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Methylviologen and dibromothymoquinone treatments of pea leaves reveal the role of photosystem I in the Chl *a* fluorescence rise OJIP

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Abstract

The effects of dibromothymoquinone (DBMIB) and methylviologen (MV) on the Chl *a* fluorescence induction transient (OJIP) were studied in vivo. Simultaneously measured 820-nm transmission kinetics were used to monitor electron flow through photosystem I (PSI). DBMIB inhibits the reoxidation of plastoquinol by binding to the cytochrome b_6/f complex. MV accepts electrons from the FeS clusters of PSI and it allows electrons to bypass the block that is transiently imposed by ferredoxin-$NADP^+$-reductase (FNR) (inactive in dark-adapted leaves). We show that the IP phase of the OJIP transient disappears in the presence of DBMIB without affecting F_m. MV suppresses the IP phase by lowering the P level compared to untreated leaves. These observations indicate that PSI activity plays an important role in the kinetics of the OJIP transient. Two requirements for the IP phase are electron transfer beyond the cytochrome b_6/f complex (blocked by DBMIB) and a transient block at the acceptor side of PSI (bypassed by MV). It is also observed that in leaves, just like in thylakoid membranes, DBMIB can bypass its own block at the cytochrome b_6/f complex and donate electrons directly to PC^+ and $P700^+$ with a donation time τ of 4.3 s. Further, alternative explanations of the IP phase that have been proposed in the literature are discussed.

Keywords: Chl *a* fluorescence; OJIP-transient; 820-nm transmission; DBMIB; Methylviologen; *Pisum sativum*

1. Introduction

The Chl *a* fluorescence induction transient (OJIP) has been studied extensively (for recent reviews, see Refs. [1–4]). In the last few years several mutually exclusive hypotheses have been put forward to explain the second and third kinetic phases of the initial fluorescence rise (JI and IP). Here, we will concentrate on the physiological processes underlying the IP phase for which at least eight hypotheses have been presented during the last 45 years. Initially, it was thought that the reduction of an electron acceptor at the acceptor side of photosystem I (PSI) was responsible for the IP phase [5–8]. Schreiber and Vidaver [9] proposed energy redistribution between PSII and PSI. The observations of Vernotte et al. [10] have been interpreted to mean that the IP phase can be explained by removal of plastoquinone (PQ) pool quenching as a consequence of reduction of the PQ pool (e.g., Refs. [11,12]). The IP phase has also been thought to represent variable PSI fluorescence [13,14], an effect of the electric field [15] or the last step in the reduction of the acceptor side of PSII [16]. Two more interpretations that have been put forward are recombinational fluorescence (with respect to the whole thermal phase of the fluorescence induction curve) [17] and slowly closing stromal PSII reaction centers [14].

Some of these interpretations may have to do with the fact that they were based on experiments with thylakoid

Abbreviations: Chl, chlorophyll; DBMIB, dibromothymoquinone, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DCMU, 3-(3,4-dichlorophenyl)-1,1′-dimethylurea; F_o and F_m, fluorescence intensity measured when all photosystem II reaction centers are open or closed, respectively; FNR, ferredoxin-$NADP^+$-reductase; I, photocurrent, a measure for the transmitted light; LED, light emitting diode; MV, methylviologen, 1,1′-dimethyl-4,4′-bipyridinium-dichloride; OJIP-curve, fluorescence induction transient defined by the names of its intermediate steps, O level, fluorescence level at 20 μs, J level, fluorescence plateau at ~2 ms, I level, fluorescence plateau at ~30 ms and P level, the maximum fluorescence level; P680 and P700, reaction center pigments of photosystem II and I, respectively; PC, plastocyanin; Q_A, primary quinone electron acceptor of photosystem II

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membranes. During the isolation of thylakoid membranes, ferredoxin on the acceptor side of PSI is lost and this changes the Chl *a* fluorescence rise. For example, no clear I-step is observed in thylakoid membranes [18–21].

Here, we have studied the effects of dibromothymoquinone (DBMIB) and methylviologen (MV) in vivo using Chl *a* fluorescence and transmission measurements at 820 nm. Both DBMIB and MV strongly affect the IP phase of the fluorescence induction transient, each for its own reason. Our results support the original hypothesis [5–8] that the IP phase is related to electron transfer through PSI and the induction of a traffic jam of electrons caused by a transient block at the acceptor side of PSI (inactive ferredoxin-$NADP^+$-reductase (FNR)).

2. Materials and methods

2.1. Plant material

For the measurements, mature leaves of 2–3-week-old pea plants (*Pisum sativum* L. cv. Ambassador) were used. Plants were grown in a greenhouse where the temperature was 20–25 °C during the day and 14–16 °C at night.

2.2. MV treatment

For the MV treatments, a 200 μM MV (1,1′ -dimethyl-4,4′ -bipyridinium-dichloride, Fluka) solution was applied to both sides of the leaf with a fine brush. The leaves were not detached and the plants were kept overnight in complete darkness before the measurements were made. Stirring clipped leaves for 12 min in 200 μM MV as done for the DBMIB treatment (see below) gave very similar results (not shown).

MV+DCMU treatment: Pea leaves were submerged into a solution containing 200 μM MV, 170 μM DCMU and 1% ethanol. Leaves were not detached and they were treated overnight in complete darkness.

2.3. DBMIB treatment

Overnight incubation of undetached leaves in a DBMIB solution was not effective. Instead, leaves with clipped edges to allow a better uptake were stirred for 12 min in a solution of 200 μM DBMIB (2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone, SIGMA) containing 1% of ethanol. The leaves were stored in the DBMIB solution until use. Leaves treated in the same way, but in the absence of DBMIB, were used as controls.

DBMIB+DCMU treatment: First, leaves were treated with DCMU (170 μM DCMU, 1% ethanol). Leaves were not detached and they were treated overnight in complete darkness. Subsequently, they were treated with DBMIB, as described above.

2.4. Measuring equipment

Chl *a* fluorescence emission was measured by a Handy PEA (Handy PEA-Plant Efficiency Analyser, Hansatech Instruments, King's Lynn, Norfolk, UK). Leaves were dark-adapted for 30 min before they were measured. Illumination (unless stated otherwise) consisted of a 1-s pulse of continuous red light (650-nm peak wavelength, 3000 μmol photons m^{-2} s^{-1} maximum light intensity) provided by an array of three light-emitting diodes (LEDs) focused on a leaf area with a diameter of 5 mm.

Transmission changes at 820 nm and Chl *a* fluorescence were recorded simultaneously using a dual channel PEA Senior instrument (Hansatech Instruments). The first reliable measuring point for fluorescence change was at 20 μs whereas the first measuring point for transmission change was at 400 μs. The time constant used for the transmission measurements was 100 μs. The light intensity used for all experiments was 1800 μmol photons m^{-2} s^{-1}. The light was produced by four 650-nm LEDs. The far-red light source was a QDDH73520 LED (Quantum Devices Inc.) filtered at 720±5 nm. The modulated (33.3 kHz) far-red measuring light was provided by an OD820 LED (Opto Diode Corp.) filtered at 830±20 nm. Executing commands like turning on and off the LEDs took about 250 μs. Turning on the red light and starting the measurement were synchronized commands. For the far-red light there was a delay of 250 μs between turning on the far-red light and the start of the measurement. Each measurement consisted of three parts. First, the transmission was measured without amplification and offset to obtain a value for total transmission (I_0). Subsequently, the transmission was measured with a gain of 50 to obtain a value for the transmission at t=0. This was followed by kinetic measurements.

3. Results

3.1. Chl a fluorescence rise

DBMIB is an artificial quinone introduced in 1970 by Trebst et al. [22] and Böhme et al. [23] as an inhibitor of photosynthetic electron transport and an antagonist of PQ. It was shown that cyt b_6/f can accept only one electron from DBMIB. As a semiquinone it remains tightly bound to the cyt b_6/f complex preventing in this way the reoxidation of other PQH_2 molecules by cyt b_6/f [24]. The DBMIB concentration used in this study inhibits the cyt b_6/f complex but it also has a limited effect on the Q_B site. In Fig. 1A several fluorescence induction curves in the presence and absence of DBMIB measured at different light intensities are shown. The main effect of DBMIB is to make the IP phase disappear, which means that under saturating light conditions the I level approaches the maximum fluorescence level. On the other hand, the F_m level is not affected by the treatment. It can also be observed