WAVELENGTH DEPENDENCE OF ELECTRON FLOW AND OXYGEN EVOLUTION IN ISOLATED CHLOROPLASTS: A POSSIBLE ROLE FOR CAROTENOIDS*

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Summary: A previously unreported stimulation of Hill activity in isolated spinach chloroplasts by long wave (>570 nm) light and an inhibition by short wave (>360 <550 nm) light has been demonstrated. The effect is particularly pronounced in the presence of the uncoupling agents, methylamine, NH_4Cl and quinacrine. Both electron transfer, and oxygen liberation, respond similarly to wavelength. It is suggested that both the stimulatory and inhibitory effects can be explained by the relative state of photoxidation of the blue-absorbing component, possibly a carotenoid. A potential regulatory role in photosynthesis is postulated for this unidentified constituent of chloroplast membranes.

Ever since Emerson and Lewis (1) reported the stimulation of respiration in Chlorella by blue light, evidence for an indirect effect in photosynthesis of a "blue"-absorbing component, possibly a carotenoid, has been accumulating. Voskresenskaya (2) subsequently demonstrated a blue-light stimulated incorporation of CO_2 into organic nitrogen compounds. From their kinetic studies of the conservation of energy in Chlorella under different wavelengths, Bell et al (3) concluded that sufficient energy was stored under blue-light, probably through the photoxidation of an absorbing component, to account for photophosphorylation, which they assumed might be used to synthesize the additional nitrogenous compounds. Lundegardh (4) reported a lower carotene: xanthophyll ratio in algae grown under blue than under red light and suggested that the blue-absorbing photoxidizable component was a carotenoid. Ogawa and his coworkers (5) showed that a pigment-protein complex, Component I, contained more reduced carotenoids than did Component II, implying that Component I represents the reducing side and Component II the oxidizing side of the photosystem-pair (PS-1 and PS-2).

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That the blue-light absorbing pigment must be intimately related to the photosynthetic apparatus seems clear in light of the above evidence. French (6) had also reached the same conclusion since the final products of photosynthesis, rate of respiration and rate of photosynthesis were all influenced by short wave light.

If the above conjectures are true, it seems logical to assume that isolated chloroplasts as well as intact cells should exhibit a wavelength sensitivity that is not related to the absorption spectrum of chlorophyll pigments. Harnischfeger and Gaffron (7) have reported such a color sensitivity in isolated chloroplasts from tobacco. Red-light was stimulatory and blue-light inhibitory for Hill activity, and the effect was dependent upon the time after isolation and consequently upon the integrity of the chloroplast. The color-related activity difference was inferred to reflect pigment separations during chloroplast degradation.

In the present paper we report a similar effect of red and blue-light on isolated spinach chloroplasts. Red light stimulated and blue light inhibited Hill activity. A major distinction between the results reported here and those reported previously (7) is that our effect could be clearly observed only in the presence of uncoupling agents. Furthermore this effect was not as obviously dependent upon time after isolation, although age of the chloroplasts, physiological state of the spinach leaves, and growth season seemed to influence the results. The effect of blue light as an inhibitor of photoactivity as well as its stimulatory action on other metabolic activities, suggests a photosynthetic regulatory function for the absorbing compound.

Materials and Methods: Chloroplasts were isolated according to Izawa and Good (8) in 0.35 M NaCl, 0.002 M EDTA, 0.05 M phosphate buffer, pH 7.4, spun at 200 x g to remove debris and then at 2000 x g to obtain the chloroplast pellet. The pellet was resuspended in 0.05 M tricine containing 0.15 M sucrose buffered at pH 7.4. Assays were performed in the tricine buffer according to the method of Becker et al (9).

Illumination was from a B & L microscope illuminator with a 100 watt tungsten filament bulb. Cinabex filters* No. 14 and 62 were used to isolate the long (>570 nm) and short (>360 < 550 nm) wavelengths, respectively. Light energy was measured with a YSI, model 65, radiation meter**. Measurements of oxygen evolution were performed in the Gilson, model KM, Oxygraph with a vibrating platinum electrode.

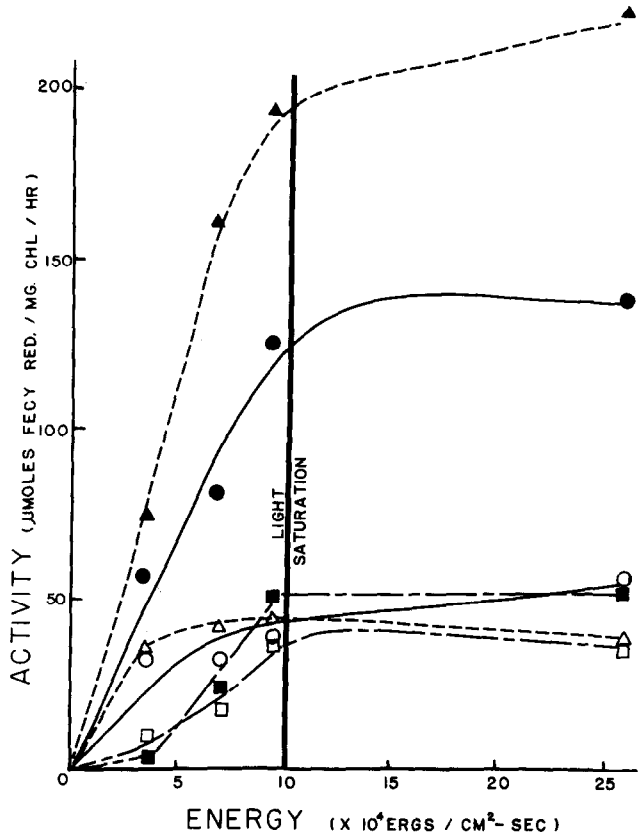

Figure 1 Hill activity of isolated chloroplasts under illumination by white light and short and long wavelength light in the presence and absence of methylamine (MA): Hollow symbols: without MA; solid symbols: with 6.6 mM MA; ——: white light; — — —: long wave ("red") light; — — —: short wave ("blue") light.

Results and Discussion: The effect of the incident energy of long ("red") and short ("blue") wavelength light in the presence and absence of the uncoupler,

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CH_3NH_2.HCl (MA) (8) at 6.6 mM is shown in Fig. 1. Clearly, "red" light is stimulatory and "blue" light is inhibitory to Hill activity when measured as ferricyanide reduced. The effect is much more clearly observed in the uncoupled than in the coupled, nonphosphorylating system. The minimal light saturation level occurs at an incident energy of about 1 x 10^5 ergs cm^{-2} sec^{-1} regardless of wavelength. In subsequent experiments, the minimal light saturation level was always exceeded by a factor of approximately 1.2.

In the presence of the uncouplers, NH_4Cl and quinacrine·HCl the same trend

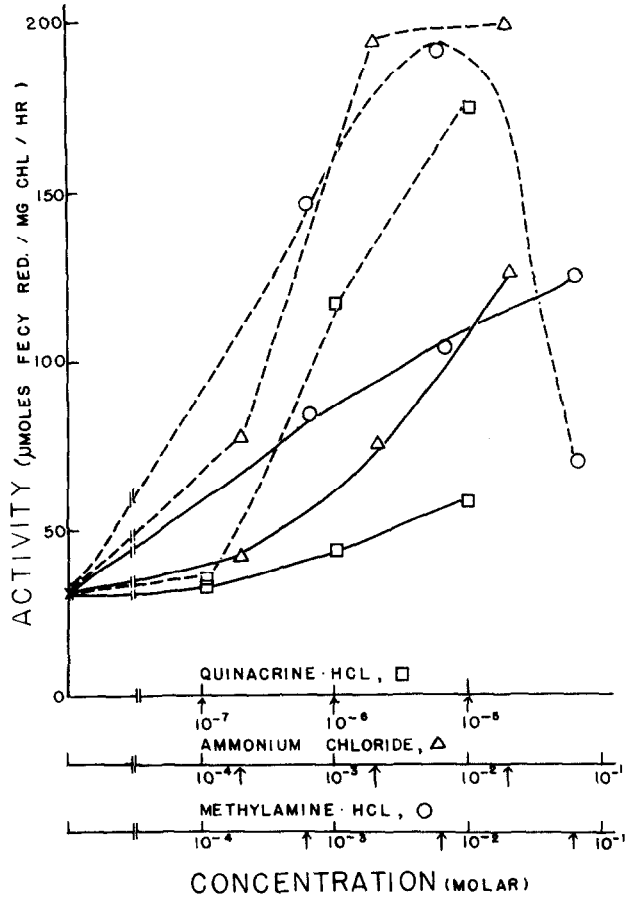


Figure 2 Effect of three uncoupling agents on Hill activity of isolated chloroplasts under saturating short ("blue") and long ("red") wavelength light. Broken line: "red" light; solid line: "blue" light; circles: methylamine; triangles: NH_4Cl; squares: quinacrine.

of activity as in MA was observed: stimulation in "red" and suppression in "blue" (Fig. 2). Hill activity in uncoupled chloroplasts was concentration and wavelength dependent, e.g. at 66.0 mM MA there was greater Hill activity under "blue" than under "red" illumination, while at 6.6 mM MA, Hill activity was far greater in "red" light than in "blue". These results suggest that there might be a permeability change in the grana lamellae induced by wavelength, since parallel dose-response curves for a given uncoupling agent should otherwise have been observed, regardless of wavelength.

Since the spectrophotometric assay used for the reduction of ferricyanide is a measure of electron transfer (9), it seemed important to examine the effect of wavelength on oxygen evolution. Determinations made by the oxygen electrode

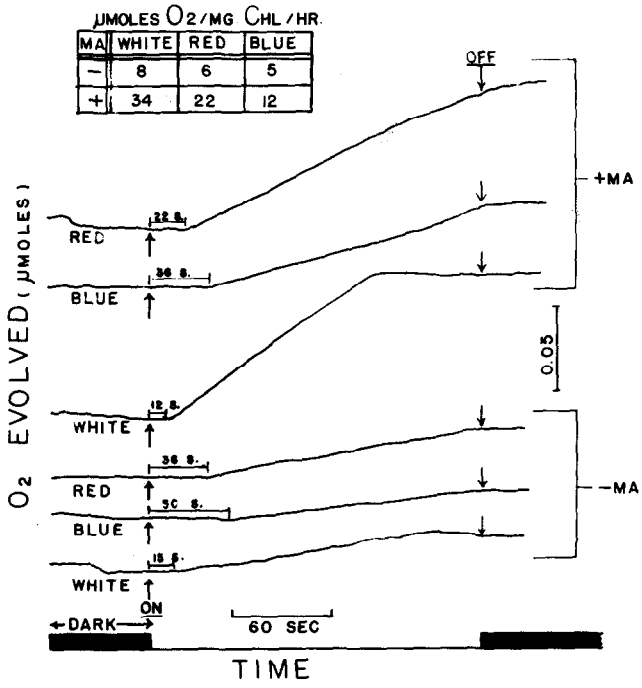


Figure 3 Oxygen evolution rates from isolated chloroplasts under illumination by saturating levels of white, "red" and "blue" light in the presence and absence of 6.6 mM methyl amine (MA). Arrows: light on and off as shown; ⊢——⊣: time delay (sec.) between light on and response; inset table: rates of O_2 evolution from slopes; Reaction mixture in μmoles: tricine, 34 (pH 7.4); KCl, 1.76; $K_3Fe(CN)_6$, 3.0; MA (when added), 6.0; chlorophyll, 164μg; total vol., 2.0 ml.

method are shown in Fig. 3. These results parallel and support those obtained from the spectrophotometric method. An interesting observation was the occurrence of a latent or induction period between the switching on of the light source and the onset of response which was considerably longer in "blue" than in white or red light (Fig. 3). In these experiments, performed during the winter months of 1969-70 on prepackaged spinach (John Henry brand), the "red" stimulation was not present; rather "red" light was inhibitory, though not as much as was "blue" light (Fig. 3); the inset table in figure 3 compares the rates of O_2 evolution. These results are suggestive of the Harnischfeger-Gaffron effect, and will be verified in subsequent studies during 1970 when fresh spinach again becomes available.

The long induction period in "blue" was eliminated by an initial illumination with white light (Fig. 4). Figure 4 also shows that there was no permanent

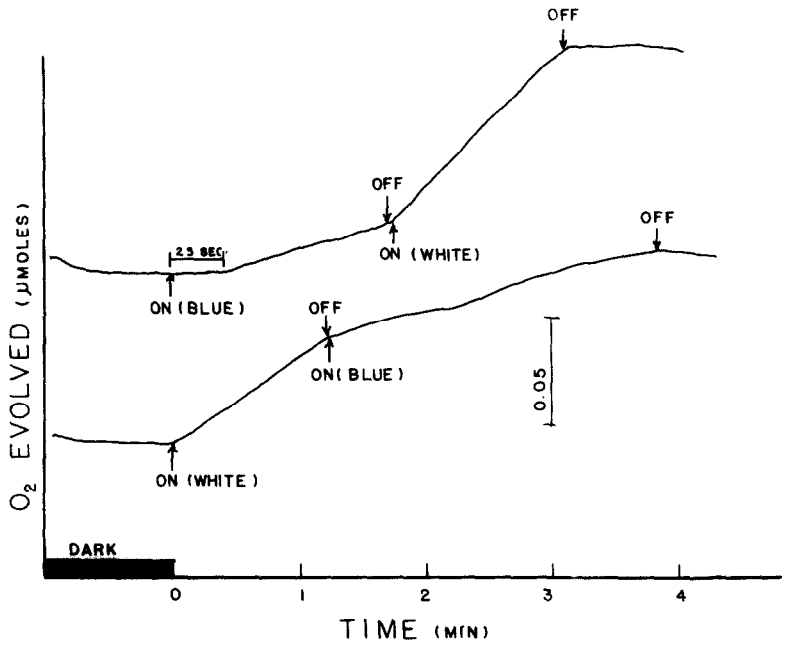
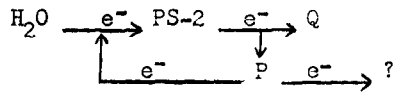


Figure 4 The effect of sequencing "blue" and white light at saturating intensities on the rate of oxygen evolution from isolated chloroplasts. Symbols and reaction mixture same as for figure 3.

or long-term transient inhibitor accumulated in "blue" light, since a lag period should then have been observed in the rate of O_2 evolution in white light following "blue". An explanation of the wavelength effect reported here is offered by two hypotheses which are not mutually exclusive: (a) the blue-light absorbing component, P, is photoxidized to a product that reduces electron flow to Q or Hill oxidant by providing an alternate route from PS-2, and perhaps indirectly returning the electron by feeding it back into the system between water and PS-2:



(b) the blue-light absorbing component, upon photoxidation, undergoes a conformational change which has an effect upon membrane permeability thus diminishing the ability of uncoupling agents to act.

Evidence favoring hypothesis (a) lies in the reduced O_2 evolution which would be expected if electrons were returned to the system since the photolysis of water would not be necessary. On the other hand, it is also possible that O_2 is consumed in the photoxidation of the hypothetical P. Furthermore, if photoxidation of P occurred only in "blue", then white light, comprising both short and long wavelength components, would cause some photoxidation and be slightly inhibitory, whereas "red" would not photoxidize P yielding the type of experimental result shown in figure 1. Thus, P could act as a photoregulatory compound.

Evidence favoring hypothesis (b) comes from some preliminary experiments which show no effect of ADP on the MA-uncoupled system when illuminated by red or white light, but some stimulation of electron transport in blue light. This result implies an incomplete uncoupling by MA in "blue" so that some photophosphorylation can still proceed in the presence of the cofactors.(10). Further investigation is required for clarification of the mechanism and identification of the responsible absorbing compound.

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