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Biochimica et Biophysica Acta 1709 (2005) 203 – 213
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
http://www.elsevier.com/locate/bba

Probing the FQR and NDH activities involved in cyclic electron transport around Photosystem I by the 'afterglow' luminescence

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Received 24 February 2005; received in revised form 16 June 2005; accepted 6 July 2005
Available online 18 August 2005

Abstract

Far-red illumination of plant leaves for a few seconds induces a delayed luminescence rise, or afterglow, that can be measured with the thermoluminescence technique as a sharp band peaking at around 40–45 °C. The afterglow band is attributable to a heat-induced electron flow from the stroma to the plastoquinone pool and the PSII centers. Using various *Arabidopsis* and tobacco mutants, we show here that the electron fluxes reflected by the afterglow luminescence follow the pathways of cyclic electron transport around PSI. In tobacco, the afterglow signal relied mainly on the ferredoxin-quinone oxidoreductase (FQR) activity while the predominant pathway responsible for the afterglow in *Arabidopsis* involved the NAD(P)H dehydrogenase (NDH) complex. The peak temperature T_m of the afterglow band varied markedly with the light conditions prevailing before the TL measurements, from around 30 °C to 45 °C in *Arabidopsis*. These photoinduced changes in T_m followed the same kinetics and responded to the same light stimuli as the state 1–state 2 transitions. PSII-exciting light (leading to state 2) induced a downward shift while preillumination with far-red light (inducing state 1) caused an upward shift. However, the light-induced downshift was strongly inhibited in NDH-deficient *Arabidopsis* mutants and the upward shift was cancelled in plants durably acclimated to high light, which can perform normal state transitions. Taken together, our results suggest that the peak temperature of the afterglow band is indicative of regulatory processes affecting electron donation to the PQ pool which could involve phosphorylation of NDH. The afterglow thermoluminescence band provides a new and simple tool to investigate the cyclic electron transfer pathways and to study their regulation in vivo.

Keywords: *Arabidopsis*; Cyclic electron transport; Ferredoxin-plastoquinone oxidoreductase; NAD(P)H dehydrogenase, Photosystem I; Thermoluminescence

1. Introduction

Far-red light induces a long-lived luminescence in plants, which manifests as a bounce superimposed on the luminescence decay in the dark [1,2]. This afterglow (AG) emission, although induced by far-red light which preferentially excites PSI, originates from PSII [3]. It is attributed to a back-flow of electrons from reducing compounds in the stroma to the plastoquinones (PQ) and the quinonic electron acceptors of PSII, oxidized by far-red light [4,5]. The reduced Q_B^- acceptors thus formed in the dark can recombine with the S2 and S3 states of the manganese oxygen-evolving complex of PSII, leading to light emission. In agreement with this interpretation, temperature elevation up to 40–45 °C has been shown to stimulate electron donation from the stroma to the acceptor side of PSII [6–8] and to enhance the AG emission [1,9]. Because the AG is stimulated by increasing temperature, this signal can be conveniently measured by thermoluminescence (TL), as a sharp band peaking between 40 and 50 °C

Abbreviations: AG, afterglow; B-band, thermoluminescence band at ca. 30 °C due to S2/3 Q_B^- recombination; PQ, plastoquinone; PS, photosystem; T_m, temperature of the maximum of the AG band; TL, thermoluminescence; E, Emerson enhancement; FQR, ferredoxin-quinone oxidoreductase; NDH, NAD(P)H dehydrogenase; PDF, photon flux density

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0005-2728/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbabio.2005.07.010

[10,11]. The AG TL band corresponds to a heat-induced stimulation of electron transfer from stromal reductants to PSII centers that are initially in the non-recombining state S2/3 Q_B and are progressively converted to light-emitting S2/3 Q_B^- states.

An important feature of the AG is its inhibition by antimycin [12] and its acceleration by N-methylphenazonium metosulphate [2], which are, respectively, an inhibitor and an activator of cyclic electron transport around PSI in chloroplasts. This suggests that the electron fluxes responsible for the AG and the cyclic electron transfer chain share a common sequence of electron carriers. In agreement with this idea, induction of cyclic electron transport by environmental stress conditions, as probed by $P700^+$ reduction and photoacoustic measurements, was found to be correlated with an acceleration of the AG luminescence in maize [13]. In green plants, electron recycling from the stroma-exposed side of PSI to the PQ pool can occur via two different routes [14–16]. One route is mediated by NAD(P)H and involves the chloroplastic NAD(P)H dehydrogenase (NDH) complex [17–19] which participates also in chlororespiration [20]. The other cyclic pathway is sensitive to antimycin and involves a putative ferredoxin-plastoquinone oxidoreductase (FQR) that catalyzes electron transfer from ferredoxin to the PQ pool [19,21]. In this study, the suggested relationship between AG luminescence and cyclic electron flow was analyzed using thermoluminometry in a series of tobacco and *Arabidopsis* mutants affected in the cyclic electron transfer pathways. The presented results show that the AG requires the activation of an electron cycle around PSI. The heat-induced electron flux reflected by the AG TL band is mediated by both the FQR and the NDH enzymatic complexes. The relative contribution of each pathway varies, however, from one species to the other.

2. Materials and methods

2.1. Plant material

Wild-type tobacco (*Nicotiana tabacum*, cv. Petit Havana) and the *ndhB*-inactivated mutant [22] were grown in a phytotron, as previously described [18]. Wild-type *Arabidopsis* (*Arabidopsis thaliana* (L.) Heynh., cv. Colombia, Landsberg erecta or Wassilewskija) and the *ndho* and *ndhn* mutants were grown under controlled conditions, as previously described [23]. A complete description of both *ndh* mutants can be found in Rumeau et al. [24]. The *pgr5* mutant, the *crr4-2* mutant and the double mutant *pgr5 crr4-2* were grown under similar conditions except that the photon flux density was reduced to 30 μmol m^{-2} s^{-1} because the *pgr5* mutant is photosensitive. Both mutations are described in Munekage et al. [16,19]. The cyanobacterium Synechocystis PCC6803 was grown at 30 °C in Allen's medium as described elsewhere [25].

2.2. Treatments

The lower epidermis of tobacco leaves was stripped off as described in [17]. Stripped leaf discs were soaked in Petri dishes containing water and 5 μM antimycin (Sigma). Time of incubation was ca. 30 min. We did not succeed in peeling *Arabidopsis* leaves. Therefore, *Arabidopsis* leaf discs were soaked in water with 5 μM antimycin for minimum 3 h under gentle stirring. With intact tobacco leaf discs, this 3-h treatment gave similar results as 30-min floating of stripped leaf discs on 5 μM antimycin. The AG band was abolished in leaf discs infiltrated under vaccum with water or with a buffer. This confirms that the AG emission occurs in intact systems only (isolated thylakoid membranes do not emit an AG luminescence). Therefore, it was not possible to use the vaccum technique to infiltrate leaves with antimycin. Detached *Arabidopsis* leaves placed on wet filter paper were driven to the light state 2 by illumination at 25 °C with a blue-green light produced by metal halide lamps (Osram, Powerstar HQI-TS, 150 W/NDL) and a BG18 Schott filter (PFD, 100 or 300 μmol photons m^{-2} s^{-1}). Far-red light (30 W m^{-2}) obtained by filtering the light through a RG715 Schott filter was used to drive leaves to state 1.

2.3. Thermoluminometry

The luminescence emitted by leaf discs (diameter, 12 mm) or cyanobacterial cells deposited on a nitrocellulose filter (diameter, 1 cm) was measured with a custom-made apparatus that has been described in detail elsewhere [26]. In brief, the sample was placed in the bottom of a cylindrical cuvette (diameter, 25 mm) made from heat-resistant plastic against a thin aluminium plate (1 mm), the temperature of which was regulated with a water-cooled Peltier system (40 × 40 mm Thermatec Peltier plate from Melcor) powered by a variable 0 to 5A current. Temperature was measured with a tiny thermocouple inserted in the aluminium plate at the center of the Peltier element at 0.5 mm under the sample. A drop of water (100 μl) was placed on the center of the aluminium plate to ensure good thermal contact with the leaf. The sample was maintained at 0 °C or 10 °C for 30 s in the dark, then it was illuminated for 60 s with far-red light (>715 nm) at this temperature. Far-red light, produced by a Schott KL1500LCD light source and a RG-715 Schott filter, was passed onto the sample with one arm of a 4-arm light guide (Walz). The fluence rate of the far-red light, measured with a Li-Cor Li185B/Li200SB radiometer, was about 50 W m^{-2}. Immediately after switching off the far-red light, temperature was increased from 0 °C or 10 °C to 70 °C, at a rate of 0.5 °C s^{-1}. The