

Investigations on a Galvanic Cell Driven by Photosynthetic Electron Transport

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A light-driven galvanic cell was constructed making use of the photosynthetic activity of isolated chloroplasts. Artificial mediators managed the transfer of electrons from the endogenous electron carriers to the platinum electrodes in each of the joined half-cells. In one the mediators were reduced by electrons originating from water. In the other the mediators were oxidized by photosystem I in the presence of an autoxidizable electron acceptor. The redox potential in the single half-cells has been studied as a function of the lipophilicity of the mediators and their concentration. Furthermore different autoxidizable acceptors and different treatments of the chloroplasts were investigated. The combined half-cells were separated by an ultrafiltration membrane. Upon illumination the system gave rise to an open circuit potential of up to 220 mV. This battery was charged with rates as high as photosynthetic electron transport rates. The results are discussed with respect to the arrangement of the cell and the properties of the components for high effectiveness and maximal potential differences.

Introduction

The challenge to realize an electrochemical device based on the photochemical charge separation in biological systems gave rise for a large number of proposals in recent years. One often used principle was to deposit the excitable system on electrodes [1–7]. Pure chlorophyll [2–4], mixtures of chlorophyll and organic redox compounds [5] or reaction centers of photosynthetic bacteria [6] have been used. The light-induced potential generated by films of thylakoid membranes was studied by an externally applied voltage [7]. Another principle of energy-converting devices was based on lipid membranes fused with the biological system. Bacteriorhodopsin-containing vesicles [8–11], photosynthetic reaction centers [12] or chloroplast lamella extracts [13] have been used for fusion. Lipid-impregnated Millipore filters increased the stability of these systems. But the current was still rather low [10, 11].

In photosynthesis of higher plants each of the two light reactions results in an electron flow of approximately 1.1 V against the thermodynamic gradient. The overall net change in redox potential for the cooperative interaction of the two light reac-

tions summarizes to about 1.6 V. It seems attractive to utilize these potential differences by an external system. Because redox couples in the thylakoid membrane do not react significantly with a metal electrode, electrical equilibrium has to be established by redox mediators [14, 15]. It is of advantage that many artificial electron donors and acceptors for electron carriers of photosynthetic electron transport are well known as mediators [16]. Extensive studies of these artificial redox compounds allowed to group them into electron donors and electron acceptors for photosystem I or photosystem II, respectively (for recent reviews see refs. [16] and [17]).

A galvanic cell driven by photosynthetic electron transport can be constructed if in one half-cell a mediator M is reduced by electrons originating from water ($M_{ox} + H_2O \xrightarrow{\text{light}} M_{red} + \frac{1}{2} O_2 + 2 H^+$) and in the other half-cell a mediator is oxidized by electron transfer to oxygen ($M_{red} + \frac{1}{2} O_2 + 2 H^+ \xrightarrow{\text{light}} M_{ox} + H_2O$). Provided the diffusion of protons through the liquid junction the net result is a potential gradient driving a current through the external circuit.

In this study we report investigations on a possible realization of these reactions utilizing the potential difference between the endogenous donor and acceptor of light reaction I. DAD, DCPIP or DCPIP sulfonate were used as mediators in both half-cells. They function as artificial electron acceptors [18–22] and as artificial electron donors of light reaction I [20, 22–25] in chloroplasts. The

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Abbreviations: AQ, sulfonate, 9,10-anthraquinone-2-sulfonate; DAD, 2,3,5,6-tetramethyl-p-phenylenediamine; DCPIP, 2,6-dichlorophenolindophenol; DCPIP sulfonate, 2,6-dichlorophenolindophenol-3'-sulfonate; E_h , potential *versus* NHE; NHE, Normal Hydrogen Electrode.



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combined half-cells were separated by an ultrafiltration membrane which enables exchange of protons and ionic conductance but prevents irreversible changes by diffusion of larger components.

Materials and Methods

Chloroplasts and reagents. Broken chloroplasts were prepared from green house grown spinach as described by Nelson *et al.* [26]. Heat treatment of these chloroplasts was for 5 min at 52 °C. Digitonin particles of chloroplasts were obtained according to the method of Anderson and Boardman [27] by incubation of chloroplasts at a chlorophyll concentration of 1 mg/ml with 1% digitonin for 1 h at 0 °C. The photosystem I fraction was collected after centrifugation at 30,000 × *g* for 1/2 h by centrifugation at 250,000 × *g* for 2 h. Ferredoxin was prepared from spinach similar to the procedure of Shin *et al.* [28]. Plastocyanin was prepared from isolated spinach chloroplasts as described by Selman *et al.* [29].

Preparation of 9,10-anthraquinone-2-carboxylic ester of polyethylene glycol: 9,10-anthraquinone-2-carboxylic acid chloride was prepared according to Mitter and Bauerjee [30] from 9,10-anthraquinone-2-carboxylic acid and SOCl₂. 1.4 g of the acid chloride and 0.58 g of polyethylene glycol (type 1550 from Serva) with a range of the molecular weight from 1300 to 1600 g/mol were stirred at room temperature for 12 h in 100 ml CH₂Cl₂. After filtration the volume of the reaction mixture was reduced to about 10 ml by evaporation in *vacuo*. The ester was separated from excess acid chloride by ascending chromatography on a column of Sephadex LH 20 in CH₂Cl₂. From the collected fractions of the ester the solvent was removed in *vacuo*. Assuming that the extinction coefficients in ethanol at 323 and 255 nm for this ester of 9,10-anthraquinone-2-carboxylic acid are comparable to those of the free acid and the acid chloride we estimated an average of 1.3 bound acid molecules per molecule of polyethylene glycol.

The preparation of 2,6-dichlorophenolindophenol-3'-sulfonate has been reported by Hauska *et al.* [22]. Quinhydrone was obtained from Merck and recrystallized twice from water at 70 °C.

Potential measurements. Potentials given *vs.* NHE were measured with a combined redox electrode Pt 65 from Schott constructed with a platinum wire and a 3.5 M KCl calomel electrode. The measured

potentials were related to that found with a saturating quinhydrone solution in 0.01 M HCl plus 0.09 M KCl of 575.4 mV at 25 °C *vs.* NHE [31]. For the studies of the light-induced redox gradient two 1 × 1 cm cuvettes as half-cells, each with a hole in one side, were combined. The facing holes with an ultrafiltration membrane UM 05 from Amicon in between were sealed with O-rings. The membrane excludes molecules of weights larger than ca. 500 g/mol. Two electrodes (P101) from Radiometer with shiny platinum flags, about 0.6 cm² in area, were used. All platinum electrodes were cleaned by anodic oxidation in 2 n HCl and rinsed extensively with distilled water before use. The potentials were measured with a PHM 64 research pH meter from Radiometer and traced with a 7047 A recorder of Hewlett Packard.

Experimental conditions. The reaction mixture contained suspended chloroplasts at a chlorophyll concentration of 20 µg/ml, 20 mM N-Tris(hydroxymethyl)methylglycine adjusted to pH 7.6 with NaOH, 20 mM KCl, 70 mM NaCl, 3 mM MgCl₂, and 3 mM NH₄Cl as uncoupler. Further additions are given in the legends of the figures. The total volume was 6 ml during the measurement with the combined redox electrode and 1.5 ml in each of the two half-cells. All measurements were carried out aerobically under vigorous stirring of the solutions. The temperature was kept constant at 25 °C through a circulating water bath. The samples were illuminated with red light of saturating intensity of 800 W·m⁻². It passed previously through 7 cm of water, a heat reflecting filter Calflex C from Balzers and a Schott filter OG 570.

Results

In favour of a large electromotive force of the anticipated galvanic cell one of the half-cells should reach a minimum and the other a maximum oxidation reduction potential in the light. In addition the electron transfer rates from the chloroplasts to the mediator should be as high as possible. The aim of the following experiments was to investigate these demands. High electron transfer rates are known for the Hill reaction as well as for the oxidation of mediators by light reaction I ([18–25], for a review cf. ref. [16]). Fig. 1 shows a possible use of these reactions for a galvanic cell. Each of the half-cells was investigated separately.

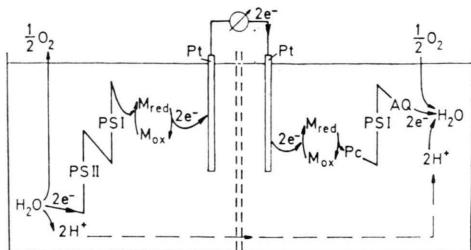


Fig. 1. Arrangement for the generation of a redox potential gradient between two half-cells by illumination of chloroplasts. In the left-hand half-cell the oxidized mediator M_{ox} is reduced as Hill acceptor. In the right-hand half-cell the activity of Photosystem I is used to oxidize the reduced mediator M_{red} at simultaneous reduction of oxygen *via* an autoxidizable electron acceptor and catalase to yield H_2O . For details see text. Electron transport chain symbolized by Z-scheme.

Abbreviations: AQ, anthraquinone derivate, stands for an autoxidizable electron acceptor; PS, Photosystem; PC, plastocyanin.

Decrease of the oxidation reduction potential during the reduction of mediators

In the left-hand half-cell the Hill reaction should be used for the reduction of oxidized mediators. Indophenols [18] and *p*-phenylenediamines [19, 20] may be suitable compounds in contrast to viologens [32] which are immediately reoxidized by oxygen. Fig. 2 shows the light-induced decrease of the redox potential of oxidized DAD, DCPIP and DCPIP sulfonate, respectively, at several concentrations. The final value reached during illumination decreased when the mediator concentration was increased. During the subsequent dark period the

redox potential remained almost constant. This indicated a negligible autoxidation of the reduced mediators. If the dark period was extended over several minutes the redox potential increased only a few millivolts and the blue colour of the oxidized indophenols returned.

The electron transfer rates can roughly be estimated if the concentration of the reduced mediator c_{red} is calculated from the redox potential E (mV) by the modified Nernst equation $c_{\text{red}} = c_0 / (1 + \text{EXP}[(E - E_m) \cdot 2.3 / 29.6 \text{ mV}])$. c_0 is the total concentration. The half-reduction potential E_m (mV) at our experimental conditions is indicated by the first point of inflection of the transients, *e.g.* in Fig. 6, if a constant rate in this concentration range is presumed. The estimated values are 250, 235 and 260 mV for DAD, DCPIP and DCPIP sulfonate, respectively. Lower values of the half-reduction potential [14, 15, 22] do not remarkably change the results. During the reduction of the first three quarters of the oxidized mediators the rates were approx. constant. Both indophenols exhibited reduction rates of about 700 $\mu\text{equiv.}/\text{mg chlorophyll/h}$ and showed lower values at a concentration of 200 μM . The reduction rate of oxidized DAD increased at increasing concentrations up to 1000 $\mu\text{equiv.}/\text{mg chlorophyll/h}$ at 200 μM . The initial delay of the potential decrease (Fig. 2, left) was due to a reduction of remaining ferricyanide which was added to oxidize DAD immediately before the measurement. The rates are similar to those found with excess of ferricyanide [21, 22].

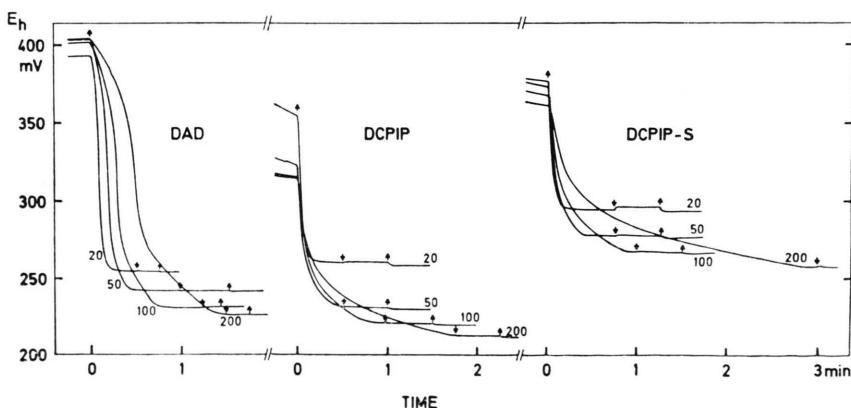


Fig. 2. Time course of the redox potential during the reduction of DAD (left), DCPIP (middle) and DCPIP sulfonate (right) by chloroplasts. At $t = 0$ light was switched on; upward open arrow indicates light on; downward full arrow indicates light off. The figures at the transients give the concentration of 20, 50, 100 and 200 μM of the mediators present during the measurement of the transient, respectively. DAD was oxidized immediately before the onset of illumination by an equivalent amount of ferricyanide. Chlorophyll content of chloroplasts was 22 $\mu\text{g}/\text{ml}$.

Increase of the oxidation reduction potential during the oxidation of mediators

In the right-hand half-cell of Fig. 1 with no active photosystem II electrons from a reduced mediator should be transferred to oxygen via photosystem I and an extremely autoxidizable electron acceptor as AQ sulfonate [33]. Efficient electron donors for photosystem I are DCPIPH₂ and DAD [19, 20, 23–25]. Not only the type and concentration of the added redox compounds have to be optimized for this half-cell but also the inactivation of photosystem II.

Fig. 3 shows the influence of inactivation of photosystem II by heat-treatment [34] on the time course of the redox potential of DAD. Addition of active (Fig. 3 A) or heat-treated chloroplasts (Fig. 3 B) showed no effect on reduced DAD as well as the first illumination period. After oxidation of DAD by an equivalent amount of ferricyanide a subsequent illumination period induced the rapid potential decrease in the presence of active chloroplasts described above. The constant potential observed with heat-treated chloroplasts indicated a lack of reduction due to a complete deactivation of the

water splitting system [34]. Therefore this sample was replaced by a fresh one. During a third illumination period the addition of autoxidizable AQ sulfonate induced an immediate oxidation of DAD. In contrast to the potential increase of approx. 200 mV induced by heat-treated chloroplasts untreated chloroplasts induced a potential increase of only 50 mV. Experiments with DCPIP showed similar traces. It is obvious that the inactivation of photosystem II was necessary for a generation of a high redox potential. The inactivation by heat-treatment could inactivate also the electron transfer from plastocyanin to P700 [35]. This would reduce the oxidation rate of the mediators [36, 37]. The used procedure was varied with respect to time and temperature and was found to be most effective for high rates and high redox potentials. Incubation of the chloroplasts with tris buffer or hydroxylamine, carried out as described in refs. [38] and [39], respectively, showed lower values.

Fig. 3 B indicates also the necessity of an autoxidizable electron acceptor for sufficient oxidation rates. In Fig. 4 the oxidation of DAD catalysed by AQ sulfonate is contrasted with that catalysed by an ester of anthraquinone carboxylic acid (AQ ester), methylviologen and ferredoxin. The rate mediated by methylviologen or benzylviologen (not shown) amounted approx. 40 per cent and by the AQ ester 70 per cent of that by AQ sulfonate. The oxidation with ferredoxin was negligible. A similar relation between the mediated oxidation rates was found with photosystem I particles and exogenous plastocyanin. Up to a concentration of 0.4 mM AQ sulfonate the oxidation rate of 200 μ M DAD increased and was almost the same at 0.7 and 1 mM (experiments not shown). With the exception of ferredoxin the potential increased to a maximal value around 440 mV.

The formation of H₂O from the superoxide anion radical O₂^{·-}, the primary product of autoxidation of AQ sulfonate [40], was accelerated by addition of 10 units of superoxide dismutase and 500 units of catalase. However, no changes in the potential increase were noted which could be expected *e.g.* from a cyclic reaction of O₂^{·-} with plastocyanin [41].

In favour of a maximal potential gradient in the cell the autoxidizable electron acceptor has to be excluded from the half-cell with reduction of the mediator by intact chloroplasts. This is concluded

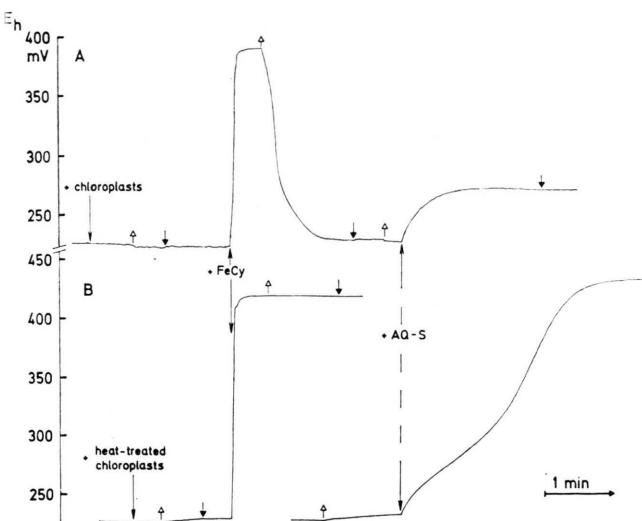


Fig. 3. The oxidation reduction potential of 2×10^{-4} M DAD as a function of time. Above: addition of broken chloroplasts. Below: addition of heat-treated chloroplasts. The starting conditions were the same for both traces. The amount of ferricyanide (FeCy) added immediately before the onset of the second illumination gave a final concentration of 4×10^{-4} M and that of 9,10-anthraquinone-2-sulfonate (AQ-S) 4×10^{-4} M. Chlorophyll content of chloroplasts was 23 μ g/ml. Upward arrow with open triangle indicates light on, downward arrow with full triangle light off.

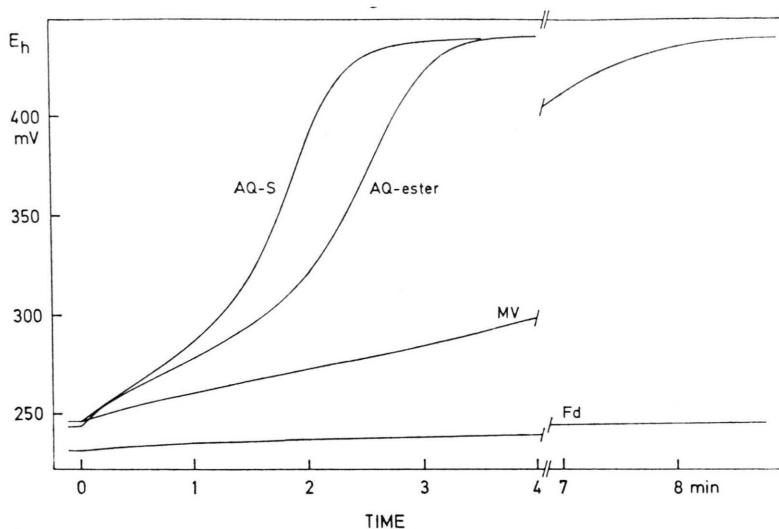
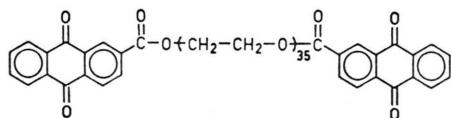


Fig. 4. Time course of the redox potential during the oxidation of 10^{-4} M DAD by heat-treated chloroplasts in the presence of different electron acceptors. The concentration was 4×10^{-4} M of AQ sulfonate (AQ-S), methylviologene (MV) and 9,10-anthraquinone-2-carboxylic ester of polyethylene glycol (AQ-ester, formula see text) on the basis of anthraquinone, respectively, and 10^{-4} M of ferredoxin (Fd). At $t=0$ light was switched on.

from the partial reoxidation of DAD after the addition of AQ sulfonate in Fig. 3 A. The ultrafiltration membrane used between the two half-cells excluded molecules of a molecular weight greater than 500 g/mol. With respect to the optimal function of AQ sulfonate we synthesized an ester of 9,10-anthraquinone-2-carboxylic acid of polyethylene glycol of approx. 2,000 g/mol:



The trace in Fig. 4 shows the fast potential increase mediated by this water-soluble ester.

In the following experiments the dependence of the potential increase on the type of the mediator

and its concentration was investigated. Fig. 5 shows the time courses in the presence of heat-treated chloroplasts and 0.4 mM AQ sulfonate. A final potential of 445–450 mV was reached with DAD and DCPIP almost independently of the concentration. The oxidation of the hydrophilic DCPIP sulfonate was negligible, in agreement with the finding of Hauska *et al.* [22]. The oxidation rates estimated as described above showed maximal values of 900 μ equiv./mg chlorophyll/h at 200 μ M DAD and approx. 700 μ equiv./mg chlorophyll/h at 100 μ M DCPIP H_2 during the beginning of the illumination period. At potentials above the half-reduction potential the oxidation rates decreased. With increasing concentrations from 20 to 100 μ M the oxidation rates of DAD and DCPIP H_2 increased in parallel. At a concentration of 200 μ M the oxidation rate of

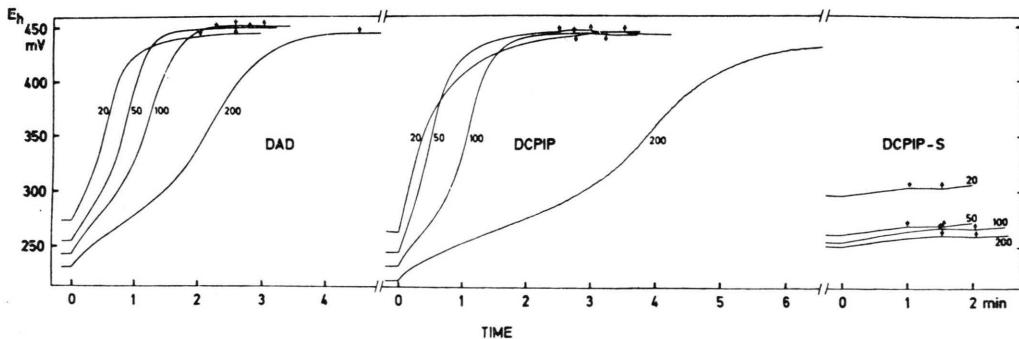


Fig. 5. Potential increase during the oxidation of mediators by heat-treated chloroplasts. The indophenols were reduced by an equivalent amount of ascorbate added immediately before the onset of illumination at the time $t=0$. The standard reaction mixture additionally contained 0.4 μ M AQ sulfonate and as mediator DAD (left), DCPIP (middle) or DCPIP sulfonate (right). Figures and arrows at the transients as in Fig. 2.

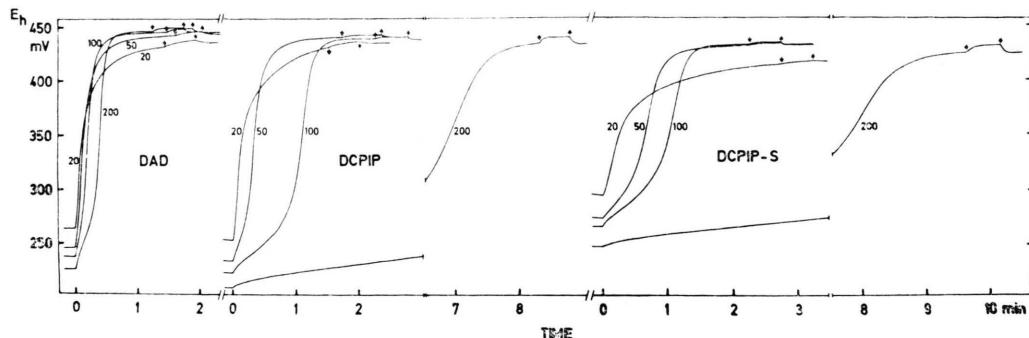


Fig. 6. Potential increase during the oxidation of mediators by photosystem I particles in the presence of $2 \mu\text{M}$ plastocyanin. The conditions were the same as in the measurements shown in Fig. 5 except that the chlorophyll content of the particles was $14.4 \mu\text{g}/\text{ml}$.

DAD showed a further increase, that of DCPIPH_2 dropped to less than 50 per cent of the maximal value. The reason for this decrease may be absorption of the exciting light by DCPIP.

Another possibility to oxidize DAD and the indophenols is by photosystem I particles in the presence of externally added plastocyanin. The results are shown in Fig. 6. Common to all mediators was the maximal final potential of $440 - 450 \text{ mV}$. The oxidation of DCPIPH_2 sulfonate showed a similar dependence on the concentration as the lipophilic DCPIPH_2 but slightly lower rates. Compared to the results in Fig. 5 the oxidation rates of DCPIPH_2 exhibited almost the same values whereas those of DAD were at least fourtimes higher. The maximal value was $3,300 \mu\text{equiv./mg chlorophyll/h}$ at $200 \mu\text{M}$ DAD. The concentration of plastocyanin was

not saturating in agreement with the results of Wood and Bendall [41]. However, this system was the most efficient for oxidation of a mediator.

Light induced generation of a redox potential difference

The experiments reported above were carried out with a reference calomel electrode. This allowed to judge the properties of the single half-cells. In the following experiments the half-cells were combined according to the scheme in Fig. 1. The initial concentration of oxidized and reduced mediator was the same in both half-cells. Electrical contact was enabled through an ultrafiltration membrane. Fig. 7 shows the time-course of the light-induced open circuit potential in the presence of DAD and DCPIP, respectively, between a half-cell with intact chloro-

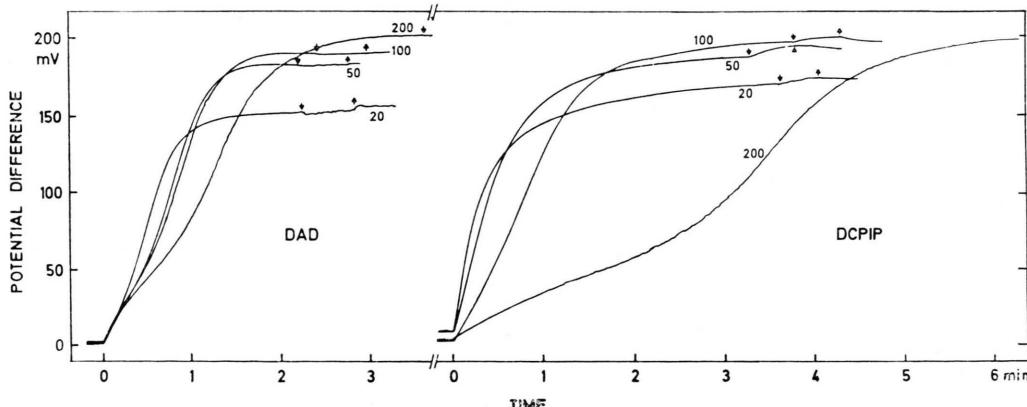


Fig. 7. Time courses of the light-induced redox potential difference of DAD (left) and DCPIP (right) between a half-cell containing heat-treated chloroplasts and 0.4 mM AQ sulfonate and a half-cell containing broken chloroplasts. Chlorophyll content of chloroplasts was $21 \mu\text{g}/\text{ml}$. The indophenol was reduced in both compartments with half the equivalent amount of sodium ascorbate added before illumination. Figures and arrows at the transients as in Fig. 2.

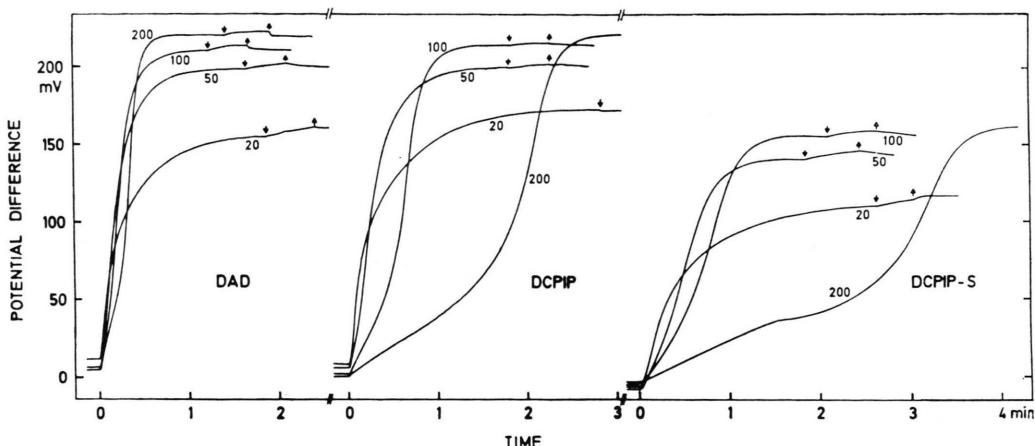


Fig. 8. Time courses of the light-induced redox potential difference of DAD (left), DCPIP (middle) and DCPIP sulfonate (right) between a half-cell containing photosystem I particles at a chlorophyll concentration of 17 $\mu\text{g}/\text{ml}$, 2 μM plastocyanin and 0.4 mM AQ sulfonate and a half-cell containing broken chloroplasts at a chlorophyll concentration of 20 $\mu\text{g}/\text{ml}$. For further details see Fig. 7.

plasts and another containing these chloroplasts after heat-treatment and 0.4 mM AQ sulfonate. DCPIP sulfonate was not investigated with respect to the negligible oxidation by heat-treated chloroplasts (*cf.* Fig. 5). The final potential difference increased with increasing concentrations of the mediators to a maximal value of 200 mV. The slow increase of the potential difference at 200 μM DCPIP is consistent with the slow oxidation observed at this concentration in Fig. 5.

For the measurements shown in Fig. 8 the half-cell with intact chloroplasts was combined with a half-cell containing photosystem I particles, soluble plastocyanin and AQ sulfonate (*cf.* Fig. 6). As in Fig. 7 the final potential difference increased with increasing concentrations. The maximal value of 220 mV was reached at a concentration of 200 μM of DAD and DCPIP after 0.5 and 2.5 min, respectively. DCPIP sulfonate was less advantageous than DCPIP.

In the dark after illumination the potential differences shown in Figs. 7 and 8 did not decrease even after several minutes. This indicates negligible losses of the light induced redox gradients. Losses would be possible by equilibration of the oxidized and reduced mediator through the junction and by autoxidation of the reduced mediator.

Discussion

The conversion of the light-induced redox gradient of photosynthetic electron carriers to a gra-

dient of an artificial oxidation reduction couple was investigated. Criteria for a suitable oxidation reduction couple are besides negligible light absorption at the absorbance bands of chloroplasts and no autoxidation of the reduced form (see above), stability of its oxidized and reduced form, effective interaction with the electron transport chain and fast equilibration with electrodes. The electron transfer from the biological to the mediator system has been mostly investigated in the presence of excess ascorbate or ferricyanide (*cf.* ref. [16]). The pathway of electrons during oxidation and that during reduction of lipophilic mediators as DAD and DCPIP are illustrated in Fig. 9. The mediator which is simultaneously present in its oxidized and reduced form in each of the half-cells should induce cyclic electron flow around photosystem I [16] via the reactions indicated by k_{ox_i} and k_{red_i} . This would reduce the effectiveness of the anticipated cell.

On the effectiveness of electron transfer between chloroplasts and mediators in the half-cells

The over-all reduction or oxidation rate of the mediator as well as the final level of the redox potential is determined by the absolute and the relative rates of the reactions indicated in Fig. 9. The oxidation of a mediator by photosystem I (k_{ox}) includes a transfer step through the thylakoid membrane. Therefore, hydrophilic mediators as DCPIP sulfonate which can hardly penetrate the membrane are not oxidized [22] (*cf.* Fig. 5).

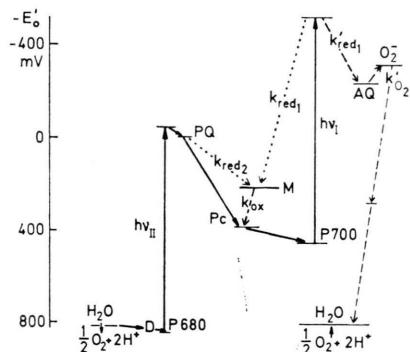


Fig. 9. Z-scheme of electron transport with simplified reactions of artificial electron acceptors and donors. Dotted arrows indicate pathways of electrons during reduction, dashed arrows during oxidation of the mediator. For further details see text. Abbreviations: AQ, derivate of anthraquinone; D, donor of photosystem II; M, redox mediator; PC, plastocyanin; PQ, plastoquinone.

electron acceptor. A slow diffusion of the mediator

In the half-cell containing chloroplasts with intact water splitting system this reoxidation by photosystem I introduces a wasteful cyclic electron flow and should diminish the reduction of the mediator. For this reason DCPIP sulfonate was expected to be reduced faster and to reach more negative potentials than DCPIP. However, DCPIP sulfonate exhibited similar rates and the decrease of the potential stopped at values which were 40 mV higher compared to DCPIP (Fig. 2). This indicates that the reoxidation (k_{ox}) at least of DCPIPH₂ was of negligible influence on its reduction. At a highly reduced level of plastocyanin and P700 the reoxidation should be prevented. This would be possible if the electron transfer to the mediator becomes rate-limiting at low concentrations of the oxidized form. The redox potential of the three mediators decreased during their reduction shown in Fig. 2 not more than 20–40 mV below their half-reduction potential. This is not fully understood because losses of electrons, *e.g.* by autoxidation of the mediators, exhibited negligible rates. Much more negative potentials than in Fig. 2 should be attainable in this half-cell. The use of mediators of lower half-reduction potential seems to be promising. However, most of these are not appropriate because of their fast reaction with oxygen [40].

In the half-cell containing chloroplasts with inactivated water splitting system the thylakoid membrane may not only prevent the oxidation of DCPIPH₂ sulfonate but also limit the oxidation rate of DAD and DCPIPH₂ by functioning as a dif-

fusion barrier. This seems to be indicated by the increased oxidation rates of DAD observed with photosystem I particles compared to chloroplasts in Fig. 6 and 5, resp. However, an increased rate could also be due to the high concentration of exogenous plastocyanin and a higher concentration of P700 at equal concentration of chlorophyll. The present experiments do not allow a discrimination between these effects.

To minimize cyclic electron transport in this half-cell the reduction rate of the electron acceptor (k_{red_1}') and the rate of its autoxidation (k_{o_2}') should be as high as possible compared to the competing reduction of the mediator (k_{red_1}). Ke [42] reported an increase of the reduction rate by photosystem I with increasing half-reduction potential of artificial electron acceptors. This could explain the faster oxidation of DAD in the presence of AQ sulfonate ($E_{m7} = -226$ mV) than methylviologen ($E_{m7} = -445$ mV) in Fig. 4, although reduced methylviologen exhibits one of the fastest autoxidations known for a radical [43]. The negligible oxidation rate of DAD in the presence of ferredoxin may be due to a limiting autoxidation rate.

Independent of cyclic electron transport any electron transferred to the electron acceptor is irreversibly removed by its autoxidation. This results in an almost complete oxidation of the photosynthetic electron carriers as well as an extremely low concentration of the reduced mediator, indicated by the final redox potential of 440–450 mV. The final potential did not depend on the type of the autoxidizable electron acceptor (Fig. 4), of the mediator and of the chloroplast preparation (Figs 5 and 6). It seems to be determined by the endogenous electron donors of photosystem I. At a potential of 450 mV 97 per cent of plastocyanin are oxidized [44].

On the junction of the combined half-cells

The junction of the cell has to be permeable for ions as well as for protons to allow a current through the cell (*cf.* Fig. 1). Therefore we have separated the half-cells by a membrane for ultrafiltration. Furthermore a relatively high ionic strength of approx. 0.1 M was used. Chloroplasts, particles and proteins in the half-cells are quantitatively separated. However, a disadvantage of the membrane is its permeability for the oxidized and reduced mediator, inhibitors and the autoxidizable

would not change the asymmetrical function of the photosystems in the two half-cells which is prerequisite for the proposed galvanic cell. This is not valid for the autoxidizable electron acceptor and inhibitors. Soluble inhibitors as 3-(3',4'-dichlorophenyl)-1,1-dimethylurea (DCMU) would inactivate the electron transfer in the other half-cell. This is avoided by special treatments of the chloroplasts, reviewed in ref. [45]. An autoxidizable electron acceptor diminishes the reduced level of the mediator in the half-cell containing chloroplasts with intact water splitting system as demonstrated in Fig. 3 A. Ferredoxin is excluded by the membrane. But for sufficiently high oxidation rates of the mediator (Fig. 4) the synthesis of a rapidly autoxidizable electron acceptor of high molecular weight, as the ester of anthraquinone-2-carboxylic acid with polyethylene glycol, was prerequisite.

Further aspects of a galvanic cell driven by chloroplasts

In addition to the investigated reactions a further possibility exists for each of the two half-cells with an equal over-all reaction. In the half-cell with light-induced reduction of the mediator the electrons could be supplied by photosystem II alone after inactivation of photosystem I, *e. g.* by KCN treatment [37]. Potentials below that of the primary electron acceptor of photosystem II near 0 V cannot be expected. It may be of advantage that an autoxidizable electron acceptor with more negative redox potential could not compete with the mediator for electrons. The further possibility for the oxidation of a mediator is by photosystem II after inactivation of the water splitting system [34, 46], *e. g.* by heat-treatment. This offers the advantage to reach potentials near that of the endogenous electron donor of photosystem II of about 0.8 V. If a half-cell with such a system is connected to a half-cell with reduction of a mediator by photosystem I a potential difference above 1 V can be expected. Two or more mediators with different half-reduction potentials

have to be used. This may also be of advantage for greater potential differences between the investigated half-cells.

Photosynthetic electron transport can be coupled to a hydrogenase reaction for light-driven hydrogen evolution [47–50]. A major problem of this reaction is to utilize the electrons from water at simultaneous oxygen evolution. It is essential for their activity to keep the oxygen and hydrogen evolving system apart from each other [47, 6]. This may be realized by a modification of the cell in Fig. 1 if in the right-hand half-cell the electrons are transferred to H^+ via ferredoxin and hydrogenase instead to oxygen. A diffusion of oxygen through the junction could be prevented by glucose oxidase and catalase captured between two membranes in place of the one shown in Fig. 1.

The interaction between mediator and electrodes becomes of great importance at a current through the cell. However, the construction of the used cell was not optimal for current measurements, which will be subject of a following paper. The monitored conversion of the redox gradient in the chloroplasts to that of artificial mediators corresponds to a light-driven charging of a battery. The rates of charging were as high as photosynthetic electron transport rates. Leaks of the charged system are only diffusion of the oxidized and reduced mediator through the membrane and autoxidation of the reduced mediator. Both turned out to be negligible. Thus, in the dark the stored light-energy is still available. Compared to systems with purified chlorophyll chloroplasts are easier isolated and not sensitive to oxygen. Other advantages are the optimal action at rather low light intensities because of the light-harvesting pigments and the effective energy transfer to the photosynthetic reaction centers.

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