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Biochimica et Biophysica Acta 1757 (2006) 155–157
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
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Discussion

Comments to water-splitting activity of photosystem II by far-red light in green plants

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Received 17 November 2005; received in revised form 22 December 2005; accepted 3 February 2006
Available online 30 March 2006

In a recent article in your journal [1] (Pettai, Oja, Freiberg, Laisk, BBA 1708 (2005), 311–321) and a parallel publication in FEBS Letters [2], oxygen production by leaves of sunflower and bean has been reported upon excitation with light in the 700 nm to 770 nm range. From this astonishing observation, the authors conclude that long-wavelength-absorbing chlorophyll *a* molecules (red forms) in the photosynthetic unit of photosystem II (PS II) deliver thermally-activated excitons to the primary donor of PS II (P680). Up till now, such strongly red-shifted forms have been thought to be uniquely due to photosystem I (PS I).

Two alternative explanations were discussed but rejected. In one of them, "reversed spill-over", the red forms of PS I supply to a certain extent PS II. However, the spatial separation of the two photosystems, the grana/stroma segregation, as well as the much shorter excited state lifetime in PS I compared to PS II, speak against such a mechanism. The other explanation, "absorption from thermally populated vibrational sub-levels of PS II chlorophylls", was rejected on the basis of the actual data. The authors estimated that the absorbance at 680 nm is 3000 times higher than at 745 nm; however, the absorptance spectrum (one minus transmittance, the units used in [1]) of the leaves indicates only a ratio of 70 [1].

Altogether, the argument against the second explanation relies critically on the accuracy of the absorbance spectrum in the red tail. However, in highly scattering samples, such as leave tissues, minute absorbances are hardly measurable with high precision, even in Ulbricht spheres as the authors did. In the following I will question the quality of the published absorptance spectrum and present several arguments in favour of the "absorption from thermally sub-populated vibrational levels of PS II chlorophylls".

Based on my article on modelling, the thylakoid membrane [3] I have developed computer-based programs (modeling modules) on photosynthesis which allow the quantitative simulation of a large variety of measurable quantities, i.e., the simulation of experiments. The modeling modules, written in Mathcad 11, comprise PS I alone, PS II alone, and the thylakoid membrane PS I+PS II. The spectral properties, like absorption and fluorescence spectra (A- and F-spectra), of the relevant complexes are properly taken into account, as are thermal effects, like the temperature dependence of the line widths. The modules can freely be copied from my homepage where also a PDF-file with a detailed description of the concepts and used formulae is found [4].

To simulate the experiments in question, I used the PS I+PS II module with the default parameters, except setting the absorption maximum of LHCII to 682 nm, the excitation intensity to 2 mW cm^{-1}, assuming 4 LHCII-trimers in a PS II unit, and the excitation wavelengths as in [1]. In particular, PS II is composed of the peripheral LHCII, the known CP complexes, and the D1D2 complex. All spectra obtained agree closely with published ones from the isolated complexes over the whole temperature range, down to 4 K [4]. The default stoichiometric ratio of PS I/PS II is one.

Let us first inspect the molar absorption spectrum (A-spectrum) of PS II (Fig. 1a, thick solid line). The maximum of the Q_y-band at around 680 nm drops rapidly to very small values at wavelengths exceeding 710 nm. It is well established that the antenna system of PS II forms a thermally equilibrated state [5–7]. Under these conditions, the fluorescence spectrum (F-spectrum) is strictly correlated to the A-spectrum by the so-called Kennard–Stepanov relation [8,9]. Applying this relation to the A-spectrum yields the F-spectrum (Fig. 1a, thin solid line). The F-spectrum computed this way looks similar to all published ones from Chl *a*-containing complexes. This demonstrates the adequacy of the assumed A-spectrum.

Next, the same A-spectrum of PS II is shown on a logarithmic scale (Fig. 1b, thick solid line). The maximum of the Q_y-band at around 680 nm appears now flat but the decline to very small

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0005-2728/$ - see front matter © 2006 Published by Elsevier B.V.
doi:10.1016/j.bbabio.2006.02.017

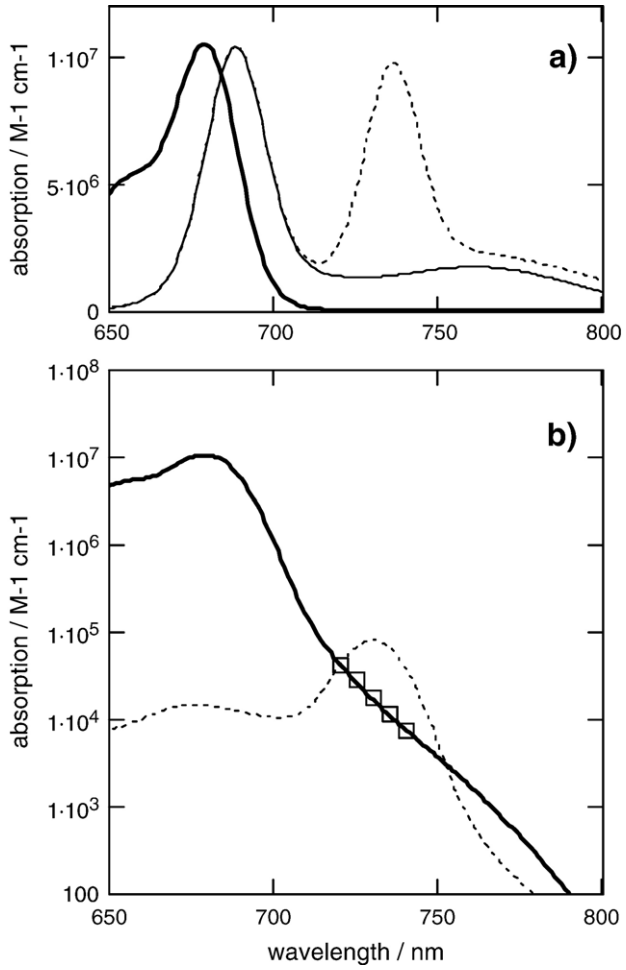

Fig. 1. (a) Molar absorption spectrum of PS II (thick solid line) and the fluorescence spectrum predicted by the Kennard–Stepanov relation (thin solid line). The dashed curve represents the Kennard–Stepanov predicted fluorescence spectrum when adding a single Chl *a* molecule with a Q_y-absorption maximum at 730 nm to the PS II absorption spectrum. (b) The above absorption spectrum and the absorption spectrum of the above red-form Chl *a* molecule (dotted line) displayed logarithmically (thick line). Oxygen evolution data reported in Fig. 1 of [1] have been adjusted by an appropriate scaling factor to fit the absorption spectrum of PS II (□).

values at wavelengths larger than 710 nm is resolved up to 785 nm. Note, that even minute changes of the shape of the red tail lead to unrealistic shapes of the Kennard–Stepanov-transformed F-spectrum. In Fig. 1 of [1], oxygen evolution rates are plotted as a function of the excitation wavelength. I have included these values in Fig. 1b (squares). They fit on the A-spectrum surprisingly well, suggesting that the observed oxygen evolution may originate from direct absorption of a normal PS II A-spectrum (not containing long-wavelength-absorbing Chl *a* forms).

The question then arises whether or not the measured absolute O_2-rates are consistent with a normal PS II A-spectrum. To find an answer to this question I take Fig. 3 of [1] in which the incident light intensity at 716 nm is quoted (120 μmol m^{-2} s^{-1}; corresponding to 2 mW cm^{-2}) and the time course of the fluorescence induction is evident. When simulating this experiment with these parameters, I get a fluorescence rise in about 4 s assuming blocked electron transfer between Q_A and Q_B. This number is well within the delayed 8 s rise in Fig. 3 of [1], which is consistent with the experimental conditions, as no inhibitor was present. Therefore, the measured O_2-rates are those expected for a normal A-spectrum, showing that the scaling factor used for adjusting the published oxygen rates in [1] to the simulatedA-spectrum (Fig. 1b, thick solid, squares) is quantitatively correct. Hence, there is no need at all for postulating novel red-shifted forms.

In Figs. 2 and 5 of ref. [1] the authors show the dependence of the absolute quantum yield of oxygen evolution on the wavelength. Between 690 nm and 710 nm there is a steep decline, though not to zero, which resembles the normal absorption decline of Chl *a*. Going further to the red the oxygen evolution levels off and remains at about 15% of the maximal value, displays a shallow side band at around 745 nm and then decays to zero. This side band may suggest a band in absorption but, this interpretation should be questioned. First, the absorptance spectrum in [1] used for the calculation is the composite of the PS II and the red forms containing PS I A-spectrum. The pure PS II A-spectrum would be needed for the calculation of the quantum yield. Second, the scattering problem mentioned above obstructs a proper calculation. Third, when the excitation of PS II is weak, as is the case at wavelengths >710 nm, turnover times exceed 10 s (see Fig. 3 in [1] or simulations with the PS I+PS II module). Then, however, charge recombination kinetics in the reaction center and relaxations of the S-states of the water splitting enzyme dictate in a complex manner the quantum yield of oxygen evolution. Hence, the quantum yield data are highly questionable.

Remains to discuss the effect of "…yet undiscovered far-red Chls of the PSII antenna…" [1]. If only a single 730 nm-Chl *a* molecule out of 180 would be present in PS II (Fig. 1b, dashed line) its Stepanov-predicted F-spectrum would display a huge peak in the far-red, which has never been reported. This is a strong argument against the presence of a significant number of red forms in PS II.

In summary, the data in [1], obtained from whole leaves of sunflower and bean, contrary to the interpretation of the authors, do not support the presence of so far unrecognised far-red Chl *a* molecules in PS II of higher plants. Rather, modelling the experimental data indicates that the far-red light directly excites vibrational sub-levels of bulk Chl *a* molecules at a rate corresponding to the residual absorption far beyond 700 nm (Fig. 1b). This interpretation predicts similar results for isolated chloroplasts or thylakoid membranes, which are less subject to scattering artefacts. At the time being, such control experiments are not available.

Independent of the interpretation of the data, the physiological relevance of far-red excitation in higher plants is modest at best, since this far-red wavelength regime under natural light conditions is always accompanied by substantial visible light and the latter dominates the excitation of any photosystem.

This does not mean that long-wavelength absorbing Chl *a* molecules >700 nm cannot or do not occur in PS II. A rare example is the green alga *Ostreobium* sp. in which peripheral

PS I light-harvesting complexes with significant red-forms (λ_{max}=715 nm) bind to PS II and 720 nm red light drives oxygen evolution by uphill energy transfer from the red-forms to P680 [10]. Note, however, that in the latter article, it was not suggested – as the authors of [1] state – "…that far-red excitations of the PS I antenna are transferred to the PS II antenna by reversed spill-over."

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