ON THE PHOTOACTIVE CHLOROPHYLL REACTION IN SYSTEM II OF PHOTOSYNTHESIS. DETECTION OF A FAST AND LARGE COMPONENT

M. GLÄSER, Ch. WOLFF, H.-E. BUCHWALD and H. T. WITT
Max-Volmer-Institut für Physikalische Chemie und Molekularbiologie, Technische Universität Berlin, 1 Berlin 12, Straße des 17. Juni 135, Germany

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1. Introduction

In photosynthesis the electron transport from H_2O to $NADP^+$ is driven by two light reactions coupled in series [1–4]. The photoactive chlorophylls within the two systems I and II were observed directly by absorption changes and identified as Chlorophyll-a_I (P 700) [5] and Chlorophyll-a_{II} (P 680) [6].

$$NADP^+ \leftarrow \text{-}\text{-} \text{ Chl-}a_I \leftarrow \begin{matrix} PC \\ Cyt\ f \end{matrix} \leftarrow \quad PQ \quad \leftarrow\text{-}\text{-} \text{ Chl-}a_{II} \leftarrow H_2O$$

Chlorophyll-a_I is oxidized in the light [5,7] in $\leqslant 20$ nsec [8]. The released electron reduces lastly $NADP^+$. Chl-a_I^+ is rereduced in the dark in three phases: 10 μsec, 200 μsec and 20 msec [9,10]. These three decay times can be explained by the release of electrons from three different electron donors (PC, Cyt f, PQ) located between Chl-a_I and Chl-a_{II} (see Materials and methods).

Chlorophyll-a_{II} (P 680) is assumed to transfer an electron lastly from H_2O to the electron donors of Chl-a_I^+. It is not yet known how this occurs in detail (photooxidation, photoreduction, sensibilisation, etc.). The half-life time of the Chl-a_{II} reaction is $\tau = 200$ μsec. A similar reaction time has been observed in an intermediate step engaged with the oxidation of water [24]. However, it is unsatisfying that the concentration of Chl-a_{II} per electron chain would be 4 times smaller than that of Chl-a_I if we assume that both compounds have the same extinction coefficient at the maximal absorption change. For details see ref. [11].

In this communication we report on a refined analysis of the photoreactions of chlorophyll in system II. The time resolution was extended to 1 μsec. We observed in system II a new fast (τ = 35 μsec) and large chlorophyll-a absorption change. The extent of the 35 μsec-absorption change corresponds to that of Chl-a_I. It may be that this new 35 μsec-component represents together with the slower 200 μsec-component the biphasic kinetics of one and the same Chlorophyll-a_{II} in system II.

Abbreviations: $NADP^+$ = nicotinamide adenine dinucleotide phosphate, Chl-a_I = chlorophyll-a_I, Chl-a_{II} = chlorophyll-a_{II}, PQ = plastoquinone pool, PC = plastocyanine, Cyt-f = cytochrome f, DCMU = 3-(3,4-dichlorophenyl)-1, 1-dimethylurea, DBMIB = 2,5-dibromo-3-methyl-6-isopropyl-1,4-benzoquinone.

2. Materials and methods

The chloroplasts were prepared from market spinach according to the method of Winget et al. [12] except that 10^{-2} M ascorbate was present during the grinding of the spinach. For the storage in liquid nitrogen, 5% dimethylsulfoxide was added. The activity of the stored chloroplasts was after thawing nearly the same as that of freshly prepared chloroplasts.

The reaction mixture containes chloroplasts (5×10^{-6} M chlorophyll), 2×10^{-2} M Tricine–NaOH buffer (pH = 7.2), 2×10^{-3} M $MgCl_2$, 2×10^{-3} M NH_4Cl and 10^{-4} M benzylviologene as electron acceptor. In special measurements with addition of

DBMIB (10^{-6} M) instead of benzylviologene $K_3[Fe(CN)_6]$ (4×10^{-4} M) was used as electron acceptor. Temperature: 20°C.

The absorption changes were recorded by the repetitive flash photometer [13] with high frequency modulated detecting light [14]. This technique allows to separate in the red region completely absorption changes from the much larger fluorescence change of chlorophyll. In a Fabri-Tek 1072 usually 16.384 signals were averaged per measurement but the sample was changed after each 4096 flashes. The electrical bandwidth of the apparatus was 1 MHz. The excitation of photosynthesis was performed by ultra short repetitive flashes [15] (duration 0.4 μsec, frequency 4 Hz). The flash light passed a Schott filter BG 23/5 mm. The optical path length of the cuvette was 20 mm, the intensity of the monitoring light < 100 erg/cm² sec, the optical bandwidth Δλ = 10–15 nm; at 690 nm, however, Δλ = 5 nm.

In the experiments of figs. 1 and 2 continuous background light (720 nm, Δλ = 15 nm) was used with an intensity of 50 000 erg/cm² sec. Far-red background light excites mainly Chl-a_I and keeps therefore the electron donors (cytochrome-f, plastocyanine) in the oxidized state. In this case rereduction of Chl-a_J^t occurs only in one slow phase (20 msec), namely by the oxidation time of plastohydroquinone (20 ms). Thus, under far-red background light conditions the fast components of Chl-a_I (10 μsec and 200 μsec) are eliminated.

3. Results

3.1. *The time course* of absorption changes at 690 nm is depicted in fig. 1. The measurements have been carried out in the presence of far-red background light to eliminate fast absorption changes (< 20 msec) of Chl-$_I$ (see 'Materials and methods'). The rise time is < 1 μsec. The decay kinetics are *three*-phasic. The slow phase has a half-life-time of τ = 14 msec, the fast one τ = 180 μsec, the very fast one τ = 34 μsec. The slow phase (averaged value 20 msec) indicates the reaction of chlorophyll-a_I [7] and the fast phase (averaged value 200 μsec) the reaction of chlorophyll-a_{II} [6] (see above). The very fast phase – averaged value τ = (35 ± 10) μsec – has not been observed before.

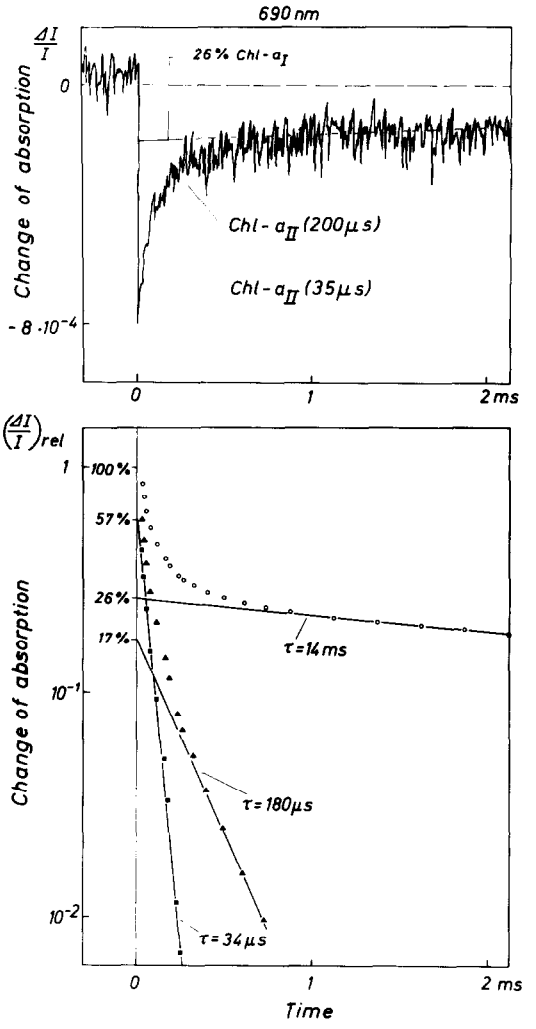

Fig. 1. *Top:* Absorption change at 690 nm as a function of time in a suspension of spinach chloroplasts. At t = 0 a single turnover flash (0.4 μsec) of half saturating intensity was fired. *Bottom:* Relative absorption changes as function of time plotted in a log scale.

3.2. The *spectrum* of the new very fast phase (35 μsec) in the red region can be derived from the results in fig. 2. Because it was not possible to separate at all wavelengths the 35 μsec-component from the 200 μsec-component, in fig. 2 the sum of both were measured. Around the maximum it is, however, possible to separate the 35 μsec- and 200 μsec-phase. The new 35 μsec-component has a maximum at about 690 nm. (The maximum of the

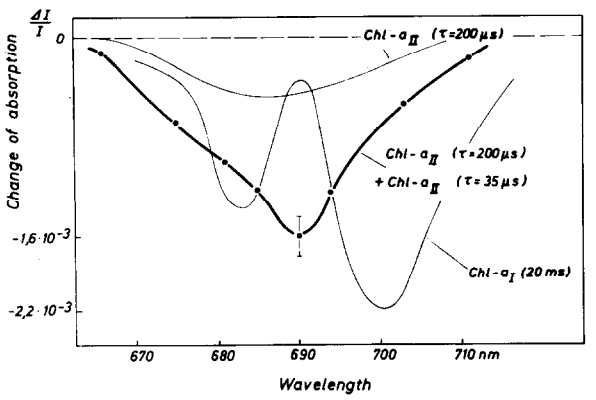

Fig. 2. Transient difference spectrum of the 35 μsec-component plus 200 μsec-component induced by saturating actinic flash light. For comparison it is also depicted the spectrum of the 200 μsec-component [16] and of Chl-a_I [23].

absorption changes of Chl-a_{II} (200 μsec) in *whole* chloroplasts have recently been determined to be located at 687 nm [16] instead of 682 nm observed in system II enriched particles, see ref. [6]). The ratio at 690 nm $\frac{\Delta I/I \ (35\ \mu sec)}{\Delta I/I\ (200\ \mu sec)} = 2.3 \pm 1$ was measured at about 50% of the saturation level.

Because no other chloroplast pigments have absorption bands in the region between 660 and 710 nm than chlorophyll-a, the result indicates that the 35 μsec-component is caused by the reaction of a chlorophyll molecule of type *a*.

3.3. With increasing flash intensity the extent of the 35 μsec-component reaches a *saturation level.* (Refined comparative measurements in relation to photosynthesis are in progress).

3.4. From DCMU it is known that it blocks the electron transfer between the two light reactions of photosynthesis. Addition of 3×10^{-7} DCMU suppresses all signals. This indicates that also the 35 μsec-component is associated with the *electron transfer reactions.*

3.5. The measurements in figs. 1 and 2 were carried out in the presence of far-red background light. This light excites mainly Chl-a_I. Because we observed that the 35 μsec-component is insensitive to this light, the 35 μsec-component does not belong to system I but probably *to system II* (see below).

3.6. From DBMIB (10^{-7} M) it is known to be an agent which blocks the electron transport chain at the plastoquinone pool [17]. DBMIB drastically decreases the rate of the electron flow from system II to Chl-a_I (W. Haehnel and G. Renger, personal communication, see also ref. [18]). In combination with $K_3[Fe(CN)_6]$ (4×10^{-4} M) the substance DBMIB acts at higher concentrations (10^{-6} M) as electron acceptor for system II [19].

In order to check that the 35 μsec-component belongs to photosystem II the absorption change was measured in the presence of DBMIB and $K_3[Fe(CN)_6]$. In fig. 3 the influence of DBMIB on the absorption changes of Chl-a_I and the sum of the 200 μsec + 35 μsec-component is shown. Addition of DBMIB causes a drastic decrease of the Chl-a_I-change at 703 nm (see fig. 3, left). In contrast to this behaviour the 200 μsec + 35 μsec-component at 690 nm are not diminished at all by addition of DBMIB (see fig. 3, right). Additionally it was checked under our measuring conditions that the oxygen production was not changed by the addition of DBMIB. Thus the photosystem II was full active.

From these results it can be concluded that the 200 μsec-component and the new 35 μsec-component are both *active in system II* of photosynthesis. Because this was already shown for the 200 μsec-component in detail [6], the 200 μsec-component was designated Chl-a_{II}. Because the 35 μsec-component is also caused by a chlorophyll of type a (see above) and is also active in system II we designate this compound also Chl-a_{II}. To distinguish both components we symbolize them as follows (see also figs. 1 and 2):

Chl-a_{II} (200 μsec) and Chl-a_{II} (35 μsec)

4. Conclusions

a) The new transient absorption change with a half life time of 35 μsec is caused by a reaction of a chlorophyll of type a. This follows from the maximal absorption change at about 690 nm.

b) Because the saturation level of the 35 μsec-

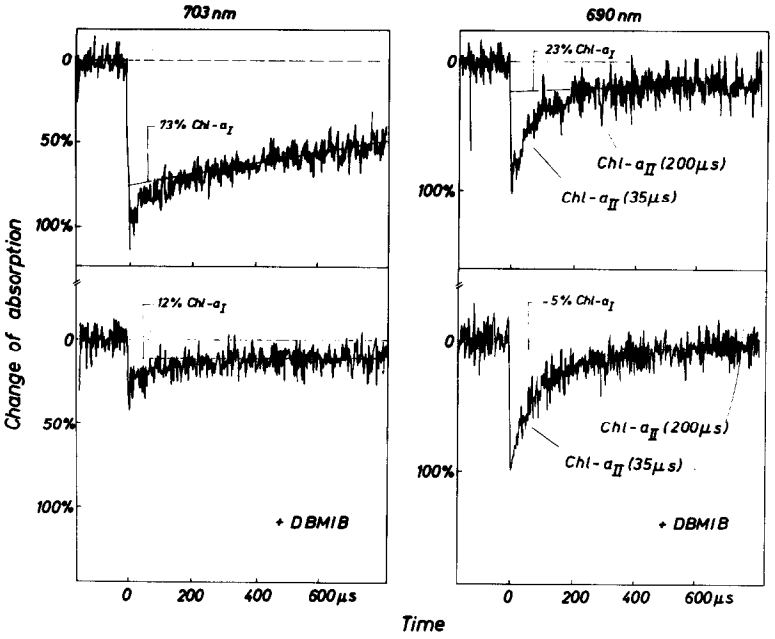

Fig. 3. Absorption changes as a function of time in the absence (top) and presence (bottom) of DBMIB (+ ferricyanide). Subject: Chloroplasts of spinach. Left: At 703 nm. Right: At 690 nm. At $t = 0$ a single turnover flash (0.4 μsec) was fired.

component corresponds to that of photosynthesis and because its absorption band is located at larger wavelengths than of the bulk chlorophylls in system II [20], the new chlorophyll-a-component differs from the normal bulk chlorophylls.

c) The insensitivity of the 35 μsec-component to far-red background light as well as to addition of DBMIB + $K_3[Fe(CN)_6]$ indicates that the new component is photoactive in system II. It is designated therefore Chl-a_{II} (35 μsec).

d) The reactions of Chl-a_{II} (35 μsec) and Chl-a_{II} (200 μsec) could represent the reaction of one and the same Chl-a_{II} with two decay kinetics (similar to the different decay kinetics of Chl-a_I, see introduction). This is supported by the location of the maxima of the absorption changes of both components in *whole* chloroplasts at approx. similar wavelengths (687 and 690 nm resp.). Both components can therefore also be designated as P 690. However, it cannot be excluded that Chl-a_{II} (35 μsec) is of a different type of reaction in system II than that of Chl-a_{II} (200 μsec).

e) Assuming a similar extinction coefficient for Chl-a_{II} (35 μsec) + Chl-a_{II} (200 μsec) as for Chl-a_I, it can be estimated that about 1 molecule Chl-a_{II} is active per electron transport chain.

f) It is of interest to note that in the photosynthetic delayed light emission (which is strongly related to the reaction center state of photosystem II) two decay kinetics have been observed with similar times: $\tau = 35$ μsec and $\tau = 200$ μsec [21, 22].

Tentatively we may suggest that the 35 μsec-component of Chl-a_{II} (P 690) (probably together with the 200 μsec-component) represents the redox-reaction of the active chlorophyll in system II.

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