The long-wavelength limit of plant photosynthesis

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Abstract It is a common knowledge that the photosynthesis efficiency drops rapidly under the long-wavelength light excitation above 680 nm. We discovered that in sunflower leaves attached to the plant the initial fall is replaced by an unexpected increase at much longer wavelengths, so that a detectable O\textsubscript{2} evolution is remained till 780 nm. The quantum yield of O\textsubscript{2} evolution at the local maximum at 745 nm reaches almost 20\% of the yield at 650 nm. We conclude that extreme long-wavelength chlorophylls may be present in the intact photosystem II antenna system, similarly to photosystem I.

Keywords: Quantum yield of photosynthesis; O\textsubscript{2} evolution; Photosystem II; Far-red chlorophyll

1. Introduction

In oxygen-evolving photosynthesis, the conversion of solar energy into electrochemical potential requires two photosystems, photosystem II (PSII) and photosystem I (PSI), operating in series. Light energy absorbed by the chlorophylls (Chls) of antenna systems of the two photosystems is rapidly transferred to the respective reaction centres where sequences of electron transfer reactions are triggered. For maximum efficiency an even distribution of absorbed light quanta between the photosystems is needed. This requirement is, however, severely degraded at the far-red end of the absorption spectrum of plants due to disparate spectral tuning of the centre pigments to about 680 and 700 nm for PSII and PSI, respectively [1]. Most of the antenna Chls are adjusted to 680 nm or shorter wavelengths, so that mainly excitation transfer occurs energetically downhill. Yet, in the PSI antenna a small number of far-red Chls is present, which absorb at even longer wavelengths than the PSI centre [2]. Occurrence of similar far-red Chls in the PSII antenna is not recognized. The unbalanced distribution of far-red light excitations between the two photosystems results in a well-known red drop of the photosynthesis quantum yield [3,4]. Though this drop has frequently been observed in the earlier work [5,6], its extension to the far-red spectral range has not been fully characterized.

Here, we revisit this classical problem by applying an ultra-sensitive zirconium electrode for the measurement of O\textsubscript{2} evolution from leaves of the growing sunflower plant and by illuminating the leaves with a tuneable laser. The improved technology allowed us to detect the light-activated O\textsubscript{2} evolution at extraordinarily far-red wavelengths up to 790 nm and to measure its spectral dependence. These measurements thus set the long-wavelength limit of plant photosynthesis.

2. Materials and methods

The PSII activity has been characterized by measuring the photosynthetic O\textsubscript{2} evolution rate as a function of excitation wavelength between 650 and 790 nm. Fully expanded sunflower (Helianthus annuus L.) leaves attached to the plant were used in the experiments. The plants were grown in a growth chamber in the laboratory. Part of a leaf was enclosed in a leaf chamber in a two-channel gas flow system (Fast-Est, Tartu, Estonia) [7]. The leaf chamber was illuminated through a multiarmed fiber-optics cable with a continuous-wave Ti:Sapphire solid state laser (Model 3900 S, Spectra Physics, Mountain View, CA, USA) with line width of ~0.03 nm (at far-red wavelengths beyond 700 nm), or with a dye laser (Model 375, Spectra Physics, Mountain View, CA, USA) (in the red spectral range between 650 and 700 nm). The incident quantum flux density in the leaf chamber was measured using a bolometric sensor calibrated against a LI-190SB quantum sensor (LiCor, Lincoln, NE, USA) in white light between 400 and 700 nm [8]. The O\textsubscript{2} evolution rate was measured with a Zr-oxide analyser Ametek S-3A (Thermox, Pittsburgh, PA, USA) [9]. After the background O\textsubscript{2} concentration was rapidly decreased from the concentration of 210–50 μmol per mol (N\textsubscript{2} buffer gas), the O\textsubscript{2} evolution rate was measured as a response to illumination of the leaf at multiple wavelengths. The light intensity was always kept on the linear section of the light response curve and carefully chosen colour filters blocked the stray light. The low O\textsubscript{2} concentration of 50 μmol per mol was maintained for no longer than 10 min, which was not harmful for the leaf. The reference O\textsubscript{2} evolution rate level was determined during brief darkening (at the very low O\textsubscript{2} concentration the respiratory O\textsubscript{2} uptake was negligible).

3. Results and discussion

An example of the recordings at different wavelengths, normalized with respect to the incident light intensity, is shown in Fig. 1. It clearly demonstrates a sufficiently high signal to noise ratio even at the longest wavelengths measured.

The resulting spectrum of the photosynthesis quantum yield (equivalently, the quantum yield of PSII electron transport) is presented in Fig. 2. This quantum yield was calculated from the measured oxygen evolution as

$$Y(\lambda) = 4 A(\lambda) I_0(\lambda) a(\lambda),$$

where $A(\lambda)$ is the O\textsubscript{2} evolution flux density in μmol m\textsuperscript{-2} s\textsuperscript{-1}, $I_0(\lambda)$ is the incident photon flux density at a given wavelength in μmol m\textsuperscript{-2} s\textsuperscript{-1}, and $a(\lambda)$ is the absorptance of the leaf (the ratio of the radiation absorbed to the total radiation falling on the surface, ranging from 0 to 1). The multiplier 4 is the
not related to the thermal activation of the energy transfer, and 716 and 740 nm far-red light. In order to eliminate the effects rates as a function of temperature were studied under the background CO₂ and O₂ concentrations were 360 and 50 μmol per mol (N₂ buffer gas), respectively.

Fig. 1. Traces of the O₂ evolution rate from a sunflower leaf at 22 °C. The data are normalized to the incident laser light intensity at different wavelengths indicated. The laser was turned off between the traces. The background CO₂ and O₂ concentrations were 360 and 50 μmol per mol (N₂ buffer gas), respectively.

Fig. 2. Spectral dependence of the photosynthesis quantum yield (left axis and data points) and leaf absorbance (right axis and solid line) for sunflower leaves. Standard deviation of the data from the mean \( n = 10 \) is indicated. The leaf absorbance spectrum was recorded using an integrating sphere and a spectroradiometer PS-2000 (Ocean Optics, Dunedin, FL, USA).

assumed number of light-driven one-electron oxidation steps required to oxidize water to molecular oxygen.

As can be seen from Fig. 2, the yield decreases significantly above 680 nm, while it remains clearly higher than the background noise level up to ~780 nm (see also Fig. 1). Apparent too is a local maximum of the yield at ~745 nm. There is no doubt that this local peak is a result of PSII photochemistry, because the enhanced O₂ evolution was accompanied by an increased yield of Chl fluorescence from the PSII antenna (data not shown), an evidence of accumulation of electrons in the quinone acceptors of PSII. Our quantum yield value well agrees with the yield for the O₂ evolution in \textit{Chlorella} at 720 nm, the maximum wavelength of the earlier systematic investigations [3–5].

Since the PSII reaction centre is located at 680 nm, energetically much uphill relative to the local maximum of the quantum yield, the far-red light-supported O₂ evolution must be highly temperature-sensitive. To check this, the O₂ evolution rates as a function of temperature were studied under the 716 and 740 nm far-red light. In order to eliminate the effects not related to the thermal activation of the energy transfer, quantum yields for the far-red light were normalized to that at 650 nm excitation, which corresponds to a weakly exothermal process. Natural logarithms of the ratios, \( \ln \left( \frac{Y(\lambda)}{Y(650)} \right) \), plotted versus inverse thermodynamic temperature \( T \) (Arrhenius plots) are shown in Fig. 3. The sample points cover the temperature range from 10 to 34 °C.

From these plots uphill activation energies of 9.9 ± 0.3 and 12.5 ± 0.8 kJ mol⁻¹ have been revealed for the 716 and 740 nm illuminations, respectively, fairly close to the corresponding energy gaps of 8.8 and 14.3 kJ mol⁻¹ between the 716 and 680 nm, and the 740 and 680 nm light quanta. This confirms that thermal activation is involved in transfer of far-red light quanta to the PSII core. The justly linear plots also imply that the energy transfer takes place from the thermally equilibrated state. The energy deficiency of light quanta is thus complemented by the thermal energy of the surroundings.

The spectral shape of the PSII activity in the far-red, distinct from the leaf absorbance spectrum (Fig. 2), indicates that the absorbance responsible for the O₂ evolution is minor relative to the bulk absorption in that region. The yield of 0.070 ± 0.005 at 745 nm implies that ~9% of absorbed far-red light quanta lead to the oxygen evolution (assuming typical fluorescence-indicated losses of about 20% hold also true for the far-red excitations), whilst the rest ~91% are apparently delivered to PSI.

Supposing that special far-red Chls are responsible for the effect, one or less such Chls per PSII complex should be present. This estimate is based on the number of the far-red Chls in the PSI antenna (total about ten, but about one at 745 nm [10]) and on the assumption of similar molecular absorption cross-sections for all Chls in PSI and PSII. The literature evidence for the far-red Chls in PSII constituents is scarce. The isolated core antenna complexes (CP43 and CP47) positively lack these varieties, while a tail of the absorption spectrum extending to 720 nm at room temperature has been observed in peripheral LHClI antenna trimers in a few cases [11,12]. Further tailing reaching ~740 nm has been found only for the bulky grana membrane fractions [13]. This list, although limited, seems to
imply that more intact preparations reveal the presence of the far-red Chl forms more frequently, reviving an old question of a possible influence of different extraction procedures on spectral properties of the relatively fragile photosynthetic complexes. Since the light absorption in the far-red region is dominated by PSI, there in principle is a possibility that Chl excitations of the PSI antenna may overflow to the PSII antenna. The mechanism known as spillover [14–16] has originally supposed to explain a transfer of surplus excitation energy from PSII to PSI, not from PSI to PSII. Although a physical contact between the PSII and PSI antennae necessary for such transfer is possible [17], the model also assumes a relatively long lifetime of far-red PSI excitations. The trapping time of light excitations in PSI determined to be between 81 and 103 ps [18], however, is considerably shorter than the lifetime of excitations in PSII equal to 300–600 ps [19]. Therefore, competition for the excitations by the 700 nm reaction centre trap of PSI is expected to become gradually more dominant with increasing wavelength that is not observed. On the contrary, as seen in Fig. 2, the quantum yield of O₂ evolution is increasing in a relatively wide spectral range between 720 and 745 nm before it finally drops.

The third possibility to elucidate our observations is based on optical excitation from thermally populated vibrational sub-levels of the PSI Chls in the ground electronic state. The optical energy needed to cover the energy gap between the ground and excited electronic states would then be reduced by the energy of vibrational quanta. Let us consider a dominant coupling of the lowest electronic transition in PSII Chls with intramolecular vibrational quanta of ≈14.7 kJ mol⁻¹ energy, which is characteristic to the LHCl antenna [20]. It then can be calculated at ambient temperature that the Boltzmann-averaged steady-state populations of first excited and ground state vibrational levels relate as ≈1/410. Absorbance from the excited vibrational state relative to that from the ground state is further reduced by a factor of 7–8 [20] due to reduced overlap of the excited and ground state vibrational wavefunctions. This determines the ratio of absorbances of PSII Chls at 680 and 745 nm equal to ≈3000. This estimate should be compared with one obtained from the measured absorbance and quantum yield data. The absorbance (optical density) spectrum of the leaf, A(λ), can be calculated from the absorbance spectrum as A(λ) = −log[1 − a(λ)]. From this spectrum (not shown) the ratio of overall (PSI+PSII) absorbances at 680 and 745 nm equal to 50–70 is obtained. Assuming that about half of the overall absorbance at 680 nm and that of ≈9% at 745 nm belong to the PSII pigments, the effective ratio between 280 and 390 is found, an order of magnitude about half of the overall absorbance at 680 nm and that of ≈9% at 745 nm belong to the PSII pigments, the effective ratio between 280 and 390 is found, an order of magnitude.

We thus are obliged to conclude that similarly to photosystem I (see [21] and references therein) there is a small amount of so far unrecognised extreme long-wavelength forms of Chls in PSI antenna.

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References