Effects of Ricinus Leaf Extracts on Light Induced Changes in Absorption of Chloroplasts Associated with System I and System II

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Absorption changes associated with photosynthetic System I and System II are (differentially) inhibited by aqueous extracts of Ricinus leaf (RLE). The absorption changes associated with thylakoid effects (e. g. those at 515 nm) are also modified by RLE; the slow component (>10−2 sec) is diminished and the fast component (<10−3 sec) is eliminated.

Fluorescence and amperometric studies were made with Zea mays chloroplasts suspended in an aqueous of Ricinus leaf (RLE)1−3. Although the influence of the extract seems to be a complex one, it has been interpreted, in part, as resulting from sequential initiation by a naturally occurring or added substrate which is directly complexed to chlorophyll. It is also possible that there is a change in energy transfer efficiency to the fluorescence center by a local "initial shock" to chlorophyll upon absorbing a light quantum. An increase in local temperature is brought about by conversion of the light energy into heating the chlorophyll so as to bring about a reorientation, deaggregation or decomplexation.

Since addition of LINO to chloroplasts containing FERRI has only a small effect on ΔΦR, LINO is tentatively assumed to interact with System II. This is in agreement with the findings of Cohen et al.13.

The presence of FERRI in chloroplast mixtures always results in a large value for ΔΦR. Addition of RLE to a mixture of FERRI and chloroplasts results in a much smaller value of ΔΦR. It would appear that a component in RLE is blocking the interaction between FERRI and the chlorophyll giving rise to fluorescence at 725 nm.

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change, the following should be recalled: the slow $(20 \times 10^{-3} \text{sec})$ kinetic changes in chloroplasts at 705 and 690 nm have been ascribed to chlorophyll $a$ of the System I reaction center (chl $a_1$) $^6$; the fast $(0.2 \times 10^{-3} \text{sec})$ changes at 690 nm (or at 684 nm in the case of the sub-chloroplast Boardman particles) is ascribed to chlorophyll $a$ of the System II reaction center (chl $a_1$) $^7$; both the fast and slow kinetic changes ($<10^{-7} \text{sec}$ and $>10^{-2} \text{sec}$) at 515 nm are attributed to both chl $b$ and carotenoids $^8$ or to carotenoids alone $^9$.

Materials and Methods

Detailed descriptions of a) the periodic flash photometer used to observe the spectral transients $^7$, and b) the experimental procedures, as well as the technique used for processing the data $^{10}$, were given earlier. The following is a summary of these descriptions. Absorption changes at either 705 or 690 nm were elicited by irradiation with repetitive flashes of blue light of saturating intensity. For absorption changes at 515 nm, repetitive flashes of red light were used. The fractional change in absorption, $\Delta I/I$, was calculated using the equation

$$\Delta I = \frac{S \cdot M \cdot X}{n \cdot V},$$

where $S$ (in cm) is the (experimentally measured) height of the signal, $M$ and $V$ are settings on the display scale of the Enhancetron and preamplifier, respectively, $X$ is a factor which depends on the sweep time of the Enhancetron, and $n$ is the number of flashes per experiment (chosen to be 8192, 1024, or 512 depending upon the magnitude of the signal). The data—originally obtained as recorder tracings of $\Delta I$ as a function of time — were replotted semi-logarithmically. The magnitudes of the slow and fast changes at time zero were determined by first extrapolating the slow portion of the semi-logarithmic curve back to zero time to obtain the value of the slow component. Then the fast component was obtained by subtracting the value of slow component from the zero time intercept of the logarithmic curve.

RLE was prepared according to the method described by Nathanson and Brody $^2$; in this form it will be referred to as “concentrated RLE”. Unless otherwise noted concentrated RLE was used for all experiments. Chloroplasts of field grown Zea mays (hybrid variety INRA 200) and of market spinach (Spinacia) were isolated according to Briantais $^{11}$, and Winget et al. $^{12}$, respectively. Chloroplast particles, rich in System II (to be referred to as “Boardman particles”), were prepared from spinach according to the method of Thornber et al. $^{13}$.

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. Concentrations of materials are given in the caption for the figures. Phenazine methosulphate (PMS) was obtained from Sigma Chemical Company (St. Louis, Mo.); benzylviologen (BV) from Serva (Heidelberg, Germany); sodium ascorbate from Fluka A.G. (Buchs, Switzerland); potassium ferricyanide (FERRI) from Merck (Darmstadt, Germany). All were used without further purification.

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 to obtain the desired concentrations. Experiments were made at 23 °C. Most measurements were completed within 10 minutes after mixing the suspensions under investigation. No systematic studies were made here of the action of RLE on chloroplasts as a function of time of incubation.

Results

System I: Addition of concentrated RLE to corn chloroplasts in the presence of PMS and BV decreases, by 87%, the slow changes at 690 nm associated with chl $a_1$ (Fig. 1—Exp. 1,3).

No spectral change can be observed when chloroplasts are suspended in BV and concentrated RLE in the absence of PMS. When PMS is added, a partial restoration of the spectral change is obtained (Fig. 1—Exp. 3). If 12 times lower concentration of RLE (i.e. “dilute” RLE) is added to chloroplasts in the presence of PMS and BV there is a 57% enhancement (compared to the control) of the chl $a_1$ absorption change at 690 nm (Fig. 1—Exp. 15). Similar effects are observed with spinach chloroplasts and with Boardman particles.

System II: Addition of dilute RLE to corn chloroplasts in the presence of FERRI diminishes by 23%,
Fig. 1. Semi-logarithmic plot of the absorption changes, $\Delta I/I$, at 690 nm, as a function of time. For observation of System I (slow component) the chloroplast suspension contained $5 \times 10^{-5}$ M chl, $2.5 \times 10^{-5}$ M phenazine methosulfate (PMS), $10^{-4}$ M benzylviologen (BV), $2 \times 10^{-3}$ M ascorbate, $2 \times 10^{-3}$ M NH$_4$Cl and 0.05 M Tris buffer pH 7.2. For observation of System II (fast component) the chloroplast suspension contained $5 \times 10^{-4}$ M ferricyanide (FERRI), $2 \times 10^{-3}$ M NH$_4$Cl and 0.05 M Tris buffer pH 7.2. A blue actinic flash (390 — 500 nm) of saturating intensity and $10^{-5}$ sec duration was fired repetitively at time zero.

The fast spectral change at 690 nm associated with chl a$_1$ (Fig. 1 — Exp. 9, 12); if 12 times greater concentration of RLE is used, the chl a$_1$ absorption change is reduced 72% (Fig. 1 — Exp. 10). Similar effects are observed with spinach chloroplasts and Boardman particles. It should be noted that in the case of Boardman particles, the slow spectral change associated with chl a$_1$ at 684 nm is positive while the fast change of the chl a$_2$ is negative; addition of dilute RLE to these particles in the presence of FERRI decreases the chl a$_2$ absorption change (Fig. 2 — Exp. 69, 74).

When RLE is added to spinach chloroplasts in the absence of any exogenously added electron donor or acceptor, instead of a decrease in absorption at 690 nm, there results an increase in absorption (Fig. 2 — Exp. 38). Such a situation leads to a spectral change that is similar to the one observed with Boardman particles rich in System II (Fig. 2 — Exp. 69).

Thylakoid effects: The slow component of the absorption change at 515 nm is decreased 40% upon addition of RLE to Boardman particles in the presence of PMS (Fig. 3 — Exp. 77, 76) — the fast component of the absorption change completely disappears.

![Fig. 3. Semi-logarithmic plot of absorption changes at 515 nm as a function of time in a suspension of Boardman particles. The concentration of all components in the suspension are the same as given in Fig. 1. A red actinic flash (620 — 720 nm) of saturating intensity and $10^{-5}$ sec duration was fired at time zero.](image)

When RLE is added to Boardman particles in the presence of FERRI, there is an 80% reduction in $\Delta I/I$ of the slow component (Fig. 3 — Exp. 71, 72). The small fast component is no longer observable after addition of RLE. Similar modifications in absorption at 515 nm are observed with chloroplasts of corn and spinach exposed to RLE.

The magnitudes at time zero of all the slow and fast changes described above are summarized in Table I.
Table I. Summary of amplitudes of absorption changes.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Fig.</th>
<th>Exp.</th>
<th>( \lambda ) [nm]</th>
<th>( \Delta I/I \times 10^6 ) at time zero</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMS + BV</td>
<td>1</td>
<td>1C</td>
<td>690</td>
<td>31</td>
</tr>
<tr>
<td>PMS + BV</td>
<td>1</td>
<td>1C</td>
<td>690</td>
<td>49</td>
</tr>
<tr>
<td>PMS + BV + RLE</td>
<td>1</td>
<td>3C</td>
<td>690</td>
<td>3.5</td>
</tr>
<tr>
<td>FERRI</td>
<td>1</td>
<td>9C</td>
<td>690</td>
<td>31</td>
</tr>
<tr>
<td>FERRI + dilute RLE</td>
<td>3</td>
<td>12C</td>
<td>690</td>
<td>90</td>
</tr>
<tr>
<td>FERRI + RLE</td>
<td>3</td>
<td>1C</td>
<td>690</td>
<td>33</td>
</tr>
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<td>515</td>
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<tr>
<td>PMS + RLE</td>
<td>3</td>
<td>77B</td>
<td>515</td>
<td>180</td>
</tr>
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</table>

**Discussion**

We have observed that when concentrated RLE is added to chloroplasts containing PMS and BV, the spectral changes associated with chl \( a_1 \) are inhibited (Fig. 1). Similarly, when RLE is added to chloroplasts containing FERRI the spectral changes associated with chl \( a_1 \) (at 690 nm) are inhibited (Fig. 1). We may conclude that RLE contains inhibitors both for System I and II.

When dilute RLE is added to chloroplasts containing only BV, all spectral changes are inhibited. Upon addition of PMS to this system, there is only a partial restoration of chl \( a_1 \) (Fig. 1 — Exp. 1, 3). This may be contrasted to the results obtained when DCMU is used in place of RLE; in this case complete restoration of chl \( a_1 \) is obtained. This finding that RLE contains specific inhibitors of System I is in agreement with those of BRODY. The action of RLE and linolenic acid are similar in some respects namely, at low concentrations both appear to have a stimulatory effect of System I, and at higher concentrations an inhibitory effect; both RLE and linolenic acid inhibit System II.

Boorman particles rich in System II show both a positive slow and a negative fast change at 684 nm (Fig. 2 — Exp. 69). Addition of concentrated RLE to a suspension of spinach chloroplasts gives rise to absorption changes that are similar to those of (control) particles rich in System II (Fig. 2 — Exp. 38). One conclusion that may be drawn from this is that RLE apparently contains a fraction which converts ordinary chloroplasts into ones which behave spectrally as if they were "rich in System II". This statement is supported not only on the basis of the data given in this work but also by the effects of RLE on fluorescence induction and fluorescence spectroscopy.

As regards the thylakoid effects, it is apparent that RLE eliminates the fast component and inhibits the slow component of the absorption change at 515 nm. This action of RLE may be contrasted with that of linolenic acid \((10^{-4} \text{m})\) which stimulates the fast component at the same time it inhibits that slow component.

WITT and JUNGE reported a definite relationship between the fast and slow components, i.e. any treatment which damaged the thylakoid converted the slow into the fast component, the sum of the fast and slow components remaining constant. In the presence of RLE, or linolenic acid, the sum is not held constant but decreases sharply. The differences in the effects of RLE and linolenic acid on the fast and slow components suggest that while the latter is most likely exerting its largely through increases in permeability of the thylakoid, the effect of the former is different or more complex. Perhaps RLE is either inhibiting transport across the thylakoid membrane or shielding chl \( b \) pigments from the potential changes associated with transport.

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