Substitution of Redox Chemicals for Radiation in Phytochrome-Mediated Photomorphogenesis

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Summary. The reducing agents, potassium ferrocyanide, β-mercaptoethylamine, cysteine, reduced DPN, ferrous sulfate, methyl viologen and ascorbic acid caused the expansion in darkness of disks of primary leaf tissue cut from dark-grown bean plants. The reducing agents interacted synergistically with low irradiances of red radiation and additively with high irradiance of red light. Exposure of disks treated with reducing agents to far red light repressed disk expansion and the decay in sensitivity to far red radiation showed the same time relations as sequential exposure to red and far red radiation.

The oxidizing agents, 1,4-naphthoquinone, ferric sulfate, hydrogen peroxide, t-butyl hydroperoxide, cystine, and potassium ferricyanide repressed the expansion of leaf disks initiated by exposure to red radiation. The oxidizing agents interacted synergistically with low irradiances of far red light and additively with irradiances of far red light.

The chain of events leading to photomorphogenesis is started by a radiation-induced shift in the phytochrome molecule. At the end of this chain are quantitative and qualitative alterations in growth substances serving as the proximate agents of photomorphogenesis (7). It is obvious that the radiation energy is inadequate by itself to be a causal factor in photomorphogenesis and that there must be some mechanism for amplifying and translating the radiation signal to mechanisms directing the alterations in growth substance balance (12, 15).

There is no direct evidence on the nature of this amplifying mechanism (4), although various investigators have suggested metabolic reactions involving changes in redox potential (5, 12), sulfhydryl-disulfide shifts (2), or interactions between Coenzyme A and pyridine nucleotides (3). Hendricks (personal communication) has found that various oxidants and reductants had no effect on the chromic shift of phytochrome in vitro, and that the photoconversion is first order with respect to energy. These facts make it unlikely that the photoconversion of phytochrome is a redox change.

As a working hypothesis, it seemed reasonable that a metabolic system immediately adjacent to phytochrome could serve to amplify the radiation signal. If this hypothesis, which is not at all original, is to have any validity, several conditions must be fulfilled. Reducing (or oxidizing) compounds should mimic red radiation by causing morphogenetic alterations and the activation should be reversed by far red radiation. Low levels of the red-mimicking agents should interact synergistically with low irradiances of red radiation and the time relation of red-far red light or red mimick-far red light should be similar. Oxidizing (or reducing) agents should reverse the effects of red radiation and interact synergistically with far red light. The oxidizing and reducing agents must be nontoxic in effective concentrations and finally, the compounds used should have no chemical similarity, possessing only reducing or oxidizing properties to link them together.

This paper reports our experimental evaluation of the above hypothesis.

![Fig. 1. Effect of concentration of various reducing agents on dark expansion of disks from primary leaves of dark-grown bean plants. Test compounds present for entire 24-hour incubation period at 25°C. Initial diameter, 3.60 mm.](image-url)
Materials and Methods

The experimental system was the bean-leaf disk expansion test (1, 9, 10, 11). The test was further modified (8) by using smaller disks to decrease variability and facilitate measurement, by reducing the incubation period to 24 hours to eliminate the necessity for an energy source, and by eliminating the nitrate ion which affects growth processes activated by red light. A few experiments were conducted under the conditions of Miller (11) with qualitatively identical results. Each experiment was repeated 3 or more times with 10 disks per variable. The reported values are the averages of all experiments; the levels of statistical significance are those previously reported (8, 14).

Test compounds were supplied to the disks in 2 x 10^{-5} M potassium phosphate buffer, pH 6.2. Iron sulfates and chlorides were equally effective; the sulfate was used routinely. Ferro(i)cyanides were potassium salts, thiosulfate was the sodium salt. In some experiments Duponol C was added at 0.001 %.

Table I. Effect of Limited Exposure of Leaf Disks to Reducing Agents.

<table>
<thead>
<tr>
<th>Reducing agent</th>
<th>m</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<tr>
<td>FeSO₄</td>
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<td>3.70</td>
<td>3.70</td>
<td>3.78</td>
<td>3.81</td>
<td>3.87</td>
<td>3.90</td>
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<tr>
<td>DPNH</td>
<td>1 x 10^{-5}</td>
<td>3.70</td>
<td>3.77</td>
<td>3.87</td>
<td>...</td>
<td>3.90</td>
<td>3.90</td>
</tr>
<tr>
<td>Methyl viologen</td>
<td>1 x 10^{-4}</td>
<td>3.70</td>
<td>3.77</td>
<td>3.87</td>
<td>...</td>
<td>3.92</td>
<td>3.91</td>
</tr>
<tr>
<td>K₄Fe(CN)₆</td>
<td>1 x 10^{-4}</td>
<td>3.71</td>
<td>3.77</td>
<td>3.85</td>
<td>...</td>
<td>3.93</td>
<td>3.93</td>
</tr>
</tbody>
</table>

![Fig. 2](image1.png)  ![Fig. 3](image2.png)

Fig. 2. Repression of effect of reducing agents on expansion of leaf disks by presentation of far red light given 30 minutes after start of treatment with reducing agents. Reducing agents present for entire incubation period. Heavy line designates leaf disks exposed to 10^4 mJoules/cm² of red light at zero time followed immediately by far red light.

Fig. 3. Interaction of red light and reducing agents on expansion of leaf disks. Heavy line represents exposure to red light alone. Reducing agents present for entire 24-hour incubation period.
to facilitate wetting of the tissues; it had no effect on disk expansion. When a test compound was re-
moved during the course of the experiment, the disks were washed several times in water under a green
safelight (6) before transfer to fresh medium. Red
radiation (625–685 mµ, peak at 660 mµ) and far red
radiation (695–800 mµ, peak at 750 mµ) were pre-
sented at 1000 µw/cm².

Results

Reducing Agents. Reducing compounds mimick red light, causing expansion of leaf disks in darkness
(fig 1). Although optimal concentration of different
reducing agents varied (penetration and side reactions
may be involved), each test compound caused the
same amount of disk expansion at its optimum con-
centration. Cysteine showed the same concentration
response curve as sodium thiosulfate, and ascorbic
acid induced responses identical to those of potassium
ferrocyanide. No other compounds were tested. In
tests of concentration, the reducing agents were pre-
sented at zero time and remained in contact with the
tissues for the entire 24-hour incubation period. If
the compounds were presented at zero time and then
removed by washing the disks and transferring them
to buffer, a 4-hour presentation time was sufficient
to cause full response (table I). We have no informa-
ton the state of reduction of test compounds
in contact with disks.

The promoting effect of reducing compounds on
disk expansion could be lessened by exposure to far
red light presented within 30 minutes after zero time
(fig 2). Except for reduced DPN, far red light
did not negate the effects of reducing agents as it
does when presented after red radiation (heavy line
in fig 2).

When reducing agents were supplied at zero time
at suboptimal concentrations and red light presented
immediately thereafter (fig 3), there was a syner-
gistic interaction of reducing agents and red light
at low irradiances (1 mJoule/cm²).

The morphogenetic response of leaf disks to red
light can be reduced by subsequent far red light for
only a limited time, after which far light is ineffective
(8). When reducing agents were supplied at the
start of the incubation period and far red light was
presented subsequently, the response of the disks to
the reducing agent was unaffected if the exposure
to far red light was delayed (fig 4), even though
the reducing agent remained in contact with the disks
for the 24-hour incubation period. The time rela-
tions of this decay in sensitivity to far red light were
approximately those noted for the interaction of red
light with far red light (heavy line in fig 4).

Oxidizing Compounds. Oxidizing compounds can
mimick far red light in preventing the expansion of
leaf disks previously exposed to red light (fig 5).
Cystine showed the same concentration response curve
as 1,4-naphthoquinone (NQ), and hydrogen peroxide
was somewhat less effective (13) than tertiary-butyl
hydroperoxide (t-BH). Disks were initially exposed
to red light and then immediately transferred to dishes
containing oxidizing compounds in concentrations
which reduced the red response by 50 %. They were
then exposed to far red light. There was a syner-
gistic interaction between oxidizing compounds and
low irradiances of far red light (fig 6).

The data presented in figure 7 parallels that of
figure 4. Concentrations of oxidizing agents which
reduced the effectiveness of red light by half (cf.
fig 5) were added subsequently to disks exposed to
red light at zero time. When the addition of NQ,
Fe₂(SO₄)₃ or t-BH (or far red light) was delayed
for more than 1.5 hours after red light treatment,
control of subsequent disk enlargement had passed
beyond the stage where it could be repressed by either
far red light or oxidizing agents. Potassium ferricy-
ane, however, continued to repress the disk re-
sponse to red light up to at least 4 hours.

Discussion

These findings are consistent with an hypothesis
that a redox change is part of the early series of
reactions leading from perception of a radiation sig-
nal by phytochrome to those events which control
photomorphogenetic responses. It is unlikely that
the chromic shift in the phytochrome molecule is
caused by a change in the redox potential of phyto-
chrome. It is more likely that reduction compounds
mimick red light by replacing the function of phyto-
chrome in its photomorphogenetically-active, far red-

![Fig. 4. Decay in sensitivity of leaf disks to repression of reducing agent, induced expansion by far red light. Disks exposed to reducing agents at zero time with far red light presented at intervals thereafter. Reducing agents present for entire incubation period. Small circles and heavy line represent time course of decay in sensitivity of leaf disks to far red light given at intervals following zero time exposure to 10⁵ mJoules/cm² red light.](image-url)
absorbing form. Thus, it is possible but not proven that the far-red absorbing form of phytochrome activates a reducing system and that the red-absorbing form activates an oxidizing system. It does not necessarily follow that the reducing and oxidizing systems are the same redox couple.

The response to reducing compounds can be repressed by far-red radiation only during the period of sensitivity to far-red radiation and a similar phenomenon is seen for the interaction of oxidizing compounds and red light. There are synergistic interactions between reducing compounds and red light (and oxidizing compounds and far-red light). All these data suggest that the step(s) involving oxidation and reduction reactions are adjacent in time or space to the chromic shift in phytochrome and are affecting those systems which are normally affected by phytochrome.

It is useless to speculate on the place where the oxidizing and reducing compounds act or on details of this action relative to either phytochrome or to the metabolic chain of events leading to photomorphogenesis. The work presented here provides no firm evidence on these or other interesting problems.

**Literature Cited**