Photochemistry and Photobiology. 1965 Vol. 4, pp. 793–801. Pergamon Press Ltd. Printed in Great Britain

ACTION SPECTRA FOR THE APPEARANCE OF DIFFERENCE ABSORPTION BANDS AT 480 AND 520 mμ IN ILLUMINATED CHLORELLA CELLS AND THEIR POSSIBLE SIGNIFICANCE TO A TWO-STEP MECHANISM OF PHOTOSYNTHESIS

GOVINDJEE and R. GOVINDJEE

Department of Botany, University of Illinois, Urbana, Illinois

(*Received* 14 *April* 1965)

Abstract—In the difference absorption spectrum of thin, actively growing* aerobic suspensions of *Chlorella pyrenoidosa*, both the 480 mμ (negative) and the 520 mμ (positive) bands are produced by light absorbed in chlorophyll *b* and chlorophyll *a*; the ratio of absorption changes caused by equal number of incident quanta of 650 mμ light and those of 680 mμ light, is about 1·2. Both effects are partially inhibited by DCMU. Upon replacing air with argon, the effects are increased several fold and become relatively insensitive to DCMU. The increase is stronger in the absorption region of chlorophyll *a*, than in that of chlorophyll *b*; the ratio of the absorption changes, caused by equal numbers of 650 mμ and 680 mμ quanta decreases to about 0·8, for both effects. Variable (as regards the exact ratios of absorption changes), but parallel results for 480 and 520 mμ bands were obtained with cultures having low quantum yield of photosynthesis. This parallelism in the behavior of the 480 mμ and the 520 mμ band suggests that at least part of these two bands have a common origin. However, many observations suggest that both difference bands may have a multiple origin; as a working hypothesis, this origin is discussed in terms of *three* reactions: Reaction A—Photoreduction of chlorophyll *a* in system II; Reaction B—Photooxidation of chlorophyll *b* in system II; and Reaction C—Photooxidation (perhaps of a carotenoid) in system I.

I. INTRODUCTION

THE RECENT hypothesis that photosynthesis involves two successive light reactions, I and II, sensitized by two different pigment systems [1] leads to the question: Which of the two systems is responsible for the difference absorption bands at 480 mμ (negative) and at 520 mμ (positive), discovered by Duysens[2] in illuminated green cells? Rubinstein and Rabinowitch[3] [cf. also Kok, *et al.*[4]] measured the action spectrum for the appearance of only 520 mμ band in thick and supposedly anaerobic Chlorella cells in low, steady light; the results suggested the predominant role of system I, containing the long-wave forms of chlorophyll *a*. Müller *et al.*[5] measured the 520 mμ band in flashing light, using a suspension of chloroplasts treated with 2,6 dichlorophenol indophenol and excess ferryicyanide (reagents supposed to supress reaction I), the action spectrum was clearly that of system II, containing chlorophyll *b* and chlorophyll *a* 670.

The action spectrum for the 480 mμ difference band has never been described before.

We describe here new measurements of the action spectra† for the appearance of both 480 mμ and 520 mμ bands in thin suspensions of Chlorella cells, suggesting a variable participation of *both* pigment systems I and II under both aerobic and anaerobic conditions.

*Having high (0·12) quantum yield of photosynthesis at 670 mμ.

†These results were first mentioned in a discussion session of the IV International Photobiology Congress, July, 1964, Oxford, England.

II. MATERIALS AND METHODS

Chlorella pyrenoidosa (Emerson's strain 3) was grown in inorganic culture media, with a continuous supply of 5% CO_2 in air, over a bank of incandescent and fluorescent lamps [see Govindjee[6] for details]. Almost all our samples were selected from actively growing cultures; the quantum yield of O_2 evolution in these samples was of the order of 0·12 at 670 mμ as measured by Emerson's manometric techniques.[6]

In optically dense (O.D. $\geq$ 1·0 at 680 mμ) suspensions, as used by Rubinstein and Rabinowitch,[3] the action spectrum can be distorted, because most of the light in the peak of the absorption band does not reach the region of the cuvette traversed by the measuring beam. Instead of correcting for this distortion, we preferred to use relatively thin suspensions (optical density was about 0·5 in the peak of the red absorption band of chlorophyll *a*).

The measuring beam, modulated at 400 cps by a rotating disc, was obtained from a 6 V, 18 A ribbon filament lamp and passed through a Bausch and Lomb monochromator (focal length, 250 mm; dispersion, 6·6 mμ per mm of slit width). The actinic beam was obtained from a similar lamp, and passed either through a large Bausch and Lomb monochromator (focal length, 500 mm; dispersion, 3·3 mμ per mm of slit width); or through a Farrand interference filter (half band width, 10–15 mμ). The actinic beam (10 mm$\times$ 20 mm) uniformly illuminated one side of the cuvette at a right angle to the measuring beam (about 2 mm $\times$ 10 mm). A blue glass filter (Corning C. S. 4–72) was placed in front of a photomultiplier (RCA 6217) with S20 response to eliminate fluorescence and scattered red actinic light [see Rubinstein[7] for details].

The absorption spectra of the cell suspensions were measured in a Bausch and Lomb Spectronic 505 spectrophotometer, equipped with an integrating sphere attachment.

The light intensity was measured by an Eppley thermopile. The changes in absorption were recorded on a Brown recorder. The sample was illuminated for 30 sec after 2-1/2 min dark intervals; the shutters were automatically controlled.

The results were normalized by dividing the Δ's (changes in absorption measured in relative units), by the thermopile readings, E (to reduce them to equal light intensity), and by the wavelength of actinic light, λ (to reduce them to equal quantum flux). In our experiments with the monochromator (band width: 3·3 mμ), the actinic light was so weak that one could assume being within the linear part of the light curve, $\Delta = f(E)$, and normalize the data, by dividing Δ by E.

In experiments with interference filters, where a sufficiently wide range of actinic light intensity was available, a few action spectra were obtained by actually plotting the light curves, $\Delta = f(E)$, determining their initial slopes and plotting them as a function of wavelength: $(\mathrm{d}\Delta/\mathrm{d}E)_0 = f(\lambda)$. No significant difference was noticed between the results obtained by the two methods.

An approximate correction was attempted for the absorption of the actinic beam in the front layer of the suspension, not traversed by the mearuring beam (an error not quite eliminated even by the use of optically thin suspensions). No precise correction was possible, because the measuring beam is scattered in the suspension, but curves 3 and 5 in Fig. show that this correction could not have changed the general picture.

III. EXPERIMENTAL RESULTS

1. *Change in absorption as function of light intensity*

Figure 1 shows the dependence of the absorption change, Δ, on light intensity, E, with the 650 mμ actinic light (obtained with a Farrand interference filter). These results confirm

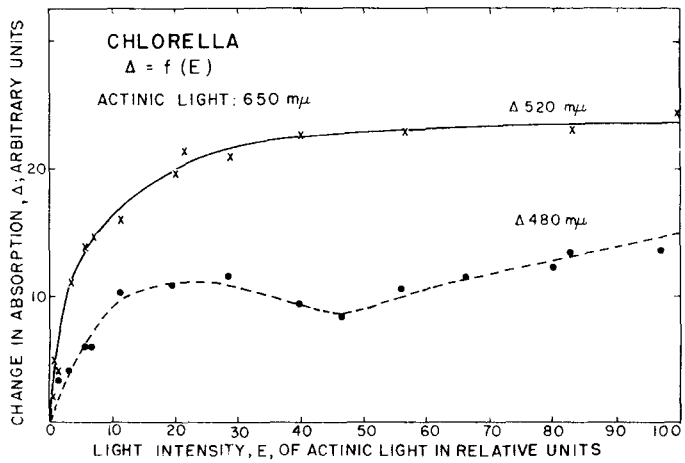

FIG. 1. Change in Absorption, Δ measured at 480 mμ (lower curve) and at 520 mμ (upper curve), as a Function of Intensity, E, of 650 mμ Actinic Light. Temperature: 22°C; Organism: *Chlorella pyrenoidosa*, Emerson's strain 3; Optical density at 680 mμ: 0·3.

the findings of Coleman and Rabinowitch.[8] They, too, found that the 480 mμ band is composed of at least two components—one that saturates early (low light component) and one that saturates only in much stronger light (high light component).

2. *Action spectra of the* 480 *mμ and* 520 *mμ difference bands in chlorella*

The action spectra for photobiological phenomena are usually presented as either the photochemical change per *incident* quanta or the change per *absorbed* quanta. The changes per incident quanta are meaningful when we are dealing with thin suspensions and when the absorption spectra (in terms of the fraction of light absorbed) are available for comparison; the kinds of pigments responsible for the effect can be easily deduced from such data. (We present such data in Fig. 2, *infra*). Our calculations for the absorption changes per absorbed quanta confirmed the conclusions obtained from Fig. 2.

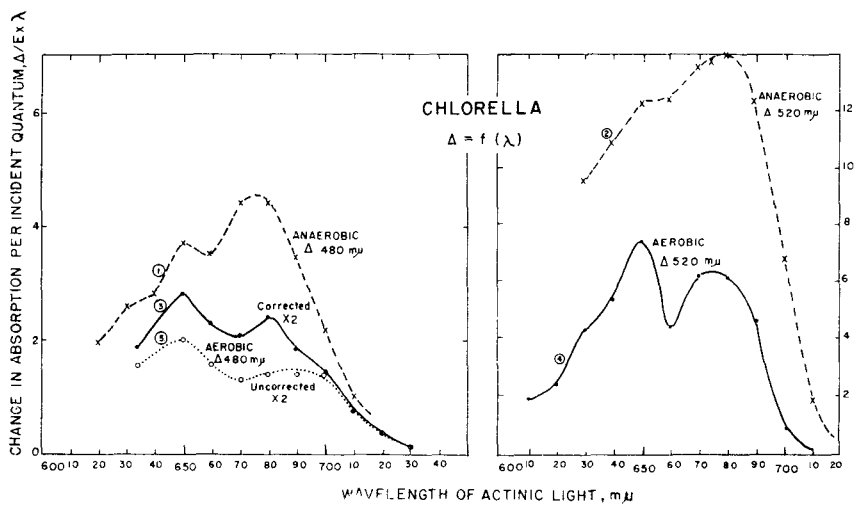

FIG. 2. Action Spectra $[\Delta/E \times \lambda = f(\lambda)]$ for the disappearance of 480 mμ band (curves 1, 3 and 5) and for the appearance of 520 mμ band (curves 2 and 4) in *Chlorella pyrenoidosa* under aerobic (curves 3, 4 and 5) and anaerobic (curves 1 and 2) conditions.

The low light component of the negative difference band at 480 mμ and the positive band at 520 mμ, is caused by absorption of light in both pigment systems, I and II. We found that under *aerobic** conditions, pigment system II is somewhat more effective, as shown by the ratio (1·2) of the chlorophyll *b* peak (650 mμ) and the chlorophyll *a* peak (680 mμ) in the action spectrum of both the 480 and the 520 mμ band (see the two solid curves, 3 and 4, in Fig. 2). Anaerobiosis (established by passing argon through the suspension) enhances both effects and changes the ratio of the two peaks from 1·2 to 0·8 (see dashed curves 1 and 2 in Fig. 2), suggesting a greater contribution of system I under anaerobic conditions. Three sets of measurements, made on different cultures grown under identical conditions and having the same pigment composition, confirmed these results.

When samples that show poor quantum yield ($\geq$0·06) of photosynthesis were used for the above experiments, results similar to that for anaerobic conditions were obtained even though they were supposedly maintained under aerobic conditions (strong light and thin suspensions). This is to be expected because low-quantum yield cells usually had high respiration rates and did not produce enough net O_2 to keep the cells under aerobic conditions. Variable, but parallel, results as regards the exact ratio of the peak at 650 mμ to that of 680 mμ in the action spectra for the 520 and 480 mμ effects were noted. However, the following general conclusions were true under all conditions:

(a) The action spectra for both 480 and 520 mμ effects were similar, but not identical.

(b) Under both aerobic and anaerobic conditions, both Systems II and I (chlorophyll *b* and chlorophyll *a*) were shown to participate.

(c) A somewhat greater contribution of System II under really aerobic conditions and somewhat greater contribution of system I under anaerobic conditions was noted.

3. *Effect of DCMU*

Contradictory results have been reported about the effect of dichlorophenyldimethyl urea (DMCU)[4,5] on the 520 mμ difference band. According to some observers, it inhibits the 520 mμ band; according to others, it has no effect.

When the action spectra for the appearance of 480 mμ and 520 mμ bands in DCMU-treated thin, aerobic cell suspensions were compared with those of untreated ones, we found a partial inhibition. At 650 mμ, the inhibition was about 60%; at 680 mμ, only 40%, in two experiments. Several cultures showed quantitatively smaller quenching. Almost no inhibition was observed when the cultures were made anaerobic, or when Chlorella with low quantum yield of photosynthesis were used. This suggests that of reactions leading to the 480 and the 520 mμ bands, only one is DMCU-sensitive; the other is not.

IV. DISCUSSION

That there are at least *two* light reactions involved in the production of the 480 and 520 mμ bands may be inferred from: (a) the complex shape of the time course of these changes—which contain a fast (temperature independent) and a slow (temperature dependent) component—under aerobic and anaerobic conditions, and under different intensities of light [Chance and Strehler;[9] Witt and co-workers[10,11]]; (b) the complex shape of the light curves [$\triangle = f(E)$, ([7,8] Fig. 1 this paper)]; (c) the action spectra for the appearance of

*The 'aerobic' condition is obtained by the use of cultures that have high (0·12) quantum yield of photosynthesis (at 670 mμ) and that are kept under aerobic conditions (strong light, thin suspensions) while action spectra are measured.

difference bands showing participation of both pigment systems I and II, and the greater contribution of system II under aerobic conditions and of system I under anaerobic conditions (Fig. 2 this paper); and (d) the different effect of DCMU under different conditions ([4,5] this paper); DCMU inhibits the appearance of 520 mμ band in the aerobic, but not in the anaerobic state. It may be further inferred that one of the reactions is sensitized by pigment *system I* and another by pigment *system II*.

Chance and Strehler[9] found that the 520 mμ band can be produced in Chlorella by the addition of oxygen to an anaerobic suspension in darkness, suggesting an *oxidation*. On the contrary, Witt and co-workers[11] observed an increase in the rate of *disappearance* of the 520 mμ band in darkness by the addition of oxidants, suggesting a *photoreduction*.

It may be inferred from the above discussion that one of the reactions leading to the difference bands is a *photoreduction* (we will call it Reaction *A*) in system II and the other is a photooxidation (we will call it *Reaction C*) in system I (Still another reaction—Reaction B—is discussed later). It may be proposed that Reaction A is photoreduction of chlorophyll *a* in system II, and Reaction C is photooxidation of an unknown compound P480–P520 (*infra*). Since the action spectra for the 520 mμ difference band measured by short flashes showed participation by system II,[5,10,11,12] we are tempted to suggest that Reaction A is the fast reaction. The Reaction C, on the other hand, may be a slow reaction (a light reaction followed by dark reactions) because the action spectra under steady state (which includes the slow decaying effect) showed participation by system I—especially under anaerobic conditions.[3]

In system II, Reaction A is suggested to be the photoreduction of chlorophyll *a*. Since the changes at 480 and 648 mμ (and even 520 mμ) have been attributed by Rumberg[12] to chlorophyll *b*, one may suggest the existence of still another reaction. We shall call it *Reaction B*. It may be a photooxidation of chlorophyll *b*. The positive band of reduced chlorophyll *b* should be around 560 mμ [cf. Evstigneev and Gavrilova [13]] and not at 520 mμ (*infra*), and since there is no band around 560 mμ, we believe that chlorophyll *b* is not photoreduced—rather, it is photooxidized, giving negative bands at 480 and 648 mμ; the 520 mμ band belongs to the photoreduced chlorophyll *a* (Reaction A).

As a working hypothesis, we describe below the three suggested reactions A, B and C, and their implications.

REACTION A

Photoreduction of chlorophyll a in system II

Reaction A may be symbolized by the following equation:

$$e^- + \text{Chl } a_2 + h\nu_2 \longrightarrow \text{Chl } a_2^-$$

Changes in Absorption: $+\,\triangle 520$; $-\,\triangle 440$?; $-\,\triangle 670$?

Chl a_2 (chlorophyll *a* trap in system II) is reduced to Chl a_2^- by light ($h\nu_2$) absorbed in system II; an electron (e) or an H-atom is added to chlorophyll *a* molecule. The expected absorption changes are: a positive change at 520 mμ ($+\,\triangle 520$) and negative changes at 440 mμ ($-\,\triangle 440$) and at 670 mμ ($-\,\triangle 670$).

Coleman and Rabinowitch[8] suggested that one part of the 520 mμ band may be due to chlorophyll *a*—perhaps to the formation of Krasnovsky's 'eosinophyll'—the 'pink'

reduction product of chlorophyll *a*. Witt *et al.*[14] had also suggested a photoreduction of chlorophyll a_2—on the basis of observed absorption changes at 520 and 430 mμ. If chlorophyll *a* were responsible for the 520 mμ band, one would expect the appearance of negative band around 440 mμ as well as at 680 mμ. While Coleman and Rabinowitch[8] and Kok[15] did observe negative difference bands in the red region, around 680 mμ, subsequent experiments by Rubinstein and Rabinowitch[16] and Karapetyan *et al.*[17] showed that these bands are due largely, if not entirely, to changes in the intensity of fluorescence. The existence of a negative difference band around 440 mμ is difficult to prove or disprove because of the complexity of the difference spectrum in this region—mainly due to changes in cytochromes. Since we attribute only a small part of the 520 mμ effect to this reaction (Reaction A), the occurrence of the corresponding small negative band in the blue and in the red cannot be excluded by available experiments. A lack of clear experimental evidence for the red and blue bands, however, weakens our case.

Since Reaction A is postulated to be a reaction of chlorophyll *a* in system II, we should observe this in all photosynthetic organisms (and it should be DCMU-sensitive). The demonstration of the 520 mμ band in *Porphyridium cruentum* by Inselberg and Rosenberg[18] is a welcome finding from this point of view.

When system II is preferentially excited (especially with high light intensities), and oxygen is present, reduced chlorophyll *a* (Chl a_2^-) may be re-oxidized by O_2. This would explain the photochemical uptake of oxygen sensitized by light absorption in system II, described by Hoch and co-workers.[19]

In our earlier publication [Krey and Govindjee[20] and Govindjee and Yang[21]], we have assumed that the energy trap of system II is of the type suggested by Franck and Rosenberg,[22] *i.e.* it is complexed with a cytochrome, and so Reaction A may be due to transformation of chlorophyll a_2-cytochrome complex instead of chlorophyll a_2 alone.

REACTION B

Photooxidation of chlorophyll b *in system II*

Reaction B may be symbolized by the following equation:

$$\text{Chl } b_2 + h\nu_2 \longrightarrow \text{Chl } b_2^+ + e^-$$

Change in Absorption: $-\triangle 480$; $-\triangle 648$

In this reaction, chlorophyll *b* (Chl b_2) is oxidized by light ($h\nu_2$) with the loss of an electron (e^-) or an H-atom and the formation of oxidized chlorophyll *b* (Chl b_2^+). Such an oxidation would cause a decrease in absorption at 480 mμ ($-\triangle 480$) and at 648 mμ ($-\triangle 648$).

Since the 520 mμ and the 480 mμ changes have been clearly observed only in green algae and higher plants (*i.e.* in chlorophyll *b*-containing organisms), Coleman and Rabinowitch[8] mentioned the possibility that at least a part of these bands could be due to chlorophyll *b*; but they pointed out that the 520 mμ band is characteristic, in solution, of a reduction product of chlorophyll *a*, while the corresponding band of the reduction product of chlorophyll *b* lies in solution at 560 mμ [cf. Evstigneev and Gavrilova [13]]. One may suggest that Reaction B, due to chlorophyll *b*, does not contribute to the 520 mμ band and is not a reduction.

Rumberg[12] observed a negative difference band at 648 mμ in chloroplasts. This is probably identical with the one discovered by Strehler and Lynch[23] and confirmed by Rubinstein and Rabinowitch.[16] Rumberg compared its behavior with that of the difference bands at 480 and 520 mμ. Similarity of the light curves of all three bands, and similar effects on them of red and far-red background light, caused Rumberg to attribute them to chlorophyll *b*. This is plausible in the case of the two negative bands, at 480 mμ and at 648 mμ, but doubtful in the case of the positive 520 mμ band. A direct photocatalytic role of chlorophyll *b* would have been plausible in Emerson's original interpretation[24] of the enhancement effect, according to which all chlorophyll *a* belongs to system I, and all chlorophyll *b*, to system II. However, subsequent observations by Govindjee and co-workers[6,25] and by French and co-workers[26] have shown that at least one form of chlorophyll *a* in green plants and diatoms, Chl *a* 670, belongs to system II. Duysens[27] similarly concluded that some chlorophyll *a* must be present in system II in red algae. If this is so, then chlorophyll *a*, with its lower excitation energy, should be the 'energy trapping center', and chlorophyll *b* only an 'energy supplier'. Duysens[28] observations on sensitized fluorescence agree with this hypothesis, by showing an almost 100 per cent efficient energy transfer from chlorophyll *b* to chlorophyll *a in vivo*.

With the role of 'energy trapping center' reserved to chlorophyll *a*, the difference bands at 480 and 648 mμ—if they are really indicative of the reversible transformation of chlorophyll *b*—must have another explanation. For example, one could suggest as a working hypothesis [cf. Rabinowitch[29] and of Arnold[30]] that chlorophyll *b* participates in the reaction step in which the photosynthetic unit recovers from water the electron it has given off, probably to a cytochrome. In other words, energy migration to this trap (trap II), and electron loss in it, may be followed by 'hole migration' to another trap—a 'hole trap'—provided by chlorophyll *b*, in which the electron is recovered. A reaction similar to 'B' must occur in red algae and the blue-green algae with phycocyanin instead of chlorophyll *b*. Recently we have observed changes in phycocyanin fluorescence causing a positive band at 669 mμ and a negative band at 660 mμ in red and blue-green algae [Krey and Govindjee[20]] appearing upon increasing light intensity.

REACTION C

Oxidation of P480–P520 in system I

The reaction C may be symbolized by the following set of equations:

$$P700 + h\nu_1 \longrightarrow P700^+ + e^- \text{ (Light Reaction I) [Kok[4]]}$$

$$P700^+ + \text{Cyt} \longrightarrow \text{Cyt}^+ + P700$$

Reaction C: $\text{Cyt}^+ + P480 \longrightarrow P520 + \text{Cyt}$

A similar result can be produced upon darkness:

$$P480 + O_2 \longrightarrow P520$$

Change in Absorption for Reaction C: $-\triangle 480; + \triangle 520$

Reaction C, as suggested above, is an oxidation in system I causing a negative band at 480 mμ ($-\triangle 480$) and a positive band at 520 mμ ($+\triangle 520$). This is a consequence of the photooxidation of the chlorophyll *a* trap in system I (P700) by light absorbed in system I ($h\nu_1$). The oxidized P700 is represented by $P700^+$; e^- is the electron or an H-atom. This is light reaction I of photosynthesis and is followed by the dark reaction in which

$P700^+$ extracts an electron from cytochrome (Cyt) and oxidized cytochrome is produced (Cyt^+). The Cyt^+ or $P700^+$ then oxidizes the pigment absorbing at 480 mμ (P480) to a pigment absorbing at 520 mμ (P520) by a dark reaction.

The P480 can also be oxidized to P520 by molecular O_2 in a dark reaction. This could explain why light absorbed in system I seems to form more of P520 under anaerobic than under aerobic conditions. In the presence of oxygen, most of P480 is converted into P520 in the dark, and the additional effect of light is relatively small.

This set of reactions predicts an inhibition of O_2 uptake by $h\nu_1$ light, as observed by Hoch and co-workers.[19] Light reaction I, followed by oxidation of P480 to P520 by one of the primary oxidation products, competes with the dark reaction [P480 + O_2 ⟶ P520], and thus slows down the uptake of O_2. The $h\nu_1$ light should also cause an uptake of O_2 after the cessation of illumination, because in the dark, the autooxidation of P480 [P480 + O_2 ⟶ P520] proceeds without competition.

The P480 may be a carotenoid with an absorption maximum at 480 mμ which upon oxidation absorbs at 520 mμ. Chance and Strehler[9] had favored the attribution of the 480 and 520 mμ bands to a carotenoid because they could not find these bands in a carotenoid-free mutant of Chlamydomonas. Coleman and Rabinowitch[8] also mentioned this possibility.

Recently Krinsky and Gordon[31] suggested that 5,6 epoxyzeaxanthin is formed in light and under aerobic conditions from zeaxanthin in *Euglena gracilis*. They also suggested that the reverse reaction occurs in dark and under anaerobic conditions. Donohue and Chichester,[32] working with New Zealand spinach leaves, have observed rapid interconversions of carotenoids (lutein, zeaxanthin, neoxanthin and violaxanthin) and observed different response under aerobic and anaerobic conditions. These results may suggest a possible correlation between the effect of anaerobiosis on the 480 and 520 mμ band and on the interconversion of carotenoids. However, we cannot exclude the possibility that the 480 and 520 mμ changes are in chlorophylls of system I.

V. CONCLUSIONS

The 480, 520, and 648 mμ difference bands in Chlorella are suggested to have a triple origin: (A) Photoreduction of chlorophyll *a* in system II, (B) Photooxidation of chlorophyll *b* in system II, and (C) Photooxidation of carotenoids (?) in system I. Reactions A and B may be simply half reactions of one single light reaction (Reaction II) leading to the reduction of chlorophyll *a* and oxidation of chlorophyll *b*, e.g.:

$$\text{Chl } a_2 + \text{Chl } b_2 + h\nu_2 \longrightarrow \text{Chl } a_2^- + \text{Chl } b_2^+$$

Changes in Absorption: + △520, — △440?, — △670?, — △480, — △648

The reduced chlorophyll a_2 (Chl a_2^-) would transfer electrons (e^-) or H-atoms to cytochrome, which is then reduced. The oxidized chlorophyll *b* must recover electrons from H_2O by a dark reaction—evolving O_2. This scheme implies that chlorophyll *b* must have an oxidation-reduction potential slightly above that of the H_2O/O_2 couple, *i.e.* + 0·8 V at pH 7·0.

The above discussion should be considered only as a working hypothesis. It may be that some of the observed absorption changes have nothing to do with the main reactions of photosynthesis.

The reaction C is actually a dark reaction, and we consider it to be a 'useless' reaction that predominates in excess $h\upsilon_1$ and under anaerobic conditions.

There are, of course, several 'gaps' in our picture. For instance, we do not have any explanation of the 657 mμ difference band [discovered by Strehler and Lynch[23] and confirmed by Rubinstein and Rabinowitch[16]]. Rumberg,[12] however, did not observe this band. Additional systematic experiments and detailed analyses are needed before a comprehensive picture can be built regarding *all* the difference bands in green plants.

Acknowledgements—We are grateful to Professor Eugene Rabinowitch for reading the manuscript. We thank Dr. D. Rubinstein and Mr. J. Spencer for showing us the operating of the difference spectrophotometer.

A research grant from the National Science Foundation is gratefully acknowledged.

REFERENCES

1. Various authors in '*Photosynthetic Mechanisms of Green Plants*', National Academy of Sciences—National Research Council, Publication No. 1145, Washington, D.C., 1963. See summary by B. KOK and A. T. JAGENDORF on page 751.
2. L. N. M. DUYSENS, *Science*, **120**, 353 (1954).
3. D. RUBINSTEIN and E. RABINOWICH, *Biophys. J.*, **4**, 107 (1964).
4. B. KOK, B. COOPER and L. YANG, in '*Studies on Microalgae and Photosynthetic Bacteria*' (*Jap. Soc. Plant Physiol.*), U. Tokyo Press, Tokyo, 1963, see p. 373.
5. A. MÜLLER, D. C. FORK and H. T. WITT, *Z. Naturforsch* **186**, 142 (1963).
6. GOVINDJEE, Ph.D. thesis, University of Illinois, Urbana, (1960); GOVINDJEE and RABINOWITCH, E., *Biophys. J.* **1**, 73 (1960).
7. D. RUBINSTEIN, Ph.D. thesis, University of Illinois, Urbana, (1964).
8. J. COLEMAN and E. RABINOWITCH, *J. Phys. Chem.* **63**, 30 (1959).
9. B. CHANCE and B. STREHLER, *Plant Physiol.* **32**, 536 (1957); C. J. P. SPRUIT, *Rec. trav. chim.* **75**, 1097, (1956).
10. B. RUMBERG, P. SCHMIDT-MENDE, J. WEIKARD and H. T. WITT, in reference (1), see p. 18.
11. A. MÜLLER, B. RUMBERG and H. T. WITT, *Proc. Roy. Soc.* **157B**, 313 (1963).
12. B. RUMBERG, *Nature* **204**, 860 (1964).
13. V. B. EVSTIGNEEV and V. A. GAVRILOVA, *Dokl. Akad. Nauk. S.S.S.R.* **91**, 899 (1953).
14. H. T. WITT, R. MORAW, A. MÜLLER, B. RUMBERG and G. ZIEGER, *Z. physik. chem.* (Frankfurt [N.S.]), **23**, 133, (1960).
15. B. KOK, *Acta Botan., Neerl.* **6**, 316, (1957).
16. D. RUBINSTEIN and E. RABINOWITCH, *Science* **142**, 681, (1963).
17. N. V. KARAPETYAN, F. F. LITVIN and A. A. KRASNOVSKY, *Biofizika* **8**, 191 (1963).
18. E. INSELBERG and J. L. ROSENBERG, *Plant Physiol.* **39**, 810 (1964).
19. G. HOCH and O. v. H. OWENS, in reference (1), see p. 409; GOVINDJEE, O. v. H. OWENS and HOCH, *Biochim. Biophys. Acta* **75**, 281 (1963).
20. A. KREY and GOVINDJEE, *Proc. Nat. Acad. Sci. U.S.A.* **52**, 1568 (1964).
21. GOVINDJEE and L. YANG, Paper presented at the X International Botanical Congress, 1964; Submitted to *J. Gen. Physiol.*
22. J. FRANCK and J. ROSENBERG, in reference (1), see p. 101.
23. B. STREHLER and B. LYNCH, *Arch. Biophys.* **70**, 527 (1957).
24. R. EMERSON, *Annl. Rev. Plant Physiol.* **9**, 1 (1958).
25. GOVINDJEE in reference (1), see p. 318.
26. C. S. FRENCH, J. MYERS and G. C. MCLEOD, in '*Comparative Biochemistry of Photoreactive Systems*' (M. B. ALLEN, Ed.), Academic Press, N.Y., see p. 361 (1960).
27. L. N. M. DUYSENS, in Colloques Intern., 119, '*La Photosynthèse*' (Editions du Centre National Recherche Scientifique, Paris), see p. 75 (1963).
28. L. N. M. DUYSENS, Ph.D. thesis, U. Utrecht, (1952).
29. E. RABINOWITCH, in Colloques, Intern., 119, '*La Photosynthèse*' (Editions du Centre National Recherche Scientifique, Paris), see p. 125 (1963).
30. W. A. ARNOLD, *J. Phys. Chem.*, **69**, 788 (1965).
31. N. I. KRINSKY and A. GORDON, Abstract No. 581 of the Federation of American Societies for Experimental Biology, Annual Meeting, Atlantic City, 1965.
32. H. V. DONOHUE and C. O. CHICHESTER, Abstract No. 2393 of the Federation of American Societies for Experimental Biology., Annual Meeting, Atlantic City, 1965.