Biochimica et Biophysica Acta 1847 (2015) 565–575

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

Oxidation of plastohydroquinone by photosystem II and by dioxygen in leaves

Agu Laisk *, Hillar Eichelmann, Vello Oja

Tartu Ülikooli Tehnoloogia Instituut, Nooruse tn. 1, Tartu 50411, Estonia

ARTICLE INFO

Article history:
Received 17 November 2014
Received in revised form 16 February 2015
Accepted 15 March 2015
Available online 20 March 2015

Keywords:
Leaves
Photosystem II
Cyclic electron transport

ABSTRACT

In sunflower leaves linear electron flow LEF = 4 O_2 evolution rate was measured at 20 ppm O_2 in N_2. PSII charge separation rate $CSR_{II} = a_{II}{\cdot}PAD{\cdot}(F_m - F) / F_m$, where a_{II} is excitation partitioning to PSII, PAD is photon absorption density, F_m and F are maximum and actual fluorescence yields. Under 630 nm LED + 720 nm far-red light (FRL), LEF was equal to CSR_{II} with $a_{II} = 0.51$ to 0.58. After FRL was turned off, plastoquinol (PQH_2) accumulated, but LEF decreased more than accountable by F increase, indicating PQH_2-oxidizing cyclic electron flow in PSII (CEF_{II}). CEF_{II} was faster under conditions requiring more ATP, consistent with CEF_{II} being coupled with proton translocation. We propose that PQH_2 bound to the Q_C site is oxidized, one e^- moving to $P680^+$, the other e^- to Cyt b_{559}. From Cyt b_{559} the e^- reduces Q_B^- at the Q_B site, forming PQH_2. About 10–15% electrons may cycle, causing misses in the period-4 flash O_2 evolution and lower quantum yield of photosynthesis under stress. We also measured concentration dependence of PQH_2 oxidation by dioxygen, as indicated by post-illumination decrease of Chl fluorescence yield. After light was turned off, F rapidly decreased from F_m to 0.2 F_v, but further decrease to F_0 was slow and O_2 concentration dependent. The rate constant of PQH_2 oxidation, determined from this slow phase, was 0.054 s^{-1} at 270 μM (21%) O_2, decreasing with $K_m(O_2)$ of 60 μM (4.6%) O_2. This eliminates the interference of O_2 in the measurements of CEF_{II}.

1. Introduction

Plastoquinone (PQ) is a lipid-soluble, diffusible in the membrane compound that stands on the central crossroad of photosynthetic electron transport pathways. The relative redox state of PQ acts as a sensor of environmental change [1], triggering various cellular responses [2], including modulated expression of genes that encode the light-harvesting apparatus of photosynthesis [3]. Plastoquinone can be stably reduced to form plastoquinol (plastohydroquinone, PQH_2) by accepting two electrons and two protons. The single-reduced radical semiquinone is relatively unstable (E_m of PQ/PQ^- is −170 mV [4]).

In photosynthesis the mainstream linear electron flow (LEF) through PSII reduces PQ, which accepts protons from the stroma, and PQH_2 is oxidized by donation of electrons to the Rieske Fe carriers in the cytochrome b_6f complex (Cyt b_6f), releasing protons into the thylakoid lumen. Such proton-coupled LEF is still insufficient to support the necessary >3ATP/2NADPH ratio requiring >14H^+/4e^- [5], therefore, PQ is also reduced by two kinds of cyclic electron flow around PSI (CEF_I), returning to PQ a fraction of electrons once passed through the Cyt b_6f complex. The (minor) chloroplast NAD(P)H dehydrogenase-related route of PQ reduction was suggested by [6] and a novel protein component CRR31 is essential for the efficient operation of Fd-dependent plastoquinone reduction by this complex [7]. The (major) antimycin A-sensitive, ferredoxin-dependent PQ reduction pathway [8] is based on a PROTON GRADIENT REGULATION protein PGR5 [9,10]. PGR5 and a "PGR5-like" protein PGRL1 were found interacting physically and being associated with PSI [11], most probably representing the elusive ferredoxin:quinone reductase (FQR) enzyme [12,13], though evidence that Q_A, but not PQ, is the direct electron acceptor was recently published [14].

The mainstream photosynthetic pathway of PQH_2 oxidation goes via the Q-cycle located in the Cyt b_6f complex, followed by light-supported electron transport through PSI. In this work, however, we focus on alternative processes oxidizing plastoquinone. PQH_2 does not remain reduced post illumination in the dark, but slowly becomes oxidized by oxygen via plastid terminal oxidase (PTOX) activity, as well as non-enzymatically by superoxide radical. Initially, PSI-less mutants of *Chlamydomonas reinhardtii* and *Chlorella pyrenoidosa* were shown to be able for oxygen-dependent PQH_2 oxidation by an enzyme that is located in electron transport chain between PSII and Cyt b_6f [15].

Abbreviations: AL, actinic light; a_{II}, relative optical cross-section of PS II; CEF, cyclic electron flow; CSR, charge separation rate; Cyt b_6f, cytochrome b_6f; $Cytb_{559}$, cytochrome b_{559}; ETR, electron transport rate; FRL, far-red light; FNR, ferredoxin:$NADP^+$ reductase; F_m, F, F_r, F_0, fluorescence yields, maximum, actual, residual and minimal; LED, light-emitting diode; LEF, linear electron flow; PFD, PAD, photon flux density, incident and absorbed; PSI, PSII, photosystems II and I; PQ, PQH_2, plastoquinone, oxidized and reduced; PTOX, plastid terminal oxidase; P680, donor pigment complex of PS II; Q_A, Q_B, Q_C, quinone binding sites in PS II; Tyr Z, tyrosine Z; Y_{III}, global quantum yield of PSII for LEF; y_{III}, yield of linear electron flow

* Corresponding author.
E-mail address: agu.laisk@ut.ee (A. Laisk).

http://dx.doi.org/10.1016/j.bbabio.2015.03.003
0005-2728/

An important finding was that in an *Arabidopsis* mutant IMMUTANS showing a variegated phenotype [16,17] the inactivated gene led to an impairment in phytoene desaturation, an essential step in carotenoid biosynthesis that requires PQ as an electron acceptor. In the mutant PQH_2 did not become sufficiently oxidized in the dark to be able to support carotenoid synthesis. Since the inactivated gene possessed sequence homologies with mitochondrial alternative oxidases, the IMMUTANS (IM) gene was named PTOX for plastid terminal oxidase [18,19]. PTOX is a 37 kDa nuclear-coded protein present in organisms that exhibit oxygenic photosynthesis [16,17] bound mostly to stroma thylakoids in a quantity of 1 PTOX per 100 PSII [20,21]. PTOX is a non-heme di-iron oxidase that can utilize plastoquinol, but not ubiquinol as a substrate [22], the end product of the oxygen reduction step being water [18]. As to the physiological role, it was proposed that PTOX may act as a "safety valve" or alternate electron sink to dissipate excess energy that might accumulate in the absence of fully functional electron transport chains in the early stage of chloroplast formation [17,23]. This model however was questioned in a study where the actual kinetics of PTOX were investigated in wild type and variegated tomato leaves [24]. In the dark, at 21% O_2 the maximum PTOX rate was smaller than 1 $e^-\ s^{-1}$ per PSII. Under all the conditions tested the enzyme activity always remained about two orders of magnitude lower than that of electron flux through the linear photosynthetic electron pathway. Therefore, PTOX generally cannot play a role of a safety valve for photo-generated electrons, though this is not excluded under stress conditions, e.g. when terminal electron acceptors are strictly limiting [25].

There is also a possibility for non-enzymatic oxidation of PQH_2, the chemistry involving activated intermediate forms of the substrates — semiquinone PQ^- and superoxide O_2^- radicals [4,26]. Oxidation of the PQ-pool after illumination with 50 or 500 μmol quanta $m^{-2}\ s^{-1}$ was measured in isolated pea thylakoids spectrophotometrically as the increase in ΔA_{263}, i.e., as the appearance of PQ. PQH_2 did not oxidize in the dark in the absence of oxygen, but under 21% O_2 the oxidation was biphasic. The authors concluded that a plastoquinone oxidase could not be involved in the biphasic process, but the initial fast phase was caused by superoxide, initially accumulated in thylakoids during illumination [27], the slow phase continuing autocatalytically.

Another interesting route has been suggested that plastoquinol might be oxidized by electron transfer to cytochrome b_{559} — a heme in the PSII complex whose metabolic function is not yet clear [28]. Though the participation of PTOX was not completely excluded in these experiments, the detected parallel redox changes in PQ and Cyt b_{559} remain a fact. By testing different quinones, rather low structural quinone specificity was observed for the Cyt b_{559} quinone-catalyzed photoreduction, the data favoring the view that the quinone-binding site active in Cyt b_{559} photoreduction is a Q_C site, different from the Q_A and Q_B sites in PSII [29].

Rather than oxygen being the terminal electron acceptor, these data are consistent with the PSII cycle, where photo-oxidized $P680^+$ is the electron acceptor. Cycling of electrons around PSII has been detected [30] in thylakoid membranes [31] and chlorella cells [32], but in leaves the fast rates detected at high light intensities have been related to donor side charge recombination rather than electron cycling via the PQ pool [33–35]. The slow cyclic pathway is based on photo-oxidation of Cyt b_{559} as follows: Cyt $b_{559} \rightarrow Car_{D2} \rightarrow Chl_{D2} \rightarrow P680^+$ [36–39]. The photo-oxidized Cyt b_{559} is re-reduced either by the PSII-bound reduced Q_B [40] or by the diffusible PQH_2 [29,41].

The physiological role of CEF_{II} involving Cyt b_{559} is still not clear, mainly because for technical reasons investigations of the secondary electron transport pathways within PSII have been carried out almost exclusively on isolates lacking the complete functional electron transport pathway. In this work we have used intact leaves possessing fully competent electron transport chain, and applied precise methods for leaf gas exchange and Chl fluorescence measurements, facilitating detection of small differences between the rates of charge separation rate within PSII (CSR_{II}), detected from Chl fluorescence, and linear electron flow through PSII (LEF), detected from O_2 evolution. The small but experimentally significant difference between CSR_{II} and LEF indicates that a part of electrons once transferred through PSII do not continue the traffic along the linear pathway, but return to the donor side of the photosystem. The cyclic process is faster in circumstances where faster extra ATP synthesis is required. We propose a mechanism for this CEF_{II}, which, similarly to the Q-cycle, is based on sequential double oxidation of one and double reduction of the other plastoquinone, using Cyt b_{559} and Q_B^- for temporary storage of the second electron.

In order to evaluate the competitive pseudo-cyclic electron turnover via PQH_2 oxidation by O_2 in our experiments, we also measured the O_2 concentration dependence of the post-illumination oxidation of intersystem electron carriers. It appears that the O_2 concentration requirement for this process is much higher than it was realistically available in our experiments. The actual rate of PQH_2 oxidation by dioxygen was by two orders of magnitude slower than the recorded rate of the light-dependent oxidation PQH_2 by PSII.

2. Materials and methods

2.1. Plant material

Sunflower (*Helianthus annuus* L.) plants were grown in a growth chamber in 4 l pots filled with peat-soil mixture with added NH_4NO_3, or an aeroponic growth system was used in the NO_3^-- or NH_4^+-nutritioned growth experiments [42]. Fully grown leaves attached to the plant were fitted to the leaf chamber for measurements.

2.2. Leaf illumination and gas exchange measurements

A laboratory-made two-channel leaf gas exchange measurement system [43] enabled control and measurement of CO_2, O_2 and water vapor concentrations (for performance see [44]). A 32 mm diameter and 3 mm thick leaf chamber, covering a part of the leaf, was illuminated by LED-based light sources through a multi-branched fiber-optic light guide (630 nm actinic, 720 nm far-red). Light intensity was measured with spectro-radiometer PC2000 (Ocean Optics, FL) calibrated against a standard lamp. The spectrum of leaf absorptivity was measured with the PC2000 in an integrating sphere and photon absorption density (PAD) was calculated by integration over the spectrum of LED emission intensity, μmol $m^{-2}\ s^{-1}\ nm^{-1}$, times leaf absorptivity. Rates of CO_2 exchange were measured with an infrared gas analyzer LI-6251 (LiCor, Lincoln, NE, USA), O_2 evolution was measured in the same gas flow with a zirconium O_2 analyzer S-3A (Ametek, Pittsburgh, PA, USA) on the background of 20 ppm O_2 in N_2 containing CO_2 as indicated. Both gas analyzers were precisely calibrated [45]. Response time of the O_2 measurement was 0.8 s and that of the CO_2 was 1.6 s.

2.3. Electron transport and quantum yields from gas exchange

Linear electron flow rates were calculated from CO_2 exchange at 21% O_2 using Eq. (1) [44],

$$LEF_C = 4(A_C + R_K)\frac{2K_sC_c + 2O_c}{2K_sC_c - O_c}, \quad (1)$$

where A_C is CO_2 gas exchange rate, R_K is Krebs cycle respiration, K_S is Rubisco specificity factor (98 at 22 °C), and C_C and O_C denote corresponding dissolved gas concentrations in chloroplast stroma. From O_2 exchange at 20 ppm O_2 LEF was calculated according to Eq. (2) equally for both photosystems

$$LEF = 4A_O, \quad (2)$$

where A_O is O_2 evolution rate. For CO_2 measurements down-regulation