Rise time of the absorption changes of chlorophyll-α<sub>i</sub> and carotenoids in photosynthesis

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With the aid of a repetitive laser giant pulse photometer the rise times of two primary events in photosynthesis have been measured: a) The oxidation of chlorophyll-α<sub>i</sub> takes place in times shorter than 20 ns. b) The formation of metastable carotenoids is faster than 25 ns.

a) Recently we reported on measurements of optical changes occurring in photosynthesis within a time range of about 10 ns. We found an upper limit of 20 ns for the “half rise time” of the field indicating absorption change at 515 nm and thus of the electric field formation across the thylakoid membrane. This field is built up by the light reactions of chlorophyll-α<sub>i</sub> and α<sub>II</sub>. One should imagine therefore that these chlorophyll-α<sub>i</sub> reactions are as fast or faster than the rise of the field. In this work we tried to prove firstly this point and measured the rise time especially of the chlorophyll-α<sub>i</sub> reaction.

b) A further fast reaction in photosynthesis has been attributed to a reaction of carotenoids which has been interpreted as a valve reaction for excess excited antenna chlorophylls: The energy of these chlorophylls is transferred with ≈ 5·10<sup>-7</sup> s to carotenoids forming a metastable state with a rate of desactivation of 3·10<sup>-6</sup> s<sup>-1</sup>. In this work we measured secondly the rise time of this state in the 10 ns range.

Materials and methods

Whole chloroplasts of spinach were prepared according to Winget et al. and used for the measurements on the metastable state of carotenoids.

Thus prepared chloroplasts were treated with digitonin according to Anderson and Boardman. The 50,000 g fraction in which reaction system I is enriched was used for the measurements of chlorophyll-α<sub>i</sub>. The spectroscopic measurements were performed by the repetitive laser giant pulse photometry. As stimulation source we used a Nd:YAG-laser model which was Q-switched and frequency doubled to λ = 530 nm. This type of laser has a repetition rate up to 10 Hz with an optical energy of 10 mJ per pulse. The half width of the pulses was 20 ns, the stability of the amplitude was within ±7 percent. Because of the low optical density of the chloroplast suspensions at 530 nm an aluminum mirror was placed on the backside of a 2×2 cm cuvette thus improving the absorption of the laser pulses. At a chlorophyll concentration of 4·10<sup>-6</sup> M and using about 3 mJ of the laser energy this arrangement was sufficient for saturating photosynthesis.

The registration technique was the same as described in the ultra short flash photometry apparatus. The rise time (10–90% of the amplitude) of the equipment was 35 ns for the measurements of the carotenoid reaction and 25 ns in the case of the chlorophyll-α<sub>i</sub> reaction.

Including the duration of the laser pulse a total rise time of the apparatus of 50 ns and 40 ns respectively has been obtained.

Results

a) Fig. 1 represents the rise of the metastable state of carotenoids at one of the negative maxima of the difference spectrum at λ = 431 nm. The photosynthetic reactions have been blocked by the addition of DCMU.

The peak of the absorption changes in fig. 1a results from the laser pulse which was not completely cut off from the photomultiplier. The absorption change is finished as the laser pulse has decayed. Fig. 1b shows the time course of the laser only, fig. 1c the difference between fig. 1a and fig. 1b. Fig. 1c therefore represents the absorption change only. It results a measured rise time of 3·10<sup>-9</sup> = 50 ns. This time is equal to the time response of the total apparatus. Therefore the real “half rise time” of
RISE TIME OF ABSORPTION CHANGES

Fig. 1. Rise of the absorption change of metastable states of carotenoids at $\lambda = 431$ nm in spinach chloroplasts. a) Time course of the measured signal. Chlorophyll content: $5 \times 10^{-6}$ M, tris-buffer pH 7.2, 0.05 M, electron acceptor benzylviologene $10^{-4}$ M, poison of photosynthetic activities DCMU $5 \times 10^{-5}$ M, temperature $22^\circ$C. Optical path: 20 mm. Excitation: Q-switched laser pulses, $\lambda = 530$ nm, halfwidth 20 ns, repetitive laser giant pulse technique, frequency 10 Hz, energy 10 mJ, 7500 pulses. Measuring beam: interference filter 431 nm, halfwidth 8 nm, intensity incident on cuvette 92 $\mu$W/cm$^2$. Rise time (10 – 90%) of the whole apparatus including laser pulse 50 ns. b) Time course of the laser pulse. Conditions as in a). Measuring beam switched off. c) Time course of the absorption change, a) minus b).

Fig. 2. Rise of the absorption change of chlorophyll-a at $\lambda = 434$ nm in digitonin treated spinach chloroplasts. Chlorophyll content: $4 \times 10^{-6}$ M, tris buffer pH 7.2, 0.05 M, electron acceptor benzylviologene $10^{-4}$ M. Electron donor: PMS $5 \times 10^{-5}$ M -- Na-ascorbate $5 \times 10^{-5}$ M. Temperature $22^\circ$C, optical path 20 mm. Excitation: Q-switched laser pulses, $\lambda = 530$ nm, halfwidth 20 ns, repetitive laser giant pulse technique, frequency 10 Hz, energy just saturating, 7500 pulses. Measuring beam: XE arc lamp, interference filter 434 nm, halfwidth 11 nm, intensity 100 $\mu$W/cm$^2$. Rise time (10 – 90%) of the whole apparatus including laser pulse 40 ns.

$\tau_{1/2} < 25$ ns.

b) One of the negative maxima of the difference spectrum of chlorophyll-a$_1$ in digitonin treated chloroplasts is centered around $\lambda = 438$ nm. In whole chloroplasts absorption changes of cytochromes occur at this wavelength too, but these are strongly reduced in digitonin treated ones. Only metastable states of carotenoids or the triplet state of chlorophyll could be superimposed. At the used laser energy (just saturating photosynthesis) the amplitude of such changes must be smaller than the noise. Therefore the time course in fig. 2 could be assigned to chlorophyll-a$_1$ only. It results a measured rise time averaged over nine measurements of $\tau_{1/2} = 42$ ns. Regarding again the response of the total apparatus the real “half rise time” of the reaction is $\tau_{1/2} < 20$ ns.

Discussion

With these results a new upper limit of the described events has been obtained. Perhaps the real value is in the sub-nanosecond time range.

It has been shown that the photooxidation of chlorophyll-a into chlorophyll-a$^\circ$ in butanol takes place from the triplet state. It may be that also in photosynthesis the photooxidation of chlorophyll-a$_1$ takes place via this state.

If we assume that at $\lambda = 434$ nm the extinction coefficients are in the same order as in butanol: $\varepsilon$(ground state) $> \varepsilon$(cation) $> \varepsilon$(triplet), then in the case of triplet intermediates the time course should show a fast absorption decrease followed by a slower absorption increase ending at the value due to chlorophyll-a$_1$.$^\circ$. This has not been observed within this set of measurements. Therefore the decay of the triplet state and the formation of chlorophyll-a$_1$.$^\circ$ must occur in times shorter than 20 ns.

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8 W. Haehnel, private communication
