The Function of Different Chlorophylls in Photosynthesis and the Action Spectra of Separated Light Reactions

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From action spectra of separated light reactions the function of different chlorophylls in photosynthesis have been estimated.

A reaction scheme derived from different short-time absorption changes has already been presented¹⁻⁷. Fig. 1 shows the scheme in a simplified form. The overall process could be separated into 7 partial reactions^{5, 6}. Among others, the two light

Fig. 1. Reaction scheme of the electron transport system in photosynthesis of green plants. TPN: triphosphopyridine nucleotide, Chl_{aI} and Chl_{aII} special chlorophylls of typ a, Cyt: cytochrome, Q: plastoquinone, red. PMS: reduced phenazine methosulfate, ox. S_I and ox. S_{II} see text. Z, E and Y are unknown.

reactions could be isolated. In the light reaction $h\nu_{II}$ the reaction of plastoquinone Q_{II} causes short-time absorption changes with a maximum at 475 and at 515 $m\mu$ ⁷ and in the light reaction $h\nu_I$ the reaction of Chl_{aI} causes absorption changes with a maximum at 433 and at 703 $m\mu$ ^{8, 3}.

In earlier experiments⁹ it was shown that in fresh chloroplasts it was possible to decouple Q_{II} (formely symbolized by X and in l. c. ^{1, 2, 6, 7} identified as

plastoquinone) from its unknown natural reaction partner E by addition of oxidized Hill-substances, ox. S_{II}, such as dichloro-, thymol-, and phenol-indophenole, toluylene blue, thionine, and methylene blue which serve as a trap for the electrons originating in water. That ox. S_{II} reacts directly with Q_{II} follows from the fact that with increasing concentration of ox. S_{II} the re-oxidation of Q_{II} can be accelerated in a first order reaction by a factor of 200 and more⁹. Under these conditions Chl_{aI} can further work by a back flow of electrons from $Z^{\oplus}$ to Chl_{aI}[⊕] (see ref. 4). To stop this reaction, ferricyanide was added which kept Chl_{aI}[⊕] oxidized and non functional (no more changes at 433 and 703 $m\mu$). This reaction mixture shows only the absorption changes at 475 and 515 $m\mu$ which are caused by Q_{II} indicating that the light reaction, $h\nu_{II}$, has been isolated^{5, 6}:

Using aged chloroplasts with reduced dichlorophenol indophenol as an electron donor (ascorbic acid in excess), VERNON, KAMEN and ZAUGG¹⁰ have shown that this system is capable of reducing Hill-substances such as triphosphopyridine nucleotide, TPN, indigocarmine etc. in the light. We observed that this system (we used also reduced phenazine methosulfate, red. PMS, as electron donor) shows

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¹ H. T. WITT, A. MÜLLER and B. RUMBERG, Nature [London] **191**, 194 [1961].

² H. T. WITT, A. MÜLLER and B. RUMBERG, Angew. Chem. **73**, 507 [1961].

³ H. T. WITT, A. MÜLLER and B. RUMBERG, Nature [London] **192**, 967 [1961].

⁴ B. RUMBERG, A. MÜLLER and H. T. WITT, Nature [London] **194**, 854 [1962].

⁵ H. T. WITT, A. MÜLLER and B. RUMBERG, Angew. Chem.,

International Edition **1**, 275 [1962]; Angew. Chem. **74**, 330 [1962].

⁶ A. MÜLLER, B. RUMBERG and H. T. WITT, Proc. Royal Soc. [London], Ser. B, in the press.

⁷ J. WEIKARD, A. MÜLLER and H. T. WITT, Z. Naturforschg. **18 b**, 139 [1963].

⁸ B. KOK, Biochim. biophysica Acta [Amsterdam] **48**, 527 [1961].

⁹ H. T. WITT and A. MÜLLER, Z. physik. Chem., Neue Folge, **21**, 1 [1959].

¹⁰ L. P. VERNON and M. D. KAMEN, Arch. Biochem. Biophysics **51**, 122 [1954]; L. P. VERNON and W. S. ZAUGG, J. biol. Chemistry **235**, 2728 [1960].

no absorption changes at 475 and 515 $m\mu$ indicating that light reaction hv_{II} becomes inactive as chloroplasts age³. The changes at 475 and 515 $m\mu$ (caused by Q_{II}) vanish also by heating fresh chloroplasts up to 50 °C and by extracting Q_{II} from fresh chloroplasts with petroleum ether^{1,7}. In all cases, nevertheless, the changes of Cyt and Chla_I still exist indicating that light reaction hv_I is intact. Electrons to support a continuing reduction of Chla_I[⊖] come from, reduced PMS via Cyt. At higher concentrations ($\geq 10^{-5}$ M) the PMS by-passes Cyt (no more absorption changes of Cyt) and reacts directly with Chla_I[⊖]. The direct interaction of PMS with chlorophyll is indicated by the observation that with increasing red. PMS concentrations ($\geq 10^{-5}$ M) the re-reduction of Chla_I[⊖] can be accelerated in a first-order reaction by a factor of 100 and more³.

Only under these conditions light reactions hv_I is isolated

When substances accept electrons from $Z^{\ominus}$, this causes a specific behaviour of the changes at 433 and 703 $m\mu$ because a back-flow of electrons from $Z^{\ominus}$ to Chla_I[⊖] is prevented (see l.c.⁴ and in more details l.c.¹²). By this method we have found that oxidized substances, ox. S_I , as indigocarmine, ferricyanide phenazine methosulfate, benzyl viologene, methyl viologene and TPN-reductase react with $Z^{\ominus}$ ¹².

These results show that it is possible to measure the two light reactions directly by the changes at 515 and at 433 $m\mu$ respectively and under conditions where they are completely separated from each other. In this communication we report the action spectra for these isolated light reactions to see directly which types of pigments are supporting them with energy by energy migration. [It has already been shown qualitatively for green plants¹ that light reaction hv_I (measured by the absorption changes of cytochrome) can be excited by longer wavelengths than can light reaction hv_{II} (measured by the absorption changes at 515 and 475 $m\mu$ which are caused by Q_{II})].

The action spectrum for the 515 $m\mu$ absorption change in chloroplast fragments in the presence of

Fig. 2. The action spectra for the production of the absorption changes at 515 and 433 $m\mu$ in spinach chloroplast fragments. The solution used for determining the action spectrum of the 515 $m\mu$ change was $1.5 \cdot 10^{-5}$ M/l dichlorophenol-indophenol, $1 \cdot 10^{-2}$ M/l $K_3Fe(CN)_6$, $5 \cdot 10^{-2}$ M/l tris buffer (pH 7.2), chlorophyll concentration of 0.115 mg/ml. The transmission at 674 $m\mu$ of this suspension was 22 per cent. The solution used for determining the action spectrum for the 433 $m\mu$ change was $9.4 \cdot 10^{-5}$ M/l PMS, $9.4 \cdot 10^{-3}$ M sodium ascorbate, $3.8 \cdot 10^{-2}$ M/l tris buffer (pH 7.2), chlorophyll concentration of 0.150 mg/ml. The transmission of this suspension at 674 $m\mu$ was 17 per cent.

2,6-dichlorophenol-indophenol (DPIP) and ferricyanide is given in Fig. 2. At each wavelength measurements of the 515 $m\mu$ absorption change were made as a function of increasing light intensity. Then a light intensity ($25 \cdot 10^{14}$ quanta $cm^{-2} sec^{-1}$) was chosen such that, for each saturation curve, it fell well within the region where the 515 $m\mu$ change was linear with light intensity. The absorption change produced by this constant quantum flux at various wavelengths was then used to plot the action spectrum. The action spectrum has a peak at 674 $m\mu$, a shoulder at 650, and a far-red limit around 705 $m\mu$.

From this action spectrum it follows that the light reaction, hv_{II} , is supported by light absorption by Chla—650 and Chla—674. This result is in agreement with the Emerson effect¹¹ (more details about the Emerson effect see l.c.¹²).

The peak of the absorption band of the energy accepting chlorophyll engaged in the light reaction hv_{II} must, according to the rule of energy migration, be at a longer wavelength than the peak of the donor molecules (i.e. $> 674 m\mu$). Absorption bands with peaks above 674 $m\mu$ belong only to chlorophylls of type a. Therefore the chlorophyll in light reaction

¹¹ C. S. FRENCH and I. MYERS, Carnegie Inst. Wash. Yearbook **58**, 323 [1959]; R. GOVINDJEE and E. RABINOWITCH, Science [Washington] **132**, 355 [1960]; Biophys. J. **1**, 73 [1960];

1, 377 [1961]; R. GOVINDJEE, J. B. THOMAS and E. RABINOWITCH, Science [Washington] **132**, 421 [1960].

¹² H. T. WITT, A. MÜLLER and B. RUMBERG, Nature [London], in the press.

$h\nu_{II}$ must be a chlorophyll a (Chla_{II}) (s. Fig. 1) and not a chlorophyll b, as has been suggested by some authors. This has been already concluded from the Emerson effect¹¹. The fluorescence emission of green plants has a maximum at $685\text{ m}\mu$. The chlorophyll active in fluorescence is therefore probably Chla-674 .

Chla_{II} has, as yet, not been seen in action by corresponding absorption changes. The reason is because the reaction of Chla_{II} takes place in $< 3 \cdot 10^{-6}$ sec which follows from the reduction of Q_{II} (causing absorption changes at 475 and $515\text{ m}\mu$, $< 3 \cdot 10^{-6}$ sec).

Aged chloroplasts (7 days) which no longer exhibited changes of absorption at 475 and $515\text{ m}\mu$ were used to determine the action spectrum for the absorption change of Chla_I at $433\text{ m}\mu$. PMS, reduced by excess sodium ascorbate, served as an electron source and, at the concentrations used, coupled directly to chlorophyll since no more absorption changes characteristic of cytochrome were detected. The action spectrum for the $433\text{ m}\mu$ absorption change given in Fig. 2 was determined in a manner similar to that described for the $515\text{ m}\mu$ action spectrum by measuring saturation curves at various wavelengths and selecting a light intensity ($100 \cdot 10^{14}$ quanta $\text{cm}^{-2} \text{sec}^{-1}$) which fell within a linear region of all the saturation curves. The action spectrum for the $433\text{ m}\mu$ absorption change, in contrast to the action spectrum for the $515\text{ m}\mu$ change, remains higher at far-red wavelengths and has a limit at $\sim 735\text{ m}\mu$. The action spectrum of Fig. 2 shows a small shoulder at $\sim 650\text{ m}\mu$, a peak at 682 , and another shoulder at $695\text{ m}\mu$. This indicates that Chla_I must be supplied by light which is absorbed by a Chla-682 , a Chla-695 and probably also by $\text{Chlb-650 m}\mu$.

Usually the chlorophyll absorption bands in green plants show only a shoulder at $650\text{ m}\mu$ reflecting chlorophyll b absorption and a broad peak at $675 - 680\text{ m}\mu$ indicating chlorophyll-a absorption. FRENCH and co-workers¹³ have been able to resolve the absorption band into three components of chlorophyll-a with peaks at 673 , $683\text{ m}\mu$ and $695\text{ m}\mu$. In comparison with results reported

here it can be seen that the shoulders and peaks of the action spectra correspond very well with the peaks of the absorption bands of the chlorophyll-a forms and with chlorophyll-b. From this it can be assumed that the function of Chlb-650 and Chla-673 is to support Chla_{II} in light reaction $h\nu_{II}$ with energy and Chla-683 and Chla-695 and probably also Chlb-650 to support Chla_I in light reaction $h\nu_I$ with energy (s. Fig. 1)*.

To sum up, there exist at least seven different chlorophylls in green plants with the following function:

Energy Donor (Bulk of Chlorophyll)	Energy Acceptor ($\sim 1/1000$ of the Bulk)
(Chl-b-650) Chl-a-682 Chl-a-695	Chla_I ($433 - 703$) in two systems*
Chl-b-650 Chl-a-674	Chla_{II}

(* It was shown previously⁴ that there exists Chla_I in two different systems.)

When the chlorophylls in the bulk are separated or both light reactions are inactivated by heating to 70°C ¹, energy migration is interrupted or prevented for photochemical consumption and the absorbed light energy should stay in the $\pi - \pi^*$ -triplet states of these molecules. This has been directly proved¹⁴.

It is important to note that under normal conditions the absorption changes at 475 and $515\text{ m}\mu$ are biphasic¹⁵. In whole cells for instance the changes at $515\text{ m}\mu$ start in permanent light with a rapid, temperature-independent increase followed by a further, but slow and strongly temperature-dependent increase. In chloroplasts this second phase is strongly reduced but does not vanish. The second phase can be eliminated from measurement by using flashing light. When we are talking about the characteristic absorption changes of light reaction $h\nu_{II}$ (caused by Q_{II}) at 475 and $515\text{ m}\mu$ we mean always the rapid changes. As yet we do not know by what reaction the second phase is produced. It may be that it is associated with longer wavelength

¹³ C. S. FRENCH, Encyclopedia of Plant Physiology, p. 252, Springer-Verlag, Berlin 1960; C. S. FRENCH, Photobiology, p. 52, Oregon State College Corvallis, Oregon 1958; J. S. BROWN and C. S. FRENCH, Plant Physiol. **34**, 305 [1959].

¹⁴ R. MORAW and H. T. WITT, Z. physik. Chem., Neue Folge, **29**, 25 [1961].

¹⁵ H. T. WITT and R. MORAW, Z. physik. Chem., Neue Folge, **20**, 283 [1959].

* The different types of Chl-a have the same chemical constitution but differ in their absorption bands probably by different arrangements and surroundings.

chlorophyll components, since action spectra for the 515 m μ absorption change, which we measured in permanent light and in a system where the light reactions are unseparated, have a limit at longer wavelengths than the separated system. Thus RABINOWITCH¹⁶ has reported action spectra for 515 m μ

changes in Chlorella with permanent light in unseparated systems which indicates also that a long wavelength chlorophyll may be associated with the production of changes at 515 m μ .

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¹⁶ E. RABINOWITCH, The symposium on photochemical processes, Duke University 1962.

Trennung und Charakterisierung cytoplasmatischer Partikel aus normaler und atmungsdefekter Bäckerhefe

Ein Beitrag zur Frage der genetischen Kontinuität der Mitochondrien von *Saccharomyces cerevisiae*

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Um die Frage nach dem Vorliegen von Mitochondrien in der atmungsdefekten, cytoplasmatischen „petite“-Mutante von *Saccharomyces cerevisiae* zu klären, wurden aus schonend hergestellten Homogenaten normaler Bäckerhefe und der „petite“-Mutante die in der Zentrifuge zwischen 1500 und 26 300 g sedimentierenden cytoplasmatischen Partikel isoliert und im Saccharose-Dichtegradienten fraktioniert. Die erhaltenen Rohsedimente sowie die einzelnen Fraktionen wurden enzymatisch und elektronenmikroskopisch untersucht. Dichtegradienten-Gleichgewichtszentrifugierung des Sedimentes aus normaler Hefe ergab bei schonendem Homogenisieren zwei Banden, deren spezifisch leichtere (Dichte 1,19 g·cm⁻³) die Mitochondrien des Rohsedimentes in gereinigter Form enthielt. Die spezifisch schwerere Bande (Dichte 1,23 g·cm⁻³) bestand aus offenen oder geschlossenen Doppelmembranen, die eine sehr aktive, Mg²⁺-stimulierte ATPase enthielten, jedoch frei von typisch mitochondrialen und mikrosomalen Enzymen waren; diese Membranen sind vermutlich Trümmer des endoplasmatischen Retikulums der Hefezelle. Nach länger dauernder Homogenisierung der Zellen konnte noch eine dritte, meist sehr schwache, Bande beobachtet werden (Dichte 1,14 g·cm⁻³), die wahrscheinlich aus Mitochondrienfragmenten besteht. Die Auftrennung des Sedimentes der „petite“-Mutante ergab ein prinzipiell gleiches Bild. Auch bei der Mutante zeigten die Atmungsenzyme Succinatdehydrogenase und DPNH-Cytochrom-c-Reduktase eine charakteristische mitochondriale Verteilung im Gradienten (Dichte 1,19 g·cm⁻³); sie sind also an Mitochondrien gebunden. Von den getesteten Enzymen fehlte lediglich Cytochromoxydase. Die Menge der Mitochondrienfragmente sowie der spezifisch schwereren Membranfraktion (Dichte 1,23 g·cm⁻³) war meist stark vermehrt.

Der Nachweis von Mitochondrien auch in der „petite“-Mutante ist mit jenen bisherigen Vorstellungen über diese erbliche Veränderung unvereinbar, die die Abwesenheit von Mitochondrien in der Mutante voraussetzen. Die bei der „petite“-Mutation ausfallenden extrachromosomalen Faktoren mit genetischer Kontinuität können also nicht mit den Mitochondrien identisch sein.

In Hefekulturen treten spontan „petite“-Mutanten auf, die auf bestimmten Nährböden Zwergkolonien bilden¹. Die Häufigkeit ihres Auftretens liegt in der Größenordnung von 1%, kann jedoch durch Acridinfarbstoffe wie Acriflavin auf nahezu 100% erhöht werden². Nach den Untersuchungen von

EPHRUSSI, SLONIMSKI und Mitarbb.³⁻⁵ handelt es sich hier um eine irreversible Mutation, deren besondere Eigenschaften nicht nach Mendelschen Gesetzen weitervererbt werden, sondern die durch Veränderung oder Verlust eines extrachromosomalen, höchstwahrscheinlich cytoplasmatischen Faktors

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³ B. EPHRUSSI u. H. HOTTINGUER, Cold Spring Harbor Sympos. quantitat. Biol. **16**, 75 [1951].

⁴ J. TAVLITZKI, Ann. Inst. Pasteur **76**, 497 [1949].

⁵ P. P. SLONIMSKI, Ann. Inst. Pasteur **76**, 510 [1949].