption and cyclic electron flow around PSII.

Most of the studies who revealed a non-linear relationship between the classical methods and the active fluorescence methods where carried out with algae. Apart from the already given explanations for the observed non-linearity another important factor is the higher plasticity of the photosynthetic machinery of phytoplankton than that of higher plants. This plasticity is mainly reflected by a different pigment composition and the intracellular arrangement of the pigments, which are furthermore highly variable under different environmental conditions (see Forster et al. this volume, Cleveland & Perry 1987, Herzig & Falkowski 1989). Therefore light absorption of phytoplankton is different to higher plants, where it is considered to be very constant. For calculation of fixed carbon or released oxygen based on fluorescence measurements a definite knowledge about the light absorption of phytoplankton is needed (Hartig et al. 1998).

It has been shown that the final conversion of irradiance into fixed C by phytoplankton is impaired due to different effects (e.g. different electron sinks, variable light absorption etc.). This complicates the comparision of photosynthetic measurements based on PAM-, O_2- and ^{14}C – methods. However in some studies carried out with phytoplankton a good correlation between O_2- or ^{14}C-based methods and the active fluorescence based methods (PAM and PNP) was found (Tab.1). Therefore we assume that the active fluorescence methods offer the potential for rapid estimation of phytoplankton photosynthesis with high spatial and temporal resolution, which is needed particularly for phytoplankton communities due to their patchy distribution.

Abbreviations

PAM: Pulse Amplitude Modulated Fluorometer
PNP: Pump and Probe Fluorometer
PM: Photomultiplier
IRGA: Infra Red Gas Analyser

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Authors:

Peter Hartig and Sebastian Lippemeier
Research and Technology Center Westcoast,
University Kiel, Hafentoern,
25761 Buesum, GERMANY
Phone: 0049-(0)4834-604209
Fax: 0049-(0)4834-604299
e-mail: hartig@ftz-west.uni-kiel.de