Effects of Linolenic Acid on System II and System I-Associated Light Induced Changes in Absorption of Chloroplasts

S. S. Brody *, M. Brody **, and G. Döring ***


The phenomological effects of exogeneous fatty acids on isolated chloroplasts, as well as chloroplast fragments and whole algal cells, are many. With increasing concentration of fatty acids: a) the three-banded steady state fluorescence emission spectrum (at 77 °K) is converted into an essentially one-banded spectrum, with maximum at 698 nm; b) fluorescence induction changes occur which have been interpreted as arising from sequential inhibition of Photosystem II and I; c) phosphorylation is uncoupled; H111 reaction is blocked, and System I electron flow ceases; d) large changes in chloroplast ultra-structure, including grana thylakoids, are observed; and e) it appears that the population of functional System II reaction centers may be decreased (amperometric studies).

In the present work we report the influence of exogenous linolenic acid on light-induced changes in absorption. Therefore, it might be well to recall the interpretations given for such changes in non-treated (control) plant material. The slow decay component of the light-induced decrease in absorption at 705 nm has been attributed to oxidized chlorophyll at the reaction center of System I (chl a1) 6,7; in the dark (or System II light) there is subsequent reduction of chl a1. The increase in absorption at 690 nm (or at 682 nm in the case of Boardman particles) has both a fast and a slow

* Dept. Biology, New York Univ., New York, N.Y.
** Dept. Biol. Science, Hunter College, City Univ. of New York, New York, N.Y.

5 S. S. Brody, in prep.
6 B. Kor, Biochim. biophysica Acta [Amsterdam] 48, 527 [1961].
decay component; the fast one \((0.2 \times 10^{-3} \text{ sec.})\) has been ascribed to chlorophyll of the System II reaction center \((\text{chl } a_{II})\)\(^8\), while the slow one \((20 \times 10^{-3} \text{ sec.})\) has been ascribed to chlorophyll of the System I reaction center \((\text{chl } a_1)\)\(^9\). Absorption change at 515 nm has been associated with ion exchange promoted by an electrical field across the thylakoid membrane\(^10\). These changes in absorption are attributed to both \(\text{chl } b\) and carotenoids\(^18\) or carotenoids alone\(^11\).

### Materials and Methods

The periodic flash photometer used in this study to observe spectral transients was described previously\(^8\). To elicit absorption changes at either 705 or 690 nm (half band widths 5 nm), suspensions of chloroplasts were irradiated at a saturating intensity of blue light using a Xenon flash lamp \((2 \times 10^{-3} \text{ sec. flash duration})\) in combination with a Schott (Hanau) heat absorbing filter (T8) plus colored filters BG 28 (4 mm) and GG 385. Actinic light was prevented from reaching the phototube by use of interference filters. To correct for fluorescence the measuring beam was extinguished and the same number of flashes repeated, but this time the signals were subtracted from the data stored in a signal averager (Enhancer 800, Nuclear Data). The flash rate was 10 Hz, and the electrical band width was 0.1 to 13.000 Hz. Spectral changes at 515 nm were induced with red light using a Xenon flash lamp in combination with Schott colored filter RG 610. The optical path through the sample cuvette was 1.2 mm.

To facilitate comparison of the effects of linolenic acid on absorption changes the original data were replotted on semi-log paper. The fractional change in absorption \(\Delta I/I\) was calculated using the equation

\[
\frac{\Delta I}{I} = \frac{S - M}{n - V} \cdot X
\]

where \(S\) (in cm) is the (experimentally measured) height of the Signal, \(M\) and \(V\) are settings of the display scale of the Enhancer and preamplifier, respectively, \(X\) is a factor which depends on the sweep time of the Enhancer and \(n\) is the number of flashes per experiment — chosen to be 8192, 1024 or 512 — depending upon the magnitude \(\Delta I\) of the signal.

The data have been analyzed in the following way. Magnitudes of the slow and fast changes at time zero were obtained by the usual technique of extrapolating the slow portion of the curve back to zero time, then substituting the extrapolated values from the experimental curve to obtain the fast component.

Chloroplasts of field grown Zea mays (hybrid variety INRA 200) and of market spinach (Spinacia) were isolated according to BRIANTAIS\(^12\) and WINGET et al\(^13\), respectively. Activity of oxygen production with the latter preparation was 111 \(\mu\text{Mol } O_2/\text{mg. chl. h.}\); on the other hand in the absence of \(\text{NH}_4 \text{Cl}\) the activity was 20 \(\mu\text{Mol } O_2/\text{mg. chl. h.}\). Chloroplasts particles rich in System II (to be referred to as "\(B o a r d m a n p a r t i c l e s\)"") were prepared from spinach according to the method of THORNBER et al\(^14\).

So that the magnitudes of the absorption changes would not be limited by depletion of the endogenous pools of electron donors and acceptors during the course of the reactions — elicited by the repetitive strobe flashes, artificial electron donors and acceptors were added in excess concentration. For observation of System I photoreaction in suspensions of chloroplasts or Boardman particles — \((5 \times 10^{-3} \text{ M chl})\) the medium contained: 2.5 \(\times 10^{-3} \text{ M phenazine methosulfate (PMS), } 10^{-4} \text{ M benzylviologen (BV), } 2 \times 10^{-3} \text{ M ascobrate, } 2 \times 10^{-3} \text{ M } \text{NH}_4 \text{Cl and } 0.05 \text{ M Tris pH 7.2; for observation of the absorption change of the System II photoreaction, the medium contained: } 5 \times 10^{-4} \text{ M ferricyanide (Ferri), } 2 \times 10^{-3} \text{ M } \text{NH}_4 \text{Cl and } 0.05 \text{ M Tris pH 7.2. (Since the uncoupling agent and buffer were always present, reference will not be made to them in the results section.)

Linolenic acid (Lino) and PMS were obtained from Sigma Chemical Company (St. Louis, Mo.), and used without further purification. BV was obtained from Serva (Heidelberg, Germany), sodium ascorbate from Fluka A.G. (Buchs, Switzerland), potassium ferricyanide (Ferri) from Merck (Darmstadt, Germany).

PMS and chloroplasts were kept, separately, at 0 °C and in total darkness, until the beginning of each experiment, at which time they were mixed. Experiments were made at 23 °C.

### Results

The results section has been divided into three parts: in part a) are included the absorption changes ascribed to \(\text{chl } a_1\) in part b) the absorption changes ascribed to \(\text{chl } a_{II}\) — as well as the dependence of \(\text{chl } a_{II}\) absorption changes on concentration of linolenic acid; in part c) effects on thylakoid membranes are considered.

#### a) System I

In the case of corn chloroplasts in BV and PMS (Fig. 1, Exp. 14C) the presence of low concentra-

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\(^{10}\) W. Junge and H. T. Witt, Z. Naturforsch. 23b, 244 [1968].

\(^{11}\) W. W. Hildret, M. Ayron, and B. Chance, Plant Physiol. 41, 983 [1966].

\(^{12}\) J. M. Briaïtaïs, Photochem. Photobiol. 6, 155 [1967].


tion of Lino (2 x 10^-5 M) brings about a small increase (15%) in ΔI/I of the slow component of the 705 spectra change (Fig. 1, Exp. 24C). The apparent stimulation of chl a1 at this concentration of linolenic acid will be considered in the discussion. The small fast component is diminished by 20 percent. The presence of fatty acid at this concentration does not significantly modify the decay constant for the slow component; however, the fast component appears to go faster.

The spectral changes which occur at 690 nm, in the presence of BV but absence of PMS, are shown in Fig. 2, Exp. 81; addition to this system of a higher concentration of Lino (2 x 10^-4 M) than that used above eliminates all spectral changes except for a small part of the fast component. Introduction of PMS restores the slow chl a1 reaction (Fig. 2, Exp. 98), but not the fast change. A similar final result is obtained if the sequence is Lino added to chloroplasts already suspended in BV and PMS.

The presence of 10^-4 M Lino in Boardman particles suspended in PMS and BV brings about a small increase (20%) in the magnitude of the slow 705 absorption change relative to the control (Fig. 1, Exp. 45B, 50B) (while the fast component completely disappears). This same stimulation was seen above at a lower concentration of Lino in the case of corn chloroplasts. The presence of 10^-4 M Lino speeds up the slow component. (The fast component observed in Fig. 1, Exp. 24C, 14C and 45B is not from chl a1, since it disappears upon irradiation with far red light.)

Similar results are obtained for the action of Lino on chl a1 of spinach chloroplasts.

b) System II

With corn chloroplasts, the amplitude of the fast chl a1 component observed at 690 nm in the presence of Ferri is attenuated (about 70%), when 10^-4 M Lino is also present (Fig. 2, Exp. 87, 86). This condition also results in a slowing of the fast component. At concentrations of Lino greater than 2 x 10^-4 M in the presence of Ferri, all spectral changes at 690 nm are eliminated.

Similar results are observed at 690 nm with spinach chloroplasts in the presence of Lino.

The spectral change observed with control Boardman particles at 682 nm, in the presence of Ferri, is somewhat different from that observed with control chloroplasts at 690 nm. Although the fast decrease in absorption of the chl a1 component is similar to that of chloroplasts, the slow chl a1 component displays an anomalous increase in absorption, which gets smaller with increasing concentration of Ferri (Fig. 3, Exp. 41 and 42). When 10^-4 M Lino is added to the system containing 5 x 10^-3 M Ferri, the fast chl a1 component disap-
Fig. 3. Absorption change as a function of time at 682 nm in a suspension of Boardman particles. The concentrations of Ferri and Lino, when present, are shown in the figure. The concentration of all other components in the suspension and the properties of the actinic flash are the same as given in Fig. 1.

pears and the slow chl a1 component is now seen as a decrease in absorption (Fig. 3, Exp. 43).

If Boardman particles are measured at 690 nm instead of 682 nm — the former wavelength being the one at which System II measurements are normally made with chloroplasts — then both components of the biphasic absorption change are negative. In this state they are more readily separated and analyzed. The relative ΔI/I for the fast chl a1 change at 690 nm as a function of Lino concentration is shown in Fig. 4, both for Boardman particles and spinach chloroplasts. Although the shapes of the curves are different in the two cases, the concentration of Lino which yields half amplitude is about the same for both, i.e. 8 × 10^{-5} M.

c) Thylakoid Effects

Addition of 10^{-4} M Lino to Boardman particles in the presence of PMS, ascorbate and BV, attenuates the slow component at 515 nm by 75%, while the relatively large fast change in absorption persists, and is even enhanced by 90% (see Fig. 5, Exp. 48, 46). When 10^{-4} M Lino is added to Boardman particles in the presence of Ferri, the slow component at 515 nm is attenuated by about 90%, and a small, rapidly decaying positive change in absorption may be seen to be present (see Fig. 5, Exp. 59, 60). In both sets of experiments the slow component is speeded upon addition of Lino.

Essentially the same results for the effects of Lino on the 515 nm spectral changes are obtained with spinach chloroplasts.

Discussion

The magnitudes at time zero of all the slow and fast changes described above are summarized in

Fig. 4. Relative change in absorption at 690 nm of the fast chl a1 component as a function of Lino concentration are shown for a suspension of Boardman particles and a suspension of spinach chloroplasts. The suspension contained 5 × 10^{-4} M Ferri; the concentration of all other components in the suspension and properties of the actinic flash are the same as given in Fig. 1.


Table 1. To determine whether Lino is blocking System I or II let us first recall that addition of DCMU ($2 \times 10^{-6} \text{m}$) to chloroplasts (in the presence of an electron acceptor) eliminates both the fast and slow components of the absorption change at 690 nm. Addition of an electron donor (PMS and ascorbate) restores the slow chl$_a$ component of the absorption change, while the fast System II component remains blocked. These results have been interpreted as indicating that DCMU blocks electron flow from System II, and that the donation of electrons to System I by PMS may be used to demonstrate that System I is functional.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Fig.</th>
<th>Exp.</th>
<th>$\lambda$ [nm]</th>
<th>$\Delta I/I \times 10^5$</th>
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<th>fast</th>
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<td>705</td>
<td>620</td>
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<td>690</td>
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<td>690</td>
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<tr>
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<td>60B</td>
<td>515</td>
<td>71</td>
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<td>Lino ($10^{-4}$) + PMS + BV</td>
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<td>48B</td>
<td>515</td>
<td>50</td>
<td>80</td>
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Table 1. Summary of Amplitudes of Absorption Changes at Time Zero.

All spectral changes at 690 nm are eliminated when $10^{-4}$ M Lino (i.e. molar concentrations of Lino/Chl $\approx 2$) is added in the presence of BV. In this case also, addition of PMS restores the slow chl$_a$ component (Fig. 2, Exp. 8, 98) but not the fast chl$_a$ component. Even at the highest concentration of Lino used in the present work, restoration of chl$_a$ was achieved by PMS; therefore it may be concluded that with concentration ratios of Lino/Chl $< 4$, inhibition of System I does not occur. A similar conclusion was previously reached by Brody et al. and Cohen et al. on the basis of System II and System I dye reduction studies and by Brody on the basis of fluorescence induction studies. One significant difference between the effectiveness of DCMU and Lino is that with the latter, considerably higher concentrations (two orders of magnitude higher) are required to block System II to the 50% level (Fig. 4).

It would appear that Lino is either acting between the reaction center of chl$_{a2}$ and Ferri or on the oxygen liberating side of System II.

The absorption change at 705 nm, which arises purely from chl$_a$ gives rise to a linear semi-log plot, with a half life time of 19 msec. (Fig. 1, Exp. 14, 45). This life time is in good agreement with the value previously published by Witt et al. Although the primary effect of Lino is on System II (at Lino/Chl $\approx 2$), we did note some effects on System I at this concentration. A speeding of the dark recovery phases of both 705 nm change and the slow component of the 690 nm change was noted (Fig. 1, Exp. 45B, 50B; Fig. 2, Exp. 98, 81). There was also a small increase in the magnitude of the spectral change of chl$_a$ (Table I, Exp. 14C, 24C, 45B, 50B). Therefore it may be suggested that at these Lino/Chl concentrations System I is beginning to be affected.

The apparent stimulation of the chl$_a$ absorption change (at 690 and 705 nm) by Lino may reflect enhanced electron flow which is associated with uncoupling of cyclic phosphorylation. Alternatively, it may be that alterations in thylakoid permeability are leading to stimulation in System I associated electron flow (see below).

Junge and Witt have shown the possibility of altering the relation between the magnitude of the slow and fast changes in absorption at 515 nm. They have been able to do so under several experimental conditions, leading them to the conclusion that the transformation of the slow phase into the fast one is brought about by an increase in membrane permeability (specifically for certain ions, or generally through osmotic effects). In this work, they suggest that the ratio of the amplitudes of the slow and fast changes represents the number of thylakoids of normal permeability compared to those of strongly increased permeability. Junge and Witt reported that during the slow to fast phase transformation, the over-all amplitude (sum of the magnitudes) remained practically constant.

The addition of $10^{-4}$ M Lino to Boardman particles results in a strong attenuation of the slow component, accompanied by an increase in the fast component. However, in contrast to the findings of Junge and Witt, the sum of the magnitudes of the
fast and slow component does not remain constant after treatment with Lino but instead decreases (Table I).

Because of the high lipid solubility of linolenic acid, it is very likely that the transformation of the slow to fast phases, here, too, results from increased permeability of the thylakoid membrane. Indeed, the light scattering changes observed by Molotkovskiy and Zheskova\(^1\) (interpreted by these workers as arising from swelling), and the configurational changes of such thylakoids\(^2\) may be in part manifestations of such increased permeability.

It should be kept in mind that under the conditions used by Junge and Witt\(^10\), both osmotic effects and exposure to gramacidin bring about increases in permeability but no changes in electron transport; in contrast, as we have seen above, linolenic acid brings about changes in both. It is of interest, therefore, to consider the possibility that the linolenic acid induced change in the sum of the magnitudes of the fast and slow phases, reflects the changes in electron transport. Indeed it may be more than a coincidence that \(10^{-4}\) M Lino, which diminishes the fast component of the 690 nm absorption changes (chl\(_{II}\)) by about 50\%, also diminishes the sum of the magnitudes of the 515 nm absorption changes by the same amount (Table 1).

While it is possible that the dual effects of Lino on permeability and electron transport are independent in regard to mechanism, it is also possible that a causal relationship exists between the two.

We (S. S. B. and M. B.) are very much obligated to Professor H. T. Witt for so graciously making his laboratory facilities available to us so that these experiments could be performed.

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