PHOTOREDUCTION AT λ 705 mμ IN ADAPTED ALGAE*

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Received July 14, 1962

Carbon dioxide reduction or adenosine triphosphate (ATP) formation are partial reactions in photosynthesis which depend upon typical water soluble enzymes found also in non-photosynthetic organisms. Therefore the capacity to reduce carbon dioxide is easily lost during the isolation of chloroplasts. The power to evolve oxygen from water in the light, on the other hand, is a remarkably durable characteristic of chloroplast fractions. As long as a certain small part of the water insoluble green lamellar system remains intact, the addition of artificial oxidants (i.e., various iron salts, quinones, dyes) suffices to evoke an oxygen production in the light. Because this property survives even freeze-drying and extraction with petroleum ether, it was possible for Bishop to show that plastoquinone is a necessary component of the pigment complex. (Bishop, 1959) The true photochemical problem in photosynthesis is now seen in the achievement of the chlorophyll complex to dehydrogenate water and to expel the rest as molecular oxygen. No less than two light quanta are necessary for a complete photolysis of one molecule of H·OH. What is the mechanism that couples two separate single quantum processes so as to make evolution of free oxygen possible?

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* These studies were aided by contracts between the Office of Naval Research, Department of the Navy [NONR 988 (10)] and the United States Atomic Energy Commission [AT-(40-1)-2687] and the Florida State University.

Emerson found in 1958 that the low yield for photosynthesis in light of a wave length around λ 700 mμ becomes normal again provided light of wave length shorter than λ 680 is given simultaneously. (Emerson, 1958, Emerson and Rabinowitch, 1960) Since then the question of two light reactions in photosynthesis has become even more intriguing. Now there appears to be a method to study this phenomenon experimentally. Several investigators have recorded the reversible spectral changes or the rates of oxygen evolution at long and short wave lengths, with the hope to be able to assign particular steps in either of the two primary light reactions to the one or the other region in the chlorophyll absorption spectrum. (cf Hoch and Kok, 1961) The dividing line, according to Myers, is around λ 685 mμ. (Myers, 1962)

In recent years we have been studying the mechanism of oxygen evolution by comparing photoreduction with photosynthesis in the same green cell. A hydrogenase containing alga stops evolving oxygen after it has been incubated a couple of hours in hydrogen. The rest of its photosynthetic activity, however, continues seemingly undisturbed. Such an adaptation to a bacterial type of photometabolism remains easily reversible unless further measures are taken. A simple incubation in hydrogen merely creates a condition where oxygen evolution and hydrogen absorption are competing for some common intermediates in the photometabolism. (Gaffron, 1960) If the cells are poisoned so that oxygen evolution becomes impossible, or are made manganese deficient, photoreduction becomes the favored reaction. A permanent change has been discovered by Bishop in a mutant of *Scenedesmus*. The new strain is a green alga incapable of evolving oxygen, while retaining all of the other properties necessary to achieve carbon dioxide reduction. (Bishop, 1962) Using interference filters to isolate light beams of ca λ 705 and ca λ 620 mμ we have now measured the rate of carbon dioxide fixation under anaerobic and adapted conditions, while submitting the cells to either the short or the long wave light or a combination of both. A few results are summarized in Tables I and II. They were obtained either by manometry of the gas exchange or the recording of pH changes in algae suspended in bicarbonate buffers. (Gaffron, 1957, Rosenberg, 1957)

TABLE I - RELATIVE RATES OF PHOTOSYNTHESIS AND PHOTOREDUCTION IN SCENEDESMUS D_3 AND ANKISTRODESMUS.(pH) .

Experimental	Arbitrary Units*			
	λ 620 mμ	λ 705 mμ	Superimposed 620 + 705	Effect 620 + 705
a) Scenedesmus D_3 Anaerobic PS (10 min. under H_2)	1.1	2.3	4.3	Enhancement
Adapted PR	1.4	2.7	3.9	No Effect
Adapted PR + DCMU (2½ hours H_2)	1.8	7.2	8.9	No Effect
b) Scenedesmus Mutant 11 Adapted (3 hours H_2)	2.8	6.3	6.9	Inhibition
c) Ankistrodesmus Manganese defic. Anaerobic PS	.9	.7	2.7	Enhancement
Adapted PR	2.9	4.6	5.6	Inhibition

* Rates always below saturation in white light

It is easy to confirm the Emerson effect in an alga like Chlorella, that is λ 705 mμ alone does much less than in combination with λ 620, for instance. It is important, however, that we can confirm Myers' observation (private communication) of a slight evolution of oxygen continuing as far out as λ 723 mμ.

With Ankistrodesmus or Scenedesmus, the Emerson effect in air is often not quite as pronounced as with Chlorella. Under anaerobic but unadapted conditions the efficiency of the long wave light tends to become

TABLE II - RELATIVE RATES OF PHOTOSYNTHESIS AND PHOTOREDUCTION IN SCENEDESMUS AND ANKISTRODESMUS (MANOMETRIC)

Experimental	λ 660 mμ*	λ 705 mμ*	$\frac{\text{Rate at 705}}{\text{Rate at 660}}$
Scenedesmus Mutant 11 PR	11	10	.91
Ankistrodesmus Normal Aerobic PS	32	13	.40
Adapted PR	61	39	.64
Ankistrodesmus Manganese Defic. Anaerobic PS	12	10	.83
Adapted PR	44	41	.93

* Equal incident light intensity at λ 660 mμ and λ 705 mμ

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better. But the most striking observation is that complete photoreduction at a high rate and for a long time is possible with light of λ 705 mμ, provided the algae have been adapted. In other words, complete photoreduction has no need of a supplement of light below λ 680 mμ. The assignment of the pigment absorbing at λ 705 mμ to the production of the photochemical reductant on account of spectroscopic studies, appears to be justified. (cf Hoch and Kok, 1961)

As long as the production of oxygen is still a potentiality of the algae, that is under conditions where oxygen evolution becomes quickly apparent at higher light intensities, light at λ 620 mμ is utilized and a superimposition of both short and long wave light gives simply the sum of the separate rates. The moment the algae are "stabilized", that is poisoned by a substance like DCMU, and are no more in a condition to evolve oxygen, but continue with photoreduction even at high light intensities, the effect of light seems to become restricted to the long wave length side of the spectrum, that is the rate at λ 705 mμ is higher but not enhanced by a superimposition of an equal (low) intensity of light λ 620 mμ or λ 547 mμ. This short wave length light seems ineffective. When after a double exposure λ 705 mμ light is turned off, while λ 620 mμ is continued, the photoreduction begins to decline and after a few minutes comes to a halt. Persistent illumination with λ 620 mμ sometimes causes the revival of a measurable photosynthetic activity, but in general it is very poor. Somewhat different is the response of the Scenedesmus mutant no. 11, the alga which cannot fix carbon dioxide in the light except under adapted conditions. Again λ 705 mμ appears to be quite sufficient for photoreduction, but λ 620 mμ and shorter wave lengths may contribute also.

Thus it appears that the long wave length region is responsible for the production of a strong reductant and that of the short wave length region for the production of a strong oxidant which eventually will yield oxygen. For the photolysis of water we need therefore a combination of the two primary systems as investigated already by Witt, Kok, French, Myers and others. (cf Hoch and Kok, 1961) The question is what connection exists between these two? The substance which is most abundant next to chlorophyll and carotene in the pigment complex is plastoquinone. (Bishop, 1959) Several suggestions have been made as to the place where the quinone should be introduced into the photochemical system (Bishop, 1960) but no place appears to be more suitable than as the main link between the photoreducing and the photooxidizing mechanisms. Thus in complete photosynthesis the production of a strong reductant and a weak oxidant with one quantum would be coupled by the quinone system to the mechanism which produces a weak reductant and a strong oxidant. This is, of course, only the bare outline of a scheme (Fig. 1) which seems to do justice to the experimental facts we have observed. The results shown in Tables I and II are examples for enhancement, no enhancement and inhibition according to circumstances.

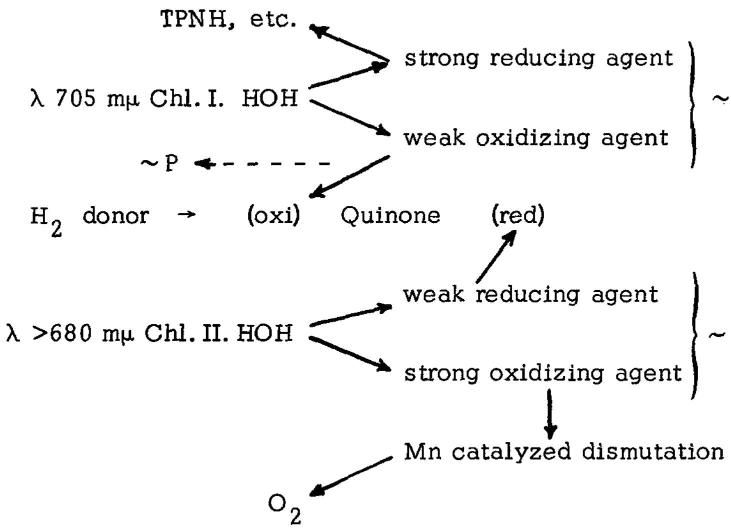

Figure 1

Just as we have no certain proof for assigning to the plastoquinone the role of a vital connecting link between the two photochemical reactions, so also we can only guess where best to place the contact (s) with the hydrogenase and the phosphorylating steps. To give our reasons for the choices indicated would need a more detailed analysis of the scheme.

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