Membrane Transport in Plants

Edited by
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With 252 Figures and 49 Tables

Springer-Verlag Berlin Heidelberg New York 1974

Structural Aspects of the Electrochemical Generator in Photosynthesis of Green Plants

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A. Introduction

Evidence has now accumulated that stimulation of photosynthetic electron transport leads to the generation of a proton electrochemical potential difference across the inner membrane of chloroplasts. Light causes H^+ uptake from the outer aqueous phase (NEUMANN and JAGENDORF, 1964), H^+ release into the inner phase (HAGER, 1969; RUMBERG and SIGGEL, 1969; ROTTENBERG et al., 1971) and the generation of an electric potential across the functional membrane of photosynthesis (JUNGE and WITT, 1968). The structural properties of the electrochemical generator are the subject of this communication.

B. Results and Discussion

I. The Electric Generator

The generation of an electric potential difference across the inner membrane of chloroplasts on illumination was detected via special absorption changes peaking around 515 nm which were sensitive to alteration of the electric conductance of the inner membrane of chloroplasts (JUNGE and WITT, 1968). Two lines of evidence led to the conclusion that these absorption changes reflect the electrochroic response of chloroplast bulk pigments to a high electric field strength across the membrane:
Kinetic evidence came from the fact that the decay of these absorption changes after excitation with a light flash was accelerated by changes in the membrane's electric conductivity (by osmotic shock, detergents, ionophores). This acceleration depended on the concentration of permeating ions in a chloroplast suspension; it was specific for certain ions in the presence of ionophores but unspecific with osmotic shock or detergent treatment. It was therefore concluded that the above absorption changes were sensitive to the electric charge but not to the chemical nature of ions. Since acceleration of the decay was observed when only one molecule of gramicidin (an ionophore) was added to a chloroplast suspension equivalent to 10^5 chlorophyll molecules, it was concluded that these absorption changes reflect a light-induced electric field across a functional membrane, the size of which is characterized by an ensemble of at least 10^5 chlorophyll molecules (JUNGE and WITT, 1968).

Spectroscopic evidence for the electrochroic nature of these absorption changes was provided by a comparison of the light flash induced difference spectrum of those absorption changes, having the above kinetic properties, resulting from isolated chloroplasts (EMRICH et al., 1969) with the spectrum of the electrochroic response of extracted chloroplast bulk pigments *in vitro* (SCHMIDT et al., 1971). Reasonable agreement was obtained. Similar absorption changes observed on excitation of bacterium chromatophores were attributed to electrochroic effects, too, because of the similarity between their difference spectrum and the difference spectrum obtained by submitting chromatophores to a salt jump causing a diffusion potential (JACKSON and CROFTS, 1969).

The rise of the electrochroic absorption changes in isolated chloroplasts is extremely fast. The 90 %-rise time on laser excitation is less than 20 ns (WOLFF et al., 1969), suggesting that the electric potential generation involves one of the primary photochemical reactions. The contribution of the two light reactions to the electric potential was studied by excitating with single-turnover-flashes (saturating and short compared with the relaxation time of the light reactions). The result, illustrated in Fig. 1, shows a relative value of 1 for the electrochroic absorption change at 515 nm, if both light reactions were active, but a relative value of about 1/2 if only one of them was active. Thus it was concluded that both light reactions contribute about equal amounts to the electric potential (SCHLIEPHAKE et al., 1969).

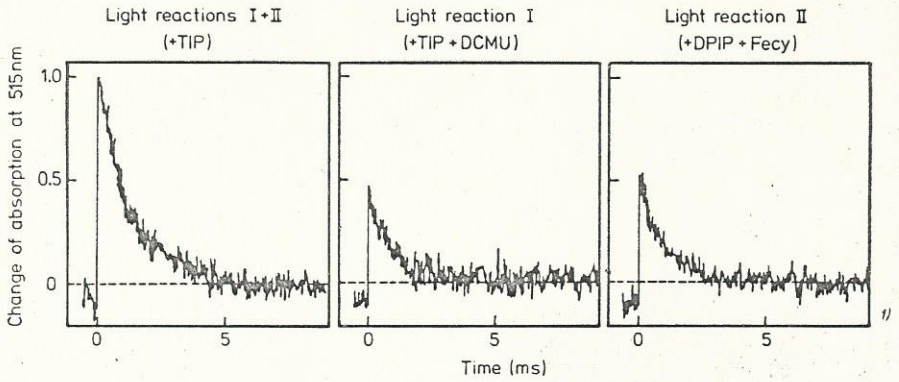

Fig. 1 Electrochroic absorption changes at 515 nm on excitation of isolated spinach chloroplasts with a short flash of light at t=0 (SCHLIEPHAKE et al., 1968).
Left: both light reactions active; middle: light reaction I active only; right: light reaction II active only. (TIP: thymolindophenol.)

II. Vectorial Electron Transport and Protolytic Reaction Sites

MITCHELL (1966) postulated that a proton electrochemical potential difference across the functional membrane of photosynthesis is generated by an alternating electron-hydrogen-transport as illustrated in Fig. 3. Light reaction II drives an electron transport from water (water-splitting system at inner side of the membrane) to the outer side of the membrane, thus causing H^+ release into the inner phase and electric potential generation. At the outer side an electron carrier (plastoquinone) becomes reduced and binds one H^+ from the outer aqueous phase. If this carrier can be oxidized at the inner side of the membrane it would act as a H^+ carrier across it. On reduction of a non-proton-binding electron acceptor at the inner side a H^+ would be released into the inner aqueous phase. Light reaction I finally drives the transport of one electron from the inner to the outer side across the membrane and so contributes to the electric potential generation as well as to H^+ binding in the outer phase as a consequence of the reduction of the terminal electron acceptor.

This model accounts for the above result that both light reactions contribute about equal amounts to the electric potential on excitation with a single-turnover-flash of light. The vectorial properties of this model are supported by the following results.
1) Electron carriers attributed to the reducing side of light reaction I have been located at the outer side of the membrane by immunological methods (BERZBORN et al., 1966; BERZBORN, 1968; REGITZ et al., 1970).

2) The rate limiting step of the electron transport between the two light reactions is sensitive to the pH of the internal phase (RUMBERG and SIGGEL, 1969).
3) Plastocyanin, an electron carrier between the light reactions seems to be loosely bound to the inner side of the membrane (HAUSKA et al., 1971).

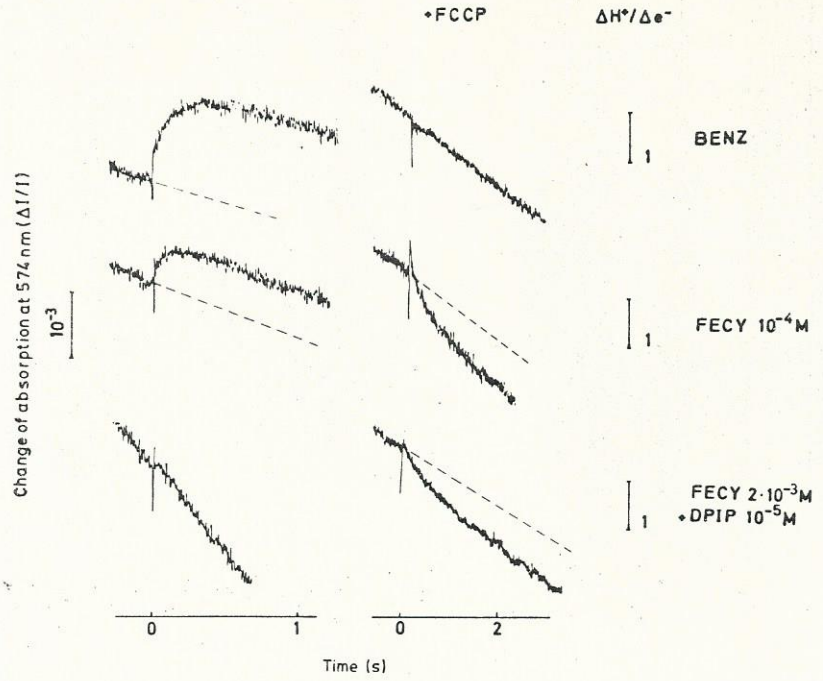

Fig. 2 pH-indicating absorption changes of cresol red at 574 nm on excitation of isolated spinach chloroplasts (broken type) with a short flash of light at t=0 (JUNGE and AUSLÄNDER, 1974).
Left: in the absence of the proton carrier FCCP; right: in the presence of the proton carrier FCCP. Different electron acceptors: BENZ: proton binding, after light reaction I; FECY (low): nonproton binding, after light reaction I; FECY + DPIP: non proton binding, after light reaction II.

The protolytic properties of the electron transport chain have been studied by excitation with single-turnover-flashes. The rather small pH-changes in the outer aqueous phase of the inner membrane system of chloroplasts were resolved by using pH-indicating dyes, the absorption changes of which, with certain precautions (JUNGE and AUSLÄNDER, 1974), are linear indicators of the number of H^+ bound or released by chloroplast inner membranes. Four different protolytic reaction sites were identified: two for H^+ binding from the outer phase (SCHLIEPHAKE et al., 1968; JUNGE and AUSLÄNDER, 1974) and two for H^+ release into the inner one (JUNGE and AUSLÄNDER, 1974). If oxygen, via benzyl viologen (BENZ), was used as terminal electron acceptor the H^+/e^- stoichiometry of each site was about one. The four protolytic reaction sites were attributed to certain redox reactions based on experiments one of which is illustrated in Fig. 2 (JUNGE and AUSLÄNDER, 1974). The traces in Fig. 2 and their interpretation (Fig. 3) will be discussed in some detail.

The traces on the left of Fig. 2 represent pH-indicating absorption changes observed on excitation of isolated chloroplasts with a short flash of light at t=0 in the presence of the dye cresol red. The extent of these absorption changes is proportional to the number of H^+

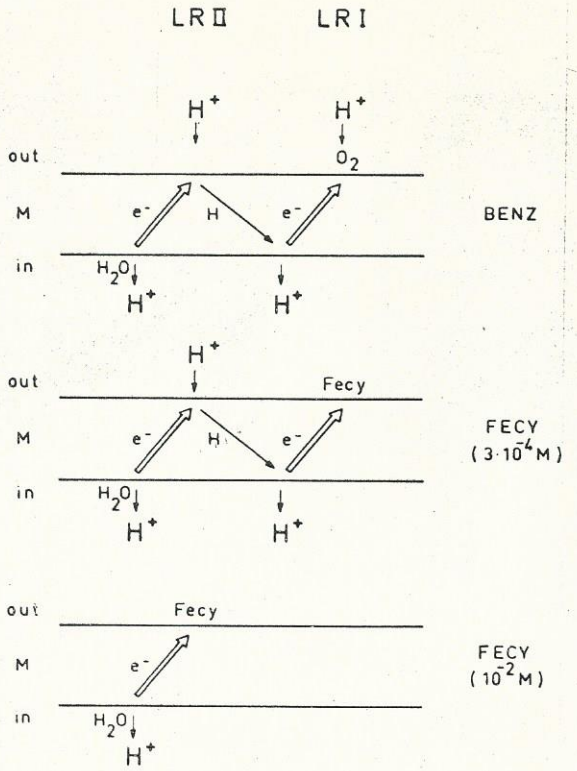

Fig. 3 Vectorial electron-hydrogen-transport scheme compatible with the experimental results depicted in Figs. 1 and 2 (JUNGE and AUSLÄNDER, 1974); see legend to Fig. 2.

bound (positive signal) in the outer aqueous phase of the inner chloroplast membrane. The bars on the right indicate the equivalent of one H^+ bound per electron transferred to the terminal acceptor. If FCCP is added to the chloroplast suspension the H^+ permeability of the functional membrane is increased and any pH-difference across the membrane relaxes within 1 s. Then the absorption changes of cresol red indicate the net H^+ production or H^+ consumption from both sides of the functional membrane (right-hand side of Fig. 2). The results depicted in Fig. 2 have been used to deduce the vectorial electron-hydrogen-transport-system illustrated in Fig. 3 (JUNGE and AUSLÄNDER, 1974). The argument being somewhat lengthy, we will only show here that the experimental results are compatible with the above model.

The upper two traces in Fig. 2 resulted if oxygen, via BENZ, was the terminal electron acceptor. The H^+ uptake from the outer phase (left) was two H^+/e^-, while the net H^+ production was zero (right), corresponding to the release of two H^+/e^- into the inner phase (see upper row in Fig. 3). The middle traces of Fig. 2 were obtained if oxygen, which binds one H^+ upon reduction, was substituted by the non-proton-binding electron acceptor ferricyanide at relatively low concentrations. Here only one H^+ was taken up from the outer phase, the net H^+ production was plus one and consequently the H^+ release into the inner phase was still two H^+/e^- (see Fig. 3, middle). If ferricyanide (FECY) in higher concentrations together with the lipophilic cofactor DPIP (2,6-dichlorophenolindophenol) was present, it accepted electrons efficiently from light reaction II thus competitively inhibiting light reaction I activity (see Fig. 3, below). Under these conditions no H^+ uptake from the

outer phase was observed but a net production of one H^+/e^- from the inner phase. These results are easily understood in terms of the model illustrated in Fig. 3, if the four protolytic reaction sites are attributed to the following redox reactions: the oxidation of water at the inner side of the functional membrane (release of 1 H^+/e^-), the reduction of plastoquinone at the outer side (binding of 1 H^+/e^-), the oxidation of plastohydroquinone at the inner side (release of 1 H^+/e^-) and the reduction of the terminal electron acceptor at the outer side (binding of $\nu H^+/e^-$) (JUNGE and AUSLÄNDER, 1974).

III. The Structure of the Functional Membrane

The above evidence for vectorial electron transport across the functional membrane stimulated studies as to the structure of the membrane across which the vectorial reactions take place. The structural models for the inner membrane of chloroplasts are conflicting, as yet. While small angle X-ray scattering studies have suggested a quasi-crystalline mosaic model – a sandwich of proteins, pigments and lipids (KREUTZ, 1970) –, electron micro-

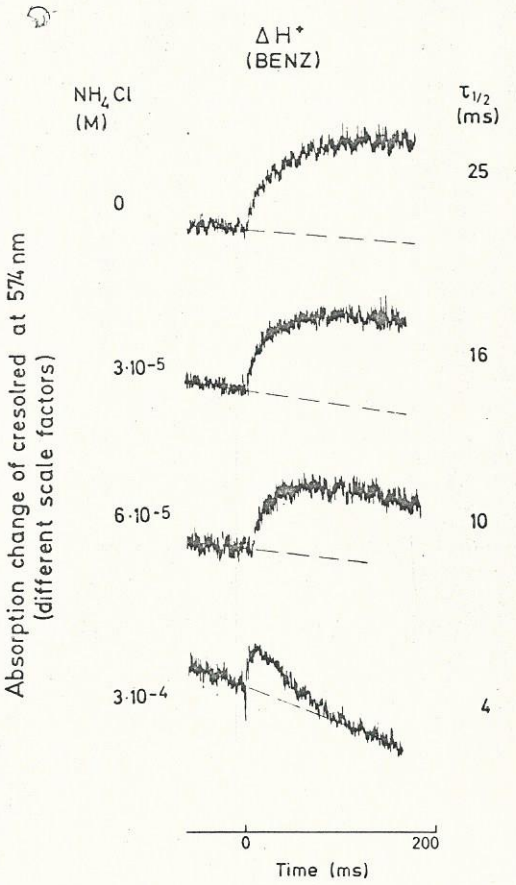

Fig. 4 Dependence of the pH-indicating absorption changes of cresol red at 574 nm on the concentration of the uncoupler NH_4Cl in chloroplasts which were fragmented by grinding with sand (AUSLÄNDER and JUNGE, 1974). An acceleration of the protolytic reaction between the reducing sites of both light reactions at the outer side of the membrane and the outer aqueous phase is obvious.

scopy suggested irregularly distributed protein 'bulbs' embedded at different depths in a lipid matrix (MÜHLETHALER, 1972).

We have chosen another approach to studying the structure of the membrane. We used the rate of H^+ uptake from the outer phase as an indication of the 'depth' of the respective redox reaction sites in the functional membrane. The two redox reactions which lead to H^+ binding in the outer aqueous phase, i.e. the reduction of plastoquinone and of the terminal acceptor, proceed in less than 1 ms. However, H^+ uptake as monitored via the absorption changes of cresol red is considerably slower (see upper left of Fig. 2). We found that mechanical (sand grinding) or chemical (digitonin treatment) disintegration of broken chloroplasts diminished the delay between redox reactions and H^+ uptake from the outer phase. The half-time of H^+ uptake shortened from 60 ms (broken chloroplasts) to 30 ms (ground chloroplasts) to 20 ms (digitonin) in the presence of BENZ (AUSLÄNDER and JUNGE, 1974). H^+ uptake was also accelerated in the presence of electron acceptors like DPIP, which can also act as uncouplers, or on addition of H^+ carriers like NH_4Cl and FCCP. The acceleration of H^+ uptake from the outer phase by NH_4Cl is illustrated in Fig. 4. The four traces show the pH-indicating absorption changes of cresol red on excitation of a suspension of ground chloroplasts with a short flash of light at t=0. The rise time of the pH-signal decreased from 25 ms (no NH_4Cl) to about 4 ms (NH_4Cl, 300 μM). At the highest concentration (lowest trace) not only H^+ binding from the outer phase, but the relaxation of the light induced pH-difference across the functional membrane was accelerated.

The observation that structural disintegration plus uncoupling agents, which can act as H^+ carriers across lipophilic barriers, reduce the delay between the redox reactions and the corresponding H^+ uptake from the outer phase, led us to postulate that these redox reaction sites are shielded from the outer phase by a diffusion barrier for H^+ (AUSLÄNDER and JUNGE, 1974). Moreover, we had to postulate the existence of a H^+ reservoir beneath this diffusion barrier providing rapid protonation of the reduced electron acceptors, while refilling by H^+ from the outer phase is considerably slower.

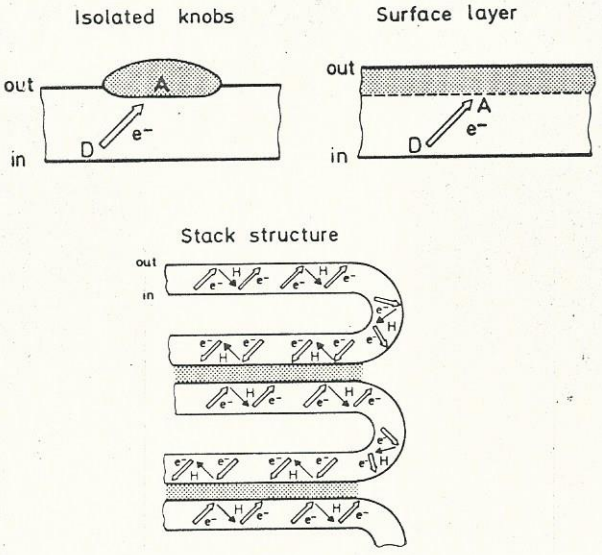

Fig. 5 Three possible structures of the diffusion barrier for H^+ which shields the redox reaction sites at the outer side of the functional membrane against the outer aqueous phase.
D: electron donor; A: electron acceptor for the primary charge translocation across the membrane; white: core layer; gray: shielding layer.

The structural and chemical nature of this diffusion barrier is still an open question. Three different structures, illustrated in Fig. 5, may be responsible for the observed delay between redox reactions and protolytic reactions. Isolated knobs covering the reducing sites for both light reactions, a continous layer covering the whole membrane, or lipophilic material filling the interthylakoid space in grana stacks are possible candidates for the H^+ diffusion barrier. No matter which of these structures is correct we must visualize the functional membrane as made up from a core layer across which light generates an electrochemical potential difference plus some shield, which delays the access of H^+ to the redox reaction sites on the outer side of the core layer. The core of the membrane and the shield are kinetically well distinguished; in broken chloroplasts (with BENZ) it takes several s for H^+ from the inner aqueous phase to leak into the outer one, while diffusion of H^+ across the shield takes only some ten ms.

IV. The Location and the Orientation of Chlorophyll a_1

It has been shown above that the vectorial electron transport crosses the core of the functional membrane but ends beneath the shielding diffusion barrier for H^+ at the outer side (see Fig. 5). Questions arise as to the location of chlorophyll a_1, which drives the vectorial electron transport of light reaction I at either side of the membrane, and to its orientation with respect to the membrane plane.

Chlorophyll a_1 is characterized by absorption changes at 700 nm (KOK, 1961), at 438 nm (RUMBERG and WITT, 1964) and by a minor component at 682 nm (DÖRING et al., 1968). It represents an aggregate of at least two chlorophyll *a* molecules interacting via excitons and charge transfer.

The location of chlorophyll a_1 can be inferred from kinetic arguments. The electric potential rises in less than 20 ns (WOLFF et al., 1969). Chlorophyll a_1 is oxidized in less than 20 ns (WITT and WOLFF, 1970), while its reduction takes some ms (WITT et al., 1963). Thus it is obvious that the oxidation of chlorophyll a_1 generates the electric potential. The polarity of the electric potential is positive inside (JUNGE and WITT, 1968). Thus we conclude that chlorophyll a_1 is located at the inner side of the membrane core.

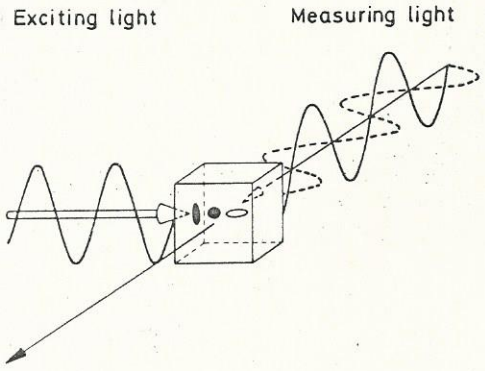

Fig. 6 The geometry in photoselection experiments.

The extremely high velocity of the electron transport across the membrane made us speculate as to the existence of highly conducting channels bridging the membrane core

which separates chlorophyll a_1 from the electron acceptors at the outer side of the membrane. Possible candidates for this bridge are special proteins (TRIBUTSCH, 1972) or possibly carotenoids or chlorophyll-aggregates with overlapping π-electron systems of their porphyrin rings. To obtain information on the architecture of the interacting chlorophyll system in the membrane we have started linear dichroism studies on the orientation of chlorophyll a_1 by photoselection (JUNGE and ECKHOF, 1973, 1974). These studies were carried out with isotropic suspensions of broken chloroplasts. The suspension was excited with a linearly polarized flash of light the E-vector of which was vertical, as illustrated in Fig. 6. Linear dichroism studies on magnetically oriented chloroplasts (GEACINTOV et al., 1971) and on chloroplasts oriented as dried films (BRETON and ROUX, 1971) have revealed that the transition moments of the antennae chlorophylls absorbing above 680 nm are almost flat in the plane of the membrane. Thus, excitation of an isotropic suspension of chloroplasts with a non-saturating polarized flash at a wavelength greater than 680 nm selects for those membrane orientations which are represented by filled circles in Fig. 6. In consequence absorption changes of chlorophyll a_1 result from the same photoselected subset, which is anisotropic. These absorption changes were observed with linearly polarized measuring light the E-vector of which was directed either parallel ($\Delta A\parallel$) or perpendicular ($\Delta A\perp$) to the E-vector of the exciting flash (Fig. 6). We observed different extents of the absorption changes of chlorophyll a_1 depending on the polarization of the measuring beam. Dichroic ratios ($\Delta A\parallel/\Delta A\perp$) greater than 1.15 were observed for both major bands of chlorophyll a_1 at 700 and 430 nm, respectively (JUNGE and ECKHOF, 1973, 1974). The dichroism of the absorption change at 705 nm is documented in Fig. 7. These dichroic ratios were interpreted on the assumption of a circular degeneration of the antennae system by multiple resonance energy transfers in the plane of the membrane (JUNGE and ECKHOF, 1974). We concluded that both major transition moments of chlorophyll a_1 are inclined at less than 25° to the plane of the membrane.

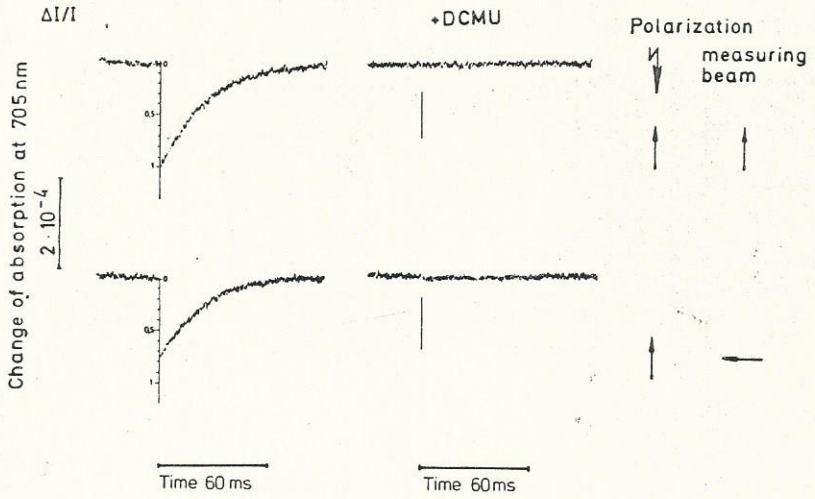

Fig. 7 Linear dichroism of the flash induced absorption changes of chlorophyll a_1 at 705 nm by photoselection (JUNGE and ECKHOF, 1974). Above: exciting beam and measuring beam polarized in parallel; below: exciting beam and measuring beam polarized perpendicularly; right: demonstration of the absence of polarization artifacts under conditions where photosynthetic electron transport is poisoned.

In order to make conclusions about the orientation of the porphyrin rings of chlorophyll a_1 to the membrane, information on the orientation of the transition moments in the

coordinate system of the dimer is required. In a chlorophyll-monomer the transition moments of the blue and the red band lie approximately perpendicular to each other in the plane of the porphyrin ring (GOUTERMAN et al., 1963). Information on the geometry of the chlorophyll a_1-dimer can be inferred from the double band at 682 and 700 nm (DÖRING et al., 1968). The intensity ratio of these bands is about 1 to 4. This has been attributed to exciton interaction in the dimer. According to KASHA's treatment of dimers under exciton interaction (KASHA, 1963) the above intensity ratio implies that the red transition moments of the two chlorophyll *a*-components of the dimer are inclined at less than 14° to the resultant transition moment of the dimer at 700 nm. This points to a rather flat structure of the dimer.

If the transition moments of chlorophyll a_1 at 430 nm and 700 nm are more-or-less parallel to the corresponding transition moments of the two monomers then their inclination at less than 25° to the membrane implies an inclination of less than 37° of the porphyrin rings to the membrane (JUNGE and ECKHOF, 1974). Since experimental imperfections in photoselection experiments tend to decrease the apparent dichroism, 37° is an upper limit. Thus a rather flat orientation of the porphyrin rings of chlorophyll a_1 at the inner side of the membrane is probable.

The red absorption bands of chlorophyll a_1 and of its antennae pigments are shifted towards the red compared to the absorption band of chlorophyll *a* in organic solvents. This can be understood by exciton interaction of the respective transition moments in a head-to-tail arrangement of the porphyrin rings (HOCHSTRASSER and KASHA, 1964). Since the red transition moment of chlorophyll a_1 lies rather flat in the membrane, a head-to-tail lining up with other chlorophyll *a* molecules will not likely bridge the membrane. Instead, some proteinaceous structure or carotenoids might be responsible for electron conduction between excited chlorophyll a_1 at the inner side of the membrane and its electron acceptor at the outer one.

C. Acknowledgements

This work was financially supported by the Deutsche Forschungsgemeinschaft.

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Round Table Discussion 5

Chairman: P.S. Nobel

VREDENBERG opened by saying that he thought we had reached a situation where we can measure the primary ionic events in intact chloroplasts by means of potential changes occuring at the enclosing membrane. He had a microelectrode inside the chloroplast with its tip probably in the stroma. A saturating light flash (rise time 5 μs, half width 2 ms) causes a positive going potential, of a few mV, across the chloroplast outer membrane with a rise time of the order of 0.5 ms. If the flash is given in the presence of continuous (717 nm) background illumination, the rate constant of the pseudo-first-order kinetics of the voltage response does not change but its amplitude depends on the intensity of the background illumination. (6.5, 13 and 29 kerg cm^{-2} s^{-1} were used.) VREDENBERG interprets these results according to the scheme of Grünhagen and Witt, in which the primary electron acceptors (X and Q) are at the stroma-facing side of the thylakoid membrane. The initial step in photosynthesis is charge separation followed by protonation of the electron acceptors to, say, XH and QH. For charge balance reasons these sites will then bind a negative ion, say OH^-, and it can be argued that this ion-binding process proceeds according to first-order kinetics. VREDENBERG suggests that this binding of a negative charge at the stroma face of the thylakoids causes a polarization of the chloroplast envelope membrane, which he is in fact measuring with his electrode. If this is so then he is measuring the kinetics of this binding and something proportional to the number of binding, i.e. electron acceptor, sites. Such an interpretation explains his results in the continuous background illumination, for such illumination will cause some of the sites to be already occupied and hence the voltage response, but not its kinetics, will be reduced.

He has also observed that, in the presence of DCMU, the potential response of a second flash, fired 250 ms after the first, is about one half of that of the first one. This is consistent with the experiments of Schliephake et al. (1968). The sites occupied by the primary acceptor Q of system II will be already in the reduced state, because of the presence of DCMU, when the second flash is fired. (If DCMU is not present the second response is equal to the first.) He thus thinks that we have an additional method of studying ionic events in the thylakoids associated with the primary events of photosynthesis, and in an intact system.

WALKER raised technical objections to the method, for he thought the electrode tip might well be among punctured thylakoids and he was worried about the sealing of the micro-electrode shaft with the chloroplast envelope. BARBER thought well of VREDENBERG's model, even if he also appeared somewhat dubious about the technique. JAFFE was puzzled that mere redistributions of charges at the surface of a thylakoid could cause potential changes across the chloroplast envelope. VREDENBERG and JUNGE tried to persuade him that it was an induction effect, essentially, that did not violate the laws of physics.

JUNGE then made some comments on the two conflicting schemes for electrochemical potential generation in photosynthesis presented by HOPE and himself. HOPE's scheme predicts an H^+/e^- ratio of 1, whereas JUNGE's scheme predicts that H^+/e^- equals 2. JUNGE then marshalled a lot of evidence in favour of his scheme. He argued, on the basis of the electrochroic absorption changes at 515 nm under various conditions, that both light reactions contribute equal amounts to the generated electric potential across the thylakoid membrane. Using cresol red as a pH indicator in the outer phase, and by means of the

different properties of benzylviologen and ferricyanide as terminal electron acceptors, and by adding FCCP when he wanted to study proton production in the inner phase, he claims to have conclusively demonstrated that $2H^+$ are produced per e^- transferred. HOPE, in reply, was somewhat sceptical about the use of P_{515} as a voltmeter measuring the PD across the thylakoid membrane; and he pointed out that the $2H^+/e^-$ ratio is found from work done with flashes and not with continuous illumination and this may reflect the differences between them. There was some argument between HOPE and JUNGE about whether the pH decay, in the ms region, after switching off continuous light was biphasic or not. JUNGE said it was and that their studies gave evidence of a diffusion potential after switching off the light, which could be influenced by changing the ratio of proton to potassium permeability of the membrane. P_{515} changes supported these results.

BARBER drew attention to the actual dimensions of the thylakoids; they are about 5000 Å in diameter and only 200 Å thick. They thus have a remarkable amount of surface area and not much volume. What do permeability, electrical neutrality, etc. mean in this kind of system? How can they maintain gradients across such large surface areas with such small volumes? JUNGE said that a lot of protons, about 20 000 per thylakoid, were accumulated in the steady state; but the inner phase had a large buffer capacity and most of these protons are buffered by the polar end groups of the lipid in the inner phase. HOPE, SMITH, and JUNGE argued for a while about the meaning of pH when only a few protons are swimming about in the inner phase volume. And SMITH also stressed the importance of BARBER's worries about the large surface area and small inner volume, particularly with respect to rates of processes across such a membrane system.

In answer to a question by BEN-AMOTZ on the influence of the outer and inner pH's on the light reactions and on whether there was any meaning to pH in the membrane itself, JUNGE spoke of the evidence for a shielding layer covering the reducing sites of the light reaction. In consequence, the pH beneath the shielding layer in the membrane will differ from the pH in the outer aqueous phase if the electron transport chain is active.

Finally KRAUSE asked: what is the counter ion in H^+ uptake? Previously HOPE had said it might be chloride, but KRAUSE believed that a more likely candidate was Mg^{2+}, for this exchange for H^+ would involve little volume change. In fact it is hard to imagine Cl^- being taken up for the thylakoid would swell; in fact, in intact systems or whole cells, the thylakoids shrink on illumination. NOBEL agreed with KRAUSE on the likelihood of Mg^{2+} (and Ca^{2+}) coming out in exchange for H^+ and suggested that more attention should be paid to this process.

The following remarks were communicated by HOPE and JUNGE. In response to a comment by JUNGE on the slowness of the response of a glass electrode which would thus underestimate the ΔH^+ signal (and thus the H^+/e^- ratio is really 2 and not 1), HOPE replied that although the response time of his glass electrode was about 0.6 s, the decay of ΔH^+ signal is approximately exponential with a decay half-time of 10 to 20 s. The speed of response does not appear to be an issue for Schröder et al. (1971) found H^+/e^- to be about 1 using fast responding cresol red to measure ΔH^+; ratios of 2 were only found when valinomycin was added. HOPE said that no doubt one day these integer values will be plausibly interpreted; perhaps, as suggested by JUNGE and others, the electrogenic effect of the H^+ influx is eliminated essentially instantaneously when the light is switched off and the driving force for passive H^+ efflux is then different from its value in the light; but this depends on the interpretation of the P_{515} changes which are controversial estimates of the PD.

JUNGE communicated the following reply.

It has been demonstrated under light flash excitation that $2H^+$ are translocated across the thylakoid membrane per electron transferred through the electron transport chain (Schliep-

hake et al., 1968; Junge and Ausländer, 1974; Junge et al., 1974). One may argue that the H^+/e^- ratio is altered under steady illumination of photosynthetic membranes. Under these conditions the proton translocation is usually measured with glass electrodes as in HOPE's experiments. A glass electrode is a rather slowly responding device. It does not follow processes as rapid as the breakdown of the electric potential across the functional membrane of photosynthesis, which under flash excitation takes about 100 ms (Junge and Witt, 1968) and becomes even faster when continuous light is switched off (Boeck and Witt, 1971). Thus a glass electrode does not get information on the rate of proton efflux from thylakoids a few milliseconds after switching off continuous light, but at best it detects the rate 0.5 s later. Since the electric potential changes during this interval, especially if the proton permeability dominates the total conductivity of the membrane, the driving force for the proton efflux is altered, too, and hence the flux rate. The polarity of the electric potential difference being positive inside (Junge and Witt, 1968) the rate of efflux slows down after the breakdown of the electric potential which follows the switching off of continuous light. Thus glass electrode measurements necessarily underestimate the steady state H^+/e^- ratio. This argument was first stressed by Schröder et al. (1971) in order to explain their finding of an $H^+/e^- = 1$ at pH 8 in the absence, and a ratio $H^+/e^- = 2$ in the presence, of the K^+ permeability increasing valinomycin. In the latter situation the electric potential persists on switching off the light as a diffusion potential until the H^+ and K^+ concentration gradients have collapsed.

A similar argument holds even if instead of a glass electrode a pH-indicating dye is used. As evident from the contribution of Junge et al. (1974) even dyes are 'slow' indicators of pH changes in the outer phase, due to the existence of a permeability barrier for protons at the outer side of the membrane covering the core across which the electric potential exists.

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